INVESTIGATING THE FEASIBILITY OF A PUBLIC HEALTH SYSTEM CARRIER-SCREENING PROGRAM IN WESTERN AUSTRALIA

Royston Ong

20322751

BSc (Molecular Biology and Biotechnology)

BScHons (Pathology)

THE UNIVERSITY OF WESTERN AUSTRALIA

This thesis is presented for the degree of

Doctor of Philosophy

The University of Western Australia

School of Medicine

2021

THESIS DECLARATION

I, Royston Ong, certify that:

This thesis has been substantially accomplished during enrolment in this degree. This thesis does not contain material which has been submitted for the award of any other degree or diploma in my name, in any university or other tertiary institutions.

In the future, no part of this thesis will be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Western Australia and where applicable, any partner institution responsible for the joint-award of this degree.

This thesis does not contain any material previously published or written by another person, except where due reference has been made in the text and, where relevant, in the Authorship Declaration that follows. This thesis does not violate or infringe any copyright, trademark, patent, or other rights whatsoever of any person. This thesis contains published work and work prepared for publication, some of which has been co-authored.

The research involving human data reported in this thesis was assessed and approved by The University of Western Australia Human Research Ethics Committee (approval #RA/4/2/04258). Written patient consent has been received and archived for the research involving patient data reported in this thesis.

Approval by the Women and Newborn Health Service Human Research Ethics Committee at King Edward Memorial Hospital for Women was obtained prior to commencing the relevant work described in this thesis (Approval number: RGS0000000946).

This thesis contains published work and/or work prepared for publication, some of which has been co-authored.

Royston Jackson-Ong

Date: 03/04/2021

DEDICATION

I dedicate this thesis to my wife, Rebecca Jackson-Ong, who stood by me throughout this journey in all my absences, my fits of pique and impatience. Your wit, humour and pearls of wisdom, made long nights easier and tough times shorter. Your unwavering support allowed me to focus and complete this Thesis without much worry as you shouldered the larger responsibility of looking after our babies, Tilly and Arthur.

For everything and more – thank you love.

ACKNOWLEDGEMENTS

Royston Ong was supported by an Australian Government Research Training Program (RTP) Scholarship and an Australian Genomics Health Alliance PhD top-up award (GNT1113531). I would like to thank Australian Genomics Health Alliance for their generous support of this work and myself over the last few years. Australian Genomics Health Alliance's aim to address issues around genomic medicine is truly inspiring and will undoubtedly play an important role in driving genomic health implementation and policies, nationally and internationally.

Undertaking this PhD has been a truly life-changing experience. As I wrap up my Thesis, I reflected in awe of the privilege to have worked alongside some of the most brilliant minds. This PhD would not have been possible without the support and guidance from these wonderful people. I am very appreciative to have this opportunity to thank you all.

To all my incredible supervisors – thank you for providing me with a once in a lifetime opportunity to make such a big difference to so many lives. Nigel and Gina, you have both gone over and beyond your call of duty for your students. Words do no justice to everything both of you have done for me and for the project. Both of you have spent much of your personal time over the last 10 months meeting every Saturday to touch base and to spur me on. For that – I am extremely grateful and thankful. I hope this work reflects well on all your efforts.

To my PhD team mates, Samantha and Georgie. Both of you came when the project was in its infancy and nurture it to what it is today. Your hard work, dedication and expertise are truly admirable. This PhD is mine as much as it is yours. Thank you.

To my extended PhD team mates, the "coalition of the willing"; the PathWest team – John, Mark, Michael, Richard, Fathin and Grace as well as the team over at Genetic Services of WA at King Edward Memorial Hospital – Nicholas, Benjamin and Karen, thank you all. This project enjoyed its successes because of all your commitment, expertise and counsel. To Mark, thank you for being my unofficial supervisor from another organisation – your tough love is well loved.

To the general practitioners in the Metro and Busselton area, thank you for supporting this project and for believing in us when many were sceptics.

To my Laing lab "family" for the past decade, thank you. Every one of you have left your mark on me: be it words of encouragement, advise or just bouncing ideas around, I don't think it's easy to find such fine company again. Special thanks to Tina who helped organise everything for the project, you have been indispensable throughout this journey and I don't think I have said "thank you" enough. To Elyshia, thanks for being such a great lab partner for many years. I still think back to those cathartic "harvesting" sessions with fondness. To my fellow PhD students Hayley and Sarah, thanks for the *fantastic* company and never-ending cynicism. To Denise Howting, thank you for all your support, kind words and patience as I trod on unfamiliar statistical grounds. To Josh and Rhonda, thanks for always being there when I needed an ear, and for asking how I am. To Emma, Georgina, Joey and Carolin, thanks for the great company and all-round fun. To the one and only Gwen – you will always have a special place amongst us. Thank you for everything. Each and every one of you have made my scientific journey very special.

To my family in Singapore – thank you for stepping up wherever and whenever possible. From flying over at short notice so you can help us with the kids, to the late-night chats and company, words cannot express my gratitude. It can sometimes be a thankless job but I'm grateful everyone was part of the journey with me.

To my Jackson family, thank you for being there whenever we needed a place or someone to distract the kids.

Second to last, to my two beautiful children – Tilly and Arthur, you will probably never read this but know that your constant giggles, smiles and laughter filled the house and my soul. You little monsters made completing this journey possible. *Thank you for filling my cup every day*.

And finally, to my beautiful and amazing wife – Rebecca, thank you for being my mountain and muse. I cannot ask for a better partner in crime.

PUBLICATIONS RESULTING FROM THIS RESEARCH TO DATE

THE CONVERSATION ARTICLES:

Royston Ong, Gina Ravenscroft, Nigel Laing. **Explainer: What is pre-pregnancy carrier screening and should potential parents consider it?** 2017, The Conversation. URL: https://theconversation.com/explainer-what-is-pre-pregnancy-carrier-screening-and-should-potential-parents-consider-it-79184

Royston Ong, Michelle Farrar, Gina Ravenscroft, Nigel Laing. **What prospective parents need to know about gene tests such as 'prepair'.** 2017, The Conversation. URL:

https://theconversation.com/what-prospective-parents-need-to-know-about-gene-tests-such-as-prepair-87083

PUBLISHED ARTICLES:

Royston Ong, Denise Howting, Alethea Rea, Hayley Christian, Pauline Charman, Caron Molster, Gianina Ravenscroft, Nigel George Laing. **Measuring the impact of genetic knowledge on intentions and attitudes of the community towards expanded preconception carrier screening.** J Med Genet. 2018;55(11):744-752.

Royston Ong, Samantha Edwards, Denise Howting, Benjamin Kamien, Karen Harrop, Gianina Ravenscroft, Mark Davis, Michael Fietz, Nicholas Pachter, John Beilby, Nigel Laing. **Study protocol of a multicentre cohort pilot study implementing an expanded preconception carrier-screening programme in metropolitan and regional Western Australia.** BMJ Open. 2019;9(6):e028209

Kirk EP, **Ong R,** Boggs K, Hardy T, Righetti S, Kamien B, Roscioli T, Amor DJ, Bakshi M, Chung CWT, Colley A, Jamieson RV, Liebelt J, Ma A, Pachter N, Rajagopalan S, Ravine A, Wilson M, Caruana J, Casella R, Davis M, Edwards S, Archibald A, McGaughran J, Newson AJ, Laing NG, Delatycki MB. **Gene selection for the Australian Reproductive Genetic Carrier Screening Project ("Mackenzie's Mission")**. Eur J Hum Genet 2021;29(1):79-87

AUTHORSHIP DECLARATION: CO-AUTHORED PUBLICATIONS

Publication 1:

Details of the work: **Explainer: What is pre-pregnancy carrier screening and should potential parents consider it?**

Authors: Royston Ong, Gina Ravenscroft, Nigel Laing

Citation: Royston Ong, Gina Ravenscroft, Nigel Laing. Explainer: What is pre-pregnancy carrier screening and should potential parents consider it? 2017, The Conversation. URL: https://theconversation.com/explainer-what-is-pre-pregnancy-carrier-screening-and-should-potential-parents-consider-it-79184

Location in the thesis: Chapter One, Chapter Five

Student contribution to work: Conceived and co-designed the publication. Drafted and edited the publication.

Publication 2:

Details of the work: **What prospective parents need to know about gene tests such as 'prepair'.**

Authors: Royston Ong, Michelle Farrar, Gina Ravenscroft, Nigel Laing

Citation: Royston Ong, Michelle Farrar, Gina Ravenscroft, Nigel Laing. What prospective parents need to know about gene tests such as 'prepair'. 2017, The Conversation. URL:

https://theconversation.com/what-prospective-parents-need-to-know-about-gene-tests-such-as-prepair-87083

Location in the thesis: Chapter Five

Student contribution to work: Conceived and co-designed the publication. Drafted and edited the publication.

Publication 3:

Details of the work: **Measuring the impact of genetic knowledge on intentions and attitudes of the community towards expanded preconception carrier screening**

Authors: Royston Ong, Denise Howting, Alethea Rea, Hayley Christian, Pauline Charman, Caron Molster, Gianina Ravenscroft, Nigel George Laing

Citation: Royston Ong, Denise Howting, Alethea Rea, Hayley Christian, Pauline Charman, Caron Molster, Gianina Ravenscroft, Nigel George Laing. Measuring the impact of genetic knowledge on intentions and attitudes of the community towards expanded preconception carrier screening. J Med Genet. 2018;55(11):744-752.

Location in the thesis: Chapter Two

Student contribution to work: Conceived study and designed survey questions, collected and performed data analysis on survey results from more than 800 people. Drafted and edited the manuscript.

Publication 4:

Details of the work: **Positive attitude of Australian health professionals towards carrier-screening**

Authors: Royston Ong, Denise Howting, Samantha Edwards, Georgina Hollingsworth, Alethea Rea, Hayley Christian, Gianina Ravenscroft, Nigel George Laing

This manuscript prepared for submission

Location in the thesis: Chapter Two

Student contribution to work: Conceived study and designed survey questions, collected and performed data analysis on survey results from more than 200 health professionals. Drafted and edited the manuscript.

Publication 5:

Details of the work: **Study protocol of a multicentre cohort pilot study implementing an expanded preconception carrier-screening programme in metropolitan and regional Western Australia**

Authors: Royston Ong, Samantha Edwards, Denise Howting, Benjamin Kamien, Karen Harrop, Gianina Ravenscroft, Mark Davis, Michael Fietz, Nicholas Pachter, John Beilby, Nigel Laing

Citation: Royston Ong, Samantha Edwards, Denise Howting, Benjamin Kamien, Karen Harrop, Gianina Ravenscroft, Mark Davis, Michael Fietz, Nicholas Pachter, John Beilby, Nigel Laing. Study protocol of a multicentre cohort pilot study implementing an expanded preconception carrier-screening prgramme in metropolitan and regional Western Australia. BMJ Open. 2019;9(6):e028209

Location in the thesis: Chapter Three

Student contribution to work: Conceived and co-designed the study. Drafted and edited the manuscript.

Publication 6:

Details of the work: **Offering an expanded couple-based carrier screening test through the public health system: The Western Australia experience**

Authors: Royston Ong, Samantha Edwards, Georgina Hollingsworth, Benjamin Kamien, Michael Fietz, Karen Harrop, Richard Allcock, Grace Androga, John Beilby, Nicholas Pachter, Mark Davis, Gianina Ravenscroft, Nigel George Laing

This manuscript prepared for submission

Location in the thesis: Chapter Four

Student contribution to work: Conceived and co-designed the study. Collected and performed data analysis on survey results. Drafted and edited the manuscript.

Publication 7:

Details of the work: **Gene selection for the Australian Reproductive Genetic Carrier Screening Project ("Mackenzie's Mission")**

Authors: Edwin P. Kirk, Royston Ong, Kirsten Boggs, Tristan Hardy, Sarah Righetti, Ben Kamien, Tony Roscioli, David J. Amor, Madhura Bakshi, Clara W. T. Chung, Alison Colley, Robyn V. Jamieson, Jan Liebelt, Alan Ma, Nicholas Pachter, Sulekha Rajagopalan, Anja Ravine, Meredith Wilson, Jade Caruana, Rachael Casella, Mark Davis, Samantha Edwards, Alison Archibald, Julie McGaughran, Ainsley J. Newson, Nigel G. Laing & Martin B. Delatycki

Citation: Kirk, E.P., Ong, R., Boggs, K. et al. Gene selection for the Australian Reproductive Genetic Carrier Screening Project ("Mackenzie's Mission"). Eur J Hum Genet 29, 79–87 (2021).

Location in the thesis: Chapter Five

Student contribution to work: Collected and performed data analysis. Edited the manuscript.

Student Signature:

Date: 03/04/2021

I, Nigel Laing certify that the student's statements regarding their contribution to each of the works listed above are correct.

As all co-authors' signatures could not be obtained, I hereby authorise inclusion of the co-authored work in the thesis.

Coordinating supervisor signature:

Date: 03/04/2021

ABSTRACT

Autosomal recessive disorders cause a significant proportion of infant and childhood morbidity and mortality. Studies have suggested that on average everyone is a carrier of more than one autosomal recessive disorder. However, most couples who have a child with a recessive disorder did not know that they were at risk of having that affected child.

Carrier-screening can inform couples of their risk of having a child affected with a recessive disorder before they become pregnant. This in turn provides these couples the best opportunity to consider their reproductive options.

In the past, carrier-screening has been offered for disorders prevalent in specific populations such as Tay-Sachs disease in the Ashkenazi population or thalassemia in Greece and Cyprus. Countries such as Israel now offer nation-wide carrier-screening programs while others such as The Netherlands have explored offering carrier-screening to their communities through their health systems. There is, however, no publicly funded nationwide carrier-screening program in Australia. Therefore, I wished to investigate how carrier-screening could be implemented in Western Australia using existing components of the Western Australian public health system.

To achieve this, I first researched the appetite for carrier-screening using questionnaires in the general population (Chapter 2, Section 1; PMID:30068663) and amongst health professionals (Chapter 2, Section 2) in Western Australia. I then designed a protocol by which carrier-screening could be implemented using existing components of the Western Australian public health system (Chapter 3; PMID:31209093). The protocol included all the methodology to offer this test such as counselling methods, targeted gene panel design, sequencing technology and post-test counselling. The protocol proposed to use couple-based screening for 474 genes associated with 420 severe recessive disorders. Recruitment

took place through selected general practices, in metropolitan Perth and the regional town of Busselton, a private genetic counselling clinic and through the Health Department genetics unit: Genetic Services Western Australia. Laboratory testing was performed by the State Health Department pathology service, PathWest. Genetic Services Western Australia counselled the high-risk couples.

The questionnaire results showed that more than two-thirds of both groups, the general population of Western Australia and West Australian health professionals, would use carrier-screening if it was available (Chapter 2). I found that genetic knowledge and attitude were key factors that influenced the public's intentions to use carrier-screening, whereas attitude was the key factor that influenced health professionals' intentions. In both groups, there was a strong preference to screen for disorders reducing the lifespan of children and infants. When asked who they would prefer accessing the test from, 80% of both groups wanted to access the test through general practitioners, however, more than 80% of health professionals would also access the test through a genetic counsellor. Many health professionals expressed concerns about discrimination, confidentiality issues and potentially doing more harm than good.

Subsequently, I ran a pilot study aiming to recruit 250 couples over a two-year period (Chapter 4). By the end of the study, 231 couples were recruited and 225 screened. Analysis indicated that implementing couple-based carrier-screening utilising components of our State's health system was effective. Results showed that 75% of samples were received within two weeks of recruitment and that 6.2% of couples fell pregnant after providing samples. Seven novel high-risk couples were identified, indicating that 1 in 32 couples in the cohort are at increased risk of having a child affected by one of the severe disorders screened for. Of the seven at high-risk couples, six women had an X-linked pathogenic variant. Three high-risk couples had pathogenic variants in genes that cause very rare disorders.

My study has identified a strong appetite for carrier-screening amongst the public and health professionals in Western Australia. The couple-based screening streamlined the workflow and decreased counselling workload compared to individual carrier testing. The study showed that carrier-screening can be provided through existing components of the public health system in Western Australia. My pilot data suggests that high-risk carrier couples are perhaps more common than previously appreciated.

This was the first trial of universal expanded carrier-screening in the Australian public health system. It provided key information for the succeeding Mackenzie's Mission, the current national Australian Reproductive Carrier-Screening project.

TABLE OF CONTENTS

THESIS STRUCTURE

Each chapter is written in a manuscript style consisting of *Introduction*, *Methods*, *Results*, *Discussion* and *References* sections.

The supplementary information for each of the chapters is included in the Appendix after the final chapter.

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.0 Mendelian Disorders

1.1 What is a Mendelian disorder?

It is estimated that there are currently more than 4,000 known Mendelian disorders[1]. Mendelian disorders are also known as single-gene disorders where changes in one protein coding gene can cause a disorder, for example cystic fibrosis (MIM# 219700). Further, many of these Mendelian disorders are considered rare or very rare. Definitions for rare disorders differ from country to country. For example, in Europe and Australia, a disorder is considered rare when it affects 1 person per 2000[2,3]. Whereas, a disorder is defined as rare when it affects less than 200,000 people in the United States[4].

Mendelian disorders are most often inherited in dominant, recessive, or X-linked manner. Mendelian disorders may also be inherited from mitochondrial DNA, showing maternal inheritance, or arise *de novo* from new mutational events. In dominantly inherited disorders, an individual who inherits one pathogenic variant develops the disorder, e.g. Huntington Disease (MIM #143100)[5]. However, if the dominant disorder has reduced penetrance, such as in amyotrophic lateral sclerosis type 1(MIM# 105400), caused by pathogenic variants in the gene *SOD1*[6], an individual may inherit the family pathogenic variant and never have the disorder.

In contrast, manifestation of recessive disorders usually arises from the inheritance of pathogenic variants in the same gene from both carrier parents, e.g. spinal muscular atrophy type 1 (MIM# 253300)[7] or nemaline myopathy 8 (MIM# 615348)[8]. On rare occasions in recessive disorders, one

variant may be inherited and the other arise *de novo*. A "carrier" of a recessive disorder is an asymptomatic individual who has generally inherited a pathogenic variant from one parent, though, again that variant may have arisen *de novo*.

From the above, *de novo* pathogenic variants may result in either dominant or recessive disorders. They are more common in some disorders than others. For example, they are particularly common in Duchenne muscular dystrophy (MIM# 310200). Due to high mutation rates, almost a third of all new cases of Duchenne muscular dystrophy are caused by *de novo* pathogenic variants[9].

Mendelian disorders may also be classified as either autosomal or X-linked[1]. Autosomal diseases are genetic disorders that arise through variants in chromosomes 1 to 22 [10-12], while X-linked disorders are genetic disorders that occur through variants in the X-chromosome (e.g., Duchenne muscular dystrophy[13] MIM# 310200).

The severity and age of onset of Mendelian disorders vary widely, from *in-utero* lethal disorders (e.g. lethal congenital contracture syndrome 9[14] MIM# 616503), through comparatively milder disorders such as nonsyndromic deafness[15] (MIM# 601072) to adult onset neurodegenerative disorders[5,6,16].

Although most Mendelian disorders are generally caused by one (dominant disorder or homozygous recessive disorder), or two pathogenic variants in a gene (compound heterozygous recessive disorder), the disorders themselves are far from straightforward to manage. For example, diagnosis of a

Mendelian disorder can be complicated by factors such as "allelic" or "genetic" heterogeneity.

Pathogenic variants in one gene causing different disorders is known as allelic heterogeneity. For example, different variants in the gene *LMNA* (MIM# 150330) may cause more than ten different disorders, including either dominant[17] or recessive inheritance[18]. When disorders with similar phenotypes result from variants in different genes, this is known as "genetic heterogeneity". An example is the Charcot-Marie-Tooth family of peripheral neuropathies which are currently associated with variants in close to 100 genes [19-22].

1.2 Identification of Mendelian disorder genes

The first human disorder genes identified were found through extensive knowledge of the disorder biology, usually including the protein involved in a process labelled "functional cloning"[23]. During the 80s and 90s, linkage studies combined with "walking" along chromosomes was used to find some disorder genes in a process called "positional cloning"[23]. Positional cloning was however very labour intensive and time consuming and was rapidly superseded by "positional candidate cloning" where disorder genes were identified by linkage combined with analysis of candidate genes within the linkage region[24]. Candidate gene cloning was achieved by analysing a number of genes when there was some knowledge of the disorder pathobiology but insufficient knowledge to pinpoint the precise gene[23].

Advances in Next Generation Sequencing (NGS) technologies in the last decade or so [25,26], have provided powerful new routes to disorder gene

discovery. Unlike previous methods, NGS methods require very little to no prior knowledge about the disorder's genetic loci or the gene or protein affected. They, in reality, make every gene in the genome a candidate gene [27-29]. However, when combined with traditional gene discovery methods such as linkage analysis, NGS rapidly became a very powerful method to identify novel disorder-causing genes[8,30-33]. Between 2013 and 2015, NGS methods identified almost three times as many novel disease-causing genes compared to traditional methods[1].

1.3 Diagnosis of Mendelian disorders

In addition to discovering many novel disorder-causing genes, NGS technologies have revolutionised the diagnosis of Mendelian disorders[26,34,35]. Diagnosis is defined here as discerning the specific molecular cause that explains the clinical features of the disorder in the patient[36].

It has long been appreciated that having an accurate diagnosis is a watershed moment for families and patients confronted with a myriad of challenges. These challenges may exist due to a lack of scientific knowledge and disorder information, or misdiagnosis due to unknown aetiology[37,38].

An accurate molecular diagnosis provides families and patients with targeted treatment, or therapies if they are available for the disorder, and disorder-appropriate management, including accurate genetic counselling. A molecular diagnosis can also lead to access to disorder-specific support groups which helps reduce feelings of isolation[39].

However, accurate diagnosis is often hampered by limited clinical knowledge[36,40], types of pathogenic variations, such as single nucleotide changes, or copy number variations, or the above mentioned genetic and allelic heterogeneity. For example, Laing distal myopathy (MIM # 160500) is often diagnosed as a peripheral neuropathy since Laing distal myopathy is rare and peripheral neuropathy is common[41].

Prior to the advent of NGS, diagnosis of Mendelian disorders was largely driven by specific disorder phenotypes[34]. Traditional methods of providing molecular diagnosis such as Sanger sequencing, whilst effective and highly accurate, were not cost-effective at scale. Furthermore, traditional methods of molecular testing were most sensitive when testing for highly distinct disorders caused by one or a few genes[36]. Testing for new genes or even potentially new pathogenic variations quickly becomes a very time-consuming and costly affair using Sanger sequencing. Thus, in the past, many rare disorders were even more underdiagnosed than they are now.

NGS technology provided a lifeline which afforded scalability and an agnostic approach that allowed diagnoses to be made for rare and ultra-rare disorders[34,36]. As part of the NGS diagnostic revolution, NGS technologies saw the transition towards a "genotype-driven" approach as opposed to the typical "phenotype-driven" method to identify causative pathogenic variants. This in turn led to an expansion in the number of genotype-phenotype relationships of many disorder genes being described[42-45]. This has provided molecular diagnosis for many previously unresolved rare and disparate Mendelian disorders.

For example, most distal arthrogryposis (DA) shows dominant inheritance [46-48]. In 2013, pathogenic variants in the gene *ECEL1* were shown to cause a new type of DA which showed recessive inheritance [49,50]. Following that, in 2014, Barnett *et al.* described two affected siblings in which compound heterozygous *ECEL1* pathogenic variants were identified to cause the disorder[51]. Both affected siblings presented with novel clinical features not previously observed. This is just one example that illustrates how NGS has become a powerful tool in the search for answers for patients and their families.

1.4 Treatment of Mendelian Disorders

Despite the successes of identifying new disorder-causing genes and expansion of knowledge on rare disorders, the rate of developing treatments for Mendelian disorders has not kept pace. It is estimated that only 5% of all Mendelian disorders have an effective treatment [52]. The lengthy development time of a drug is one issue. On average, it takes about a decade and USD2.5B to bring a new drug to market[52]. Further, the process of developing drugs is often limited by constraints such as having only a handful of available trial participants, the time required to increase the knowledge base for the disorder and development and identification of outcome measures [52]. All of this drives up costs. As a result, many academic and industry researchers tend to place their focus on more common disorders with a higher potential for return-on-investment, such as Duchenne muscular dystrophy or spinal muscular atrophy.

Furthermore, effective therapies once developed, are usually very expensive for the reasons described previously. For example, the drug Ataluren developed for Duchenne muscular dystrophy patients with nonsense mutations, costs around AU$356,000 annually per patient[53]. Similarly, the cost of treating one of the most common recessive disorders, spinal muscular atrophy, using the antisense oligonucleotide Nusinersen is approximately USD$750,000 for the first year and USD$350,000 per year thereafter[54].

Globally, few countries including Australia, have programs to subsidise or provide treatments for Mendelian disorders, at nominal or no out of pocket expense[55]. In Australia, the Pharmaceutical Benefits Scheme (PBS) began as a limited scheme in 1948, providing free medicines for pensioners and a list of 'life-saving and disorder preventing' medicines to the community[56]. The PBS scheme remains active and in 2018, the Federal Department of Health invested AUD$2.4 billion in new medicines, of which, $241.3 million was provided to list Nusinersen (Spinraza®) to treat Spinal Muscular Atrophy[57] in the PBS for four years. Thus, Nusinersen treatment for just one rare disorder for four years, was 10% of the increased expenditure on pharmaceuticals by Australia in 2018.

As well as the expense, several other issues complicate the benefits of treatments.

Firstly, the effectiveness of treatments varies between disorders and may also be restricted to specific pathogenic variants [58]. Secondly, whilst treatment may avert a catastrophic early outcome, previously unrecognized morbidities have been uncovered and long-term health outcomes do not necessarily

improve quality of life[58]. For example, the drug nitisinone is administered for the treatment of hereditary tyrosinemia type 1 (HT1, OMIM# 276700)[59]. The treatment reduces toxic metabolites production thereby preventing liver failure in most cases. However, the residual risk of developing liver complications such as hepatocellular carcinoma remains and affects some children. Further, long-term management of HT1 requires substantial dietary modifications, with frequent monitoring and tailoring of care[59], resulting in reduced adherence to dietary requirements, which eventually impacts on health outcomes[58].

Geographical limitation and isolation may further compound the issue of access to treatments. This is certainly the case for Western Australia with a land mass of 2.5 million square kilometres. Limited access to health facilities in regional and rural communities is a known problem in Australia[60].

As research and development into treatments continue, early identification of couples at high risk of having an affected child with a Mendelian disorder, provides the opportunity to remove some of the emotional and psychological burden from these families. In order to achieve this, the molecular basis of the disorder in the family needs to be known.

2.0 Genetic carrier-screening

Daphne Esquivel-Sada and Minh Thu Nguyen recently demonstrated that receiving an accurate diagnosis had a profound impact on patients medically, socially and personally[37]. In particular, adult and parent participants stressed the importance of the diagnosis for life-planning such as making reproductive decisions. In fact,

many of these participants viewed informing their extended family of their genetic result as a way of helping relatives with their own family planning decisions.

The limited number of treatments for Mendelian disorders means among other things, that an accurate diagnosis is important to detect family members who are carriers of a family recessive disorder, and therefore at risk of having a child affected with the recessive disorder.

The identification of couples in the general population at high risk of having an affected child with a recessive disorder can be achieved through carrier-screening. Genetic carrier-screening thus allows couples with no family history to know their risk of having a child with a recessive disorder before they have children[61,62].

2.1 Global Carrier-Screening Programs – Past, Present and the Future

2.1.1 Pioneering carrier-screening programs

According to Antonarkis' 2019 review, historically, carrier-screening for recessive disorders began with a program screening for sickle cell anaemia performed in a village in Greece by George Stamatoyannopoulos starting in 1966[63].

Thereafter, one of the larger early programs was for the autosomal recessive lysosomal storage disorder Tay-Sachs disease (MIM# 272800). It was first discovered in 1969 that biochemical deficiency of the enzyme hydrolase β-hexosaminidase could cause Tay-Sachs disease[64]. Accurate and affordable methods were quickly developed to rapidly diagnose Tay-Sachs disease and to differentiate between those affected by Tay-Sachs disease and carriers of the disease[65]. These methods allowed provision of prenatal

diagnosis[66]. The test cost then was about $50 (about AUD450 in 2020 terms), making it relatively affordable.

These rapid advancements paved the way for the first multiphasic pilot program designed to prevent Tay-Sachs disease in Ashkenazi Jewish communities in the Baltimore-Washington area starting in 1970[67]. The program comprised a framework of:

1) a public education component
2) a voluntary and affordable carrier-screening test
3) provision of genetic counselling to at risk couples

Similar programs were then initiated in other parts of the world to screen for Tay-Sachs disease. As a result of implementing this test in seven countries, including Australia, the incidence of Tay-Sachs disease within the Ashkenazi Jewish communities in these countries combined, was reduced by more than 90% between 1974 to 1993[68]. Prior to the screening program, the incidence of TSD, in the general population amongst the seven countries mentioned above, was 1:4,000[68].

A similar framework would again prove effective when Mediterranean countries introduced carrier-screening for β-thalassaemia (MIM# 613985) by haemoglobin electrophoresis, as part of their national health programs in the mid-1970s[69-71]. These screening programs may be viewed as highly successful, as they led to a dramatic decrease in births of affected individuals with β-thalassaemia around the region, especially in Cyprus[72]. The situation was particularly critical in Cyprus with a significant proportion of the population being affected by β-thalassaemia. In a 1986 World Health

Organisation meeting memorandum, recommendations for a carrier-screening program were made in hopes of not only reducing the number of affected children, but also with reducing the costs of treatment for thalassaemia for their health departments, which threatened to consume a large part of the available health budget[73].

An affordable and accurate diagnostic test capable of identifying carriers in a well-considered community program underscore the requirements for a successful screening program. Lessons learnt from the failed sickle cell screening program introduced in 1972 in the United States [74] further highlighted the importance of community education, proper and consistent messaging and an appropriate and efficient triage system for identified carriers and at risk couples.

A lack of forethought on implementation and messaging of the sickle cell screening program left many in the African American community confused about health risks in the 1970s[75]. As a result, a high level of mistrust developed amongst the African American community of the underlying intentions of screening. In addition, many in the community felt forced to undergo testing and experienced employment and health insurance discrimination[76]. Even after 20 years, only 16% of individuals were aware of their sickle cell status[77].

From the above, screening programs are more likely to be successful if they are designed in consultation with the community, even for very religious communities with specific requirements. For example, the not-for-profit organization Dor Yeshorim was introduced to Orthodox Jewish communities

in the 1980s as a means to circumvent the tragedy of losing children to fatal but preventable disorders[78].

During the early days of the Tay-Sachs disease screening programs, the test was largely used during pregnancy or by married couples mostly because of convenience[68]. As a result, many ultraorthodox families perceived that the Tay-Sachs disease screening programs were focused on testing individuals who were already married, if not pregnant. For people who would not consider termination, the orthodox religious communities viewed these tests as inappropriate and therefore never got tested[79].

It took one determined ultraorthodox rabbi, and the loss of four of his own children to Tay-Sachs disease, to galvanize the idea of creating a screening program specifically for his very religious Jewish community. Thus, the Dor Yeshorim program was born in 1983. However, there were many objections to its initial proposal due to widespread fear of stigmatisation and issues with confidentiality of being identified as a carrier. The latter was most prominent amongst adolescent students since the initial proposal included screening of students in these communities who were usually married by age 20[79].

Continued dialogues and discussions with the grassroot leaders of the Ashkenazi community led to the success story of Dor Yeshorim[79]. These days, Dor Yeshorim screens for 10 recessive disorders in its Ashkenazi panel, and is offered strictly to couples with results indicating only whether they are compatible or not. When both members of the couple are carriers for the same recessive disorder, they are deemed incompatible and offered genetic counselling separately[78]. No individual results are provided and hence,

issues with stigmatisation and confidentiality about being a carrier are reduced.

2.2 Carrier-screening programs in the 21st century

2.2.1 Current screening programs around the world

Around the world today, many countries are offering screening for recessive disorders in one form or another. Globally, haemoglobinopathy has become the most frequently publicly funded recessive disorder being screened. At least ten countries now offer screening for haemoglobinopathy at different life stages, e.g., school years to antenatal screening[80,81]. Other than haemoglobinopathy, some countries have publicly funded screening programs for additional disorders. This includes Friedreich ataxia in Cyprus[82], and cystic fibrosis in certain regions in Italy[83] and in Scotland [84].

Meanwhile, many countries such as England continue to increase Mendelian disorders being offered to pockets of the community, e.g., Tay-Sachs disease or cystic fibrosis, based on ancestry or family history[80,81,85]. In a similar vein, the number of genes screened for in a publicly funded program tend to increase in countries where consanguinity is common. For example, the most comprehensive preconception carrier-screening program to-date is offered in Israel. Starting with the screening of Tay-Sachs disease amongst Ashkenazi-Jewish populations in 1980s, Israel expanded their national screening program in 2002 to include Arab and Druze communities[81]. The expanded national screening program screened for a handful of disorders with an incidence of 1 in 1000 or higher in these sub-populations[86]. The program now includes more

than 50 genes and has provided more than 900,000 carrier-screening tests to Israel's population of 9 million people between 2015 and 2017[87,88].

However, public screening programs, in general, although successfully implemented, have remained largely static despite recent advances in genetic screening technologies. Programs continue to screen only for a handful of disorders, offered to certain high-risk groups based on ancestry and family history. The very nature of recessive disorders means that couples without *a priori* risks will not know that they are carriers of the same recessive disorder. Thus, couples who neither have a family history, nor come from specific ancestries, have had to seek out alternative solutions offering carrier-screening services, such as commercial providers, in order to determine their risks of having an affected child.

2.2.2 Expanded carrier-screening programs and tests

In 2011, Bell *et al.* published a seminal study in which they explored the use of next generation sequencing (NGS) to screen a cohort of patients for 448 severe recessive disorders[89]. Screening for an increased number of genes at once has been termed "Expanded Carrier-Screening" or ECS.

In 2017, Chokoshvili *et al.* reported that as many as 16 providers offered carrier-screening tests to multiple countries – 13 commercial entities, 2 medical hospitals and 1 academic diagnostic laboratory[90]. Most of these providers operated out of the United States. Their report also highlighted the considerable discrepancies between the different providers, including that the tests screened between 41 and 1792 disorders[90]. Only cystic fibrosis,

maple syrup urine disease 1b (MIM # 248600), and Niemann–Pick disease (MIM # 607616, 257200) were consistently screened for by all 16 providers.

The rise in the number of expanded carrier-screening providers coincided with increase in interest of using ECS panels both within the community[91-98] and amongst healthcare professionals[99,100]. Research in different countries, indicated the level of interest was as high as 76%, with both communities having a positive attitude towards ECS[91,93,98].

Plantinga *et al.* showed that the sentiment of wanting to spare their children a life of a serious disorder was the most common in those who would use carrier-screening if it was available. In addition, their study found that younger generations were more often "undecided" and that religious individuals would more often choose to not use carrier-screening if offered[91].

More recently, some European countries have also begun exploring the possibility of offering carrier-screening to their populations on a larger scale[91,101,102]. For example, in the Netherlands, the University Medical Centre Groningen has explored offering carrier screening through selected general practitioners[103], and the University of Amsterdam Department of Clinical Genetics an ECS panel for 50 autosomal recessive disorders [81]. In the Netherlands, for some couples with high-risk because of consanguinity or ethnic background, the cost of carrier screening is part-reimbursed by health insurance [81].

In 2019, following the 2017 recommendations by the Superior Health Council of Belgium[101], all Belgian genetic clinics started to offer the Belgian Genetic Expanded Carrier-Screening (BeGECS) test to the Belgian community [104].

The BeGECS test panel screens more than 1,000 genes with a focus on both autosomal and X-linked recessive disorders.

3.0 International Guidelines

The rise in public interest in using expanded carrier-screening resulted in several recommendations on carrier-screening being published by professional organizations over the last few years. These recommendations addressed a wide range of topics and offered guidelines to countries or organisations considering providing carrier-screening services to the public.

Beginning in 2015, a joint statement between the American College of Medical Genetics and Genomics (ACMG), the American College of Obstetricians and Gynaecologists (ACOG), the National Society of Genetic Counsellors, the Perinatal Quality Foundation and the Society for Maternal-Foetal Medicine stated that 'women of reproductive age should ideally be offered carrier-screening before conception'[105].

Other key recommendations included:

1) screening tests had to be voluntary
2) consideration had to be given to defining "severity" of recessive disorders
3) the need for post-test genetic counselling for all carriers, especially if the patient is pregnant and lastly
4) considerations had to be given for guidelines for interpreting molecular findings.

This joint statement set the tone and approach for other recommendations that followed.

In 2016, the European Society of Human Genetics (ESHG) issued recommendations regarding the responsible implementation of ECS panels. Like the 2015 joint statement from the US, the ESHG recommendations reinforced the need for such tests to be voluntary and the need to focus on severe childhood-onset disorders. Further, the recommendation also emphasized that the primary objective of a program should be to increase reproductive autonomy. The recommendations highlighted key challenges when trying to implement a carrier-screening program for the community, such as dialogues, public education and the provision of an end-to-end service through an appropriate governance plan[106].

While reduced prevalence of the disorders screened for is likely to be an outcome of carrier-screening programs, the EHSG guidelines are clear that effectiveness of any carrier-screening programs should not be measured by reduced prevalence. However, measuring the level of reproductive autonomy remains challenging for purposes of calculating cost-effectiveness of a program.

In 2017, the American College of Obstetricians and Gynaecologists (ACOG) published *Committee Opinion 690* that clarified and updated key carrier-screening recommendations for Obstetricians and Gynaecologists wanting to offer ECS panels to their patients[107]. A key recommendation to clinicians was that "all carrier-screening tests are acceptable strategies, each obstetrician-gynaecologist or other health care provider or practice should establish a standard approach that is consistently offered to and discussed with each patient, ideally before pregnancy."

4.0 Implementing a universal carrier-screening program – Considerations

Implementing a universal carrier-screening program expands on socio-technological related issues that previous screening programs do not necessarily

deliberate, with some aspects coming under heavy scrutiny. For example, the advent of Next Generation Sequencing (NGS) technologies and the tendency to increase the number of genes and disorders being screened for has significant ethical and social implications. Further, increasing the number of genes being screened also increases difficulties in variant interpretation mostly due to Variants of Unknown Significance (VOUS), which inherently makes genetic counselling more challenging.

The section below discusses these challenging aspects of implementing a universal carrier-screening program.

4.1 Gene panel designs

Deciding on the number of disorders, and therefore genes, to include in a carrier-screening panel is currently under considerable debate[108-113]. Yet, current guidelines do not sufficiently address this issue mainly because considerations are multifaceted.

The common narrative for screening guidelines has focused on the application of the key principles of the Wilson and Jungner criteria published in 1968 under the auspices of the World Health Organisation[114]. However, strict application of the original Wilson and Jungner principles is problematic in relation to carrier-screening. This is because the criteria on which the original principles were based had not included all possible considerations required for carrier-screening today[38].

For example, adherence to the original Wilson and Jungner principles, because they include the necessity of a treatment being available, would only allow carrier-screening for recessive disorders for which there is a

treatment. As mentioned above, there are only a small proportion of known recessive genetic disorders which currently have treatments. Screening only for recessive genetic disorders for which there is a treatment would not provide information to a majority of couples at high risk of having a child affected with a recessive disorder. Since the original Wilson and Jungner publication, others, notably Andermann and colleagues[115,116], have suggested modified criteria by which proposed screening programs should be assessed, which are more relevant for screening for genetic disorders.

Studies over the last few years have argued that screening for more genes can be advantageous. With the advent of NGS-based research, estimates put the proportion of couples in the general population at high risk of having a child affected with a severe recessive disorder to be between 0.5% and 4.5% [91,108,109,111,117]. All but one of these studies, have a sample size between 23,000 to 475,000 individuals and screened between 50 to 415 recessive disorders.

As expected, increasing the number of genes in a panel correlated with increased identification of at-risk couples[108]. However, identifying at-risk couples will tend to saturate at some point despite increasing the number of genes[118]. Some health professionals have argued that when offering a carrier-screening test to a community without a history of recessive disorders, increasing the number of genes increases the harms couples are exposed to unnecessarily[80,119]. Others have suggested that the inclusion of additional genes beyond a point may not necessarily translate to immediate benefits[120,121].

To this end, ACOG Opinion 690[107] recommended that the disorders included in carrier-screening should:

1) have a carrier frequency of 1 in 100 or greater

2) have a well-defined phenotype

3) have a detrimental effect on quality of life, cause cognitive or physical impairment, require surgical or medical intervention

4) have an onset early in life

Some groups have argued that the 1% carrier frequency threshold should be reconsidered. Ben-Shachar *et al.* demonstrated that adhering to the recommendation missed 11% of at-risk couples when based on their 176-gene panel[118]. Guo and Gregg also demonstrated that modelling for disorders with a carrier frequency of >1% from their panel of 415 genes would identify only between 76–97% of carrier couples[109]. For example, including only carrier frequency of >1% will detect 76% of high-risk couples amongst South Asians as opposed to detecting 97% of high-risk couples amongst African Americans.

As a result, some researchers have also cautioned against offering carrier-screening programs too hastily[119-122]. Others argued that a program should not be a top-down approach initiated by the healthcare system but rather a response to an actual need expressed by the community[68].

Recent studies also show that the number of high-risk couples identified differ largely based on ethnicity and the number of genes being screened[109-111]. For example, the study by Guo and Gregg showed that the highest high-risk couple rate was 2.5% amongst Ashkenazi Jewish couples, and 1.9% for

couples with African ancestry using their 415-gene panel. Conversely, their study showed the lowest frequency of high-risk couples was in inter-ancestry couples, at 0.17%[109]. Similarly, Haque *et al.* showed that the potential number of affected fetuses born when using their 90-gene panel was between 0.09 – 0.3% of their study population, or that between 0.36 – 1.2% of couples would be identified to be high-risk[111].

Taken together, these studies show that ethnicity, severity of disorder and carrier frequency of variants, are factors that should be considered when deciding what genes to include in a carrier-screening panel for any community.

4.2 Gene and disorder inclusion criteria

The seminal study by Bell *et al.* in 2011[89] showed that it is possible to screen multiple genes and for multiple disorders at once while keeping costs low with NGS, something not achievable by traditional screening methods.

By 2018, the number of disorder genes included in expanded carrier-screening panels was between 70 and 1,700[90,91,113,123-125]. However, deciding which disorders are clinically significant enough to offer to couples in an expanded carrier-screening panel can be challenging and ethically charged[126-128]. Inclusion criteria suggested by the medical community included severe life-threatening disorders[127,128], disorders that causes severe physical and mental impairment or disorders that have treatment[127].

During the time of my PhD, the general consensus amongst health professionals (including genetic counsellors, clinical geneticists or obstetricians) [63,100,105,127-130] and the community[91,125,131] has been

to screen for recessive disorders that are life-threatening and those that may cause significant physical and mental impairment. Most also indicated that they would prefer to screen for a larger panel if costs were the same[100]. However, there is no agreement regarding the inclusion of adult-onset and comparatively milder disorders[91,127,131].

Some health professionals warn that knowing you are at high-risk of having a child with a mild disorder may cause more psychological harm than benefit[127] and that the inclusion of milder disorders runs the risk of sending the wrong message to those living with disabilities and their parents: that they are of lesser value[128]. It has also been argued that including disorders with variable penetrance may decrease the clinical utility of carrier-screening programs[105,132,133].

To address the selection dilemma, a few taxonomies of recessive disorders were created[110,128,130]. These taxonomies provide a systematic classification of recessive disorders having similar disorder traits into groups – for example life-limiting disorders. This broad description for a group of recessive disorders, allows a simpler way of providing genetic counselling as well as a basis for selecting a subset of disorder genes for screening.

4.3 Variant Interpretation

The number of variants identified in an individual will increase as the number of genes included on a screening panel increases. Because each next generation sequencing (NGS) test can identify hundreds of variants, many of which are benign changes, it quickly becomes challenging to distinguish pathogenic or likely pathogenic variants from benign variants. Distinguishing

pathogenic and benign variants is achieved in the process known as variant interpretation.

As a result, the American College of Medical Genetics and Genomics (ACMG) collaborated with the Association for Molecular Pathology (AMP) to provide a framework to classify variants[134], in part to address the increasing issue of Variant of Unknown Significance (VOUS) and false positive results[127]. VOUS are variants that had insufficient evidence available during the variant review process to support pathogenicity at that stage.

Increasing the complexity during variant interpretation is the limited knowledge about pathogenic variants in rare and very rare disorders[111]. Accordingly, there was strong support among clinicians for reporting only clinically significant variants[105,129,133]. However, the challenge to reporting only clinically significant variants is the increasing numbers of reports that raised concerns about variants previously reported as pathogenic that now appear to be benign[89,99,125,127,133].

In addition, Beauchamp *et al.* showed that the differences in sequencing methodology employed, e.g., genotyping specific selected variants vs whole gene sequencing, will vary the number of high-risk couples detected. These differences they said will go on to influence the number of fetuses predicted to be affected by a recessive disorder[110].

Further, studies of variant interpretation using the ACMG guidelines showed that concordance of experts sat between 80%[135] and 88%[136]. Notably, Harrison *et al.* discussed that persistent interpretation differences were due to a lack of gene or disease-specific knowledge[136].

These studies underlined the huge variability in variant interpretation despite the increased widespread adoption of NGS technology and guidelines for variant interpretation. When laboratories were presented with the same variants and asked to use the ACMG guidelines to classify variants, there was still considerable variability with variant interpretation between centres [135,136]. Pilot studies might be the key to understanding the efficacy and competency of local pathology laboratories in variant interpretation prior to large scale population carrier-screening programs using expanded screening panels.

4.4 Genetic counselling requirements

The ever-increasing popularity and demand for carrier-screening in the general population and amongst health professionals, has placed a larger emphasis on genetic counselling; in part due to the continued push to focus on reproductive autonomy[137]. Genetic counselling is usually included at two points during the carrier-screening journey: before the test is administered, (also known as pre-test counselling), and when receiving results (post-test counselling).

Pre-test counselling facilitates the informed decision-making process for individuals or couples by providing key information about the test, the disorders included, and the limitations of the test being offered[99,105,106,124,132]. An informed decision has been described as, "a capable person makes a reasonable choice based on the benefits and risks of the decision to be made and his or her personal values"[138].

That decision is dependent on two basic elements – proper comprehension of the medical facts (including the test itself) and making the choice without coercion[139]. Methods to increase comprehension of the test and medical facts can be provided verbally or through other means such as pamphlets, videos or online resources[105,126]. It has been suggested that making information available before the pre-test counselling session is preferable, since it can make the counselling more efficient[120].

Genetic counsellors, obstetricians[129] and general practitioners (GP)[91,140,141] have been suggested as suitable health professionals to provide counselling for carrier-screening programs. Importantly, the study by Schuurmans *et al.* showed that the time taken for pre-test couple-based counselling by a GP was, in combination with general preconception advice such as folic acid supplementation, only approximately 20 minutes [103]. Based on this, Delatycki *et al.* [142] calculated that it would take 16 GPs working 40-hour weeks and doing no other work for a year, in order to meet the demand for pre-test counselling for 100,000 pregnancies annually in the Netherlands. Delatycki *et al.* [142] then suggested that such traditional face-to-face pre-test counselling presents a labour-intensive challenge that may not be practical at a population level.

Post-test counselling helps individuals or couples cope with the test results by exploring options available to either an individual or a couple. Options for couples vary depending on when a couple take the test, with the best time for carrier-screening being the preconception period. This allows the greatest number of reproductive options for couples including preimplantation

genetic diagnosis using *in-vitro* fertilisation[142]. For high-risk couples, these post-test counselling sessions help them navigate through a very difficult and often challenging and overwhelming period. On the other hand, individual post-test counselling for every positive result identified will be extremely challenging[124] in carrier-screening programs using NGS technologies. Lynch *et al.* demonstrated that the median time for individual result disclosure was 64 minutes and that preparation work was the most time-consuming activity[143]. This means that traditional post-test counselling may also not be feasible when required at a population level.

The path going forward to providing adequate levels of pre-test and post-test counselling on a population level will remain a challenge. Innovative means of managing education[142] and counselling requirements[143] will be critical in the provision of carrier-screening on a population level. Methods such as pamphlets, websites, and brochures have been suggested as an alternative to detailed pre-test genetic counselling[144]. Further, post-test counselling has also been suggested to only be offered to couples who were identified to have pathogenic variants in the same gene (e.g., high-risk carrier couples)[91,99,105,124,129,131].

4.5 Technical limitations of an expanded carrier-screening test

A major limitation to carrier-screening using Next Generation Sequencing technologies is that NGS mainly focuses on the coding region. Rare disorders caused by abovementioned *de novo* previously unknown pathogenic variants or by complex genomic events such as chromosomal abnormalities (e.g., Down syndrome MIM# 190685) will not be detected. Specifically, copy

number variations (CNVs) are of particular importance, which is the main cause of the common recessive disorder spinal muscular atrophy (SMA). SMA is caused by deletion of exon 7 in *SMN1*. Whilst sequencing of exons in *SMN1* is possible, the pseudogene *SMN2* causes significant mapping issues resulting in erroneous data being produced[145]. Other limitations may include VOUS or allelic heterogeneity in which a pathogenic variant in a gene causes a novel type of disorder[146].

5.0 Australian Carrier-screening Programs – Past and Present

5.1 Carrier-screening programs in Australia

There is currently no national population-based carrier-screening programs established in Australia. However, Australia does have a long history of offering carrier-screening to select populations, usually based on initiatives in individual Australian states.

Australia started its carrier-screening efforts more than five decades ago, during the 1970s, when it participated in the global efforts to reduce prevalence of Tay-Sachs disease in Ashkenazi-Jewish populations[68]. At around the same time, Australia also began screening for hemoglobinopathy, as migration from Mediterranean countries post World War 2 and South East Asia in the 1970s increased the number of beta- and alpha-thalassemia carriers in Australia[81].

Haemoglobinopathy carrier-screening continues to be offered to everyone with specific ancestries tested through a variety of methods[147].

Systematic Tay-Sachs disease screening was established in Jewish high schools and communities in Sydney in 1995[148,149] and in Melbourne in

1997[150]. In 2016, the Melbourne program ceased while the Sydney program continues to operate[151]. At their peak, the programs offered screening for seven disorders including Canavan disease(MIM #271900), Bloom syndrome (MIM #210900) and Nieman Pick disease type A (MIM # 257200)[152]. Through the Tay-Sachs disease screening programs in Melbourne and Sydney, the ratio of TSD-affected births for Jewish births in 2011 was half that of all other ethnicities[153].

On a population level, screening for cystic fibrosis was available to women during pre-pregnancy and early stages of pregnancy from 2007 in Victoria, Australia[154]. This fee-for-service program continued until 2012 when spinal muscular atrophy and fragile X syndrome were added to the screening program. Results from this three-disorder screening service found that around 1 in 20 women screened were carriers of at least one of the three disorders[155]. Such a fee-for-service program exists in New-South Wales[156] and Victoria[157] but not any of the other states.

Interest in a population-wide CF screening program bubbled briefly in Western Australia in 2000 because of a research project, but the program ceased after the study[158]. Honnor *et al.* showed that, population carrier-screening for cystic fibrosis offered in a community setting in Western Australia was acceptable to almost half of those offered testing. Acceptability was especially important for younger participants and those planning for a family, for whom knowledge of their carrier status could be useful in reproductive planning[158].

Currently in Australia, cascade screening remains the only means of accessing a publicly funded carrier-screening test[159]. Cascade screening refers to testing of relatives of an affected person who is a carrier of a genetic disorder[160]. More recently in May 2020, cascade screening for childhood syndromes was provided a Medicare Benefits Schedule (MBS) number[161]. An MBS number allows rebate to patients for an approved test through the Australian health insurance agency, Medicare, which provides government funded universal access to healthcare for all Australians. This then opens up opportunities for family members with a family history of childhood disorders to be tested.

5.2 Australian Recommendations

In Australia, carrier-screening has garnered a similar level of interest to many European countries in the last few years. The Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) recommended in 2015 that screening for the more common genetic disorders such as cystic fibrosis and spinal muscular atrophy may be offered to women planning a pregnancy[162].

This statement was updated in July 2018 under *Recommendation 15* to, "Information on carrier-screening for the more common genetic disorders that affect children (e.g. cystic fibrosis, spinal muscular atrophy, fragile X syndrome) should be offered to all women planning a pregnancy or in the first trimester of pregnancy."[163].

Within a year, RANZCOG released another statement (March 2019) titled, "Genetic Carrier-Screening" to further recommend that information on

screening with "an expanded panel that contains many disorders (up to hundreds)" should be offered to all women planning a pregnancy or in the first trimester of pregnancy[164].

The series of changes may be interpreted as resulting from the increased interest in population carrier-screening within the community and/or recognition of its benefits amongst healthcare professionals[141].

Also notable was the 2018 recommendation from the Royal Australian College of General Practitioners (RACGP) that, "Carrier-screening for common recessive genetic disorders (e.g. cystic fibrosis [CF]) may be offered to low-risk women or couples (i.e. regardless of family history and ethnicity)."[165]

The flurry of recommendations by various Australian peak bodies suggested that there was an increasing appetite for an expanded universal carrier-screening program in Australia.

5.3 Inequitable screening practices in Australia

The current piecemeal offer of carrier-screening in Australia had left many couples without a family history, or not from a specific ancestry, wanting more. In Australia, it was estimated that approximately 94% of newborns with CF are born to families without any family history of the disorder[166]. More recently, Archibald *et al.* highlighted that approximately 88% of the carriers identified in their fee-for-service three-disorder screen, had no family history. Their study also showed that 1 in 240 couples were at risk of having a child affected with either CF, Fragile X or SMA. The high number of carriers and couples at risk, emphasised the benefits of a population-wide carrier-

screening program rather than relying on family history to guide screening decisions[167].

Inequitable screening practices based on ethnicity have also led to the worldwide phenomenon where more families not from specific ethnic backgrounds were affected by disorders that were typically more prevalent in specific populations. For example, in a 2013 conference for Canavan disorder, only 1 in 15 families affected by Canavan disease was of reported Jewish ancestry[168]. Similarly, as mentioned previously, the ratio of Tay-Sachs disease-affected births for Jewish births in 2011 was half that of all other ethnicities through the Tay-Sachs disease screening programs in Melbourne and Sydney[153].

In Australia, there are no surveillance or monitoring mechanisms to record and report on the collective impact of rare disorders[169]. Unpublished data from the WA Health Department informs that about 50% of infant deaths before 1 year of age are due to rare disorders. A study by the Office of Population Health Genomics in Western Australia indicates that 2% of the population admitted to hospital had rare disorders, but accounted for 10.5% of the annual WA hospitalisation expenditure. This amounted to $395 million dollars and the study included only 467 rare disorders[170]. This study therefore is only a partial estimate of the total health costs of rare disorders which would include additional expenditure such as outpatient and general practitioner visits, emergency and allied health services costs[53].

Additionally, as demand for genetic screening provided by commercial entities increases, certain sectors of society miss out. This is because cost is

prohibitive for the disadvantaged. It is also likely that people in higher socio-economic groups tend to be more educated and therefore also tend to learn more about these disorders and the options to screen for them. Recent studies in Australia have shown that couples living in more advantaged suburbs across Australia were significantly more likely to have accessed carrier-screening than those living in the most disadvantaged suburbs[171].

These examples point toward a need for a publicly-funded universal carrier-screening program to overcome the highly inequitable and inadequate nature of carrier-screening practices in Australia.

6.0 Australian Carrier-screening Programs – The Future

Acknowledging the benefits and growing screening appetite, I set out to identify the best way to introduce a universal carrier-screening program into the public health system in Western Australia.

In June 2015, Prof Nigel Laing (Head of the then Neurogenetic Diseases Group at the Harry Perkins Institute) in collaboration with Genetic Services WA and Office of Population Health Genomics organised a Preconception Carrier-Screening satellite workshop of the European Society of Human Genetics conference in Glasgow.

The specific aim was to harness the experience of world leaders in carrier-screening and understand what requirements were needed to successfully implement a government-funded carrier-screening program in Australia[38]. A second workshop was held in the Harry Perkins Institute of Medical Research in 2017 to discuss practical issues of implementing a carrier-screening program in Western Australia. The outcomes from these meetings, in addition to key points identified in my literature review, helped shape the way my PhD study was implemented.

Like the first multiphasic pilot program developed in 1970 for the screening of Tay-Sachs disease, I endeavoured to design my PhD project such that:

1) There is a public education and outreach component, especially for the community and for general practitioners (GP).

2) The screening program operates within the existing health infrastructure in WA, including 1) the network of GPs and genetic counsellors, 2) the clinical genetics arm of the Western Australian State Health Department (Genetic Services WA), and 3) the Department of Health Pathology Services: PathWest.

3) The testing panel developed remains affordable, with both variant interpretation and reporting standardised.

6.1 PhD Aims

The overall aim of my study was to determine whether it was feasible to implement an expanded carrier-screening program using components of the public health system in Western Australia that could effectively deliver carrier screening results to participating couples for them to make reproductive decisions in line with their values.

I wanted to establish through my study whether Western Australia could consider offering a population-wide carrier-screening test and how should Western Australia go about offering such a test.

6.2 PhD Objectives

The objectives of the study were:

1) To study the preferences and attitudes of the Western Australian community and health professionals about carrier-screening in Western Australia.
2) To develop a working end-to-end protocol, using existing healthcare infrastructures, to implement a carrier-screening program in Western Australia.
3) To design and validate a targeted next generation sequencing panel for use during the pilot study.
4) To perform a pilot study of PCS in Western Australia using the proposed study protocol.
5) To evaluate the effectiveness of the tools developed for health professionals during the study.
6) To refine the initial protocol until the protocol was optimised and could be implemented in the Western Australian public health system.

This will be the first study to investigate introducing an expanded carrier-screening test into a public health system in Australia.

7.0 PRESENTATIONS AND AWARDS:

Invited as a guest speaker to the 2016 Combined Biological Sciences Conference, Perth to talk about my PhD study.

8.0 References

1. Chong JX, Buckingham KJ, Jhangiani SN, Boehm C, Sobreira N, Smith JD, Harrell TM, McMillin MJ, Wiszniewski W, Gambin T, Coban Akdemir ZH, Doheny K, Scott AF, Avramopoulos D, Chakravarti A, Hoover-Fong J, Mathews D, Witmer PD, Ling H, Hetrick K, Watkins L, Patterson KE, Reinier F, Blue E, Muzny D, Kircher M, Bilguvar K, Lopez-Giraldez F, Sutton VR, Tabor HK, Leal SM, Gunel M, Mane S, Gibbs RA, Boerwinkle E, Hamosh A, Shendure J, Lupski JR, Lifton RP, Valle D, Nickerson DA, Centers for Mendelian G, Bamshad MJ. The Genetic Basis of Mendelian Phenotypes: Discoveries, Challenges, and Opportunities. Am J Hum Genet 2015;**97**(2):199-215 doi: 10.1016/j.ajhg.2015.06.009published
2. Nguengang Wakap S, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, Murphy D, Le Cam Y, Rath A. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. Eur J Hum Genet 2020;**28**(2):165-73 doi: 10.1038/s41431-019-0508-0published
3. Australian Federal Department of Health. What we're doing about rare diseases? 2020. Date Accessed 26/01/2021. https://www.health.gov.au/health-topics/chronic-conditions/what-were-doing-about-chronic-conditions/what-were-doing-about-rare-diseases#:~:text=In%20Australia%2C%20a%20disease%20is,life%20threatening%20or%20chronically%20debilitating.
4. U.S.Food and Drug Administration. Rare Diseases at FDA. 2020. Date Accessed 26/01/2021. https://www.fda.gov/patients/rare-diseases-fda#:~:text=The%20Orphan%20Drug%20Act%20defines,people%20in%20the%20United%20States.
5. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell 1993;**72**(6):971-83 doi: 10.1016/0092-8674(93)90585-epublished
6. Suthers G, Laing N, Wilton S, Dorosz S, Waddy H. "Sporadic" motoneuron disease due to familial SOD1 mutation with low penetrance. Lancet 1994;**344**(8939-8940):1773 doi: 10.1016/s0140-6736(94)92913-0published
7. Thomas NH, Dubowitz V. The natural history of type I (severe) spinal muscular atrophy. Neuromuscul Disord 1994;**4**(5-6):497-502 doi: 10.1016/0960-8966(94)90090-6published
8. Ravenscroft G, Miyatake S, Lehtokari VL, Todd EJ, Vornanen P, Yau KS, Hayashi YK, Miyake N, Tsurusaki Y, Doi H, Saitsu H, Osaka H, Yamashita S, Ohya T, Sakamoto Y, Koshimizu E, Imamura S, Yamashita M, Ogata K, Shiina M, Bryson-Richardson RJ, Vaz R, Ceyhan O, Brownstein CA, Swanson LC, Monnot S, Romero NB, Amthor H, Kresoje N, Sivadorai P, Kiraly-Borri C, Haliloglu G, Talim B, Orhan D, Kale G, Charles AK, Fabian VA, Davis MR, Lammens M, Sewry CA, Manzur A, Muntoni F, Clarke NF, North KN, Bertini E, Nevo Y, Willichowski E, Silberg IE, Topaloglu H, Beggs AH, Allcock RJ, Nishino I, Wallgren-Pettersson C, Matsumoto N, Laing NG. Mutations in KLHL40 are a frequent cause of severe autosomal-recessive nemaline myopathy. Am J Hum Genet 2013;**93**(1):6-18 doi: 10.1016/j.ajhg.2013.05.004published
9. Aartsma-Rus A, Ginjaar IB, Bushby K. The importance of genetic diagnosis for Duchenne muscular dystrophy. J Med Genet 2016;**53**(3):145-51 doi: 10.1136/jmedgenet-2015-103387published
10. Laing NG, Laing BA, Meredith C, Wilton SD, Robbins P, Honeyman K, Dorosz S, Kozman H, Mastaglia FL, Kakulas BA. Autosomal dominant distal myopathy: linkage to chromosome 14. Am J Hum Genet 1995;**56**(2):422-7
11. Donkervoort S, Papadaki M, de Winter JM, Neu MB, Kirschner J, Bolduc V, Yang ML, Gibbons MA, Hu Y, Dastgir J, Leach ME, Rutkowski A, Foley AR, Kruger M, Wartchow EP, McNamara

E, Ong R, Nowak KJ, Laing NG, Clarke NF, Ottenheijm C, Marston SB, Bonnemann CG. TPM3 deletions cause a hypercontractile congenital muscle stiffness phenotype. Ann Neurol 2015;**78**(6):982-94 doi: 10.1002/ana.24535published
12. Ravenscroft G, Zaharieva IT, Bortolotti CA, Lambrughi M, Pignataro M, Borsari M, Sewry CA, Phadke R, Haliloglu G, Ong R, Goullee H, Whyte T, Consortium UK, Manzur A, Talim B, Kaya U, Osborn DPS, Forrest ARR, Laing NG, Muntoni F. Bi-allelic mutations in MYL1 cause a severe congenital myopathy. Hum Mol Genet 2018;**27**(24):4263-72 doi: 10.1093/hmg/ddy320published
13. Boland BJ, Silbert PL, Groover RV, Wollan PC, Silverstein MD. Skeletal, cardiac, and smooth muscle failure in Duchenne muscular dystrophy. Pediatr Neurol 1996;**14**(1):7-12 doi: 10.1016/0887-8994(95)00251-0published
14. Ravenscroft G, Nolent F, Rajagopalan S, Meireles AM, Paavola KJ, Gaillard D, Alanio E, Buckland M, Arbuckle S, Krivanek M, Maluenda J, Pannell S, Gooding R, Ong RW, Allcock RJ, Carvalho ED, Carvalho MD, Kok F, Talbot WS, Melki J, Laing NG. Mutations of GPR126 are responsible for severe arthrogryposis multiplex congenita. Am J Hum Genet 2015;**96**(6):955-61 doi: 10.1016/j.ajhg.2015.04.014published
15. Veske A, Oehlmann R, Younus F, Mohyuddin A, Muller-Myhsok B, Mehdi SQ, Gal A. Autosomal recessive non-syndromic deafness locus (DFNB8) maps on chromosome 21q22 in a large consanguineous kindred from Pakistan. Hum Mol Genet 1996;**5**(1):165-8 doi: 10.1093/hmg/5.1.165published
16. Beecroft SJ, Cortese A, Sullivan R, Yau WY, Dyer Z, Wu TY, Mulroy E, Pelosi L, Rodrigues M, Taylor R, Mossman S, Leadbetter R, Cleland J, Anderson T, Ravenscroft G, Laing NG, Houlden H, Reilly MM, Roxburgh RH. A Maori specific RFC1 pathogenic repeat configuration in CANVAS, likely due to a founder allele. Brain 2020;**143**(9):2673-80 doi: 10.1093/brain/awaa203published
17. Kirschner J, Brune T, Wehnert M, Denecke J, Wasner C, Feuer A, Marquardt T, Ketelsen UP, Wieacker P, Bonnemann CG, Korinthenberg R. p.S143F mutation in lamin A/C: a new phenotype combining myopathy and progeria. Ann Neurol 2005;**57**(1):148-51 doi: 10.1002/ana.20359published
18. De Sandre-Giovannoli A, Chaouch M, Kozlov S, Vallat JM, Tazir M, Kassouri N, Szepetowski P, Hammadouche T, Vandenberghe A, Stewart CL, Grid D, Levy N. Homozygous defects in LMNA, encoding lamin A/C nuclear-envelope proteins, cause autosomal recessive axonal neuropathy in human (Charcot-Marie-Tooth disorder type 2) and mouse. Am J Hum Genet 2002;**70**(3):726-36 doi: 10.1086/339274published
19. Rudnik-Schoneborn S, Auer-Grumbach M, Senderek J. Hereditary Neuropathies: Update 2017. Neuropediatrics 2017;**48**(4):282-93 doi: 10.1055/s-0037-1603518published
20. Hayasaka K, Himoro M, Sato W, Takada G, Uyemura K, Shimizu N, Bird TD, Conneally PM, Chance PF. Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin P0 gene. Nat Genet 1993;**5**(1):31-4 doi: 10.1038/ng0993-31published
21. Hoogendijk JE, Hensels GW, Gabreels-Festen AA, Gabreels FJ, Janssen EA, de Jonghe P, Martin JJ, van Broeckhoven C, Valentijn LJ, Baas F, et al. De-novo mutation in hereditary motor and sensory neuropathy type I. Lancet 1992;**339**(8801):1081-2 doi: 10.1016/0140-6736(92)90668-spublished
22. Street VA, Bennett CL, Goldy JD, Shirk AJ, Kleopa KA, Tempel BL, Lipe HP, Scherer SS, Bird TD, Chance PF. Mutation of a putative protein degradation gene LITAF/SIMPLE in Charcot-Marie-Tooth disease 1C. Neurology 2003;**60**(1):22-6 doi: 10.1212/wnl.60.1.22published
23. Collins FS. Positional cloning moves from perditional to traditional. Nat Genet 1995;**9**(4):347-50 doi: 10.1038/ng0495-347published

24. Douar AM, Mosser J, Sarde CO, Lopez J, Mandel JL, Aubourg P. X-linked adrenoleukodystrophy gene: identification of a candidate gene by positional cloning. Biomed Pharmacother 1994;**48**(5-6):215-8 doi: 10.1016/0753-3322(94)90135-xpublished
25. Ong RW, AlSaman A, Selcen D, Arabshahi A, Yau KS, Ravenscroft G, Duff RM, Atkinson V, Allcock RJ, Laing NG. Novel cofilin-2 (CFL2) four base pair deletion causing nemaline myopathy. J Neurol Neurosurg Psychiatry 2014;**85**(9):1058-60 doi: 10.1136/jnnp-2014-307608published
26. Beecroft SJ, Lamont PJ, Edwards S, Goullee H, Davis MR, Laing NG, Ravenscroft G. The Impact of Next-Generation Sequencing on the Diagnosis, Treatment, and Prevention of Hereditary Neuromuscular Disorders. Mol Diagn Ther 2020;**24**(6):641-52 doi: 10.1007/s40291-020-00495-2published
27. Ghaoui R, Cooper ST, Lek M, Jones K, Corbett A, Reddel SW, Needham M, Liang C, Waddell LB, Nicholson G, O'Grady G, Kaur S, Ong R, Davis M, Sue CM, Laing NG, North KN, MacArthur DG, Clarke NF. Use of Whole-Exome Sequencing for Diagnosis of Limb-Girdle Muscular Dystrophy: Outcomes and Lessons Learned. JAMA Neurol 2015;**72**(12):1424-32 doi: 10.1001/jamaneurol.2015.2274published
28. Todd EJ, Yau KS, Ong R, Slee J, McGillivray G, Barnett CP, Haliloglu G, Talim B, Akcoren Z, Kariminejad A, Cairns A, Clarke NF, Freckmann ML, Romero NB, Williams D, Sewry CA, Colley A, Ryan MM, Kiraly-Borri C, Sivadorai P, Allcock RJ, Beeson D, Maxwell S, Davis MR, Laing NG, Ravenscroft G. Next generation sequencing in a large cohort of patients presenting with neuromuscular disease before or at birth. Orphanet J Rare Dis 2015;**10**:148 doi: 10.1186/s13023-015-0364-0published
29. Beecroft SJ, Yau KS, Allcock RJN, Mina K, Gooding R, Faiz F, Atkinson VJ, Wise C, Sivadorai P, Trajanoski D, Kresoje N, Ong R, Duff RM, Cabrera-Serrano M, Nowak KJ, Pachter N, Ravenscroft G, Lamont PJ, Davis MR, Laing NG. Targeted gene panel use in 2249 neuromuscular patients: the Australasian referral center experience. Ann Clin Transl Neurol 2020;**7**(3):353-62 doi: 10.1002/I3.51002published
30. Martin PB, Kigoshi-Tansho Y, Sher RB, Ravenscroft G, Stauffer JE, Kumar R, Yonashiro R, Muller T, Griffith C, Allen W, Pehlivan D, Harel T, Zenker M, Howting D, Schanze D, Faqeih EA, Almontashiri NAM, Maroofian R, Houlden H, Mazaheri N, Galehdari H, Douglas G, Posey JE, Ryan M, Lupski JR, Laing NG, Joazeiro CAP, Cox GA. NEMF mutations that impair ribosome-associated quality control are associated with neuromuscular disease. Nat Commun 2020;**11**(1):4625 doi: 10.1038/s41467-020-18327-6published
31. Senderek J, Lassuthova P, Kabzinska D, Abreu L, Baets J, Beetz C, Braathen GJ, Brenner D, Dalton J, Dankwa L, Deconinck T, De Jonghe P, Drager B, Eggermann K, Ellis M, Fischer C, Stojkovic T, Herrmann DN, Horvath R, Hoyer H, Iglseder S, Kennerson M, Kinslechner K, Kohler JN, Kurth I, Laing NG, Lamont PJ, Wolfgang NL, Ludolph A, Marques W, Jr., Nicholson G, Ong R, Petri S, Ravenscroft G, Rebelo A, Ricci G, Rudnik-Schoneborn S, Schirmacher A, Schlotter-Weigel B, Schoels L, Schule R, Synofzik M, Francou B, Strom TM, Wagner J, Walk D, Wanschitz J, Weinmann D, Weishaupt J, Wiessner M, Windhager R, Young P, Zuchner S, Toegel S, Seeman P, Kochanski A, Auer-Grumbach M. The genetic landscape of axonal neuropathies in the middle-aged and elderly: Focus on MME. Neurology 2020;**95**(24):e3163-e79 doi: 10.1212/WNL.0000000000011132published
32. Olive M, Engvall M, Ravenscroft G, Cabrera-Serrano M, Jiao H, Bortolotti CA, Pignataro M, Lambrughi M, Jiang H, Forrest ARR, Benseny-Cases N, Hofbauer S, Obinger C, Battistuzzi G, Bellei M, Borsari M, Di Rocco G, Viola HM, Hool LC, Cladera J, Lagerstedt-Robinson K, Xiang F, Wredenberg A, Miralles F, Baiges JJ, Malfatti E, Romero NB, Streichenberger N, Vial C, Claeys KG, Straathof CSM, Goris A, Freyer C, Lammens M, Bassez G, Kere J, Clemente P, Sejersen T, Udd B, Vidal N, Ferrer I, Edstrom L, Wedell A, Laing NG. Myoglobinopathy is an

adult-onset autosomal dominant myopathy with characteristic sarcoplasmic inclusions. Nat Commun 2019;**10**(1):1396 doi: 10.1038/s41467-019-09111-2published
33. Lassuthova P, Rebelo AP, Ravenscroft G, Lamont PJ, Davis MR, Manganelli F, Feely SM, Bacon C, Brozkova DS, Haberlova J, Mazanec R, Tao F, Saghira C, Abreu L, Courel S, Powell E, Buglo E, Bis DM, Baxter MF, Ong RW, Marns L, Lee YC, Bai Y, Isom DG, Barro-Soria R, Chung KW, Scherer SS, Larsson HP, Laing NG, Choi BO, Seeman P, Shy ME, Santoro L, Zuchner S. Mutations in ATP1A1 Cause Dominant Charcot-Marie-Tooth Type 2. Am J Hum Genet 2018;**102**(3):505-14 doi: 10.1016/j.ajhg.2018.01.023published
34. Bamshad MJ, Nickerson DA, Chong JX. Mendelian Gene Discovery: Fast and Furious with No End in Sight. Am J Hum Genet 2019;**105**(3):448-55 doi: 10.1016/j.ajhg.2019.07.011published
35. Hartman P, Beckman K, Silverstein K, Yohe S, Schomaker M, Henzler C, Onsongo G, Lam HC, Munro S, Daniel J, Billstein B, Deshpande A, Hauge A, Mroz P, Lee W, Holle J, Wiens K, Karnuth K, Kemmer T, Leary M, Michel S, Pohlman L, Thayanithy V, Nelson A, Bower M, Thyagarajan B. Next generation sequencing for clinical diagnostics: Five year experience of an academic laboratory. Mol Genet Metab Rep 2019;**19**:100464 doi: 10.1016/j.ymgmr.2019.100464published
36. Wright CF, FitzPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children. Nat Rev Genet 2018;**19**(5):325 doi: 10.1038/nrg.2018.12published
37. Esquivel-Sada D, Nguyen MT. Diagnosis of rare diseases under focus: impacts for Canadian patients. J Community Genet 2018;**9**(1):37-50 doi: 10.1007/s12687-017-0320-xpublished
38. Molster CM, Lister K, Metternick-Jones S, Baynam G, Clarke AJ, Straub V, Dawkins HJ, Laing N. Outcomes of an International Workshop on Preconception Expanded Carrier Screening: Some Considerations for Governments. Front Public Health 2017;**5**:25 doi: 10.3389/fpubh.2017.00025published
39. Garrino L, Picco E, Finiguerra I, Rossi D, Simone P, Roccatello D. Living with and treating rare diseases: experiences of patients and professional health care providers. Qual Health Res 2015;**25**(5):636-51 doi: 10.1177/1049732315570116published
40. Liu Z, Zhu L, Roberts R, Tong W. Toward Clinical Implementation of Next-Generation Sequencing-Based Genetic Testing in Rare Diseases: Where Are We? Trends Genet 2019;**35**(11):852-67 doi: 10.1016/j.tig.2019.08.006published
41. Lamont PJ, Wallefeld W, Hilton-Jones D, Udd B, Argov Z, Barboi AC, Bonneman C, Boycott KM, Bushby K, Connolly AM, Davies N, Beggs AH, Cox GF, Dastgir J, DeChene ET, Gooding R, Jungbluth H, Muelas N, Palmio J, Penttila S, Schmedding E, Suominen T, Straub V, Staples C, Van den Bergh PY, Vilchez JJ, Wagner KR, Wheeler PG, Wraige E, Laing NG. Novel mutations widen the phenotypic spectrum of slow skeletal/beta-cardiac myosin (MYH7) distal myopathy. Hum Mutat 2014;**35**(7):868-79 doi: 10.1002/humu.22553published
42. Cabrera-Serrano M, Coote DJ, Azmanov D, Goullee H, Andersen E, McLean C, Davis M, Ishimura R, Stark Z, Vallat JM, Komatsu M, Kornberg A, Ryan M, Laing NG, Ravenscroft G. A homozygous UBA5 pathogenic variant causes a fatal congenital neuropathy. J Med Genet 2020;**57**(12):835-42 doi: 10.1136/jmedgenet-2019-106496published
43. Beecroft SJ, Olive M, Quereda LG, Gallano P, Ojanguren I, McLean C, McCombe P, Laing NG, Ravenscroft G. Cylindrical spirals in two families: Clinical and genetic investigations. Neuromuscul Disord 2020;**30**(2):151-58 doi: 10.1016/j.nmd.2019.12.006published
44. Gonzalez-Mera L, Ravenscroft G, Cabrera-Serrano M, Ermolova N, Dominguez-Gonzalez C, Arteche-Lopez A, Soltanzadeh P, Evesson F, Navas C, Mavillard F, Clayton J, Rodrigo P, Servian-Morilla E, Cooper ST, Waddell L, Reardon K, Corbett A, Hernandez-Lain A, Sanchez A, Esteban Perez J, Paradas-Lopez C, Rivas-Infante E, Spencer M, Laing N, Olive M. Heterozygous CAPN3 missense variants causing autosomal-dominant calpainopathy in

seven unrelated families. Neuropathol Appl Neurobiol 2020 doi: 10.1111/nan.12663published
45. Ravenscroft G, Clayton JS, Faiz F, Sivadorai P, Milnes D, Cincotta R, Moon P, Kamien B, Edwards M, Delatycki M, Lamont PJ, Chan SH, Colley A, Ma A, Collins F, Hennington L, Zhao T, McGillivray G, Ghedia S, Chao K, O'Donnell-Luria A, Laing NG, Davis MR. Neurogenetic fetal akinesia and arthrogryposis: genetics, expanding genotype-phenotypes and functional genomics. J Med Genet 2020 doi: 10.1136/jmedgenet-2020-106901published
46. Bamshad M, Watkins WS, Zenger RK, Bohnsack JF, Carey JC, Otterud B, Krakowiak PA, Robertson M, Jorde LB. A gene for distal arthrogryposis type I maps to the pericentromeric region of chromosome 9. Am J Hum Genet 1994;**55**(6):1153-8
47. Tajsharghi H, Kimber E, Holmgren D, Tulinius M, Oldfors A. Distal arthrogryposis and muscle weakness associated with a beta-tropomyosin mutation. Neurology 2007;**68**(10):772-5 doi: 10.1212/01.wnl.0000256339.40667.fbpublished
48. Coste B, Houge G, Murray MF, Stitziel N, Bandell M, Giovanni MA, Philippakis A, Hoischen A, Riemer G, Steen U, Steen VM, Mathur J, Cox J, Lebo M, Rehm H, Weiss ST, Wood JN, Maas RL, Sunyaev SR, Patapoutian A. Gain-of-function mutations in the mechanically activated ion channel PIEZO2 cause a subtype of Distal Arthrogryposis. Proc Natl Acad Sci U S A 2013;**110**(12):4667-72 doi: 10.1073/pnas.1221400110published
49. McMillin MJ, Below JE, Shively KM, Beck AE, Gildersleeve HI, Pinner J, Gogola GR, Hecht JT, Grange DK, Harris DJ, Earl DL, Jagadeesh S, Mehta SG, Robertson SP, Swanson JM, Faustman EM, Mefford HC, Shendure J, Nickerson DA, Bamshad MJ, University of Washington Center for Mendelian G. Mutations in ECEL1 cause distal arthrogryposis type 5D. Am J Hum Genet 2013;**92**(1):150-6 doi: 10.1016/j.ajhg.2012.11.014published
50. Dieterich K, Quijano-Roy S, Monnier N, Zhou J, Faure J, Smirnow DA, Carlier R, Laroche C, Marcorelles P, Mercier S, Megarbane A, Odent S, Romero N, Sternberg D, Marty I, Estournet B, Jouk PS, Melki J, Lunardi J. The neuronal endopeptidase ECEL1 is associated with a distinct form of recessive distal arthrogryposis. Hum Mol Genet 2013;**22**(8):1483-92 doi: 10.1093/hmg/dds514published
51. Barnett CP, Todd EJ, Ong R, Davis MR, Atkinson V, Allcock R, Laing N, Ravenscroft G. Distal arthrogryposis type 5D with novel clinical features and compound heterozygous mutations in ECEL1. Am J Med Genet A 2014;**164A**(7):1846-9 doi: 10.1002/ajmg.a.36342published
52. Kaufmann P, Pariser AR, Austin C. From scientific discovery to treatments for rare diseases - the view from the National Center for Advancing Translational Sciences - Office of Rare Diseases Research. Orphanet J Rare Dis 2018;**13**(1):196 doi: 10.1186/s13023-018-0936-xpublished
53. Teoh LJ, Geelhoed EA, Bayley K, Leonard H, Laing NG. Health care utilization and costs for children and adults with duchenne muscular dystrophy. Muscle Nerve 2016;**53**(6):877-84 doi: 10.1002/mus.24965published
54. Acmg Board of Directors. Insuring patient access and affordability for treatments for rare and ultrarare diseases: a policy statement of the American College of Medical Genetics and Genomics. Genet Med 2018;**20**(11):1303-04 doi: 10.1038/s41436-018-0059-8published
55. The McKell Institute. Funding Rare Disease Therapies in Australia, 2014.
56. Australian Federal Department of Health. The Pharmaceutical Benefits Scheme (PBS). 2021. Date Accessed https://www.pbs.gov.au/info/about-the-pbs#What_is_the_PBS.
57. Federal Australian Department of Health. Portfolio Budget Statements 2018-19, Budget Related Paper No. 1.9 - Health Portfolio. In: Health Do, ed. Canberra, 2018.
58. Potter BK, Khangura SD, Tingley K, Chakraborty P, Little J. Translating rare-disease therapies into improved care for patients and families: what are the right outcomes, designs, and

engagement approaches in health-systems research? Genet Med 2016;**18**(2):117-23 doi: 10.1038/gim.2015.42published
59. de Laet C, Dionisi-Vici C, Leonard JV, McKiernan P, Mitchell G, Monti L, de Baulny HO, Pintos-Morell G, Spiekerkotter U. Recommendations for the management of tyrosinaemia type 1. Orphanet J Rare Dis 2013;**8**:8 doi: 10.1186/1750-1172-8-8published
60. Australian Institute of Health and Welfare. Rural & remote health, 2019.
61. Nigel Laing GR, Michelle Farrar, Royston Ong. What prospective parents need to know about gene tests such as 'prepair'. Analysis 2017. https://theconversation.com/what-prospective-parents-need-to-know-about-gene-tests-such-as-prepair-87083.
62. Nigel Laing GR, Royston Ong. Explainer: what is pre-pregnancy carrier screening and should potential parents consider it? 2017. https://theconversation.com/explainer-what-is-pre-pregnancy-carrier-screening-and-should-potential-parents-consider-it-79184.
63. Antonarakis SE. Carrier screening for recessive disorders. Nat Rev Genet 2019;**20**(9):549-61 doi: 10.1038/s41576-019-0134-2published
64. Okada S, O'Brien JS. Tay-Sachs disease: generalized absence of a beta-D-N-acetylhexosaminidase component. Science 1969;**165**(3894):698-700 doi: 10.1126/science.165.3894.698published
65. Robinson D, Stirling JL. N-Acetyl-beta-glucosaminidases in human spleen. Biochem J 1968;**107**(3):321-7 doi: 10.1042/bj1070321published
66. O'Brien JS, Okada S, Fillerup DL, Veath ML, Adornato B, Brenner PH, Leroy JG. Tay-Sachs disease: prenatal diagnosis. Science 1971;**172**(3978):61-4 doi: 10.1126/science.172.3978.61published
67. Rogers JF, Hogan EL, Jorgenson RJ. Tay-Sachs disease: a pilot screening program for the detection of the heterozygote in the Charleston Jewish community. South Med J 1976;**69**(11):1453-5
68. Kaback M, Lim-Steele J, Dabholkar D, Brown D, Levy N, Zeiger K. Tay-Sachs disease--carrier screening, prenatal diagnosis, and the molecular era. An international perspective, 1970 to 1993. The International TSD Data Collection Network. JAMA 1993;**270**(19):2307-15
69. Angastiniotis MA, Hadjiminas MG. Prevention of thalassaemia in Cyprus. Lancet 1981;**1**(8216):369-71 doi: 10.1016/s0140-6736(81)91682-2published
70. Loukopoulos D. Current status of thalassemia and the sickle cell syndromes in Greece. Semin Hematol 1996;**33**(1):76-86
71. Cao A, Furbetta M, Galanello R, Melis MA, Angius A, Ximenes A, Rosatelli C, Ruggeri R, Addis M, Tuveri T, Falchi AM, Paglietti E, Scalas MT. Prevention of homozygous beta-thalassemia by carrier screening and prenatal diagnosis in Sardinia. Am J Hum Genet 1981;**33**(4):592-605
72. Cao A. Results of programmes for antenatal detection of thalassemia in reducing the incidence of the disorder. Blood Rev 1987;**1**(3):169-76 doi: 10.1016/0268-960x(87)90032-4published
73. World Health Organisation. Community control of hereditary anaemias: memorandum from a WHO meeting. Bull World Health Organ 1983;**61**(1):63-80
74. Gerhard Peters and John T. Woolley. Statement on Signing the National Sickle Cell Anemia Control Act. Date Accessed 17/12/2020. https://www.presidency.ucsb.edu/documents/statement-signing-the-national-sickle-cell-anemia-control-act.
75. Naik RP, Haywood C, Jr. Sickle cell trait diagnosis: clinical and social implications. Hematology Am Soc Hematol Educ Program 2015;**2015**:160-7 doi: 10.1182/asheducation-2015.1.160published
76. Culliton BJ. Sickle cell anemia: national program raises problems as well as hopes. Science 1972;**178**(4058):283-6 doi: 10.1126/science.178.4058.283published

77. Treadwell MJ, McClough L, Vichinsky E. Using qualitative and quantitative strategies to evaluate knowledge and perceptions about sickle cell disease and sickle cell trait. J Natl Med Assoc 2006;**98**(5):704-10
78. Yeshorim D. History & Achievements. 2019. Date Accessed 17/12/2020. https://doryeshorim.org/history-achievements/.
79. Ekstein J, Katzenstein H. The Dor Yeshorim story: community-based carrier screening for Tay-Sachs disease. Adv Genet 2001;**44**:297-310 doi: 10.1016/s0065-2660(01)44087-9published
80. Rowe CA, Wright CF. Expanded universal carrier screening and its implementation within a publicly funded healthcare service. J Community Genet 2020;**11**(1):21-38 doi: 10.1007/s12687-019-00443-6published
81. Delatycki MB, Alkuraya F, Archibald A, Castellani C, Cornel M, Grody WW, Henneman L, Ioannides AS, Kirk E, Laing N, Lucassen A, Massie J, Schuurmans J, Thong MK, van Langen I, Zlotogora J. International perspectives on the implementation of reproductive carrier screening. Prenat Diagn 2020;**40**(3):301-10 doi: 10.1002/pd.5611published
82. Kolnagou A, Kontoghiorghe CN, Kontoghiorghes GJ. Transition of Thalassaemia and Friedreich ataxia from fatal to chronic diseases. World J Methodol 2014;**4**(4):197-218 doi: 10.5662/wjm.v4.i4.197published
83. Picci L, Cameran M, Marangon O, Marzenta D, Ferrari S, Frigo AC, Scarpa M. A 10-year large-scale cystic fibrosis carrier screening in the Italian population. J Cyst Fibros 2010;**9**(1):29-35 doi: 10.1016/j.jcf.2009.10.003published
84. Lewis PA. Influence of five years of antenatal screening on the paediatric cystic fibrosis population in one region. Arch Dis Child 1999;**80**(5):493 doi: 10.1136/adc.80.5.493cpublished
85. Alhamdan NA, Almazrou YY, Alswaidi FM, Choudhry AJ. Premarital screening for thalassemia and sickle cell disease in Saudi Arabia. Genet Med 2007;**9**(6):372-7 doi: 10.1097/gim.0b013e318065a9e8published
86. Zlotogora J. Genetics and genomic medicine in Israel. Mol Genet Genomic Med 2014;**2**(2):85-94 doi: 10.1002/mgg3.73published
87. Singer A, Sagi-Dain L. Impact of a national genetic carrier-screening program for reproductive purposes. Acta Obstet Gynecol Scand 2020 doi: 10.1111/aogs.13858published
88. Shalev SA, Zlotogora J, Shalata A, Levy-Lahad E. Medical genetics in Israel's diverse population. Lancet 2017;**389**(10088):2453-55 doi: 10.1016/S0140-6736(17)30875-9published
89. Bell CJ, Dinwiddie DL, Miller NA, Hateley SL, Ganusova EE, Mudge J, Langley RJ, Zhang L, Lee CC, Schilkey FD, Sheth V, Woodward JE, Peckham HE, Schroth GP, Kim RW, Kingsmore SF. Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med 2011;**3**(65):65ra4 doi: 10.1126/scitranslmed.3001756published
90. Chokoshvili D, Vears D, Borry P. Expanded carrier screening for monogenic disorders: where are we now? Prenat Diagn 2018;**38**(1):59-66 doi: 10.1002/pd.5109published
91. Plantinga M, Birnie E, Abbott KM, Sinke RJ, Lucassen AM, Schuurmans J, Kaplan S, Verkerk MA, Ranchor AV, van Langen IM. Population-based preconception carrier screening: how potential users from the general population view a test for 50 serious diseases. Eur J Hum Genet 2016;**24**(10):1417-23 doi: 10.1038/ejhg.2016.43published
92. Shiroff JJ, Nemeth LS. Public Perceptions of Recessive Carrier Testing in the Preconception and Prenatal Periods. J Obstet Gynecol Neonatal Nurs 2015;**44**(6):717-25 doi: 10.1111/1552-6909.12764published
93. Ekstrand Ragnar M, Tyden T, Kihlbom U, Larsson M. Swedish parents' interest in preconception genetic carrier screening. Ups J Med Sci 2016:1-6 doi: 10.1080/03009734.2016.1218575published

94. Holtkamp KCA, Mathijssen IB, Lakeman P, van Maarle MC, Dondorp WJ, Henneman L, Cornel MC. Factors for successful implementation of population-based expanded carrier screening: learning from existing initiatives. Eur J Public Health 2017;**27**(2):372-77 doi: 10.1093/eurpub/ckw110published
95. Voorwinden JS, Buitenhuis AH, Birnie E, Lucassen AM, Verkerk MA, van Langen IM, Plantinga M, Ranchor AV. Expanded carrier screening: what determines intended participation and can this be influenced by message framing and narrative information? Eur J Hum Genet 2017;**25**(7):793-800 doi: 10.1038/ejhg.2017.74published
96. Propst L, Connor G, Hinton M, Poorvu T, Dungan J. Pregnant Women's Perspectives on Expanded Carrier Screening. J Genet Couns 2018;**27**(5):1148-56 doi: 10.1007/s10897-018-0232-xpublished
97. Spencer S, Ewing S, Calcagno K, O'Neill S. Adopted Individuals' Views on the Utility and Value of Expanded Carrier Screening. J Genet Couns 2018;**27**(6):1341-48 doi: 10.1007/s10897-018-0256-2published
98. Chokoshvili D, Belmans C, Poncelet R, Sanders S, Vaes D, Vears D, Janssens S, Huys I, Borry P. Public Views on Genetics and Genetic Testing: A Survey of the General Public in Belgium. Genet Test Mol Biomarkers 2017;**21**(3):195-201 doi: 10.1089/gtmb.2016.0418published
99. Lazarin GA, Haque IS. Expanded carrier screening: A review of early implementation and literature. Semin Perinatol 2016;**40**(1):29-34 doi: 10.1053/j.semperi.2015.11.005published
100. Lazarin GA, Detweiler S, Nazareth SB, Ashkinadze E. Genetic Counselors' Perspectives and Practices Regarding Expanded Carrier Screening after Initial Clinical Availability. J Genet Couns 2016;**25**(2):395-404 doi: 10.1007/s10897-015-9881-1published
101. SUPERIOR HEALTH COUNCIL BELGIUM. Expanded carrier screening in a reproductive context. Towards a responsible implementation in the healthcare system. In: Superior Health Council, ed. Belgium, 2017.
102. Matar A, Hansson MG, Hoglund AT. "A perfect society"- Swedish policymakers' ethical and social views on preconception expanded carrier screening. J Community Genet 2019;**10**(2):267-80 doi: 10.1007/s12687-018-0389-xpublished
103. Schuurmans J, Birnie E, van den Heuvel LM, Plantinga M, Lucassen A, van der Kolk DM, Abbott KM, Ranchor AV, Diemers AD, van Langen IM. Feasibility of couple-based expanded carrier screening offered by general practitioners. Eur J Hum Genet 2019;**27**(5):691-700 doi: 10.1038/s41431-019-0351-3published
104. Belgian Genetic Expanded Carrier Screening (BeGECS). Genetic carrier screening. In: Antwerp University Hospital, ed., 2019.
105. Edwards JG, Feldman G, Goldberg J, Gregg AR, Norton ME, Rose NC, Schneider A, Stoll K, Wapner R, Watson MS. Expanded carrier screening in reproductive medicine-points to consider: a joint statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine. Obstet Gynecol 2015;**125**(3):653-62 doi: 10.1097/AOG.0000000000000666published
106. Henneman L, Borry P, Chokoshvili D, Cornel MC, van El CG, Forzano F, Hall A, Howard HC, Janssens S, Kayserili H, Lakeman P, Lucassen A, Metcalfe SA, Vidmar L, de Wert G, Dondorp WJ, Peterlin B. Responsible implementation of expanded carrier screening. Eur J Hum Genet 2016;**24**(6):e1-e12 doi: 10.1038/ejhg.2015.271published
107. American College of Obstetricians and Gynecologists. Committee Opinion No. 690: Carrier Screening in the Age of Genomic Medicine. Obstet Gynecol 2017;**129**(3):e35-e40 doi: 10.1097/AOG.0000000000001951published

108. Rowe CA, Wright CF. Expanded universal carrier screening and its implementation within a publicly funded healthcare service. J Commun Genet 2020;**11**(1):21-38 doi: 10.1007/s12687-019-00443-6published
109. Guo MH, Gregg AR. Estimating yields of prenatal carrier screening and implications for design of expanded carrier screening panels. Genet Med 2019;**21**(9):1940-47 doi: 10.1038/s41436-019-0472-7published
110. Beauchamp KA, Muzzey D, Wong KK, Hogan GJ, Karimi K, Candille SI, Mehta N, Mar-Heyming R, Kaseniit KE, Kang HP, Evans EA, Goldberg JD, Lazarin GA, Haque IS. Systematic design and comparison of expanded carrier screening panels. Genet Med 2018;**20**(1):55-63 doi: 10.1038/gim.2017.69published
111. Haque IS, Lazarin GA, Kang HP, Evans EA, Goldberg JD, Wapner RJ. Modeled Fetal Risk of Genetic Diseases Identified by Expanded Carrier Screening. JAMA 2016;**316**(7):734-42 doi: 10.1001/jama.2016.11139published
112. Balzotti M, Meng L, Muzzey D, Johansen Taber K, Beauchamp K, Curation Team MG, Curation Team BG, Mar-Heyming R, Buckley B, Moyer K. Clinical validity of expanded carrier screening: Evaluating the gene-disease relationship in more than 200 conditions. Hum Mutat 2020;**41**(8):1365-71 doi: 10.1002/humu.24033published
113. Punj S, Akkari Y, Huang J, Yang F, Creason A, Pak C, Potter A, Dorschner MO, Nickerson DA, Robertson PD, Jarvik GP, Amendola LM, Schleit J, Simpson DK, Rope AF, Reiss J, Kauffman T, Gilmore MJ, Himes P, Wilfond B, Goddard KAB, Richards CS. Preconception Carrier Screening by Genome Sequencing: Results from the Clinical Laboratory. Am J Hum Genet 2018;**102**(6):1078-89 doi: 10.1016/j.ajhg.2018.04.004published
114. Wilson JM, Jungner YG. [Principles and practice of mass screening for disease]. Bol Oficina Sanit Panam 1968;**65**(4):281-393
115. Andermann A, Blancquaert I, Beauchamp S, Dery V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ 2008;**86**(4):317-9 doi: 10.2471/blt.07.050112published
116. Andermann A, Blancquaert I, Beauchamp S, Costea I. Guiding policy decisions for genetic screening: developing a systematic and transparent approach. Public Health Genomics 2011;**14**(1):9-16 doi: 10.1159/000272898published
117. Ropers HH. On the future of genetic risk assessment. J Community Genet 2012;**3**(3):229-36 doi: 10.1007/s12687-012-0092-2published
118. Ben-Shachar R, Svenson A, Goldberg JD, Muzzey D. A data-driven evaluation of the size and content of expanded carrier screening panels. Genet Med 2019;**21**(9):1931-39 doi: 10.1038/s41436-019-0466-5published
119. Cho D, McGowan ML, Metcalfe J, Sharp RR. Expanded carrier screening in reproductive healthcare: perspectives from genetics professionals. Hum Reprod 2013;**28**(6):1725-30 doi: 10.1093/humrep/det091published
120. Matar A, Kihlbom U, Hoglund AT. Swedish healthcare providers' perceptions of preconception expanded carrier screening (ECS)-a qualitative study. J Community Genet 2016;**7**(3):203-14 doi: 10.1007/s12687-016-0268-2published
121. Janssens S, Chokoshvili D, Vears D, De Paepe A, Borry P. Attitudes of European Geneticists Regarding Expanded Carrier Screening. J Obstet Gynecol Neonatal Nurs 2017;**46**(1):63-71 doi: 10.1016/j.jogn.2016.08.012published
122. Wilfond BS, Goddard KA. It's complicated: criteria for policy decisions for the clinical integration of genome-scale sequencing for reproductive decision making. Mol Genet Genomic Med 2015;**3**(4):239-42 doi: 10.1002/mgg3.130published
123. Nazareth SB, Lazarin GA, Goldberg JD. Changing trends in carrier screening for genetic disease in the United States. Prenat Diagn 2015;**35**(10):931-5 doi: 10.1002/pd.4647published

124. Lazarin GA, Goldberg JD. Current controversies in traditional and expanded carrier screening. Curr Opin Obstet Gynecol 2016;**28**(2):136-41 doi: 10.1097/GCO.0000000000000256published
125. Abuli A, Boada M, Rodriguez-Santiago B, Coroleu B, Veiga A, Armengol L, Barri PN, Perez-Jurado LA, Estivill X. NGS-Based Assay for the Identification of Individuals Carrying Recessive Genetic Mutations in Reproductive Medicine. Hum Mutat 2016;**37**(6):516-23 doi: 10.1002/humu.22989published
126. Lau TK. Obstetricians should get ready for expanded carrier screening. BJOG 2016;**123 Suppl 3**:36-8 doi: 10.1111/1471-0528.14196published
127. Chokoshvili D, Janssens S, Vears D, Borry P. Designing expanded carrier screening panels: results of a qualitative study with European geneticists. Per Med 2016;**13**(6):553-62 doi: 10.2217/pme-2016-0018published
128. Korngiebel DM, McMullen CK, Amendola LM, Berg JS, Davis JV, Gilmore MJ, Harding CO, Himes P, Jarvik GP, Kauffman TL, Kennedy KA, Simpson DK, Leo MC, Lynch FL, Quigley DI, Reiss JA, Richards CS, Rope AF, Schneider JL, Goddard KA, Wilfond BS. Generating a taxonomy for genetic conditions relevant to reproductive planning. Am J Med Genet A 2016;**170**(3):565-73 doi: 10.1002/ajmg.a.37513published
129. Benn P, Chapman AR, Erickson K, Defrancesco MS, Wilkins-Haug L, Egan JF, Schulkin J. Obstetricians and gynecologists' practice and opinions of expanded carrier testing and noninvasive prenatal testing. Prenat Diagn 2014;**34**(2):145-52 doi: 10.1002/pd.4272published
130. Lazarin GA, Hawthorne F, Collins NS, Platt EA, Evans EA, Haque IS. Systematic Classification of Disease Severity for Evaluation of Expanded Carrier Screening Panels. PLoS One 2014;**9**(12):e114391 doi: 10.1371/journal.pone.0114391published
131. Holtkamp KC, van Maarle MC, Schouten MJ, Dondorp WJ, Lakeman P, Henneman L. Do people from the Jewish community prefer ancestry-based or pan-ethnic expanded carrier screening? Eur J Hum Genet 2016;**24**(2):171-7 doi: 10.1038/ejhg.2015.97published
132. Rose NC. Expanded carrier screening: too much of a good thing? Prenat Diagn 2015;**35**(10):936-7 doi: 10.1002/pd.4638published
133. Langlois S, Benn P, Wilkins-Haug L. Current controversies in prenatal diagnosis 4: pre-conception expanded carrier screening should replace all current prenatal screening for specific single gene disorders. Prenat Diagn 2015;**35**(1):23-8 doi: 10.1002/pd.4532published
134. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;**17**(5):405-24 doi: 10.1038/gim.2015.30published
135. Amendola LM, Jarvik GP, Leo MC, McLaughlin HM, Akkari Y, Amaral MD, Berg JS, Biswas S, Bowling KM, Conlin LK, Cooper GM, Dorschner MO, Dulik MC, Ghazani AA, Ghosh R, Green RC, Hart R, Horton C, Johnston JJ, Lebo MS, Milosavljevic A, Ou J, Pak CM, Patel RY, Punj S, Richards CS, Salama J, Strande NT, Yang Y, Plon SE, Biesecker LG, Rehm HL. Performance of ACMG-AMP Variant-Interpretation Guidelines among Nine Laboratories in the Clinical Sequencing Exploratory Research Consortium. Am J Hum Genet 2016;**99**(1):247 doi: 10.1016/j.ajhg.2016.06.001published
136. Harrison SM, Dolinsky JS, Knight Johnson AE, Pesaran T, Azzariti DR, Bale S, Chao EC, Das S, Vincent L, Rehm HL. Clinical laboratories collaborate to resolve differences in variant interpretations submitted to ClinVar. Genet Med 2017;**19**(10):1096-104 doi: 10.1038/gim.2017.14published

137. van der Hout S, Dondorp W, de Wert G. The aims of expanded universal carrier screening: Autonomy, prevention, and responsible parenthood. Bioethics 2019;**33**(5):568-76 doi: 10.1111/bioe.12555published
138. Bekker H, Thornton JG, Airey CM, Connelly JB, Hewison J, Robinson MB, Lilleyman J, MacIntosh M, Maule AJ, Michie S, Pearman AD. Informed decision making: an annotated bibliography and systematic review. Health Technol Assess 1999;**3**(1):1-156
139. Reeves A, Trepanier A. Comparison of Informed Consent Preferences for Multiplex Genetic Carrier Screening among a Diverse Population. J Genet Couns 2016;**25**(1):166-78 doi: 10.1007/s10897-015-9854-4published
140. Schuurmans J, Birnie E, Ranchor AV, Abbott KM, Fenwick A, Lucassen A, Berger MY, Verkerk M, van Langen IM, Plantinga M. GP-provided couple-based expanded preconception carrier screening in the Dutch general population: who accepts the test-offer and why? Eur J Hum Genet 2020;**28**(2):182-92 doi: 10.1038/s41431-019-0516-0published
141. Delatycki MB, Laing NG, Moore SJ, Emery J, Archibald AD, Massie J, Kirk EP. Preconception and antenatal carrier screening for genetic conditions: The critical role of general practitioners. Aust J Gen Pract 2019;**48**(3):106-10
142. Delatycki MB, Laing N, Kirk E. Expanded reproductive carrier screening-how can we do the most good and cause the least harm? Eur J Hum Genet 2019;**27**(5):669-70 doi: 10.1038/s41431-019-0356-ypublished
143. Lynch FL, Himes P, Gilmore MJ, Morris EM, Schneider JL, Kauffman TL, Shuster E, Reiss JA, Dickerson JF, Leo MC, Davis JV, McMullen CK, Wilfond BS, Goddard KAB. Time Costs for Genetic Counseling in Preconception Carrier Screening with Genome Sequencing. J Genet Couns 2018;**27**(4):823-33 doi: 10.1007/s10897-017-0205-5published
144. Janssens S, Chokoshvili D, Vears DF, De Paepe A, Borry P. Pre- and post-testing counseling considerations for the provision of expanded carrier screening: exploration of European geneticists' views. BMC Med Ethics 2017;**18**(1):46 doi: 10.1186/s12910-017-0206-9published
145. Trier C, Fournous G, Strand JM, Stray-Pedersen A, Pettersen RD, Rowe AD. Next-generation sequencing of newborn screening genes: the accuracy of short-read mapping. NPJ Genom Med 2020;**5**:36 doi: 10.1038/s41525-020-00142-zpublished
146. Kraft SA, Duenas D, Wilfond BS, Goddard KAB. The evolving landscape of expanded carrier screening: challenges and opportunities. Genet Med 2019;**21**(4):790-97 doi: 10.1038/s41436-018-0273-4published
147. The Royal Australian College of General Practitioners. Genomics in general practice. 2018. Date Accessed 17.09. www.racgp.org.au/download/Documents/Guidelines/Genomics-in-general-practice.pdf
148. Barlow-Stewart K, Burnett L, Proos A, Howell V, Huq F, Lazarus R, Aizenberg H. A genetic screening programme for Tay-Sachs disease and cystic fibrosis for Australian Jewish high school students. J Med Genet 2003;**40**(4):e45 doi: 10.1136/jmg.40.4.e45published
149. Burnett L, Proos AL, Chesher D, Howell VM, Longo L, Tedeschi V, Yang VA, Siafakas N, Turner G. The Tay-Sachs disease prevention program in Australia: Sydney pilot study. Med J Aust 1995;**163**(6):298-300
150. Gason AA, Sheffield E, Bankier A, Aitken MA, Metcalfe S, Barlow Stewart K, Delatycki MB. Evaluation of a Tay-Sachs disease screening program. Clin Genet 2003;**63**(5):386-92 doi: 10.1034/j.1399-0004.2003.00074.xpublished
151. WOLPER Jewish Hospital. School Screening Program. 2020. Date Accessed 20/11/2020. https://www.genetics.wolper.com.au/our-programs/school-screenings.

152. Ioannou L, Massie J, Lewis S, Petrou V, Gason A, Metcalfe S, Aitken MA, Bankier A, Delatycki MB. Evaluation of a multi-disease carrier screening programme in Ashkenazi Jewish high schools. Clin Genet 2010;**78**(1):21-31 doi: 10.1111/j.1399-0004.2010.01459.xpublished
153. Lew RM, Proos AL, Burnett L, Delatycki M, Bankier A, Fietz MJ. Tay Sachs disease in Australia: reduced disease incidence despite stable carrier frequency in Australian Jews. Med J Aust 2012;**197**(11):652-4 doi: 10.5694/mja12.11010published
154. Ioannou L, Massie J, Collins V, McClaren B, Delatycki MB. Population-based genetic screening for cystic fibrosis: attitudes and outcomes. Public Health Genomics 2010;**13**(7-8):449-56 doi: 10.1159/000276544published
155. Archibald AD, Smith MJ, Burgess T, Scarff KL, Elliott J, Hunt CE, Barns-Jenkins C, Holt C, Sandoval K, Siva Kumar V, Ward L, Allen EC, Collis SV, Cowie S, Francis D, Delatycki MB, Yiu EM, Massie RJ, Pertile MD, du Sart D, Bruno D, Amor DJ. Reproductive genetic carrier screening for cystic fibrosis, fragile X syndrome, and spinal muscular atrophy in Australia: outcomes of 12,000 tests. 2017
156. Sonic Genetics. Beacon Expanded Carrier Screen. Date Accessed 21/04/2019. https://www.sonicgenetics.com.au/rcs/beacon/.
157. Victorian Clinical Genetic Services. Prepair Genetic Carrier Screening. 2019. Date Accessed 22/04/2019. https://www.vcgs.org.au/tests/prepair.
158. Honnor M, Zubrick SR, Walpole I, Bower C, Goldblatt J. Population screening for cystic fibrosis in Western Australia: community response. Am J Med Genet 2000;**93**(3):198-204 doi: 10.1002/1096-8628(20000731)93:3<198::aid-ajmg7>3.0.co;2-qpublished
159. Burns BL, Bilkey GA, Coles EP, Bowman FL, Beilby JP, Pachter NS, Baynam G, Dawkins HJS, Weeramanthri TS, Nowak KJ. Healthcare System Priorities for Successful Integration of Genomics: An Australian Focus. Front Public Health 2019;**7**:41 doi: 10.3389/fpubh.2019.00041published
160. RANZCOG. Genetic carrier screening C-obs 63. Genetic carrier screening The Royal Australian and New Zealand College of Obstetricians and Gynaecologists, 2019.
161. Federal Australian Department of Health. Changes to the MBS May 2020. In: Medicare Benefits Schdule (MBS) Online, ed., May 2020.
162. Royal Australian and New Zealand College of Obstetricians and Gynaecologists. Prenatal screening and diagnosis of chromosomal and genetic abnormalities in the fetus in pregnancy (C-Obs 59). 2015. Date Accessed 20/11/2020. https://www.geneticclinic.com.au/wp-content/uploads/Prenatal-screening-and-diagnosis-of-chromosomal-and-genetic-conditions-C-Obs-59-Amended-May-2016.pdf.
163. Royal Australian and New Zealand College of Obstetricians and Gynaecologists. Prenatal screening and diagnostic testing for fetal chromosomal and genetic conditions. 2018. Date Accessed 20/11. https://ranzcog.edu.au/RANZCOG_SITE/media/RANZCOG-MEDIA/Women%27s%20Health/Statement%20and%20guidelines/Clinical-Obstetrics/Prenatal-screening_1.pdf?ext=.pdf.
164. Royal Australian and New Zealand College of Obstetricians and Gynaecologists. Genetic carrier screening (C-Obs 63). 2019. Date Accessed 20/11/2020. https://ranzcog.edu.au/RANZCOG_SITE/media/RANZCOG-MEDIA/Women%27s%20Health/Statement%20and%20guidelines/Clinical-Obstetrics/Genetic-carrier-screening(C-Obs-63)New-March-2019_1.pdf?ext=.pdf.
165. The Royal Australian College of General Practitioners. Genomics in general practice., East Melbourne, Vic: RACGP, 2018.
166. McClaren BJ, Metcalfe SA, Amor DJ, Aitken M, Massie J. A case for cystic fibrosis carrier testing in the general population. Med J Aust 2011;**194**(4):208-9

167. Archibald A, Smith M, Burgess T, Amor D. Reproductive Genetic Carrier Screening for Cystic Fibrosis, Fragile X Syndrome and Spinal Muscular Atrophy in Australia: Outcomes of 12,000 Tests. Australian & New Zealand Journal of Obstetrics & Gynaecology 2017;**57**:12-12
168. Gabrielle Birkner. Non-Jews Hit by 'Jewish' Diseases Fall Through the Cracks of Genetic Screening. 2013. Date Accessed 20/11/2020. https://forward.com/culture/181751/non-jews-hit-by-jewish-diseases-fall-through/?p=all.
169. Office of Population Health Genomics. Scoping paper on the need for a national rare disease plan for Australia. In: Western Australia Department of Health OoPHG, ed., 2013.
170. Walker CE MT, Davis G, Miller LJ, Girschik J, Brameld K, Sun W, Rath A, Aymé S, Zubrick SR, Baynam GS, Molster C, Dawkins HJ, Weeramanthri TS. The collective impact of rare diseases in Western Australia: an estimate using a population-based cohort. Genetic Medicine doi: doi: 10.1038/gim.2016.143.published
171. Robson SJ, Caramins M, Saad M, Suthers G. Socioeconomic status and uptake of reproductive carrier screening in Australia. Aust N Z J Obstet Gynaecol 2020 doi: 10.1111/ajo.13206published

CHAPTER 2

Investigating the appetite for a carrier-screening program in Western Australia

CHAPTER 2 PREFACE

In Chapter 1, I discussed the possible benefits of having a carrier-screening program in Western Australia (WA) and how that may empower couples wanting a family, by increasing their reproductive autonomy. I also discussed recent studies that showed that the community prefer screening for recessive disorders that are life-threatening and those that may cause significant physical and mental impairment[1-3]. These and other studies also show that attitudes towards PCS are complicated and influenced by many factors including genetic knowledge, feelings of vulnerability and concerns regarding the impact of the test[1,4-6]. In addition, these studies investigated themes such as the intention to use preconception carrier-screening testing if it was available, attitudes towards the carrier-screening test, who the community would prefer accessing the test from and how much they were willing to pay for the test.

However, there was no research investigating themes surrounding carrier-screening in Australia in the last two decades. Of the studies that did, almost all investigated attitudes and knowledge retention about single gene disorders such as cystic fibrosis (OMIM 219700)[7-9] or Fragile X (OMIM 300624)[10]. In 2009, Molster *et al.* investigated the genetic knowledge of Western Australians and concluded that Western Australians were aware of basic genetic concepts, but few understood the biological mechanisms of genes, inheritance and disorders[11]. The paucity of recent information about attitudes towards, knowledge of and preferences regarding carrier-screening of Western Australians made implementing a pilot study in WA challenging. Therefore, I set out to investigate the knowledge of and attitudes towards carrier-screening of Western Australians. The two sections in Chapter 2 focus

on knowledge of and attitudes towards carrier-screening in the WA community (Section 1) and of WA health professionals (Section 2) using quantitative methods.

The quantitative methods included surveys that contained several pre-existing scales measuring themes such as genetic knowledge, attitudes towards carrier-screening, intention to take a carrier-screening test and follow-up considerations. Established scales included in the questionnaire were modified to be specific to rare disorders. A detailed methodology of the measures and data analysis is described within each section. Both cohorts were given the same questionnaire, with specific questions modified to capture cohort specific information, such as "*Please specify your main professional role*" for health professionals.

Finally, this chapter concludes with a discussion on the findings from both studies and how these findings would influence the way in which the proposed pilot study will be implemented in Western Australia.

Supplementary Information (SI) for this Thesis have been placed in the Appendix on Page 212.

CHAPTER 2:

Section 1

Measuring the impact of genetic knowledge on intentions and attitudes of the community towards a carrier-screening test

Ong R, Howting D, Rea A, Christian H, Charman P, Molster C, Ravenscroft G, Laing NG. Measuring the impact of genetic knowledge on intentions and attitudes of the community towards expanded preconception carrier screening. J Med Genet 2018;55(11):744-52 doi: 10.1136/jmedgenet-2018-105362

Royston Ong

Denise Howting

Alethea Rea

Hayley Christian

Pauline Charman

Caron Molster

Gina Ravenscroft

Nigel Laing

INTRODUCTION

Autosomal recessive disorders are genetic disorders that most often arise when both parents in a couple are carriers of the same recessive disorder. In this situation, the couple have a 1-in-4 risk of an affected child in each pregnancy. Since carriers of recessive disorders are usually unaffected, children with recessive disorders are most often born into families with no history of the disorder[10]. The risk of having a child with these severe recessive disorders is higher than[1] or equal[10] to the birth prevalence of children with Downs syndrome[12]. Genetic disorder is responsible for a significant proportion of infant morbidity and mortality[13] and the burden of genetic disorder on patients, families and society in terms of suffering and cost is large. For example, Walker *et al.* investigated 437 rare disorders defined by orpha codes and demonstrated that these disorders affected 2% of the population in Western Australia; however accounted for 9.9% of hospitalisations and 10.5% of hospitalisation costs, at AUD395m a year[14].

Carrier-screening aims, according to the European Society of Human Genetics Public and Professional Policy Committee, "to facilitate informed reproductive decision making by identifying those couples at risk of having an affected child with an (autosomal or X-linked) recessive disorder"[15]. Carrier-screening programs have historically been for severe recessive disorders in high-risk populations, such as Tay-Sachs disease (TSD [OMIM #272800]) in the Ashkenazi-Jewish community,[16] or thalassemia in Mediterranean countries[17]. These programs reduced the birth prevalence of Tay-Sachs disease by over 90%[16] in the Ashkenazi community and of thalassemia, from 1:250 to 1:4,000 in Sardinia[17]. However, recommendations have been in place for some time for carrier-screening of selected recessive genetic

disorders in the general population. For example, the American College of Medical Genetics recommended carrier-screening for cystic fibrosis (CF [MIM 219700]) to all couples regardless of ethnicity in 2001[18] and for spinal muscular atrophy (SMA [MIM 253300]) in 2008[19].

It has been deduced, and now shown experimentally, that everyone is a carrier of two to eight severe recessive lethal pathogenic variants[20,21]. Therefore, screening for multiple recessive disorders through expanded carrier-screening has the potential to identify more couples at risk of an affected pregnancy[22]. Haque *et al.* in 2016 modelled an expanded panel of around 100 genes covering multiple recessive disorders and demonstrated it could detect carrier status for many more severe disorders than screening based on the guidelines then in place[23].

In 2017, the American College of Obstetricians & Gynaecologists recommended that all patients should be offered preconception carrier-screening, according to their values, including expanded carrier-screening for multiple genetic disorders[24]. Currently, Israel performs one of the most comprehensive pan-ethnic population-wide carrier-screening programs, screening >60,000 citizens annually for an expanded list of disorders[25].

In Western Australia, the largest ethnic population, at 62%, is of European descent, with therefore the same carrier risks as other European countries. There is however an increasing proportion of individuals of African, Asian and Middle Eastern descent, with higher frequencies of some recessive disorders such as thalassemia and higher rates of consanguinity than in the general population. This demography is similar to the rest of Australia[26]. There is no population-based carrier-screening program available in Western Australia currently, the only screening available through the

public health system is cascade screening in families of individuals affected by a genetic disorder. Screening from commercial entities is available but not subsidised by the healthcare system[27]. Reproductive options available to at-risk Australian couples include using assisted reproductive technologies such as in-vitro fertilisation (IVF) with pre-implantation diagnostic genetic diagnosis (PGD)[28]. However, the cost per IVF cycle in Australia is complex with partial subsidy from Medicare and some private health insurance cover, resulting in considerable out of pocket expenses.[29] Prenatal diagnosis is also available for at-risk couples and is fully subsidised by Medicare. Other options for at-risk couples include adoption, foregoing having children, or not intervening in pregnancies[30].

Increasing interest in providing carrier-screening to the general population has led to multiple investigations into various aspects of carrier-screening such as the opinions of target patient groups in various countries[1,5,6,31] or to the use of publicly-available databases such as ExAC[32] and 1000genomes[33] to theoretically determine carrier frequencies of disorders of interest[4,23,34]. However, previous studies have shown that the public's willingness to use carrier-screening is limited. Attitudes towards carrier-screening are multifaceted and influenced by a range of factors including lack of knowledge, feelings of vulnerability and concerns regarding the impact of the test[1,4-6]. These international studies explored various factors such as preferences, familiarity and perceived benefits or risk. Previous research in Australia has included obstetricians[35] or qualitatively explored themes surrounding carrier-screening[36].

Since knowledge is known to be an important factor influencing a person's intention to participate in a carrier-screening program[37,38], I sought to explore baseline

levels of genetic knowledge and awareness regarding carrier-screening in Western Australia prior to the implementation of any public health information campaigns, without specifying what carrier-screening meant in the survey. I also aimed to investigate factors that might influence knowledge and attitudes to participation in any future carrier-screening program implemented in Western Australia.

METHODS

Study design and participant recruitment

Ethics approval for the study was granted by the Human Research Ethics Committee of the University of Western Australia (RA/4/1/8847).

The study was a cross-sectional study of 832 adults who participated in an online survey conducted by a third-party market research company over a two-week period in March 2017. Eligibility criteria were residing in Western Australia and being aged 18 years or older. The survey took approximately 15 minutes to complete. A total of 24,530 individuals on four online panels of Western Australian residents were invited to participate through emails from the market research company.

As we wished to measure current baseline knowledge and attitudes towards carrier-screening of the Western Australian community, no information on carrier-screening was provided to the participants.

Measures

The questionnaire contained several existing scales measuring *prior knowledge of carrier-screening*, *genetic knowledge*, *attitudes towards carrier-screening*, *intention to take a carrier-screening test* and *follow-up considerations of carrier-screening*. Established scales included in this questionnaire were modified for this study to be

specific to rare disorders. For example, the question '*Will lead to discrimination of people with CF*'[9] was modified to '*Will lead to discrimination of people with rare disorders*'.

Genetic knowledge and prior knowledge of carrier-screening

Questions about genetic knowledge were obtained from three studies[11,39,40]. A total of 21 questions were used to test participants' level of genetic knowledge such as '*Unaffected parents can have a child with an inherited disorder*' and '*A gene is part of a chromosome*' (Table 1 & Supplementary Information 1: Table S1). Participants' responses to the genetic questions were consistent, with an alpha score of 0.89. A Pearson product-moment correlation was run to determine the validity of the genetic questions tested. There was a strong positive correlation between all questions, which was statistically significant (p=<0.001). Individual results were stratified into interquartile ranges based on the total number of correctly answered genetic questions: high, good, some, and low.

Attitudes towards carrier-screening

Items such as '*Should be made available for all couples planning a pregnancy*' and '*I will be discriminated against if I am identified as a carrier*' measured participants' attitudes towards carrier-screening and were obtained and modified from existing scales[9,39] and measured on a six-point Likert scale (1=strongly disagree; 3=neither agree nor disagree; 5=strongly agree; and 6=don't know or not applicable).

Principal factor analysis with varimax rotation, with an Eigenvalue greater than 1.0 was used to identify possible underlying dimensions in the individual statements measuring attitudes. Values lower than 0.4 were suppressed and not displayed.

Reliability analysis was also used to determine the homogeneity of each set of statements (Cronbach's α). To investigate how each factor was associated with intention to take the carrier-screening test and genetic knowledge, statements from each item in each factor were combined to create a mean score. All items in each factor were measured on the same scale. The 22 items used to measure various aspects of attitudes loaded onto four factors and explained 61% of the total variance in those items. Factor 1 to Factor 4 explained 33.7%, 16.4%, 6.1% and 4.8% of the variance respectively (SI 1: Table S2). Each factor was labelled to best reflect the items grouped in that factor and the main construct/s it was measuring. Factor 1 included 11 items measuring *apprehension and religious beliefs* (α=0.899). Factor 2 included five items measuring *equity of access and feelings of empathy* (α=0.848). Factor 3 included three items that measured *feelings about individuals with a genetic disorder* (α=0.848). Factor 4 consisted of three items measuring *test related concerns* (α=0.784). All four Factors were included in the overall model (SI 1: Table S2).

Intention to take a carrier-screening test

Intention to take a carrier-screening test was measured using the existing item[1] '*If you are offered preconception carrier-screening, would you accept the test?*' on a three-point Likert scale (1=yes; 2=no; and 3=unsure).

Follow-up considerations of carrier-screening

Existing items such as 'I *will do the test if the disorders detected are very severe*' and '*I will do a preconception carrier-screening test if it costs me less than $50*', which

measured preferences on the way the carrier-screening test is offered,[1] were included and were measured on a dichotomous scale (Yes/No).

Confounders

Socio-demographic and other potential confounders were included in the online survey and comprised age, gender, location of residence, education level, religiosity or spirituality, individual annual income, relationship status, parenthood experience and intention to be parents (SI 1: Table S3 and SI 1: Table S4).

Data analysis

Descriptive analysis was run to understand participants' characteristics. Chi-square tests of independence were used to examine the association between intention to take a carrier-screening test and i) sociodemographic and other potential confounders; ii) prior knowledge about the screening program and iii) genetic knowledge.

Multinomial logistic regression and ordinal logistic regression were used to identify factors associated with intention to take a carrier-screening test and genetic knowledge and attitudes towards carrier-screening. Socio-demographic variables that were significantly associated with intention to take the test were included in each logistic regression analysis.

RESULTS

Sociodemographic characteristics

A total of 832 participants completed the survey and 84.5% (n=719) were of reproductive age (defined as 18-44 years of age). There were approximately the same proportion of males and females. More than 36% had completed a university

degree. At least 42% of participants had an annual income less than the Western Australian median income of $52,504 per annum;[41] indicating that in regards to income, the respondents were a good representation of the Western Australian population. Almost half of the responders were not religious. Most participants (71.3%) were in a relationship, 49.9% were parents and 70.6% of participants reported their intention to become parents (Table 2).

Intention to take a carrier-screening test and follow-up considerations

Accepting the test:

Overall, 67.5% (n=562) of participants indicated that they would take the test if preconception carrier-screening was offered to them (Table 2). Of these, 92.0% said they would take the test if the disorders screened affected the lifespan of children or infants and 78.8% said they would take the test if the disorders screened for were chronic and required them to be a full-time carer. Sixty percent said they would take the test if the test screened for adult-onset disorders (Table 3A). Of those participants willing to take the test, 79.7% indicated that they would want to access the test through their general practitioner. Most participants (85.4%) reported that they would not access the test and results via the mail and/or online ordering, midwives (81.3%) or gynaecologist or obstetricians (57.8%). Finally, 75.1% reported that they would take the test if it cost less than AUD200. (Table 3A).

Declining the test:

Only 10.1% of participants reported that they would decline the carrier-screening test if it were offered to them. A third of these participants had no interest in finding

out their genetic information and 28.6% believed that the test would not be useful for them (Table 3B).

Being unsure about the test:

Overall, 22.4% of participants indicated that they were unsure about taking the test if carrier-screening was offered to them. As a follow-up to this question, 67.7% said they would like more information about the disorders tested, 46.8% said they would like more information about the technology used and 43.5% said they would like more information about post-screening options (Table 3C).

Level of genetic knowledge amongst participants

Most participants (n= 645; 77%) correctly answered at least ten out of the 21 genetic knowledge questions. Two thirds of participants answered key concepts pertaining to carrier-screening correctly (Table 1). Participants did not fare well in advanced genetic concepts regarding probability (answered correctly=13%, Question 6) and inheritance of mutations (answered correctly=35%, Question 13). Almost half of participants correctly answered that their child may still have a genetic disorder even if both parents tested negative for the disorder. Misconceptions about disorders associated with lifestyle choices were also identified, with 63% thinking that one cannot develop harmful genetic mutations from lifestyle choices and 83% thinking that spina bifida is caused only by genetic mutations (Table 1 & SI 1: Table S1).

Factors associated with the intention to take a carrier-screening test

Sociodemographic factors:

Education level was positively associated with intention to take the test. Those who had completed post-school vocational education were twice as likely to reject the test than take it compared to those who had completed year 12 or equivalent (OR=2.18, 95% CI (1.09 – 4.32), p=0.03) (SI 1: Table S5). Income was also significantly associated with taking the test. Participants who earned an annual income ofAUD$80,000-AUD125,000 compared to participants with an annual income of $0-$30,000 were two times more likely to take the test (OR=2.27, 95% CI (1.07 – 4.83), p=0.033). Those who were religious, or spiritual were three times more likely to reject the test when compared to those who were not religious or spiritual (OR=3.05, 95% CI (1.06 – 8.83), p=0.039) (SI 1: Table S5). Age, gender, relationship status and intentions of becoming a parent were *not significantly* associated with taking the test (Table 2).

Prior knowledge and genetic knowledge factors:

A third of participants (n=239) had heard about carrier-screening, reflecting prior knowledge or awareness of the screening test. Prior knowledge was shown to be significantly associated with intention to take the carrier-screening test (SI 1: Table S6). Participants who had prior awareness of the test, were more likely to either take or reject the test, compared with those who were unsure of their intentions (Take the test: OR= 2.53, 95% CI (1.65 – 3.89), p=<0.001; Reject the test: OR=2.20, 95% CI (1.20 – 4.05), p=0.011) (SI 1: Table S7). Knowing about carrier-screening from family members or searching through the internet were strongly associated with intention to take a carrier-screening test (p=<0.05). Amongst participants who had heard

about the carrier-screening test from family members, 93.2% would take the test compared with 6.8% who were unsure. Similarly, amongst participants who know the test through internet searches, 91.1% will take the test compared with 8.8% who are unsure (SI 1: Table S6).

The likelihood of an individual accepting the carrier-screening test compared with rejecting it was significantly higher for people who had 'high', 'good' and 'some' genetic knowledge compared to those who had 'low' genetic knowledge (all p=<0.05) (Table 4A and SI 1: Table S8). The participants who had 'good' genetic knowledge were seven times more likely to take the test (OR=7.62, 95% CI (3.04–19.14), p=<0.001) while those with 'high' genetic knowledge, were only four times more likely to take the test (OR=4.15, 95% CI (1.68– 10.28), p=0.002) (Table 4A).

Intention not to use carrier-screening in individuals with "high" genetic knowledge was associated with four concerns: 1*) negative impact on my family members*, 2) *confidentiality of genetic information*, 3) *discrimination based on genetic result* and 4) *negative implications to obtain health, life and/or disability insurance* (SI 1: Table S9).

Attitude factors:

Individuals were more likely to take the carrier-screening test than reject it with every one unit increase in the score (i.e. from 4 to 5 on the Likert scale) for Factor 2 "*equity of access and empathy*", Factor 3 "*feelings about individuals with a genetic disorder*" and Factor 4 "*test related concerns*" (all p=<0.001).

Individuals were less likely to take the test with every one unit increase in the score for Factor 1 "*apprehension and religious beliefs*" (OR=0.20, 95% CI (0.13 – 0.32), p=<0.001).

There were also some individuals who were more likely to be unsure about their intentions to take the test rather than rejecting the test with every one unit increase in the *feelings about individuals with a genetic disorder* score (OR=1.43, 95% CI (1.02 – 2.01), p=0.037) (Table 4B).

Association between genetic knowledge and attitudes towards carrier-screening

Increases in genetic knowledge (e.g., from 'some' genetic knowledge to 'good' genetic knowledge) were positively correlated with individuals' scores on the *equity of access and empathy* factors and *test related concerns* factor (OR=2.36, 95% CI (1.96 – 2.84), p=<0.001; OR=2.72, 95% CI (2.19 – 3.39), p=<0.001, respectively).

Individuals who had 'high' genetic knowledge but were less likely to take the test had higher mean scores for statements in attitude Factor1 "*apprehension about the test and religious beliefs*" compared with those with 'high' genetic knowledge who said they intended to use carrier-screening. In addition, these individuals also had lower mean scores for statements in Attitude Factors 2 "*equity of access and empathy*", 3 "*feelings about individuals with a genetic disorder*" and 4 "*tests related concerns*" (SI 1: Table S10).

As genetic knowledge decreased, scores for Factor 1 "*apprehension about the test and religious beliefs*" increased (OR=2.78, 95% CI (2.26 – 3.43), p=<0.001) (Table 4C).

Table 1: Participants' level of genetic knowledge and knowledge about concepts related to genetics and carrier-screening (n=832)

Level of genetic knowledge	Number of questions answered correctly	n=	Percent
High	16 – 21	284	34.10%
Good	11-15	361	43.40%
Some	6-10	104	12.50%
Low	0 – 5	83	10.00%
Total		832	100%

Genetic Questions (correct answer)	Correct answer	Incorrect answer
Genetic questions testing basic concepts (True/False/Don't know)		
Question 1. An individual with a genetic mutation for a recessive disorder is known as a carrier (True)	542 (65%)	290 (35%)
Question 2. A carrier of a genetic disorder carries a mutation for that disorder but does not have the disorder (True)	548 (66%)	284 (34%)
Question 5. Individuals in certain ethnic groups have an increased risk of being carriers of certain abnormal genes (True)	609 (73%)	223 (27%)
Question 7. Healthy parents can have a child with an inherited disorder (True)	658 (79%)	174 (21%)
Question 9. Half your genes come from your mother and half from your father (True)	518 (62%)	314 (38%)
Question 10. A gene is part of a chromosome (True)	505 (61%)	327 (39%)
Question 11. Genes are segments of DNA that encode information critical for development (True)	645 (78%)	187 (22%)
Question 12. Genetic mutations may either harm or have little to no effect on an organism (True)	542 (65%)	290 (35%)
Question 14. Some harmful genetic mutations can be inherited (True)	668 (80%)	164 (20%)
Genetic questions testing understanding (True/False/Don't know)		
Question 3. If both my partner and I test negative for a specific disorder, our baby will definitely not have that disorder (False)	400 (48%)	432 (52%)
Question 4. I can be a carrier for a genetic disorder even though there is no history of the disorder in my family (True)	555 (67%)	277 (33%)
Question 6. If both my parents are carriers, I have a 75% chance of becoming a carrier (False)	111 (13%)	721 (87%)
Question 8. If a person is the carrier of a disorder gene, it means that they have the disorder (False)	581 (70%)	251 (30%)
Question 13. Genetic mutations in the DNA of any cells will be passed on to offspring (False)	289 (35%)	543 (65%)
Question 15. You cannot develop harmful genetic mutations from lifestyle choices (False)	312 (37%)	520 (63%)
Genetic question testing misconceptions (Genetic Mutations/Environmental Factors/Mixture/Don't know)		
Question 16. Eye colour (Genetic Mutations)	623 (75%)	209 (25%)
Question 17. Food poisoning (Environmental Factors)	685 (82%)	147 (18%)
Question 18. Spina bifida (Mixture)	144 (17%)	688 (83%)
Question 19. Frost bite (Environmental Factors)	704 (85%)	128 (15%)
Question 20. Cystic fibrosis (Genetic Mutations)	554 (67%)	278 (33%)
Question 21. Diabetes (Mixture)	599 (72%)	233 (28%)

Table 2: Demographics of study participants and intention groups

	Would you take the test?					
	Yes (n=562,68%)	No (n=84,10%)	Unsure (n=186,22%)	Total (n=832,100%)	Value	p-value
Age (years)						
18 – 24	72 (13%)	12 (14%)	28 (15%)	112 (13%)	2.38	0.89
25 – 44	409 (73%)	62 (74%)	136 (73%)	607 (73%)		
45 – 64	59 (10%)	6 (7%)	17 (9%)	82 (10%)		
65+	22 (4%)	4 (5%)	5 (3%)	31 (4%)		
Gender						
Male	252 (45%)	39 (46%)	90 (48%)	381 (46%)	19.10	0.076
Female	309 (55%)	43 (51%)	96 (52%)	448 (54%)		
Religiosity						
Yes	221 (39%)	50 (60%)	74 (40%)	345 (41%)	13.49	**0.01**
No	341 (61%)	34 (40%)	112 (60%)	487 (59%)		
Education						
Completed university	220 (39%)	27 (32%)	57 (31%)	304 (37%)	13.32	**0.039**
Completed vocational education	146 (26%)	32 (38%)	52 (28%)	230 (28%)		
Currently studying university or vocational education	67 (12%)	12 (14%)	21 (11%)	100 (12%)		
Completed high school or equivalent	129 (23%)	13 (16%)	56 (30%)	198 (24%)		
Annual individual income?						
$125,000 and over	41 (8%)	12 (17%)	9 (6%)	62 (8%)	18.58	**0.016**
$80,000 - $124,999	129 (25%)	11 (15%)	30 (18%)	170 (23%)		
$50,000 - $79,999	135 (26%)	14 (19%)	55 (34%)	204 (27%)		
$30,000 -$49,999	89 (17%)	12 (16%)	30 (18%)	131 (17%)		
$0 - $29,999	124 (24%)	24 (33%)	40 (24%)	188 (25%)		
What is your relationship status?						
In a relationship	405 (72%)	61 (73%)	127 (68%)	593 (71%)	1.06	0.59
Not in a relationship	157 (28%)	23 (27%)	59 (32%)	239 (29%)		
Are you, or have you been, a parent (including adoptive or step)?						
Yes	277 (49%)	49 (58%)	89 (48%)	415 (50%)	12.09	0.076
No	273 (49%)	32 (38%)	95 (51%)	400 (48%)		
No, we are expecting a child soon.	12 (2%)	3 (4%)	2 (1%)	17 (2%)		
Do you intend to be a parent?						
Yes	399 (71%)	65 (77%)	123 (66%)	587 (71%)	3.69	0.160
No	163 (29%)	19 (23%)	63 (34%)	245 (29%)		

Bold number indicates significant associations

Number indicates the responses while parentheses indicate percentages.

Table 3A: Considerations when participants want to take the test

Will take the test (n=562; 67.5%)						
Statement			**NO**		**YES**	
I will do the test if the disorders that are screened affects lifespan of any children or infants.			45 (8%)		517 (92%)	
I will do the test if the disorders that are screened is chronic and requires me to be a full-time carer.			119 (21%)		443 (79%)	
I will do the test if the disorders that are screened first show symptoms when my child is an adult but still able to look after himself/herself.			221 (39%)		341 (61%)	
I would want to access this test through my: General Practitioner (GP)			114 (20%)		448 (80%)	
I would want to access this test through my: Midwife			457 (81%)		105 (19%)	
I would want to access this test through my: Gynaecologist/Obstetrician			325 (58%)		237 (42%)	
I would want to access this test through my: Genetic counsellor			328 (58%)		234 (42%)	
I would want to access this test through my: Through mail or online ordering			480 (85%)		82 (15%)	
I will do a preconception carrier-screening test if it costs me	**Free**	**< AUD50**	**AUD50 to AUD200**	**AUD200 to AUD500**	**AUD500 to AUD1000**	**Any amount**
	109 (19%)	121 (22%)	192 (34%)	75 (13%)	19 (3%)	46 (8%)

Table 3B: Considerations when participants do not want to take the test

Will not take the test (n=84; 10.1%)		
Statement	**NO**	**YES**
I will not take the test because if we take it, pregnancy becomes less natural.	73 (87%)	11 (13%)
I will not take the test because I am concerned about my privacy regarding my genetic information.	76 (90%)	8 (10%)
I will not take the test because I don't trust the test results.	76 (90%)	8 (10%)
I will not take the test because I am not interested in finding out my genetic information.	57 (68%)	27 (32%)
I will not take the test because I don't believe it would be useful to me.	60 (71%)	24 (29%)
I will not take the test because I am concerned the information will have a negative impact on my life.	66 (79%)	18 (21%)
I will not take the test because I am concerned the information will have a negative impact on my family members.	69 (82%)	15 (18%)
I will not take the test because I don't trust the organisations/companies/people offering the test.	80 (95%)	4 (5%)
I will not take the test because I am opposed to genetic testing.	78 (93%)	6 (7%)
I will not take the test because I am concerned what other people might do with my genetic information.	75 (89%)	9 (11%)
I will not take the test because I am concerned, I could be discriminated against based on my personal genetic test results.	73 (87%)	11 (13%)
I will not take the test because I am concerned that obtaining my personal genetic information will have negative implications on my ability to obtain health, life and/or disability insurance.	68 (81%)	16 (19%)
I will not take the test because I am concerned that my employer could discriminate based on my personal genetic results.	78 (93%)	6 (7%)

Number indicates the responses while parentheses indicate percentages.

Table 3C: Considerations when participants are unsure about taking the test

Will not take the test (n=186; 22.4%)		
Statement	**NO**	**YES**
More information about specific disorders tested.	60 (32%)	126 (68%)
More information about the technology used for the screening program.	99 (53%)	87 (47%)
More information about post screening pathways including adoption and surrogacy options and IVF subsidies.	105 (56%)	81 (44%)

Number indicates the responses while parentheses indicate percentages.

Table 4: Logistic regression with significant associations comparing genetic knowledge, attitudes and taking the test (adjusting for social demographics)

A: Genetic knowledge and taking the test

Would you take the test?	Genetic knowledge levels	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
Yes[a]	High genetic knowledge	1.42	**0.002**	4.15	1.68	10.28
	Good genetic knowledge	2.03	**<0.001**	7.62	3.04	19.14
	Some genetic knowledge	1.12	**0.032**	3.06	1.1	8.52
	Low genetic knowledge[b]	0	.	.	.	.
Unsure[a]	Good genetic knowledge	-1	**0.028**	0.366	0.15	0.9
	Low genetic knowledge[b]	0	.	.	.	.

a. The reference category is: No.
b. Reference variable.

B: Attitudes and taking the test

Attitudes	Would you take the test?	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
Factor1: *Apprehension about the test and religious beliefs*	Yes[a]	-1.59	**<0.001**	0.2	0.13	0.31
Factor2: *Equity of access and empathy*	Yes[a]	1.77	**<0.001**	5.84	3.9	8.76
Factor3: *Feelings about individuals with a genetic disorder*	Yes[a]	0.7	**<0.001**	2.01	1.48	2.74
	Unsure[a]	0.36	**0.04**	1.43	1.02	2.01
Factor4: *Tests related concerns*	Yes[a]	1.04	**<0.001**	2.82	1.82	4.35

a. The reference category is: No.

C: Genetics knowledge and attitudes

Genetic knowledge	Attitude	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
Decreasing genetic knowledge: *i.e., High genetic knowledge to Low genetic knowledge*	Factor1*: Apprehension about the test and religious beliefs*	1.02	**<0.001**	2.78	2.26	3.43
Increasing genetic knowledge: *i.e., Low genetic knowledge to High genetic knowledge*	Factor2: *Equity of access and empathy*	0.86	**<0.001**	2.36	1.96	2.84
	Factor4: *Tests related concerns*	1	**<0.001**	2.72	2.19	3.39

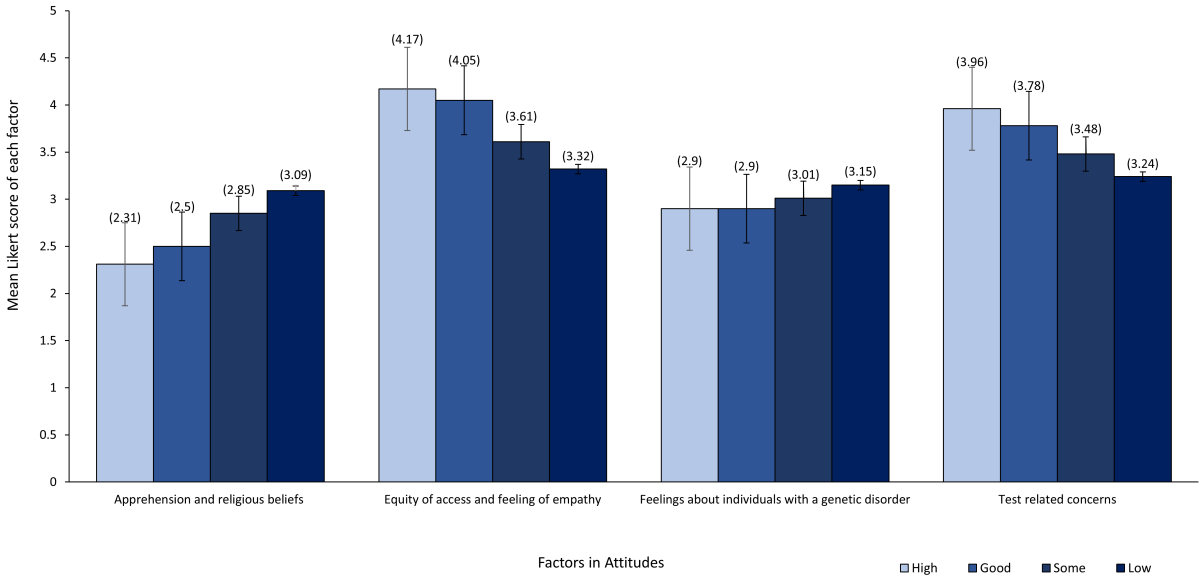


Figure 1: Distribution of the mean Likert score per factor across different levels of genetic knowledge.

The X-axis represents various factors identified in questions related to attitudes. The Y-axis measures the mean Likert score for each factor. Colours represent each of the four levels of genetic knowledge: 'high'; 'good'; 'some' and 'low'. Genetic knowledge is measured according to how many genetic questions an individual answered correctly. Error bars indicate standard error. The number in parentheses above each bar indicates the mean Likert score of each factor from individuals with a specific genetic knowledge. Likert scores range from 1 to 5 with 1 being strongly disagree and 5 being strongly agree.

DISCUSSION

This study identified key factors associated with intention to participate in a carrier-screening program. Higher levels of genetic knowledge correlated significantly with carrier-screening participation, consistent with previous studies[37,38]. Of interest is the comparative decrease in intention to participate in a carrier-screening test in participants who had 'high' genetic knowledge compared with those with 'good' genetic knowledge. Our results show that those that have 'good' genetic knowledge were seven times more likely to take the test while individuals with 'high' genetic knowledge, were only four times more likely to take the test. This finding appears to be explained in part by participants concerns related to: 1*) negative impact on my family members*, 2) *confidentiality of genetic information*, 3) *discrimination based on genetic result* and 4) *negative implications to obtain health, life and/or disability insurance*. In addition, individuals with 'high' genetic knowledge who said they would not take the test scored more highly on Factor 1 attitudes "apprehension about the test and religious beliefs" and less highly on attitudes in relation to statements in the other three Factors. This indicates that high genetic knowledge has limited influence on certain attitudes.

Issues around privacy and insurance received the greatest number of responses among those who had 'high' levels of genetic knowledge and had no intention of taking the test. Since the introduction of expanded gene panels in screening programs, similar concerns have been raised and identified in studies amongst health professionals and communities[15,22,39,42]. This has resulted in calls for more transparent methods of ensuring confidentiality and privacy in order to minimise stigmatisation and social discrimination[43]. Community education, public campaigns and more extensive pre- and post-test counselling have been

suggested as methods to reduce social discrimination. Some authors have suggested that the introduction of an expanded carrier-screening program may reduce social stigma through the "universal test" approach as opposed to targeting a single ethnic group[15].

Similarly, genetic discrimination is recognised as an international phenomenon[44] and can occur in different types of insurance covers such as health or life insurance[43]. As such, legislation including a moratorium, or the Oviedo Convention, (which prohibits insurance companies from asking for any genetic test results from their applicant) are in place in certain countries, to protect their citizens from genetic discrimination[44]. Moratoria temporarily restricting insurers' use of genetic information exist in Australia and the UK[45]. Under the moratoria, insurers cannot request their applicants to undergo a genetic test or request previous results for policies under certain amounts, but applies only to health and not life insurance in Australia. As a result, even though participants in our study may have an understanding that being a carrier does not implicate or have an impact on their health, there is no legal framework in Australia to safeguard and protect consumers against discrimination by life insurance companies. As shown, this fear may reduce intention to participate in carrier-screening programs. Otlowski *et al.* suggested that continuous monitoring of policies on insurance, through any available common metrics and instruments, will aid in the comparative studies of long-term impact on individuals, families and the community[44].

Previous studies have highlighted that knowledge, attitude and personal values affect informed decision-making[38,46]. Consistent with other studies[11,38,47], our data showed that 77.5% of participants had at least 'good' genetic knowledge. Highly educated individuals tend to have higher levels of genetic knowledge and

a deeper understanding of genetic concepts. However, a proportion of individuals with 'good' genetic knowledge answered incorrectly questions that tested understanding (SI 1: Table S11) such as "*if both members of a couple test negative for a specific disorder, their child may still have a disorder*". These statements reflect core principles in preconception carrier-screening and without a sound understanding, informed decision-making may be compromised. This result suggests that having 'good' knowledge may not be sufficient to understand and appreciate core concepts of preconception carrier-screening and may impact the ability to make informed decisions. The community may benefit from a tailored education program to reduce misconceptions and improve genetic literacy.

Participants who had positive attitudes towards the test tended to agree with statements such as '*Provides couples with reproductive choices*' or '*It is difficult for a person with a severe recessive disorder to have a very good life*'. These individuals were at least twice as likely to take the test, consistent with previous studies showing that positive attitudes towards a screening test generally correlates significantly with participation rates[9,38]. Conversely, individuals who were more agreeable to statements such as '*Is morally unacceptable*' or '*Will do more harm than good*' were less likely to take the test. These individuals were also more likely to have lower levels of genetic knowledge (Table 4C). Similarly, we found that with increasing genetic knowledge, individuals tend to agree more with statements such as *'Provides couples with reproductive choices'* as well as statements about *'A post-test consultation with a genetic counsellor would be essential'* (Figure 1). However, deeply held personal values and beliefs such as '*I think it is wrong to knowingly bring a child with a severe recessive disorder into the world*' and religious values are not influenced by genetic knowledge (Table 4C). Our results also show that religious

individuals are three time more likely to reject the test than participate. Overall, these findings highlight that increasing genetic knowledge may have a positive effect on certain attitudes, but not personal values and beliefs, which go on to influence participation rates.

We also show that prior knowledge of carrier-screening before taking the test is associated with increased likelihood of participation (p=<0.001) (SI 1: Table S6). Further investigation indicates those who had prior awareness of carrier-screening reported that they would either take the test or reject it (SI 1: Table S7). This conflicting result may suggest that those who will take the test probably have a positive attitude towards the test, perceived susceptibility to the disorder or probably want to avoid having an affected child, as studies have suggested[48]. Conversely, those who decline the test may feel that they are not at risk, or that a lack of family history is sufficient to convince them that such tests are unnecessary[42].

Our results show that who individuals learn about the test from is important. Although numbers are small, if an individual learned about the test through a family member, none would not take the test (SI 1: Table S6). The high level of intention to participate in those who had heard about carrier-screening from a family member, suggests the social environment is strongly associated with an individual's intention to participate in carrier-screening and is consistent with other studies examining how an individual's beliefs about a particular behaviour are influenced by the judgement of significant others (e.g., family)[49].

More than two-thirds of our participants indicated intentions to use a carrier-screening test. Three previous studies have shown about a third of their participants were willing to take the test[1,5,42]. The significant increase in media coverage of

carrier-screening in Australia in the months prior to the survey, (for example,[50,51]), may have raised awareness about carrier-screening testing, and highlighted the benefits of adopting carrier-screening before pregnancy. This may have encouraged more participants to consider taking carrier-screening. It was not surprising that most participants in the Netherlands study preferred to access the test through their general practitioner (GP) and trust their opinions, given the strong primary healthcare structure in the Netherlands. Similarly, in Western Australia, almost 80% of our participants preferred to access the test through their GP. Most healthy Australians will see a GP at least annually, whereas interactions with medical specialists (e.g. obstetricians) are less frequent. Interestingly, most of our participants rejected all other options including accessing the test through a gynaecologist or obstetrician, or accessing the test and results directly via mail and/or online ordering. This may suggest confidence in our primary healthcare structure, or simply that GPs provide the greatest convenience to the community.

This cross-sectional study provides comprehensive data on key factors affecting intentions to participate and attitudes towards carrier-screening in Western Australia. We show that increased genetic knowledge and a positive attitude to genetic testing are instrumental in influencing intentions to participate and whether those decisions are informed. Concerns surrounding social issues because of screening were also raised.

The study nevertheless has limitations which might bias the findings. The demographics may not fully represent the Western Australian population; though it is indicative of the cohort to whom carrier-screening would be most relevant. As participants could choose whether to participate, self-selection bias may mean that the respondents included an overrepresentation of individuals both strongly for or

against carrier-screening. In addition other variables that may affect the uptake interest, such as perceived behavioural control (how easy or difficult it is for an individual to perform the particular behaviour)[49]. Availability of reproductive options, or considerations around termination of pregnancy were not directly measured.

It is well known that intentions to do a behaviour and actual participation are not always in alignment, and may be influenced by factors such as social barriers (e.g. stigmatization, discrimination), familiarity of disorders tested and awareness or perceived benefit[6]. Consequently, tailored community education programs addressing the issues identified in this study would be required to ensure individuals with different levels of genetic knowledge are sufficiently informed to make decisions regarding carrier-screening testing. This study highlights that continuous education of GPs, and thus the community, is crucial to reduce misconceptions and to raise awareness about preconception carrier-screening in the community. Increasing genetic literacy amongst those who have a positive attitude towards screening in turn might improve uptake. Our findings thus inform how carrier-screening might best be implemented into the future.

Supplementary information for this Chapter is available on page 213 in the Appendix section.

PRESENTATIONS AND AWARDS:

An abstract on this study was selected as an oral presentation during the 2018 Human Genetics Society of Australasia conference (Sydney).

Results from this study were awarded the *BEST POSTER* in the Young Statistics Western Australian workshop and the 2018 Combined Biological Sciences Meeting.

CHAPTER 2:

Section 2

Attitudes towards and knowledge of an expanded carrier-screening test amongst Western Australian health professionals

Prepared manuscript

Royston Ong
Samantha Edwards
Denise Howting
Alethea Rea
Hayley Christian
Gina Ravenscroft
Nigel Laing

INTRODUCTION

Carrier-screening aims to identify couples who are at risk of having a child with a monogenic recessive disorder[52]. In communities with a high prevalence of recessive disorders, carrier-screening programs were available as early as the 1970s[16,17] and have successfully reduced disorder incidence[16]. Currently most carrier-screening programs are recommended for specific communities or ancestries[53]. For example, Israel's pan-ethnic population carrier-screening programs screen >60,000 citizens annually for known mutations in an expanded list of disorders[25].

Financial limitations restrict most traditional screening programs to identifying only known pathogenic mutations associated with a set of disorders[25]. As a result, only disorders that have well-defined phenotypes and a high prevalence within a population have been targeted by traditional carrier-screening. However, recent studies have shown that the risk of having a child with a severe recessive disorder in the general population is higher than[1] or equal to[10] the birth prevalence of Down syndrome[12]. Furthermore, the recent advent of massively-parallel sequencing (MPS) technologies has prompted a new type of screening program in which genes associated with hundreds of recessive disorders can be investigated and screened simultaneously[1,20,23]. This new form of screening program is known as an expanded carrier-screening program and differs from traditional screening programs where only known pathogenic mutations associated with a set of disorders[25] are screened for.

As MPS declines in cost, this form of sequencing has become more cost-effective than traditional methods[54] and as a result, the use of MPS technology for

screening is gaining traction and popularity[20,23,55,56]. Haque *et al.* recently demonstrated that an expanded carrier-screening panel could detect carrier status for hundreds of severe disorders at once; much more than the current carrier-screening recommendations[23]. This suggests that expanded screening panels have a place in reproductive healthcare. In response to the increasing popularity of using MPS for carrier-screening, the American College of Obstetricians & Gynaecologists recommended in 2017 that all patients should be offered preconception carrier-screening, according to their values, including expanded carrier-screening for multiple genetic disorders[57].

In the last few years commercial entities have also started offering carrier-screening testing using MPS technology[58-60]. The gene panels used sometimes include disorders that might be very rare or considered mild[34]. Consequently, some health professionals warn about the possibility of limited utility of an expanded carrier-screening test, and a lack of immediate benefits[61,62] when offering carrier-screening to a community without a history of recessive disorders. Researchers have also warned that offering an expanded carrier-screening program does not automatically translate to meaningful reproductive choices[63], or necessarily provide equity of access to screening[62]. Couples may misunderstand the limitations of the test, thereby potentially causing more harm than good to those who decide to use carrier-screening[64]. Researchers have also cautioned against offering carrier-screening too hastily[61,62,64,65] and argue that a carrier-screening program should not be a top-down approach initiated by the healthcare system but rather a response to an actual need by the community, such was the case for screening for Tay-Sachs disease in the Ashkenazi Jewish population[16].

Advocates of expanded carrier-screening programs, however, argue that it increases reproductive autonomy as well as improving equitable access to reproductive information, which may reduce the stigma associated with being a carrier or having a genetic disorder[63,66] or even detect more at-risk couples[10,23,55].

It was in the context of these opposing views that we sought to investigate the attitude and knowledge of carrier-screening amongst a representative sample of Western Australian (WA) health professionals. In addition, we investigated if there was support for or concerns against implementing carrier-screening and how these perceptions differ between health professionals and the general community.

METHODS

Recruitment

All the participating health care professionals were recruited via the snowball sampling method.

Measures

Information about items measuring genetic knowledge and prior knowledge of carrier-screening, attitudes towards carrier-screening, intentions to use carrier-screening and follow-up considerations on those who would use the test have been reported previously[52]. Concerns around carrier-screening implementation were included in items measuring attitudes towards carrier-screening. Established scales about specific rare disorders were modified for this study to rare disorders in general. For example, the question "*Will lead to discrimination of people with CF*"[9] was modified to "*Will lead to discrimination of people with rare disorders*".

Questions about genetic knowledge are shown in Supplementary Information 2: Table S1 and were measured on a three-point Likert scale (1=True; 2=False; 3=Don't know). A total of 20 questions were used to test participants' level of genetic knowledge such as '*Unaffected parents can have a child with an inherited disorder*'. Knowledge items measured basic understanding, advance concepts related to carrier-screening and misconceptions. Individual results were summed and stratified into interquartile ranges based on the total number of correctly answered genetic questions: *high, good, some,* and *low*.

Items measuring health professionals' attitude towards carrier-screening were modified from existing scales[9,47] and measured on a six-point Likert scale (1=Strongly disagree; 3=Neither agree nor disagree; 5=Strongly agree; and 6=Don't know or not applicable). Responses were grouped into three sub-scales: 1) seven statements that supported the use of carrier-screening (e.g. "carrier-screening *provides couples with reproductive options*"); 2) 12 statements that reflected fear and distrust in the implementation of carrier-screening (e.g. "carrier-screening *is morally unacceptable"*); and 3) one statement that reflected ambivalence (e.g. "carrier-screening *may result in an increase in my insurance rates")* (Figure 1). Items in the 'fear and distrust' sub-scale (12 items) were reverse coded and summed with the items from the 'supported the use of carrier-screening sub-scale (7 items) to give a combined attitude towards carrier-screening score. Scores greater than or equal to 12 reflected a more positive attitude towards carrier-screening (Figure 2). The statement measuring ambivalence was not included in the positive attitudes towards carrier-screening score.

Intention to take a carrier-screening test was measured on a three-point Likert scale (1=Yes; 2=No; and 3=Unsure). Follow-up considerations on intentions to use carrier-screening such as "*I will do the test if the disorders detected are very severe*" were included and were also measured (1= Yes; 2=No).

Socio-demographic and other potential confounders collected included: age, gender, religion, education level, individual annual income, relationship status, parenthood experience, intention to be parents and profession (Table 1).

Data analysis

Descriptive analyses were conducted. Chi-square tests of independence were used to examine the association between intention to take a carrier-screening test and i) sociodemographic and other potential confounders; ii) prior knowledge about the screening program and iii) genetic knowledge.

Multinomial logistic regression was used to identify factors associated with health professionals' intention to take a carrier-screening test as well as genetic knowledge and attitudes towards carrier-screening. The Wilcoxon rank-sum test was used to determine whether there were significant differences associated with basic genetic concepts and genetic questions that tested understanding. Socio-demographic variables that were significantly associated with intention to take the test were included in each logistic regression analysis (parenthood experience and profession). Tableau Desktop Professional Edition software (*v.2018.2.0*) was used to visualise participants' preference on attitudes and intentions to take the test.

Finally results from the health professionals were contrasted with our previous study analysing community attitudes[52] (Table 2) in a summary table comparing the

proportion of health professionals and the community who intended to take a carrier-screening test, their genetic knowledge and attitudes.

RESULTS

Socio-demographic characteristics

Almost three quarters (73.4%, n=149) of health professionals were within the reproductive age group (defined as 18-44 years of age). There were similar numbers of practitioners and non-practitioners, with 80.0% of respondents being female. 60.6% of health professionals had an income between $50,000 and $124,000 AUD per annum. More than half of the responders (68.5%) were not religious and 80.9% were in a relationship. 46.8% were parents and 30% of those who were not parents said they would like to be parents. Finally, 65.0% of health professionals knew someone who was affected with a genetic disorder(s) (Table 1).

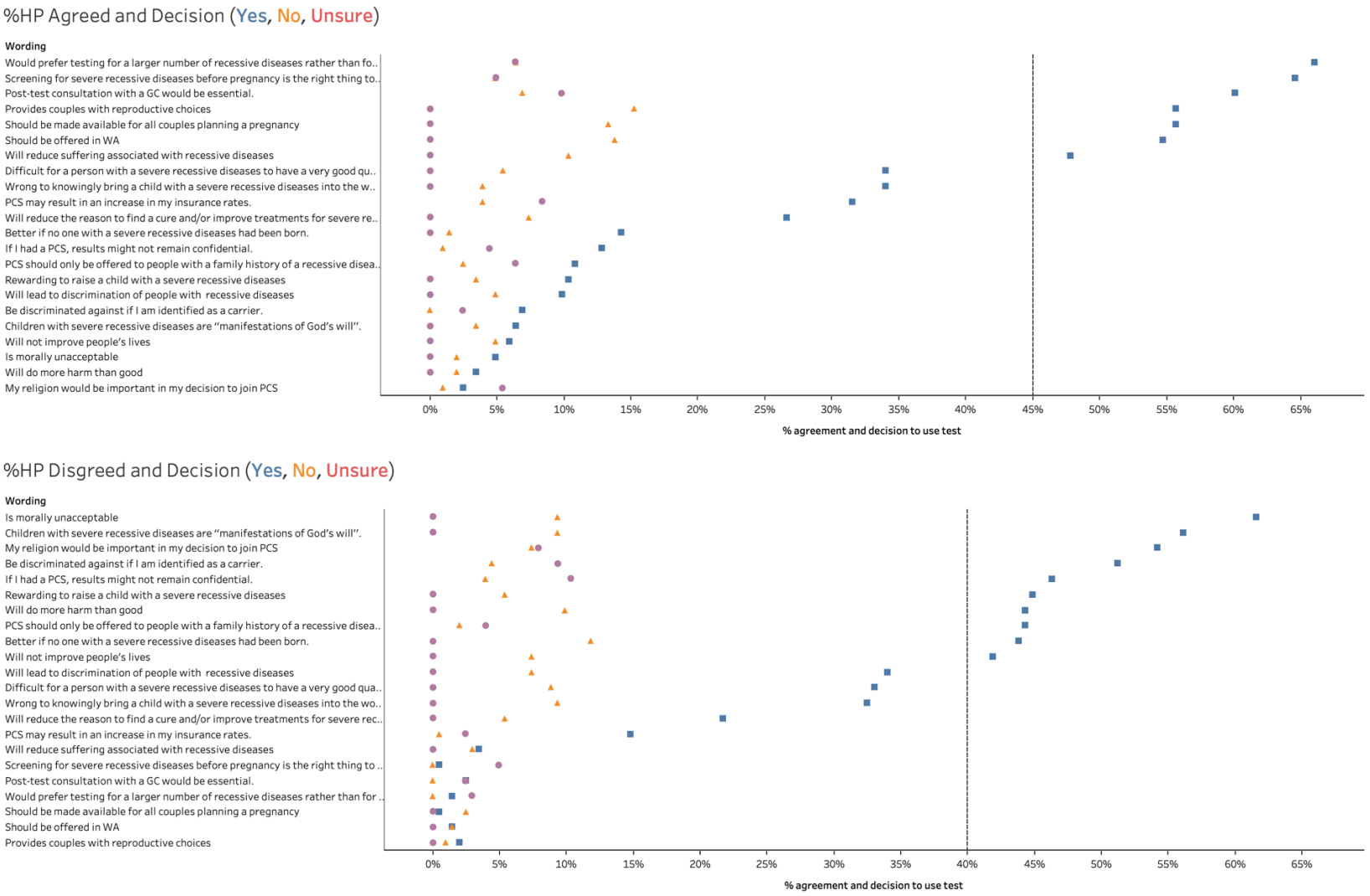


Figure 1: Distribution of attitude statements amongst health professionals and their intention to use carrier-screening themselves.
Dots represent proportion of health professionals who either agree (top figure) or disagree (bottom figure) to different attitude statements and their intentions to use carrier-screening. Blue squares represented health professional with intentions to use carrier-screening, purple dots represented health professional who have no intention to use carrier-screening, and orange triangles represented health professional who are unsure.

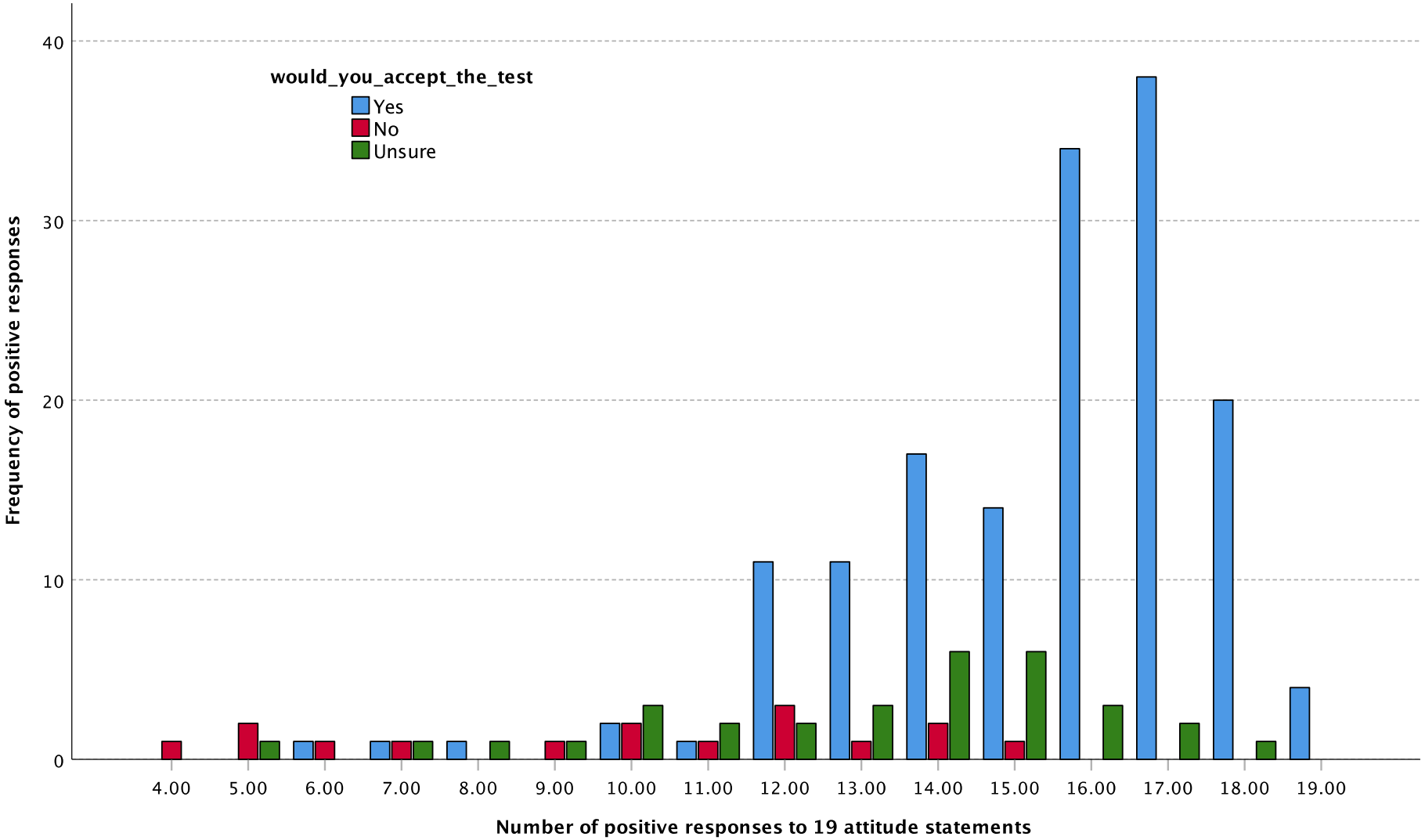


Figure 2: Number of positive responses from health professionals and their intentions to use carrier-screening test themselves.
The X-axis represents the number of positive responses of health professionals to 19 attitude statements while the Y-axis represents the frequency of positive responses. Blue bars represent health professionals with intentions to use carrier-screening, red bars represent health professionals who have no intention to use carrier-screening, and green bars represent health professionals who are unsure.

Intention to take a carrier-screening test and follow-up considerations to use carrier-screening

Accepting the test:

Overall, 76.4% (n=155) of health professional indicated that they would take the test if carrier-screening were offered to them (Table 2). Of these, 96.8% (n=150) said they would take the test if the disorders screened affected the lifespan of children or infants and if the disorders screened for were chronic and required them to be a full-time carer. More than 70% said they would take the test if the test screened for adult-onset disorders (Table 2, SI 2: Table S1A). When given a choice of who they would prefer accessing the test from, 83.9% reported that they would prefer accessing the test through their general practitioners (GP), 83.9% through their gynaecologists or obstetricians and 80.6% through a genetic counsellor. 65.8% reported that they would take the test if it cost between AUD0 to AUD200. (SI 2: Table S1A).

Declining the test:

Only 7.9% of health professional reported that they would decline the carrier-screening test if offered to them (Table 2). Amongst those who declined taking the test, 81.3% (n=13) were practitioners. The five main considerations or concerns for not wanting to take the test included: 1) 75% did not want to take the test because they do not believe it to be useful to them; 2) 56% were not interested in finding out the results; 3) 56% believed that the results would have a negative impact on their life; 4) 38% thought it would have a negative impact on their family; and 5) 44% had concerns about insurance (SI 2: Table S1B).

Unsure about the test:

Overall, 15.7% of health professional indicated that they were unsure about taking the test if carrier-screening was offered to them (Table 2). As a follow-up to this question, 65.6% said they would like more information about the disorders tested, 43.8% said they would like more information about the technology used and post-screening options (SI 2: Table S1C).

Table 1: Demographics of participants by intentions to take the carrier-screening test

	Would you take the test?				Value	p-value
	Yes (n=155,76%)	No (n=16,8%)	Unsure (n=32,16%)	Total (n=203,100%)		
Age (years)						
18 – 24	14 (9%)	0 (0%)	3 (9%)	17 (8%)	2793	0.25
25 – 44	103 (66%)	9 (56%)	20 (63%)	132 (65%)		
45 – 64	35 (23%)	7 (44%)	7 (22%)	49 (24%)		
65+	3 (2%)	0 (0%)	2 (6%)	5 (3%)		
Gender						
Male	32 (21%)	0 (0%)	7 (22%)	39 (19%)	4.16	0.14
Female	123 (79%)	16 (100%)	25 (78%)	164 (81%)		
Religiosity						
Yes	45 (29%)	9 (56%)	10 (31%)	64 (31%)	4.98	0.09
No	110 (71%)	7 (44%)	22 (69%)	139 (69%)		
Education						
Completed vocational education	7 (5%)	1 (6%)	3 (9%)	11 (5%)	1.25	0.64
Currently studying university	6 (4%)	0 (0%)	0 (0%)	6 (3%)		
Completed university	57 (37%)	12 (75%)	16 (50%)	85 (42%)		
Currently pursuing post-graduate studies (Masters or PhD)	25 (16%)	2 (13%)	4 (13%)	31 (15%)		
Completed post-graduate studies (Masters or PhD)	60 (38%)	1 (6%)	9 (28%)	70 (35%)		
Annual individual income?						
I prefer not to say	7 (5%)	1 (6%)	3 (9%)	11 (5%)	6.20	0.82
$125,000 and over	20 (13%)	0 (0%)	3 (9%)	23 (11%)		
$80,000 - $124,999	53 (34%)	5 (31%)	13 (41%)	71 (35%)		
$50,000 - $79,999	40 (26%)	4 (25%)	8 (25%)	52 (26%)		
$30,000 -$49,999	19 (12%)	3 (19%)	3 (9%)	25 (12%)		
$0 - $29,999	16 (10%)	3 (19%)	2 (7%)	21 (10%)		
What is your relationship status?						
In a relationship	125 (81%)	15 (94%)	24 (75%)	164 (81%)	14.25	0.17
Not in a relationship	30 (19%)	1 (6%)	8 (25%)	39 (19%)		
Are you, or have you been, a parent (including adoptive or step)?						
Yes	62 (40%)	10 (63%)	16 (50%)	88 (43%)	9.83	**0.041**^
No	88 (57%)	4 (25%)	16 (50%)	108 (53%)		
No, we are expecting a child soon.	5 (3%)	2 (12%)	0 (0%)	7 (4%)		
Do you intend to be a parent?						
Yes	52 (59%)	2 (50%)	7 (44%)	61 (57%)	3.69	0.17
No	9 (10%)	2 (50%)	3 (19%)	14 (13%)		
Not sure	22 (25%)	0 (0%)	6 (37%)	28 (26%)		
Unable to conceive	5 (6%)	0 (0%)	0 (0%)	5 (4%)		
Profession						
Practitioner	82 (53%)	13 (81%)	23 (72%)	118 (58%)	7.74	**0.021**^
Non-practitioner	73 (47%)	3 (19%)	9 (28%)	85 (42%)		

^ represent significant association ($p<0.05$) with intentions of each potential confounders to use carrier-screening

Table 2: Summary comparison between health professionals and the community

Questions	Response	Community (n=832)	Health Professional (n=203)	p-value
Genetic Knowledge	High Knowledge *(>15 correct answers)*	34.1%	89.7%	**<0.001**^
	Good Knowledge *(10 – 15 correct answers)*	43.4%	9.4%	
	Some Knowledge *(5 – 9 correct answers)*	12.5%	0.5%	
	Low Knowledge *(<5 correct answers)*	10.0%	0.5%	
Intention to take the test	Yes	67.5%	76.4%	**0.05**^
	No	10.1%	7.9%	
	Unsure	22.4%	15.7%	
Disorders screened affected the lifespan of children or infants	Yes	92.0%	96.8%	**0.039**^
Chronic disorders and required to be full time carer	Yes	78.9%	96.7%	**<0.001**^
Adult onset disorders	Yes	60.6%	73.5%	**0.003**^
Access through GP	Yes	79.7%	83.9%	0.247
Access through a midwife	Yes	18.7%	43.2%	**<0.001**^
Access through a gynaecologist/obstetrician	Yes	42.2%	83.9%	**<0.001**^
Access through a genetic counsellor	Yes	41.6%	80.6%	**<0.001**^
Access through mail or online ordering	Yes	14.6%	20.0%	0.10
Willingness to pay	Free	19.4%	0%	**<0.001**^
	<AUD50	21.5%	7.1%	
	AUD50 to AUD200	34.2%	21.9%	
	AUD200 to AUD500	13.3%	29.7%	
	AUD500 to AUD1000	3.4%	21.9%	
	Any amount	8.2%	19.4%	
Social factors and decision to take test	Will take test	Religiosity	-	N/A
	Will take test	Education	Parenthood experience	N/A
	Will take test	Income	Occupation	N/A
Prior Awareness about carrier-screening	Yes	28.7%	98.5%	**<0.001**^
Where from?	Will take test	Family members	Friends	N/A
	Will take test	Internet searches	Healthcare professionals	N/A
Provides couples with reproductive choices.	Agree	76.8%	98.5%	**<0.001**^
A post-test consultation with a genetic counsellor would be essential.	Agree	67.5%	94.1%	**<0.001**^
Should be offered in Western Australia.	Agree	76.2%	92.1%	**<0.001**^
Will reduce suffering associated with recessive disorders.	Agree	66.8%	81.3%	**<0.001**^
If the costs were the same, I would prefer to be tested for a larger number of recessive disorders rather than for a smaller number.	Agree	70.8%	79.8%	**0.010**^
Should be made available for all couples planning a pregnancy.	Agree	74.3%	78.3%	0.232
I think that being screened for severe recessive disorders before pregnancy is the right thing to do.	Agree	61.3%	69.0%	**0.043**^
	Mean	70.5%	84.7%	

^: represent significant associations.

Table continues next page.

Table 2: Summary comparison between WA community and health professionals (continue)

Is morally unacceptable.	Disagree	60.0%	88.2%	
Will do more harm than good.	Disagree	58.5%	87.2%	
I think that children with severe recessive disorders are ‘‘manifestations of God’s will’’.	Disagree	55.3%	86.2%	
Will not improve people’s lives.	Disagree	55.2%	82.3%	
I will be discriminated against if I am identified as a carrier.	Disagree	47.1%	74.4%	
If I had a preconception carrier-screening test, I would worry that the results might not remain confidential.	Disagree	45.2%	73.4%	
My religion would be important in my decision to participate in carrier-screening.	Disagree	54.8%	70.4%	**<0.001‡**
Will reduce the reason to find a cure and/or improve treatments for severe recessive disorders.	Disagree	36.2%	66.0%	
Carrier-screening should only be offered to people with a family history of a recessive disorder.	Disagree	43.3%	65.0%	
Will lead to discrimination of people with recessive disorders.	Disagree	41.1%	64.5%	
It would be better if no one with severe recessive disorders had been born.	Disagree	45.9%	61.6%	
Raising a child with a severe recessive disorder would be rewarding for me.	Disagree	40.4%	54.7%	
	Mean	48.6%	72.8%	

‡: all associations between health professionals and the community were significant

Level of genetic knowledge amongst health professionals

Most health professional had high genetic knowledge with 89.7% (n=182/203) correctly answering at least 16 out of 20 genetic knowledge questions. There was no significant difference (p=0.083) in the average number of correct answers for questions testing basic genetic concepts (Mean=94%) and questions that tested understanding (80%) (S2: Table S2).

Almost all health professional (93.6%) answered key carrier-screening concepts correctly. Almost half of the health professional answered genetic concepts about probability incorrectly (46.3%; Question 6) while 27.6% answered genetic concepts about result interpretation incorrectly (Question 3). Misconceptions about disorders associated with lifestyle choices were also identified, with 42.4% responding that spina bifida is caused only by genetic mutations (SI 2: Table S2).

Attitudes amongst health professionals

Collectively, 88.2% of health professional responded positively (λ_2=0.781) towards having an expanded carrier-screening program in Western Australia.

Items measuring support for the use of carrier-screening showed that 98.5% of health professional agreed that carrier-screening provides couples with reproductive choices; 94.1% agreed that a post-test consultation with a genetic counsellor would be essential; and 92.1% agreed that carrier-screening should be offered in Western Australia (Table 2, SI 2: Table S3). However, practitioners were generally more conservative in their support for carrier-screening (78.8%) compared with non-practitioners (89.2%) (Table 3). In addition, non-practitioners were significantly more supportive of the statements, "*If the costs were the same, I would prefer to be tested for a larger number of recessive disorders rather than for a smaller number*" (p=0.04) and "*I think that being screened for severe recessive disorders before pregnancy is the right thing to do*" (p=0.01) (Table 3).

Items measuring fear and distrust in the implementation of carrier-screening, showed that 88.2% of health professional disagreed that carrier-screening is morally unacceptable, 87.2% disagreed that carrier-screening will do more harm than good or that it will not improve people's lives (82%) (Table 2, SI 2: Table S3). There was no difference in attitudes amongst practitioners and non-practitioners in items measuring fear and distrust in the implementation of carrier-screening (Non-practitioners and Practitioners =72.8%) (Table 3). However, fewer non-practitioners disagreed with the statements "*I will be discriminated against if I am identified as a carrier*" (p=0.03) and "*It would be better if no one with a severe recessive disorder had been born*" (p=<0.001) while fewer practitioners disagreed with the statement

"*Will reduce the reason to find a cure and/or improve treatments for severe recessive disorders*" (p=0.04) (Table 3).

Health professional factors associated with the intention to take a carrier-screening test

Sociodemographic factors:

Health professionals who were not parents were 3.5 times more likely to take the test compared to those who were parents (OR=3.55, 95% CI (1.06 – 11.83), p=0.039). Non-practitioners were 3.9 times more likely to take the test than not take the test compared to practitioners (OR=3.86, 95% CI (1.06 – 14.08), p=0.041) (Table 4A). All other sociodemographic factors were *not significantly* associated with taking the test (Table 1).

Prior knowledge and genetic knowledge factors:

The majority of health professional (80.3%) had heard about carrier-screening. However, there was no significant association between prior awareness and intentions to take the test (SI 2: Table S4). Of those who were aware of carrier-screening, knowing about carrier-screening from friends or healthcare professionals was strongly associated with intention to take a carrier-screening test (p=<0.05). Amongst health professional who had heard about the carrier-screening test from friends, 58.6% would take the test, 24.1% would not take the test and 17.2% were unsure. In contrast, amongst health professional who had heard about carrier-screening through other healthcare professionals, 96.2% would take the test compared with 3.8% who were unsure (SI 2: Table S5). There was no significant association between genetic knowledge and intentions to take the test (p=0.11).

Attitude:

Of those who indicated that they would take the test, 64.3% of health professionals supported the use of carrier-screening while 55.9% disagreed with fear and distrust statements about having carrier-screening in WA (SI 2: Tables S6). Collectively, 88.2% of health professionals who responded positively to 12 or more attitude statements were 31.9 times more likely to take the test than those who responded less positively (OR=31.9, 95% CI (8.86 – 114.9), p=<0.001) (Table 4B).

Differences in attitude and preferences between health professionals and the WA community

Data comparing both cohorts are summarised in Table 2. Results showed that both health professionals and the community were equally interested in using carrier-screening, with more than two-thirds intending to use carrier-screening. Both study cohorts agreed that carrier-screening should be made available for all couples planning a pregnancy. More health professionals preferred to screen for chronic disorders than the community (p=<0.001) with more than 95% of health professionals who said they would use carrier-screening indicating that they would use screening for disorders that will affect the lifespan of children or for chronic debilitating disorders. In contrast, the community had a stronger preference to screen for disorders that affect the lifespan of children and infants and less so for chronic debilitating disorders.

Health professionals' genetic knowledge (p=<0.001), intentions to use carrier-screening test (p=0.05) and various preferences (p=<0.05) were all significantly different from the WA community. More health professionals preferred to access the test through gynaecologists or genetic counsellors in addition to GP practices than

the WA community (p=<0.001). Religious individuals from the community were less likely to take the test whereas religiosity is not significantly associated with intentions to take the test in health professionals. Education and income correlated with potential uptake rates in the community while parenthood experience and occupation correlated with uptake rates in health professionals. Our results show that health professionals were significantly more positive about carrier-screening than the community with 84.7% of health professionals agreeing to items measuring support for the use of carrier-screening compared to 70.5% of the community. A significant difference (p=<0.001) was observed in items measuring fear and distrust with 72.8% of health professionals disagreeing with these statements compared to 48.6% of the community (Table 2).

Table 3: Practitioners' and non-practitioners' attitudes to different statements

Agreed to attitude statements				
Questions	Attitude Statements	Non-Practitioner	Practitioner	p-value
1	Provides couples with reproductive choices	100.0%	97.5.%	0.33
20	A post-test consultation with a genetic counsellor would be essential.	97.6%	91.5%	0.19
2	Should be offered in Western Australia	94.1%	90.7%	0.60
4	Will reduce suffering associated with recessive disorders	80.0%	82.2%	0.92
21	***If the costs were the same, I would prefer to be tested for a larger number of recessive disorders rather than for a smaller number.***	**88.2%**	**73.7%**	**0.04**^
3	Should be made available for all couples planning a pregnancy.	85.9%	72.9%	0.55
16	***I think that being screened for severe recessive disorders before pregnancy is the right thing to do.***	**78.8%**	**61.9%**	**0.01**^
	Mean	89.2%	78.8%	-

Disagreed to attitude statements				
Questions	Attitude Statements	Non-Practitioner	Practitioner	p-value
9	Is morally unacceptable	88.2%	88.1%	0.54
8	Will do more harm than good	91.8%	83.9%	0.21
14	I think that children with severe recessive disorders are ''manifestations of God's will''.	89.4%	83.9%	0.34
7	Will not improve people's lives	88.2%	78.0%	0.10
15	***I will be discriminated against if I am identified as a carrier.***	**65.9%**	**80.5%**	**0.03**^
17	If I had a preconception carrier-screening test, I would worry that the results might not remain confidential.	64.7%	79.7%	0.06
18	My religion would be important in my decision to participate in preconception carrier-screening.	69.4%	71.2%	0.87
5	***Will reduce the reason to find a cure and/or improve treatments for severe recessive disorders***	**71.8%**	**61.9%**	**0.04**^
22	Preconception carrier-screening should only be offered to people with a family history of a recessive disorder.	69.4%	61.9%	0.53
6	Will lead to discrimination of people with recessive disorders	69.4%	61.0%	0.32
11	***It would be better if no one with a severe recessive disorder had been born.***	**44.7%**	**73.7%**	**<0.001**^
12	Raising a child with a severe recessive disorder would be rewarding for me.	61.2%	50.0%	0.10
	Mean	72.8%	72.8%	-

^: represent significant associations.

Table 4: Logistic regression of factors and intentions to use carrier-screening amongst health professionals (adjusting for social demographics)

A: Socio-demographic factors and taking the test

Would you take the test[a]	Socio-demographics factors	B	p-value	Exp(B)	95% CI for Exp(B)	
					Lower Bound	Upper Bound
Yes	Expecting a child	-0.91	0.32	0.4	0.07	2.37
	No parenthood experience	1.27	**0.039**^	3.55	1.06	11.83
	Had parenthood experience	0[b]	-	-	-	-
Yes	Non-Practitioner	1.35	**0.041**^	3.86	1.06	14.08
	Practitioner	0[b]	-	-	-	-

B: Attitude factors and taking the test

Would you take the test[a]	Attitude	B	p-value	Exp(B)	95% CI for Exp(B)	
					Lower Bound	Upper Bound
Yes	[More positive Attitude= >12]	3.464	**<0.001**^	31.93	8.86	114.90
	[Less positive attitude= <12]	0[b]	-	-	-	-
Unsure	[More positive Attitude= >12]	1.19	0.063	3.29	0.93	11.50
	[Less positive attitude= <12]	0[b]	-	-	-	-

a. Reference category is: No.
b. This parameter is set to zero because it is redundant.

^: represent significant correlations.

DISCUSSION

The study demonstrated that the majority of health professionals in Western Australia have positive attitudes towards expanded preconception carrier-screening and indicated that they would use carrier-screening. Health professionals reported that they would like to have access to carrier-screening through GPs, genetic counsellors and gynaecologists/obstetricians. In contrast, results from our prior survey of the WA community[52] found that the community preferred accessing carrier-screening through their GPs. This difference in who to access carrier-screening through may be, in part, due to health professionals being more aware of the complexities of carrier-screening and that accessing the test through a specialist may more adequately address any potential issues. Awareness about such complexities was also highlighted with the increased proportion of allied health practitioners and genetic counsellors who were unsure about increasing the number of disorders in a panel (SI 2: Table S7).

Health professional's comprehension and awareness about the complexities of carrier-screening may be a reflection of their high genetic knowledge level. However, levels of genetic knowledge amongst health professionals were not significantly associated with intentions to take the test. In addition, more than a quarter of health professionals were less informed about genetic concepts involving probability and result interpretation, despite answering key carrier-screening concepts correctly. This is consistent with a previous study where only a third of obstetricians were comfortable with counselling patients prior to the test and even less were comfortable explaining the results[66]. Qualitative studies have also

highlighted the lack of necessary expertise amongst practitioners who can offer carrier-screening to inform and counsel on various aspects of the test[62,64].

Therefore, while practitioners, especially GPs and gynaecologists or obstetricians, should be able to offer carrier-screening, access to resources such as laboratory scientists and genetic counsellors to clarify issues, would provide better support for non-genetic clinicians. Training non-genetic health professionals, such as GPs, in pre-test counselling has been identified as a strategy for better equipping them with the tools to deal with common issues such as the limitations of carrier-screening and misconceptions about carrier-screening [52,64]. In addition, supporting resources for health professionals to clarify their concerns around result interpretation and limitations of the test would help to reduce barriers related to offering carrier-screening and potentially increase comprehension about carrier-screening amongst the community. A similar lack of knowledge in genetic concepts involving probability and result interpretation was also observed in the WA community and a tailored education program for the community was proposed to address this issue[52]. We postulate that a tailored education program for the community will not only empower them in their decision-making processes but enable more effective use of consultation time for GPs to address questions specific to each couple's needs.

Our data also show that practitioners and non-practitioners had very similar attitudes towards carrier-screening. This is particularly important because it provides some insight as to how different health professionals may respond to the implementation of carrier-screening in the health system and how they may advise individuals in regards to using carrier-screening.

Health professionals' concerns

Significantly different opinions and concerns regarding carrier-screening were report amongst different groups of health professionals. For example, genetic counsellors and allied health practitioners were more cautious about agreeing to the statement "*screening for severe recessive disorders is the right thing to do before pregnancy*". In addition, the statement, "*it would be better if no one with a severe recessive disorder had been born*" is a contentious topic amongst non-practitioners. A quarter of researchers agreed with the statement while more than 30% of researchers and diagnostic laboratory scientists were unsure about it. In contrast, more than 70% of all practitioners, including clinicians, disagreed with the statement (SI 2: Table S7). Ready *et al.* previously investigated similar attitudes amongst women's healthcare providers and reported that 88.1% disagreed that it would be better if no one with a severe recessive disorders had been born[47]. Therefore, while our clinicians have similar attitudes to those in Ready *et al.*, more studies are required to understand why particular groups of health professionals have different attitudes – given the high overall support for carrier-screening and what these attitudes might mean for carrier-screening programs during implementation.

Furthermore, differences in concerns about confidentiality and discrimination were observed in statements such as, "*I will be discriminated against if I am identified as a carrier*" and "*If I had a preconception carrier-screening test, I would worry that the results might not remain confidential*". Non-practitioners, in particular diagnostic laboratory scientists and technicians were unsure about whether they would be discriminated against, while some diagnostic lab scientists and researchers reported that they are worried the result might not be confidential (SI 2: Table S7). A previous

study had found similar apprehension amongst some health professionals – that carrier-screening might lead to stigmatisation of, or discrimination against, individuals due to their carrier status[63]. Interestingly, in our study, about 81% of practitioners disagreed that they will be subjected to discrimination or that the results will not be confidential (SI 2: Table S7). This difference may suggest that non-practitioners lack experience in the processes of delivering genetic results to patients or have witnessed patients being discriminated against after receiving their genetic results.

Concern about discrimination by health professionals based on the results from carrier-screening is not isolated within non-practitioners. In our study of the community's attitudes to carrier-screening, we found that half of the community also had concerns about discrimination and stigmatisation[52] (Tables 4, SI 2: Table S8). Furthermore, studies in other countries had previously reported similar proportions of participants being worried about stigmatization[67] or discrimination[5,42,56]. However, long-term studies have shown little evidence of discrimination against individuals identified as carriers[37,68]. To protect patients from such discrimination, a moratorium currently restricts insurers' use of genetic information in Australia[44]. Health insurance companies in Australia cannot discriminate individuals based on their genetic information, however better policies are required to protect Australians, as there is no legal framework currently to safeguard consumers against potential discrimination by life insurance companies[44]. We have previously highlighted that more transparent methods of ensuring confidentiality and privacy are needed in addition to increasing community education, public awareness campaigns and comprehensive pre- and post-test counselling to help reduce social discrimination[52]. Future studies of

expanded carrier-screening should identify if any discrimination around carrier status exists in the short and longer term.

Differences between health professionals' and the community's responses

Our results showed that the community were more apprehensive and uncertain about implementing carrier-screening than health professionals. Significant differences in attitudes that reflected the apprehension between the two cohorts were identified. This was most prominent in statements measuring fear and distrust in the implementation of carrier-screening (Table 2) whereby 48% of the community disagreed with these statements, compared to 72% of health professionals who disagreed. Previous studies have argued that participants may struggle to find screening results meaningful, or to make informed choices, due to a lack of understanding of the results from the disorders tested[5,63] or even comprehend the amount of information presented to them[69]. These issues could also manifest in increased anxiety due to inconclusive results[62] or the seemingly little clinical utility (e.g. further actions as a result of knowing their carrier status), for the vast majority of couples who use carrier-screening [70]. Nonetheless, studies in disorders such as cystic fibrosis or haemoglobinopathies have shown little evidence of long-term psychological harm associated with being identified as a carrier for a recessive disorder[37,68]. More studies are required to determine actual levels of apprehension and anxiety in the community using pilot studies as opposed to studies presenting a hypothetical situation.

This study has limitations, which may have introduced bias. The small cohort of health professionals may not be representative of all Western Australian health professionals' views and opinions. As participants could choose whether to

participate, self-selection bias may have led to an over-representation of individuals either strongly for or against carrier-screening. In addition, other variables such as barriers to taking the test, or anxiety were not directly measured.

Conclusion

Our results indicated that the majority of West Australian health professionals have positive attitudes towards carrier-screening and that it is acceptable to the medical community in Western Australia. In addition, this study has identified that researchers and diagnostic scientists were concerned about discrimination and confidentiality issues, while genetic counsellors were worried about doing more harm than good. Furthermore, the WA community were more concerned than health professionals about the outcomes of implementing carrier-screening. Further research is required to provide clarity on issues such as anxiety, apprehension and potential discrimination raised in this study. Overall, the findings highlight that implementing carrier-screening requires a multidisciplinary team of primary health care practitioners including GPs, obstetricians and gynaecologists, diagnostic laboratory medical scientists and genetic pathologists, allied with genetic services in clinical geneticists and genetic counsellors.

Supplementary information for this Chapter is available on page 230 in the Appendix section.

PRESENTATIONS AND AWARDS:

An abstract on this study was selected for a poster presentation at the 2019 Human Genetics Society of Australasia conference (Wellington, New Zealand).

CHAPTER 2 SUMMARY DISCUSSION

Results from these two studies show that there is significant support from both the community and health professionals in Western Australia (WA) for the use of carrier-screening if it was available. Both cohorts responded positively to the concept of having a carrier-screening test available in WA and in both cohorts the majority preferred accessing the test through general practitioners (GPs). However, non-genetic specialists, including GPs, offering ECS need access to genetic specialists such as genetic counsellors or clinical geneticists, to clarify any issues around the screening. As such, there are clear benefits to upskilling the work force, improving genetic literacy amongst health professionals and the community.

The results from both cohorts show that almost everyone who wanted to use ECS would screen for disorders that affect the lifespan of infants and children. This finding aligns with current disorder selection recommendations for carrier-screening, as stated in Chapter 1. Lastly, if given a choice, the majority of both cohorts who would use the test, would prefer screening for more disorders than less.

A number of key concerns were identified in the studies despite the general support. Issues surrounding discrimination and confidentiality were raised repeatedly. Specifically, non-practitioners and the community were more concerned than practitioners about being discriminated against, or that the results would not be confidential. I discussed that this may, in part, be due to the lack of experience of the community and non-practitioners, in the processes of delivering genetic results to patients. Some health professionals also indicated concerns that couples may experience an increased amount of anxiety due to inconclusive results, or lack of an available post-test support network pertaining to a couple's carrier status. Finally,

there are concerns regarding couples with incomplete genetic knowledge and whether their decisions were truly informed. This is based on the increased genetic misconceptions in core preconception carrier-screening principles observed amongst this sub-population.

Overall, the findings from the two studies define some requirements needed to implement a successful carrier-screening pilot program in WA, such as:

1) The preconception carrier-screening test should be offered at least through GPs and genetic counsellors. Since there are both public and private genetic counsellors (who feed patients into the public health system), in Western Australia, both would have to be included in any pilot study.

2) A comprehensive pre-test counselling session for each couple should be designed to clarify misconceptions surrounding the test and potentially reduce anxiety surrounding privacy and confidentiality.

3) Life-limiting disorders affecting children and infants should be screened for.

The next two chapters describe the preparation (Chapter 3) and implementation phase (Chapter 4) of the pilot ECS study in Western Australia. These are informed largely on the findings of this chapter.

REFERENCES

1. Plantinga M, Birnie E, Abbott KM, et al. Population-based preconception carrier screening: how potential users from the general population view a test for 50 serious diseases. *Eur J Hum Genet.* 2016;24(10):1417-1423.
2. Holtkamp KC, van Maarle MC, Schouten MJ, Dondorp WJ, Lakeman P, Henneman L. Do people from the Jewish community prefer ancestry-based or pan-ethnic expanded carrier screening? *Eur J Hum Genet.* 2016;24(2):171-177.
3. Abuli A, Boada M, Rodriguez-Santiago B, et al. NGS-Based Assay for the Identification of Individuals Carrying Recessive Genetic Mutations in Reproductive Medicine. *Hum Mutat.* 2016;37(6):516-523.
4. Shiroff JJ, Nemeth LS. Public Perceptions of Recessive Carrier Testing in the Preconception and Prenatal Periods. *J Obstet Gynecol Neonatal Nurs.* 2015;44(6):717-725.
5. Ekstrand Ragnar M, Tyden T, Kihlbom U, Larsson M. Swedish parents' interest in preconception genetic carrier screening. *Ups J Med Sci.* 2016:1-6.
6. Holtkamp KCA, Mathijssen IB, Lakeman P, et al. Factors for successful implementation of population-based expanded carrier screening: learning from existing initiatives. *Eur J Public Health.* 2017;27(2):372-377.
7. Honnor M, Zubrick SR, Walpole I, Bower C, Goldblatt J. Population screening for cystic fibrosis in Western Australia: community response. *Am J Med Genet.* 2000;93(3):198-204.
8. Gordon C, Walpole I, Zubrick SR, Bower C. Population screening for cystic fibrosis: knowledge and emotional consequences 18 months later. *American journal of medical genetics Part A.* 2003;120A(2):199-208.
9. Maxwell SJ, Kyne G, Molster C, Barker NM, Ormsby J, O'Leary P. Perceptions of population cystic fibrosis prenatal and preconception carrier screening among individuals with cystic fibrosis and their family members. *Genetic testing and molecular biomarkers.* 2011;15(3):159-164.
10. Archibald AD, Smith MJ, Burgess T, et al. Reproductive genetic carrier screening for cystic fibrosis, fragile X syndrome, and spinal muscular atrophy in Australia: outcomes of 12,000 tests. 2017.
11. Molster C, Charles T, Samanek A, O'Leary P. Australian study on public knowledge of human genetics and health. *Public Health Genomics.* 2009;12(2):84-91.
12. World Health Organisation. Births with Down's syndrome per 100 000 live births. 2017; https://gateway.euro.who.int/en/indicators/hfa_603-7120-births-with-downs-syndrome-per-100-000-live-births/. Accessed 1/11, 2017.
13. Kingsmore SF, Lantos JD, Dinwiddie DL, et al. Next-generation community genetics for low- and middle-income countries. *Genome Med.* 2012;4(3):25.
14. Walker CE, Mahede T, Davis G, et al. The collective impact of rare diseases in Western Australia: an estimate using a population-based cohort. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2016.
15. Henneman L, Borry P, Chokoshvili D, et al. Responsible implementation of expanded carrier screening. *Eur J Hum Genet.* 2016;24(6):e1-e12.
16. Kaback M, Lim-Steele J, Dabholkar D, Brown D, Levy N, Zeiger K. Tay-Sachs disease--carrier screening, prenatal diagnosis, and the molecular era. An international perspective, 1970 to 1993. The International TSD Data Collection Network. *JAMA.* 1993;270(19):2307-2315.
17. Cao A, Rosatelli MC, Monni G, Galanello R. Screening for thalassemia - A model of success. *Obstet Gyn Clin N Am.* 2002;29(2):305-+.

18. Grody WW, Cutting GR, Klinger KW, et al. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2001;3(2):149-154.
19. Prior TW, Professional P, Guidelines C. Carrier screening for spinal muscular atrophy. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2008;10(11):840-842.
20. Bell CJ, Dinwiddie DL, Miller NA, et al. Carrier testing for severe childhood recessive diseases by next-generation sequencing. *Sci Transl Med.* 2011;3(65):65ra64.
21. Morton NE, Crow JF, Muller HJ. An Estimate of the Mutational Damage in Man from Data on Consanguineous Marriages. *Proc Natl Acad Sci U S A.* 1956;42(11):855-863.
22. Edwards JG, Feldman G, Goldberg J, et al. Expanded carrier screening in reproductive medicine-points to consider: a joint statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine. *Obstet Gynecol.* 2015;125(3):653-662.
23. Haque IS, Lazarin GA, Kang HP, Evans EA, Goldberg JD, Wapner RJ. Modeled Fetal Risk of Genetic Diseases Identified by Expanded Carrier Screening. *JAMA.* 2016;316(7):734-742.
24. American College of Obstetricians and Gynecologists. Committee Opinion No. 690 Summary: Carrier Screening in the Age of Genomic Medicine. *Obstet Gynecol.* 2017;129(3):595-596.
25. Zlotogora J, Grotto I, Kaliner E, Gamzu R. The Israeli national population program of genetic carrier screening for reproductive purposes. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2016;18(2):203-206.
26. *Australian Bureau of Statistics. Census of Population and Housing: Reflecting Australia - Stories from the Census.* 2016.
27. Laboratory AC. Counsyl - Family Prep Screen. https://www.clinicallabs.com.au/patient/our-tests/pregnancy-testing/counsyl/.
28. Delatycki MB, Burke J, Christie L, et al. Human Genetics Society of Australasia position statement: population-based carrier screening for cystic fibrosis. *Twin Res Hum Genet.* 2014;17(6):578-583.
29. IVF A. IVF Treatment Costs. 2017; https://www.ivf.com.au/ivf-fees/ivf-costs. Accessed 30/04/2018, 2018.
30. Lew RM, Burnett L, Proos AL, Delatycki MB. Tay-Sachs disease: current perspectives from Australia. *Appl Clin Genet.* 2015;8:19-25.
31. Voorwinden JS, Buitenhuis AH, Birnie E, et al. Expanded carrier screening: what determines intended participation and can this be influenced by message framing and narrative information? *Eur J Hum Genet.* 2017;25(7):793-800.
32. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature.* 2016;536(7616):285-291.
33. The Genomes Project C. A global reference for human genetic variation. *Nature.* 2015;526:68.
34. Lazarin GA, Haque IS, Nazareth S, et al. An empirical estimate of carrier frequencies for 400+ causal Mendelian variants: results from an ethnically diverse clinical sample of 23,453 individuals. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2013;15(3):178-186.
35. Stark Z, Massie J, McClaren B, et al. Current practice and attitudes of Australian obstetricians toward population-based carrier screening for inherited conditions. *Twin Res Hum Genet.* 2013;16(2):601-607.

36. Archibald AD, Hickerton CL, Wake SA, Jaques AM, Cohen J, Metcalfe SA. "It gives them more options": preferences for preconception genetic carrier screening for fragile X syndrome in primary healthcare. *J Commun Genet.* 2016;7(2):159-171.
37. Ioannou L, McClaren BJ, Massie J, et al. Population-based carrier screening for cystic fibrosis: a systematic review of 23 years of research. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2014;16(3):207-216.
38. Metcalfe SA, Martyn M, Ames A, et al. Informed decision making and psychosocial outcomes in pregnant and nonpregnant women offered population fragile X carrier screening. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2017.
39. Ready K, Haque IS, Srinivasan BS, Marshall JR. Knowledge and attitudes regarding expanded genetic carrier screening among women's healthcare providers. *Fertil Steril.* 2012;97(2):407-413.
40. Lazarin GA, Detweiler S, Nazareth SB, Ashkinadze E. Genetic Counselors' Perspectives and Practices Regarding Expanded Carrier Screening after Initial Clinical Availability. *Journal of genetic counseling.* 2016;25(2):395-404.
41. *Australian Bureau of Statistics. Estimates of Personal Income for Small Areas, 2011-2016: OVERVIEW OF PERSONAL INCOME IN AUSTRALIA.* 2016.
42. Gilmore MJ, Schneider J, Davis JV, et al. Reasons for Declining Preconception Expanded Carrier Screening Using Genome Sequencing. *Journal of genetic counseling.* 2017.
43. Keogh LA, Otlowski MF. Life insurance and genetic test results: a mutation carrier's fight to achieve full cover. *The Medical journal of Australia.* 2013;199(5):363-366.
44. Otlowski M, Taylor S, Bombard Y. Genetic discrimination: international perspectives. *Annu Rev Genomics Hum Genet.* 2012;13:433-454.
45. ABI. Concordat and Moratorium on Genetics and Insurance. *Her Majesty Government.* 2014.
46. Ames AG, Metcalfe SA, Dalton Archibald A, Duncan RE, Emery J. Measuring informed choice in population-based reproductive genetic screening: a systematic review. *Eur J Hum Genet.* 2015;23(1):8-21.
47. Ready K, Haque IS, Srinivasan BS, Marshall JR. Knowledge and attitudes regarding expanded genetic carrier screening among women's healthcare providers. *Fertil Steril.* 2012;97(2):407-413.
48. Ioannou L, Massie J, Collins V, McClaren B, Delatycki MB. Population-based genetic screening for cystic fibrosis: attitudes and outcomes. *Public Health Genomics.* 2010;13(7-8):449-456.
49. Sheeran P, Conner M, Norman P. Can the theory of planned behavior explain patterns of health behavior change? *Health Psychol.* 2001;20(1):12-19.
50. White N. Family devastated after two of their three children are suddenly struck down with an incurable rare degenerative disorder that can kill young sufferers within years. *Daily Mail Australia.* December 6, 2016.
51. Aubusson K. The tragedy of Aviana. *The Sydney Morning Herald* March 3, 2017.
52. Ong R, Howting D, Rea A, et al. Measuring the impact of genetic knowledge on intentions and attitudes of the community towards expanded preconception carrier screening. *J Med Genet.* 2018;55(11):744-752.
53. Nazareth SB, Lazarin GA, Goldberg JD. Changing trends in carrier screening for genetic disease in the United States. *Prenatal diagnosis.* 2015;35(10):931-935.
54. Schofield D, Alam K, Douglas L, et al. Cost-effectiveness of massively parallel sequencing for diagnosis of paediatric muscle diseases. *NPJ Genom Med.* 2017;2.

55. Mathijssen IB, Holtkamp KCA, Ottenheim CPE, et al. Preconception carrier screening for multiple disorders: evaluation of a screening offer in a Dutch founder population. *Eur J Hum Genet.* 2018;26(2):166-175.
56. SUPERIOR HEALTH COUNCIL BELGIUM. Expanded carrier screening in a reproductive context. Towards a responsible implementation in the healthcare system. In: Superior Health Council, ed. Belgium2017.
57. American College of Obstetricians and Gynecologists. Committee Opinion No. 690: Carrier Screening in the Age of Genomic Medicine. *Obstet Gynecol.* 2017;129(3):e35-e40.
58. Sonic Genetics. Beacon Expanded Carrier Screen. https://www.sonicgenetics.com.au/rcs/beacon/. Accessed 21/04/2019.
59. Counsyl. FORESIGHT™ CARRIER SCREEN. https://www.counsyl.com/provider/foresight-carrier-screen/. Accessed 06/12/2018, 2018.
60. Invitae. Comprehensive Carrier Screen. https://www.invitae.com/en/physician/tests/60100/?cat=CAT000239. Accessed 06/12/2018, 2018.
61. Matar A, Kihlbom U, Hoglund AT. Swedish healthcare providers' perceptions of preconception expanded carrier screening (ECS)-a qualitative study. *J Community Genet.* 2016;7(3):203-214.
62. Janssens S, Chokoshvili D, Vears D, De Paepe A, Borry P. Attitudes of European Geneticists Regarding Expanded Carrier Screening. *J Obstet Gynecol Neonatal Nurs.* 2017;46(1):63-71.
63. van der Hout S, Holtkamp KC, Henneman L, de Wert G, Dondorp WJ. Advantages of expanded universal carrier screening: what is at stake? *Eur J Hum Genet.* 2016;25(1):17-21.
64. Cho D, McGowan ML, Metcalfe J, Sharp RR. Expanded carrier screening in reproductive healthcare: perspectives from genetics professionals. *Human reproduction.* 2013;28(6):1725-1730.
65. Wilfond BS, Goddard KA. It's complicated: criteria for policy decisions for the clinical integration of genome-scale sequencing for reproductive decision making. *Mol Genet Genomic Med.* 2015;3(4):239-242.
66. Benn P, Chapman AR, Erickson K, et al. Obstetricians and gynecologists' practice and opinions of expanded carrier testing and noninvasive prenatal testing. *Prenatal diagnosis.* 2014;34(2):145-152.
67. Chokoshvili D, Belmans C, Poncelet R, et al. Public Views on Genetics and Genetic Testing: A Survey of the General Public in Belgium. *Genetic testing and molecular biomarkers.* 2017;21(3):195-201.
68. Lakeman P, Plass AM, Henneman L, Bezemer PD, Cornel MC, ten Kate LP. Three-month follow-up of Western and non-Western participants in a study on preconceptional ancestry-based carrier couple screening for cystic fibrosis and hemoglobinopathies in the Netherlands. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2008;10(11):820-830.
69. Reeves A, Trepanier A. Comparison of Informed Consent Preferences for Multiplex Genetic Carrier Screening among a Diverse Population. *Journal of genetic counseling.* 2016;25(1):166-178.
70. Komal Bajaj SJG. Carrier Screening: Past, Present, and Future. *J Clin Med.* 2014;3(3):10.

CHAPTER 3

Preparing for the carrier-screening pilot study in Western Australia

CHAPTER 3 PREFACE

Results from Chapter 2 identified key requirements to successfully implement a carrier-screening pilot study in Western Australia which included:

1) The carrier-screening test should be offered at least through GPs and genetic counsellors. Since there are both public and private genetic counsellors (who feed patients into the public health system), in Western Australia, both would have to be included in any pilot study.
2) A comprehensive pre-test counselling session for each couple should be designed to clarify any misconceptions surrounding the test and potentially reduce anxiety over privacy and confidentiality.
3) Life-limiting disorders affecting children and infants should be screened for.

Chapter 3, Section 1 describes the development of the panel of genes to be screened. Based on the findings from Chapter 2, all life limiting recessive disorders affecting children and infants should be screened for and the associated genes included in the targeted gene panel for screening. The first part of Section 1 describes how the genes were selected, starting from the entire catalogue of disorders in the Online Mendelian Inheritance in Man (OMIM) database at that time. The rest of Section 1 describes the technical details of the carrier-screening gene panel and concludes with the optimisation results.

Chapter 3, Section 2 is the published study protocol[1] for the multicentre pilot study of carrier-screening using the selected expanded gene panel, carried out in metropolitan and regional Western Australia. The protocol includes description of the public and private health infrastructures within Western Australia and why each site for recruitment of couples was selected. The protocol also covers the development of the pre-test counselling materials, such as the health professionals'

checklist and training materials. In addition, the qualitative and quantitative instruments developed to track the efficacy of the training modules and counselling methods are described.

CHAPTER 3:

Section 1

Designing and optimising a comprehensive carrier-screening gene panel

Royston Ong

INTRODUCTION

In 2011, a seminal study by Bell *et al*. explored the use of next generation sequencing (NGS) to screen a cohort of patients for 448 severe recessive disorders[2]. The authors demonstrated that it is feasible to detect carriers of pathogenic variants using NGS methods affordably. By 2019, the number of disorder genes included in carrier-screening panels varied between 70 to 728[3-8]. However, deciding which genes should be offered to couples in a carrier-screening test can be challenging and ethically charged[9-11].

Recommendations by the European Society of Human Genetics[12] suggest that any severe childhood-onset disorder included in an expanded carrier-screening panel should be evidence based, while achieving high clinical validity. The guidelines set out in the American College of Obstetricians and Gynaecologists (ACOG) Opinion 690[13] were also considered in relation to which disorders should be included in the screen. The ACOG Opinion recommends that only disorders that 1) have a detrimental effect on quality of life, 2) cause cognitive or physical impairment, 3) require surgical or medical intervention, or 4) have an onset early in life, should be included in a carrier-screening test. This is congruent with consensus amongst genetic counsellors, clinical geneticists and obstetricians and gynaecologists in The United States of America (USA) and many European countries[10,11,14-17]. In addition, community surveys in The Netherlands and USA had demonstrated that public preferences were to screen for recessive disorders that are life-threatening and those that may cause significant physical and mental impairment[6-8,18]. Many health professionals also state that they would prefer to screen for a larger panel if costs were the same[17]. Inclusion criteria were also

discussed at length in two carrier-screening workshops organised by the Group I work in: one held as a Satellite Meeting of the European Society of Human Genetic Annual Congress in Glasgow in June 2015[19] and another in Western Australia in November 2016. In particular, the outcomes from the Glasgow workshop(19) were similar to current recommendations of screening for severe disorders, though the authors highlighted that what a "severe disorder" was had to be clearly defined.

From all of the above, the conclusion was clear that the carrier-screening panel designed for the study must focus on life-limiting disorders. Given that there was no agreement on the number of disorders to screen for[20], I aimed to explore and report on the clinical utility of a large panel. I planned to achieve this aim by maximising the number of genes included in the study within bounds set by the pathology laboratory where the testing was to be performed – PathWest Department of Diagnostic Genomics, the pathology laboratory arm of the Western Australian Department of Health. The PathWest laboratory did not want the total number of genes to at that time exceed 500 genes. As early as 2013[21], Illumina Inc. marketed Bell *et al.*'s gene list encompassing 448 recessive disorders, under the brand name "Trusight Inherited Disease Sequencing Panel" (TruSight inherited panel). The TruSight inherited panel was the largest commercially available panel, however, it included nonlife-limiting disorders such as Cholestasis (MIM 605479) or Fructose intolerance (MIM 229600). Given this, I decided to design a carrier-screening panel based on the collective guidelines and recommendations from ESHG, ACOG and the Glasgow and Perth workshops.

Therefore, the selection criteria for the inclusion of a gene as the core of the targeted panel were genes associated with recessive disorders that cause infant

and childhood mortality, or are common in the general population in Australia and offered in Australian carrier-screening programs, namely cystic fibrosis [22], spinal muscular atrophy [23] and Fragile X [24]. This was later modified in an attempt to produce a standardised carrier-screening test across Australia in collaboration with the Victorian Clinical Genetics Service (VCGS) in Melbourne.

The research group in which I did my PhD, the Neurogenetic Diseases Group in the Centre for Medical Research, University of Western Australia, has had a long and close collaborative relationship with the Department of Diagnostic Genomics, Neurogenetic Unit, PathWest Laboratory Medicine, Department of Health Western Australia. The PathWest Neurogenetic Unit was an early adopter of NGS in the diagnostic setting, beginning such testing in 2013. The Neurogenetic Unit now exclusively uses the Illumina NGS Platform. To date, they have run >4,000 patients across a number of iterated bespoke, custom-designed gene panels for neurogenetic disorders[25,26] as well as panels for other disorder categories such as cardiomyopathies and cancer susceptibility genes. The Diagnostic Genomics services are compliant with international and national pathology requirements and accredited by the National Association of Testing Authorities (NATA): Australia's laboratory accreditation body. PathWest's experience and expertise with troubleshooting and analysing data using Illumina NGS platforms led me to design and construct the carrier-screening panel based on Illumina's NGS chemistry.

This Section 1 of Chapter 3 of my Thesis describes the processes I used for design and construction of the carrier-screening targeted gene panel, as well as the validation of the panel to define the acceptable quality metrics for the study.

METHODS

Disorder and gene selection process

The entire OMIM disease database was defined as five groupings: 1) Disorders with a molecular basis; 2) Disorders without a molecular basis; 3) Unconfirmed or spurious mapping; 4) Multifactorial and susceptibility disorders genes; 5) a Miscellaneous group including cancer susceptibility genes, disorders without a MIM number and genomic regions with chromosomal aberrations.

All disorders in OMIM with a known molecular basis were reviewed. Disorders associated with large insertions or deletions, repeat expansions, and chromosomal aberrations were excluded due to the technological limitations of next generation sequencing to detect such variants. Many cancer-associated genes are not inherited in a simple Mendelian manner. They often predispose an individual to a particular cancer subjected to environmental and lifestyle factors. These reasons led me to omit cancers from the final carrier-screening targeted panel.

The remaining disorders were grouped into either dominantly- or recessively-inherited disorders. To determine disorder severity, the OMIM application-programming interface (API) was used to analyse the OMIM database for specific terms such as "Death", "Mortality", and "Dead" under the heading of "Description", "Clinical Synopsis" and "Clinical Features". All recessive disorders not categorised via API were then manually categorised based on the criteria stated below, using information from "Clinical Synopsis", "Description" and "Clinical Features". Duplicated gene symbols were removed from the final gene list.

Definition of disorder selection criteria and classification

Recessive disorders in OMIM were then classified into three categories:

1) Category 1 included any recessive disorders causing childhood and infant mortality;

2) Category 2 included any recessive disorders causing a severe reduction in quality of life. The 2003 International Classification of Functioning, Disability and Health (ICF) checklist[27] was used as a guide to provide a phenotypic ranking for Category 2 disorders.

3) All other recessive disorders that did not fulfil Categories 1 and 2 criteria were classified as Category 3.

Designing the targeted panel

Genomic start and end positions of each exon of the genes included in the carrier-screening panel were generated using UCSC's table browser function. To identify whether certain regions of the selected genes might not be well captured, I compared the probe capture efficiency of genes present in the carrier-screening panel and panels then in use in PathWest such as TruSight one and the muscle-, neuro- and cardiac-targeted panel versions in use at that time(Table 1). This allowed me to provide Illumina Inc with a list of regions that had a mean coverage of less than 99%. These regions had additional probes added to increase the amount of sequencing data obtained. Further to increasing probes in certain regions, I also utilised Illumina's Concierge Service[28] to validate my probe design. The Illumina concierge service is a suite of services providing assistance and customization for every step in the Illumina workflow (Figure 1). Results from the concierge services were comparable to other panels that PathWest had previously designed and, in

consultation with the PathWest Neurogenetic Unit senior Scientist-in-Charge, Dr. Mark Davis, the final carrier-screening probe design was accepted.

Table 1: Percentage coverage obtained for the carrier-screening genes present in the TS1, Muscle, Neuro and Cardiac panel

%Coverage across genes	# of overlapping genes in each PathWest panel and the study panel			
	TS1	Muscle	Neuro	Cardiac
<90%	26	2	4	0
90.0-94.9%	42	1	0	1
95.0-97.9%	69	5	4	1
98.0-98.9%	56	3	0	0
≥99.0%	244	13	12	4
Total	437	24	20	6

TS1: TruSight1 panel

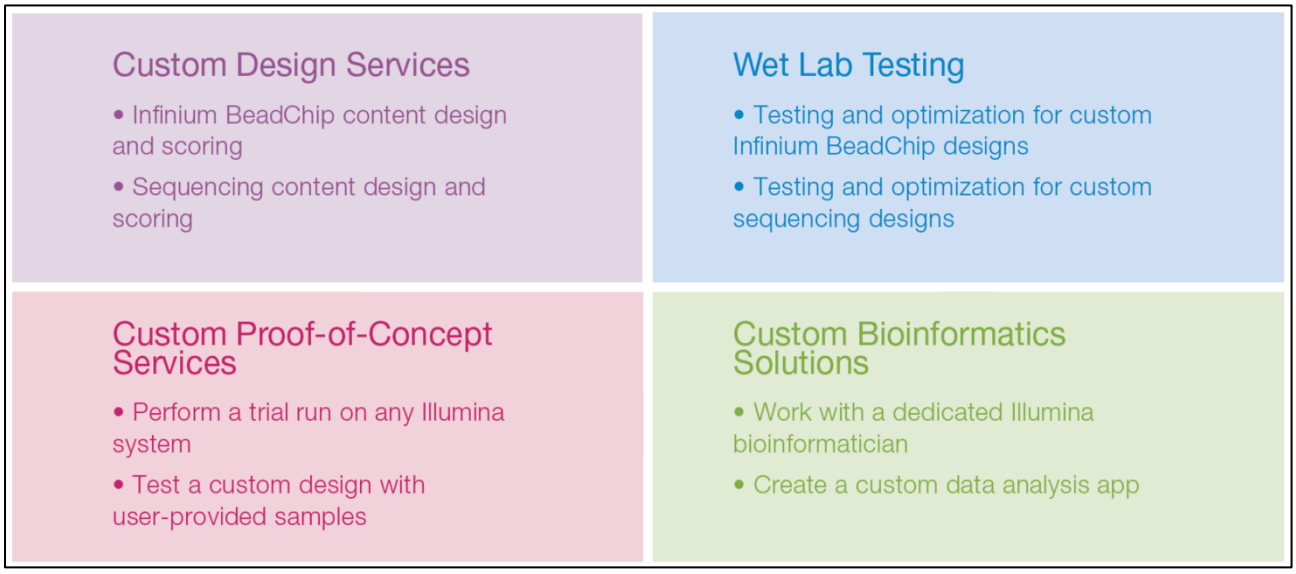


Figure 1: Services Illumina Concierge Service provide.
Figure taken from: https://www.illumina.com/content/dam/illumina-marketing/documents/services/concierge-services-data-sheet-070-2017-009.pdf

Study panel probe optimisation

PathWest's typical acceptable quality metrics for a run were: 1) having an average read depth of more than 100-fold, 2) having a uniformity of more than 98% and 3) having an average 20-fold (20x) coverage at more than 99% of the targeted regions. However, it is important to note that the acceptable quality metric of each targeted panel depends on a number of factors such as the footprint of the panel, the number of samples on each run, the number of difficult regions to sequence in the panel, probe volume and how efficiently the probes bind to each targeted

region. Therefore, it is crucial to perform optimisation runs to ascertain the quality range for any new panel. A panel is considered acceptable after optimisation, if the overall performance is sufficiently close to the typical acceptable quality metric. In an iterative process a standard PathWest protocol is first used to determine baseline values of a new targeted panel. After obtaining the baseline values, probe volume is then adjusted to optimise the new panel.

Bioinformatic pipeline

After sequencing was completed, sequencing data (FASTQ files) were uploaded into BaseSpace. Reads were aligned and variant calling performed using BaseSpace Software (Illumina). A variant call format (vcf) file was generated for each sample after this step. Each vcf file was annotated, filtered and analysed in Alissa Interpret (Agilent Technologies).

Data for each sample was first compared to a list of previously observed pathogenic variants in Alissa known as the "*Managed Variant List*" (MVL). Any known pathogenic variants identified at this step were retained and known benign variants were removed. During the early days of my PhD, the acceptable cut-off in PathWest for recessive variants in population databases like gnomAD (https://gnomad.broadinstitute.org/) or ExAC (http://exac.broadinstitute.org/) was 0.5%. Hence variants with more than 0.5% minor allele frequency in population databases were then removed from each vcf file. Rare potential loss of function variants, canonical splice site variants or variants ±5bp from a donor or acceptor splice site, and missense variants were retained in the final variant list along with any known pathogenic variants from the MVL. A final variant list was generated for every sample sequenced in the study (Figure 2). The individual final variant lists for both

partners were analysed together to pull out genes with variants in both members of the couple. ACMG guidelines were used to curate variants present in the same gene in each couple for pathogenicity[29]. Only when likely pathogenic or pathogenic (Class4 or Class5) variants were identified in the same gene in both members of a couple, or identified in an X chromosome gene in the female partner, were the couple considered as "high-risk" and reported as such (Figure 2).

A total of three trial runs were sequenced. Eighteen positive controls (i.e. individuals known to carry pathogenic or likely-pathogenic variants within genes that were present in the carrier-screening panel) were sequenced in the first two runs to determine accuracy and detection rate of the analysis pipeline. The last trial run was to simulate a run with actual study participants.

DNA extraction and Fragile X and SMA testing

DNA was extracted at PathWest from the blood samples using QIASymphony for analysis using the targeted gene panel. Spinal muscular atrophy (*SMN1)* and Fragile X testing were performed as per standard diagnostic practice in PathWest Diagnostic Genomics using qPCR[30] and repeat-primed PCR respectively[31].

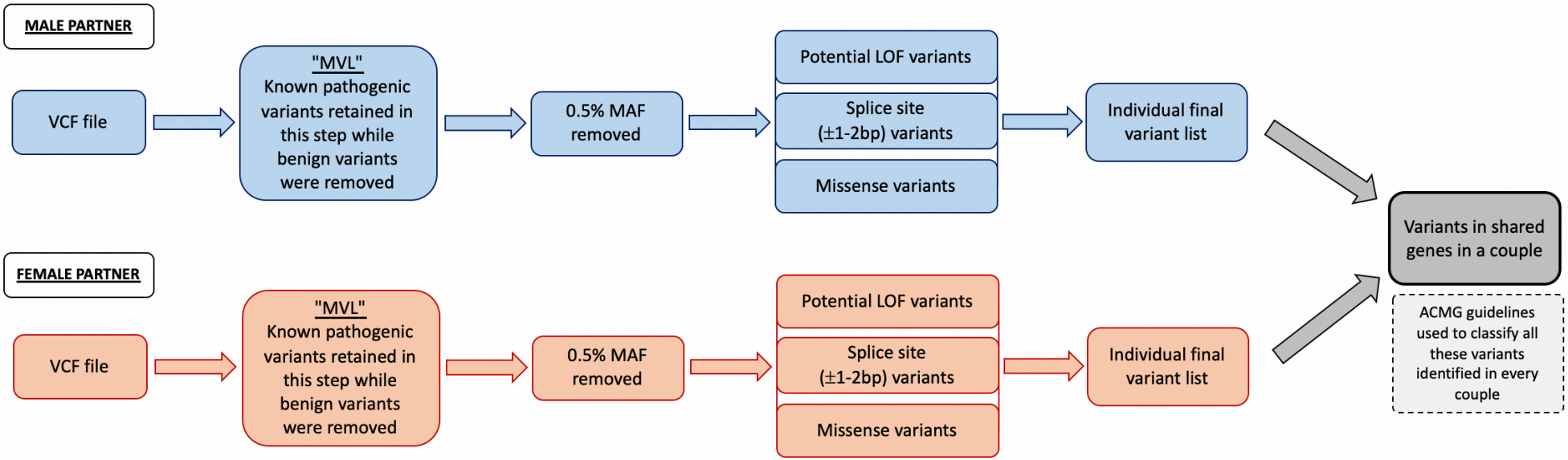


Figure 2: Alissa Interpret bioinformatic pipeline for the pilot study

A typical sample variant processing pipeline using Alissa Interpret. VCF files are parsed through the pipeline with filters applied to the variants being processed before obtaining a final variant list for one sample. Known pathogenic variants, potential LOF variants, canonical splice site variants and missense variants of less than 0.5% allele frequency were retained in the final variant list.

VCF: Variant Call Format; MVL: Manage Varian List; MAF: Minor Allele Frequency; LOF: Loss of Function

RESULTS

The study panel disorder and gene selection

There were 2,248 recessive disorders identified in OMIM of which 451 were found to be associated with infant and childhood mortality (Category 1); 758 disorders were early onset and chronic as well as severely reducing the quality of life for patients (Category 2); and 1,039 disorders were adult-onset disorders or disorders that neither affect infant or childhood mortality nor severely limit the quality of life (Figure 3).

A total of 403 genes associated with the 451 recessive disorders met the criteria for Category 1. These genes associated with Category 1 disorders were considered the core of the panel design. In late 2017, I collaborated with Victorian Clinical Genetic Services (VCGS) and merged their Prepair plus gene list of 117 genes that they had developed, with the 403 I had identified. This was in a bid to produce a semi-standardised carrier-screening test in at least two Australian States (personal communication with Professor Martin Delatycki). As a result of that collaboration, the final carrier-screening panel consisted of 474 genes associated with mainly childhood and infant lethal disorders(SI 3, Table S1). The total capture area or "*footprint*" of the targeted carrier-screening panel is 1.23Mbp.

Quality metric of carrier-screening panel

A total of three runs were performed to optimise the carrier-screening panel: varying the probe volume around the volume suggested in the manufacturer's instructions. In the first run 16.6µl of probes were used, then 10µl for the second run and finally 6.6µl for the third run. As shown in Figure 4, the mean read depth of trial 2 (average of 185.5-fold) and trial 3 (average of 156.4-fold) exceeds the acceptable quality threshold of >100-fold, while uniformity (96.7% & 96.2% respectively) was close to

PathWest's acceptable range of 98%. Lastly, 99% of the target region had coverage to >20x.

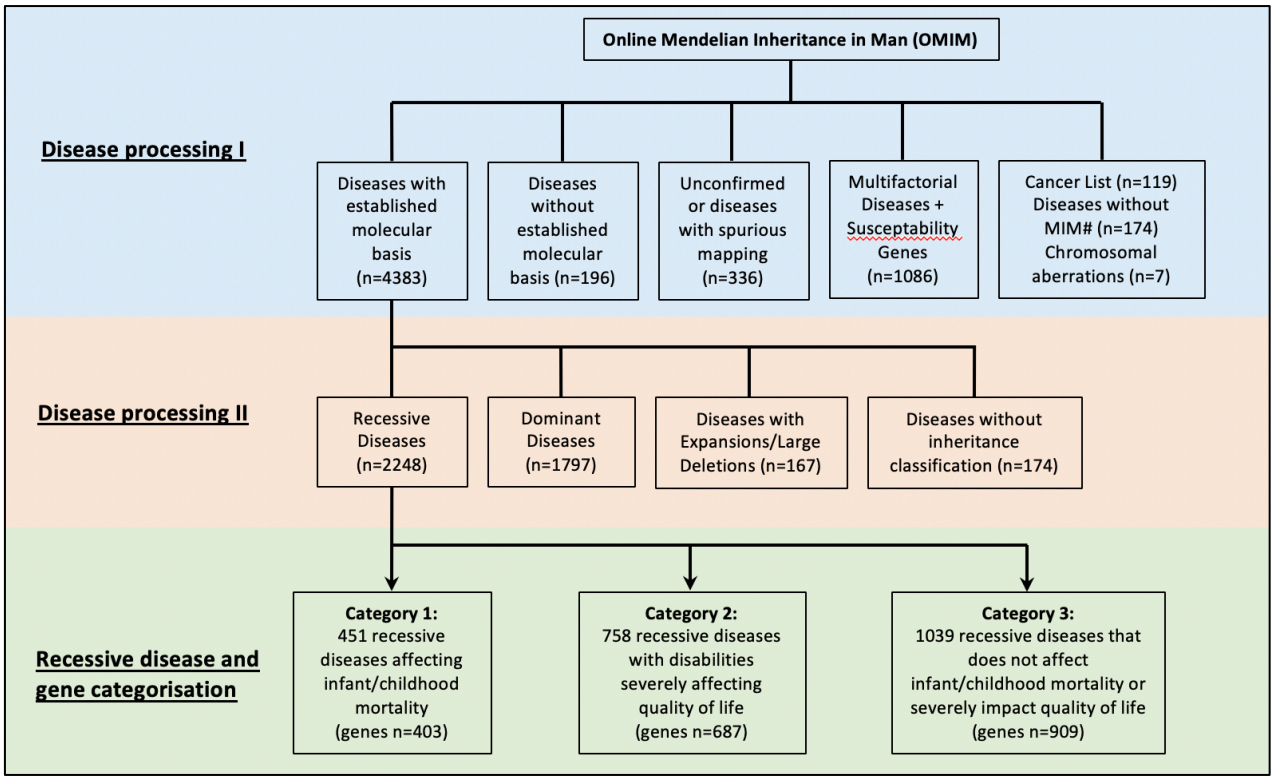


Figure 3: The description of the entire categorising workflow.
A total of 451 recessive disorders affecting infant and childhood mortality (Category 1); 758 recessive disorders were early onset and chronic as well as severely reducing the quality of life for patients (Category 2); and 1,039 recessive disorders are adult onset disorders or disorders that neither affect infant or childhood mortality nor severely limit the quality of life.

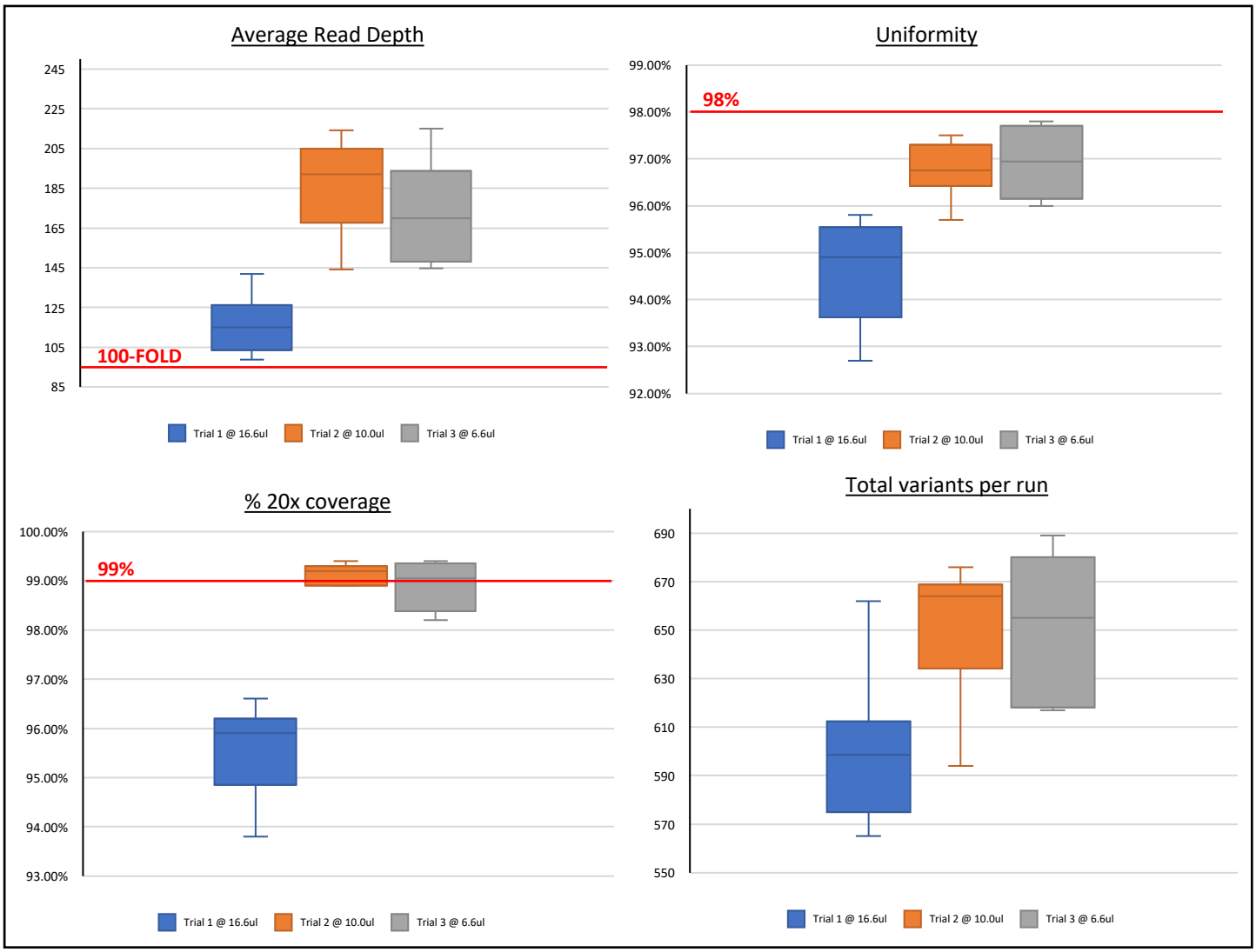


Figure 4: Carrier-screening panel optimisation results
Data of the three carrier-screening panel trials with different probe volumes (blue, orange and gray) compared to the PathWest accepted QC data for each metric (red line). Black line shows the average number of variants called across the three trial runs.

Positive control detection rate and finalised bioinformatic pipeline

All known carriers were identified from the data and correctly assessed in the first two runs (Table 2). The last trial run (Trial 3) was to simulate a run with actual study participants and produced very similar quality parameters to Trial 2 (Figure 4). This indicated that using 6.6µl of probes, while running 8 samples per run, following one of PathWest's standard NGS protocols produced a balanced outcome of reagents used and results outcome. The PathWest standard NGS protocol used is a modified version of Illumina's Nextera™ DNA Flex Library Prep protocol (Nextera™ DNA Flex Library Prep Reference Guide).

Table 2: Number of known carriers with pathogenic variants in the carrier-screening gene panel identified during optimising process

Trial Sample #	Gene	cDNA position	protein position	variant type	Identified in data?	Present in study gene list?
Sample 1	*POMT1*	c.2167dup	p.Asp723fs	missense	Yes	Yes
Sample 2	*POMT1*	c.598G>C	p.Ala200Pro	missense	Yes	Yes
Sample 3	*CAPN3*	c.649G>A	p.Glu217Lys	missense	No	No
Sample 4	*CAPN3*	c.1401_1403delGGA	p.Glu467del	missense	No	No
Sample 5	*DOK7*	c.1263dupC	p.Ser422fs	missense	No	No
Sample 6	*DOK7*	c.1124_1127dupTGCC	p.Ala378fs	missense	No	No
Sample 7	*DYSF*	c.107_108del	p.Lys36fs	missense	Yes	Yes
Sample 8	*DYSF*	c.5698_5699del	p.Ser1900fs	missense	Yes	Yes
Sample 9	*SGCB*	c.341C>T	p.Ser114Phe	missense	Yes	Yes
Sample 10	*SGCB*	c.31C>T	p.Gln11*	missense	Yes	Yes
Sample 11	*NEB*	c.11610C>A	p.Tyr3870*	missense	Yes	Yes
Sample 12	*NEB*	c.18024_18027delAGTC	p.Val6009fs	missense	Yes	Yes
Sample 13	*ARSA*	c.1108-3C>G	-	missense	Yes	Yes
Sample 14	*ARSA*	c.465+1G>A	-	missense	Yes	Yes
Sample 15	*CHRNG*	c.459dupA	p.Val154fs	missense	Yes	Yes
Sample 16	*CHRNG*	c.56-1G>A	-	missense	Yes	Yes
Sample 17	*GAA*	c.-32-13T>G	del ex 18	missense	Yes	Yes
Sample 18	*DMD*	N/A	del ex 45 to 50	CNV	Yes	Yes

CNV: Copy Number Variation

DISCUSSION

The results indicated that the optimised protocol for the carrier-screening panel was to use 6.6µl of probes, while running 8 samples per run and using one of PathWest's standard NGS protocols. We also confirmed that the bioinformatics pipeline should allow detection of most, if not all of the pathogenic variants that would be encountered in the actual pilot study.

Supplementary information for this Chapter is available on page 242 in the Appendix section.

REFERENCES

1. Ong R, Edwards S, Howting D*, et al.* Study protocol of a multicentre cohort pilot study implementing an expanded preconception carrier-screening programme in metropolitan and regional Western Australia. BMJ Open. 2019;9(6):e028209.
2. Bell CJ, Dinwiddie DL, Miller NA*, et al.* Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med. 2011;3(65):65ra64.
3. Nazareth SB, Lazarin GA, Goldberg JD. Changing trends in carrier screening for genetic disease in the United States. Prenat Diagn. 2015;35(10):931-935.
4. Matar A, Kihlbom U, Hoglund AT. Swedish healthcare providers' perceptions of preconception expanded carrier screening (ECS)-a qualitative study. J Community Genet. 2016;7(3):203-214.
5. Lazarin GA, Goldberg JD. Current controversies in traditional and expanded carrier screening. Curr Opin Obstet Gynecol. 2016;28(2):136-141.
6. Plantinga M, Birnie E, Abbott KM*, et al.* Population-based preconception carrier screening: how potential users from the general population view a test for 50 serious diseases. Eur J Hum Genet. 2016;24(10):1417-1423.
7. Abuli A, Boada M, Rodriguez-Santiago B*, et al.* NGS-Based Assay for the Identification of Individuals Carrying Recessive Genetic Mutations in Reproductive Medicine. Hum Mutat. 2016;37(6):516-523.
8. Wilfond BS, Kauffman TL, Jarvik GP*, et al.* Lessons Learned From A Study Of Genomics-Based Carrier Screening For Reproductive Decision Making. Health Aff (Millwood). 2018;37(5):809-816.
9. Lau TK. Obstetricians should get ready for expanded carrier screening. BJOG. 2016;123 Suppl 3:36-38.
10. Chokoshvili D, Janssens S, Vears D*, et al.* Designing expanded carrier screening panels: results of a qualitative study with European geneticists. Per Med. 2016;13(6):553-562.
11. Korngiebel DM, McMullen CK, Amendola LM*, et al.* Generating a taxonomy for genetic conditions relevant to reproductive planning. Am J Med Genet A. 2016;170(3):565-573.
12. Henneman L, Borry P, Chokoshvili D*, et al.* Responsible implementation of expanded carrier screening. Eur J Hum Genet. 2016;24(6):e1-e12.
13. American College of Obstetricians and Gynecologists. Committee Opinion No. 690: Carrier Screening in the Age of Genomic Medicine. Obstet Gynecol. 2017;129(3):e35-e40.
14. Edwards JG, Feldman G, Goldberg J*, et al.* Expanded carrier screening in reproductive medicine-points to consider: a joint statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine. Obstet Gynecol. 2015;125(3):653-662.
15. Benn P, Chapman AR, Erickson K*, et al.* Obstetricians and gynecologists' practice and opinions of expanded carrier testing and noninvasive prenatal testing. Prenat Diagn. 2014;34(2):145-152.
16. Lazarin GA, Hawthorne F, Collins NS*, et al.* Systematic Classification of Disease Severity for Evaluation of Expanded Carrier Screening Panels. PLoS One. 2014;9(12):e114391.
17. Lazarin GA, Detweiler S, Nazareth SB*, et al.* Genetic Counselors' Perspectives and Practices Regarding Expanded Carrier Screening after Initial Clinical Availability. J Genet Couns. 2016;25(2):395-404.
18. Holtkamp KC, van Maarle MC, Schouten MJ*, et al.* Do people from the Jewish community prefer ancestry-based or pan-ethnic expanded carrier screening? Eur J Hum Genet. 2016;24(2):171-177.

19. Molster CM, Lister, K., Metternick-Jones, S., Baynam, G., Clarke, A. J., Straub, V., Dawkins, H. J. S., and Laing, N. Outcomes of an International Workshop on Preconception Expanded Carrier Screening: Some Considerations for Governments. . Frontiers Public Health
20. Ben-Shachar R, Svenson A, Goldberg JD*, et al.* A data-driven evaluation of the size and content of expanded carrier screening panels. Genet Med. 2019;21(9):1931-1939.
21. Chang F, Li MM. Clinical application of amplicon-based next-generation sequencing in cancer. Cancer Genet. 2013;206(12):413-419.
22. Massie J, Curnow L, Gaffney L*, et al.* Declining prevalence of cystic fibrosis since the introduction of newborn screening. Arch Dis Child. 2010;95(7):531-533.
23. Smith M, Calabro V, Chong B*, et al.* Population screening and cascade testing for carriers of SMA. Eur J Hum Genet. 2007;15(7):759-766.
24. Archibald A, Smith M, Burgess T*, et al.* Reproductive Genetic Carrier Screening for Cystic Fibrosis, Fragile X Syndrome and Spinal Muscular Atrophy in Australia: Outcomes of 12,000 Tests. Australian & New Zealand Journal of Obstetrics & Gynaecology. 2017;57:12-12.
25. Todd EJ, Yau KS, Ong R*, et al.* Next generation sequencing in a large cohort of patients presenting with neuromuscular disease before or at birth. Orphanet J Rare Dis. 2015;10:148.
26. Beecroft SJ, Yau KS, Allcock RJN*, et al.* Targeted gene panel use in 2249 neuromuscular patients: the Australasian referral center experience. Ann Clin Transl Neurol. 2020;7(3):353-362.
27. WHO. International Classification of Functioning, Disability and Health (ICF) 2003 [Available from: www.who.int/classifications/icf/training/icfchecklist.pdf)
28. Illumina Inc. Illumina Professional Product Services brochure [Available from: https://www.illumina.com/content/dam/illumina-marketing/documents/services/professional-product-services-handout-070-2016-019-web.pdf.
29. Richards S, Aziz N, Bale S*, et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424.
30. Feng Y, Ge X, Meng L*, et al.* The next generation of population-based spinal muscular atrophy carrier screening: comprehensive pan-ethnic SMN1 copy-number and sequence variant analysis by massively parallel sequencing. Genet Med. 2017;19(8):936-944.
31. Biancalana V, Glaeser D, McQuaid S*, et al.* EMQN best practice guidelines for the molecular genetic testing and reporting of fragile X syndrome and other fragile X-associated disorders. Eur J Hum Genet. 2015;23(4):417-425.

CHAPTER 3:

Section 2

Study protocol of a multicentre cohort pilot study implementing an expanded carrier-screening programme in metropolitan and regional Western Australia

Ong R, Edwards S, Howting D, Kamien B, Harrop K, Ravenscroft G, Davis M, Fietz M, Pachter N, Beilby J, Laing N. Study protocol of a multicentre cohort pilot study implementing an expanded preconception carrier-screening programme in metropolitan and regional Western Australia. BMJ Open 2019;9(6):e028209

doi: 10.1136/bmjopen-2018-028209

Royston Ong
Samantha Edwards
Denise Howting
Ben Kamien
Karren Harrop
Gina Ravenscroft
Mark Davis
Michael Fietz
Nicholas Pachter
John Beilby
Nigel Laing

CHAPTER 3 SECTION 2 – PREAMBLE

The follow Section is an author accepted manuscript of the published article [1] that covers the full end-to-end protocol of the pilot study, including the panel and bioinformatic design discussed in the previous Section, recruitment criteria, site selection, associated educational materials, analysis methods and reporting rules.

ARTICLE SUMMARY:

Strengths

1. Participation of various service providers such as the Western Australian Health Department's genetics arm, Genetic Services Western Australia (GSWA), will allow the research team to understand limitations in infrastructure and bottlenecks in integrating carrier-screening into the West Australian public health system.
2. The study will evaluate stakeholders' experiences to understand different requirements between metropolitan and regional areas.
3. Evaluating training materials and tools developed during the study will allow refinements and improvements for future implementation.

Limitations

4. The small study will likely identify only major issues encountered in different settings within Western Australia. Other limitations may include individuals wishing to receive individual carrier reports rather than a couple report.

INTRODUCTION

Carrier-screening involves screening individuals or couples for recessive variants. These couples do not usually have an a priori increased risk of being a carrier based on their or their partners' personal or family history of the disorder. The aim of carrier-screening is therefore not for early diagnosis/prevention and treatment, but to facilitate reproductive decision-making[32].

Recently, the American College of Obstetricians and Gynecologists recommended that: "each obstetrician–gynecologist or other health care provider or practice

should establish a standard approach (to carrier-screening) that is consistently offered to and discussed with each patient, ideally before pregnancy."[13].

As a result of falling sequencing costs and increased cost-effectiveness of sequencing technologies[33,34], pilot studies have been suggested as a means to research: i) implementing a carrier-screening test including deciding on which and how many genes to screen, ii) population and health practitioner attitudes to screening, iii) counselling requirements and iv) laboratory infrastructure requirements[35]. It has also been proposed that pilot studies should be carried out in multiple countries[36] since best practice methods for carrier-screening will vary depending on each country's health system.

In recent years, some countries have begun researching implementation of pan-ethnic expanded carrier-screening programs into their health systems[6,37]. For example, a gene panel that covers 50 serious, early-onset, autosomal-recessive disorders was developed in the Netherlands[6], while Belgium has been actively working towards implementing an expanded carrier-screening program into their healthcare system[37].

Australia lags behind world best practice methods in carrier-screening and what is available is highly variable in different States. All available services are consumer-pays, including tests offered by commercial entities. In Victoria for example, the Victorian Clinical Genetics Service offers the Prepair™ screening program for three common recessive disorders: cystic fibrosis, spinal muscular atrophy (SMA) and fragile X syndrome, and carrier-screening is being offered through some general practitioner (GP) group practices providing a route to access the commercial programs. In Western Australia (WA), carrier-screening is generally only performed

as cascade screening for families with a history of a recessive disorder. The piecemeal nature of carrier-screening in Australia means that there are inequities in access and outcomes across States and populations.

Our research in WA shows that there is considerable support for carrier-screening. Two-thirds of nearly 1,000 Western Australians surveyed said they would use carrier-screening if it were available to them[38]. Of those who said they would use it, 80% said they would like to access the test and obtain results from their GP and 40% through a genetic counsellor. This clearly indicates that there is an appetite for carrier-screening in the WA community as well as suggesting possible delivery methods we can evaluate[38].

This study protocol seeks to identify the most effective way of delivering carrier-screening in WA, given that WA spans over 2.5 million square kilometres. To do this, we are leveraging existing research and health infrastructures including GSWA, PathWest Laboratory Medicine (PathWest) in the Department of Health, and the Busselton Population Medical Research Institute. In addition, we will explore the role GPs and private genetic counselling clinics can play in providing carrier-screening in the metropolitan region.

METHOD

Study design

This is a multicentre cohort study offering carrier-screening to couples planning on starting, or extending a family (Figure 1). The study will be conducted through three routes: GSWA, a private genetic counselling practice and a general practice in the metropolitan area, and participating general practices in Busselton, Western Australia. Recruitment for the study started in early September 2018 and will

continue for two years or until study numbers are reached. The study is advised by the WA Consumer and Community Health Research Network.

Metropolitan Region

Genetic Services of Western Australia (GSWA)

GSWA provides genetic counselling for individuals and couples with a family history of a genetic disorder. In this study, GSWA will recruit such couples who are planning more children. This will enable us to determine the specific requirements necessary for carrier-screening for this subset of couples.

Private genetic counselling practice

A private genetic counselling practice, will offer carrier-screening to interested couples. This recruitment arm will provide the study an opportunity to recruit couples from the metropolitan area who wish to be screened for possible genetic disorders, but who do not have a family history of a genetic disorder.

The genetic counsellor will offer the research test protocol as an option in addition to the testing from commercial suppliers currently made available. Participants will pay for any pre-test consult as per the genetic counsellor's rates.

If a couple choose the research protocol, the laboratory testing will be free of charge to the couple.

Local GP practice

A local GP practice will offer carrier-screening to interested couples planning to have children. Recruiting couples through a metropolitan GP practice will provide perspective from a different demographic, complementing data from recruitment of couples through the private genetic counselling practice.

Regional Areas

GP practices in the Busselton region

The Busselton community and their GPs have a 50-year history of participation in research studies[39]. The voluntary participation rate in Busselton Population Medical Research Institute (BPMRI) projects is very high. Participating GP practices will recruit couples that are planning to have children. Recruiting couples in the Busselton region will enable us to determine the specific requirements necessary for successful implementation of carrier-screening in regional communities. The Busselton Population Medical Research Institute will facilitate dissemination of awareness of the study in the Busselton region.

Procedure

Participant recruitment

We aim to recruit 250 couples (500 individuals) between the metropolitan and Busselton sites.

Metropolitan Region (Genetic Counsellors)

The aim is to recruit 100 couples in the metropolitan region.

Posters and leaflets will be placed in GSWA and the private genetic counselling practices to raise awareness about the study and to prompt potential participants to ask about the study during their visits. Potential participant couples will also be made aware of the study through genetic counsellors and clinical geneticists during clinic visits.

Potential participants will be given a study information pack, consisting of the patient information sheet and consent form, to take home and consider with their partner at their leisure.

Couples interested in the study will have to return for couples-based pre-test counselling. If the couple decides to participate in the study, they will sign the consent form. Couples will then be asked if they are willing to participate in evaluation of the carrier-screening program to inform researchers about their experience and how the program can be improved.

If potential participants decide not to participate, they can voice their reasons through an online survey link provided in the leaflet.

Couples will be reminded that that they can withdraw from the study at any time.

Busselton Region (GP practices)

The aim is to recruit 150 couples in the Busselton region. Posters and leaflets will be placed in each participating GP clinic, and at the Busselton Population Medical Research Institute, to raise awareness about the study. In addition, newspaper articles, and other media, will be used to increase awareness of the study.

Potential eligible participants will also be made aware of the study during GP visits. Interested potential participants will be given a study information pack, including the patient information sheet and consent form and study pamphlet, to take home and consider with their partner, at their leisure.

Potential participants interested in the study will return for couples-based pre-test counselling provided by their GP. If the couple decide to participate in the study, they will sign the consent form. Couples will also be asked if they are willing to

participate in the evaluation of the carrier-screening program to inform researchers about their experience and how the program can be improved.

If potential participants decide not to participate, they can voice their opinions through an online survey provided in the leaflet.

Couples will be reminded that they can withdraw from the study at any time.

Participant inclusion criteria

Each participating couple MUST meet the following requirements to be enrolled in this study:

1) Not be pregnant at the time of recruitment but considering having children in the future
2) Couples must participate in the study together
3) Both members of the couples must be at least 18 years of age
4) Couples who have had a pregnancy loss or a child with a serious genetic disorder who are planning more children or who have a family history of a genetic disorder (only applicable to GSWA)

Participant exclusion criteria

Couples meeting ANY of the following criteria will be excluded from the study:

1) Are pregnant at the time of recruitment
2) Only one member of the couple agrees to participate in the study
3) The couple (or one of them) are younger than 18 years of age

4) Have had a pregnancy loss, or a child with a serious genetic disorder, or a family history of a genetic disorder (applicable to recruiters other than GSWA)

5) Same sex couples

Health professional recruitment

Metropolitan Region (Genetic Counsellors)

An email inviting potential participating genetic counsellors and/or clinical geneticists to an information session will be sent to GSWA staff and the private genetic counsellor prior to the session. The session will inform genetic counsellors (GCs) and clinical geneticists about the study, recruitment criteria. Reporting methods and questions pertaining to the study will also be clarified.

Any health professional that expresses an interest will be given a health professional information pack, consisting of an information sheet and a consent form, to take away to deliberate. Signed consent forms can either be collected by the study GC or sent via a stamped addressed envelope to the study principal investigator.

Busselton (regional GP practices)

An email inviting potential participating GPs to an information session with the study GC and PI will be sent to all GPs within the Busselton region. The Busselton Population Medical Research Institute (BPMRI)[39] will facilitate recruitment of Busselton GPs. The session will inform GPs about the study, recruitment criteria, training involved and reporting methods and questions pertaining to the study will be clarified.

Any GP who expresses an interest in participating will be given a health professional information pack, consisting of an information sheet and a consent form, to take

away to consider. Signed consent forms can be sent via a stamped addressed envelope to the study PI.

Participating GPs will be trained by the study GC to provide pre-test counselling to potential participant couples.

Biospecimen collection

For all sites, following appropriate informed consent, 4 ml of venous blood, will be collected at any PathWest collection centre.

Collected blood will be sent to the Department of Diagnostic Genomics, PathWest, QEII Medical Centre for DNA extraction and storage. The DNA will be handled, prepared and sequenced within the Department of Diagnostic Genomics, which has been accredited by the National Association of Testing Authorities (NATA) for massively parallel sequencing.

Massively parallel sequencing (MPS) and variant curation

DNA will be sequenced using a custom enrichment capture panel, which was developed by the research team and synthesised by Illumina Inc. The panel consists of 474 genes associated with 440 childhood and infant lethal and debilitating recessive disorders. Sequence data will be mapped, annotated and interrogated with Alissa Interpret (Agilent Technologies), as used routinely in the Department of Diagnostic Genomics, PathWest.

Only pathogenic or likely pathogenic (ACMG guidelines: Class 4 and 5)[29] recessive variants identified in the same gene in both members of a couple or identified on the X-chromosome in the female partner will be reported.

Quality control & quality assurance

All methods will be conducted and results analysed according to NATA-accredited protocols. Senior Scientists-in-Charge at PathWest will have responsibility for the laboratory data quality control.

The Head of the Department of Diagnostic Genomics, PathWest will address any quality control issues.

Reporting

There will be two reporting options: "high-risk" or "low-risk". In addition, if couples agree to participate in the study, but choose not to receive any results, this option is available in the consent form. No results will be communicated to such couples.

"High-risk" couples

PathWest will generate a "high-risk" report if Class 4 or 5 variants are identified in the same gene in both partners, or in an X-linked gene in the female partner. High-risk couples will receive their result initially by telephone through the study genetic counsellor. They will then be offered an appointment with GSWA for further counselling about the specific genetic disorder, their reproductive options and the impact on their relatives. A copy of the report will be forwarded to the referring clinician for their records.

"Low-risk" couples

PathWest will notify the study genetic counsellor for "low-risk" couples, i.e., those with no pathogenic recessive variants identified in the same gene. Low-risk couples will receive a letter generated by the study genetic counsellor outlining their result and

providing contact details of the study genetic counsellor for further clarification if needed. The referring clinician will receive a copy.

A low-risk result means that the couple's risk of having a child with a severe recessive disorder amongst those screened for by the carrier-screening test has been significantly reduced.

Quality control & quality assurance

GSWA clinical geneticists will be in charge of ensuring consistency in counselling provided for "high-risk" couples and will address any counselling issues.

STUDY AIMS AND OBJECTIVES

Aims

The primary aim of the study is to identify the most effective way of delivering carrier-screening in WA through the public health system.

Secondary aims of the study include evaluating (1) reproductive autonomy of couples who participate in the study; and (2) the effectiveness of the tools developed during the study.

Objectives

The primary objective is to perform and evaluate a pilot study of carrier-screening in Western Australia.

Outcomes

The primary outcome will be to develop a working end-to-end carrier-screening program compatible with the WA health care system.

Secondary outcome measures include: evaluating the psychosocial impact on couples using a carrier-screening test; identifying areas within the health system that had difficulties in implementing the carrier-screening program; evaluating the tools developed during the research study; and evaluating participants' and stakeholders' experiences of the program.

EVALUATION AND ANALYSIS

The evaluation of the study will include assessment of the technical aspects of the carrier-screening program, as well as determining the GP, clinical geneticist, genetic counsellor, laboratory personnel and participant experience of the program, and reviewing factors that affect uptake of the program.

Evaluation and anticipated publications

A. Evaluating the delivery experience of carrier-screening in the healthcare system

1) An end-to-end carrier-screening program in Western Australia within the health system – an evaluative study

- Evaluate turnaround time and result delivery for the metropolitan and regional sites.
- Evaluate resources developed during the pilot study, for example websites, short videos explaining key study principals and the GP counselling syllabus.
- Evaluate accuracy and diagnostic value of the targeted NGS panel.
- Evaluate workload and challenges of providing counselling for metropolitan and regional Western Australia.
- Evaluate workload and challenges of providing sequencing results for samples coming from metropolitan and regional Western Australia.
- Evaluate problems faced during the pilot study and how they were resolved.

2) General practitioners' experience and challenges in providing pre-test counselling for an expanded carrier-screening program in regional Western Australia (Figure 1)

   - Tools include: GP pre-test counselling education package, pre-training questionnaire, end-of-training questionnaire and a follow up post-training questionnaire plus telephone interview.

   - Mix-method study evaluating the training syllabus for general practitioners and their experience, including limitations of the pilot study.

3) Genetic counsellor experience and challenges in providing pre-test counselling for the expanded carrier-screening program in metropolitan Western Australia (Figure 1)

   - Tools include: telephone interview.

   - Qualitative study evaluating genetic counsellor experience, including limitations of the pilot study.

4) Implementing a State-wide carrier-screening program using next-generation sequencing technologies – outcomes and lessons learnt

   - Tools include: Targeted MPS panel.

   - Evaluate the targeted MPS panel for accuracy including calling and identifying copy number variations using metrics such as Phred Score, call quality and average coverage.

   - Evaluate number of couples at risk of having an affected child.

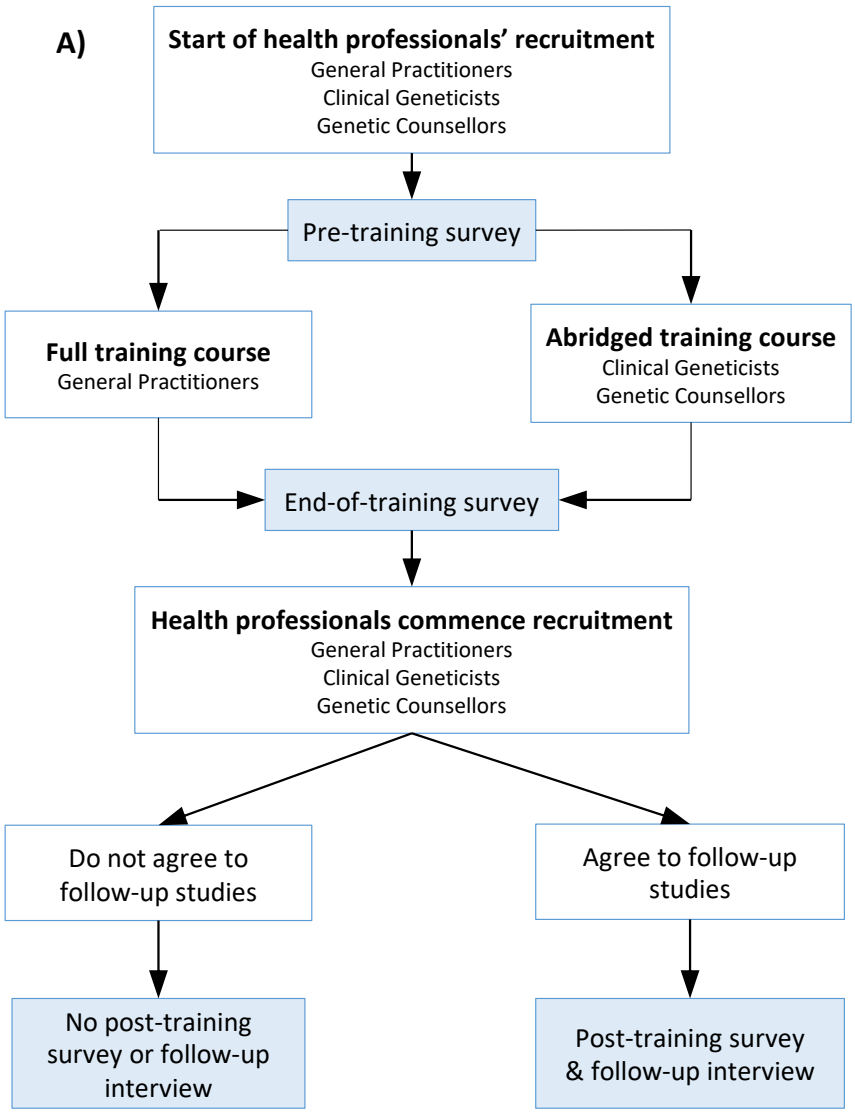


Figure 1: Health professionals will be invited to participate in the study. Full training programme consists of aspects of genetic counselling and the technology used for the test and its limitations. Full training programme will be provided to health professionals who are not used to genetic counselling, such as general practitioners, while an abridged training programme will be provided to health professionals who are used to genetic counselling but require additional information on technology used and its limitations. Mixed-methods longitudinal study will be conducted, in which assessments are made at three time points through either questionnaires and/or semi-structured interviews, with both participating couples and recruiters.

B. Evaluating factors that influence reproductive autonomy and uptake rates

5) Factors that influence informed decision making, anxiety, genetic knowledge and uptake rates of carrier-screening test in WA (Figure 2)
   - Tools include: Participant pre-test counselling questionnaire, post-result questionnaire and decliner questionnaire.
      - This quantitative study will evaluate participant informed-decision making measured by the Multi-dimensional Measure of Informed Choice (MMIC) as well as correlation studies between genetic knowledge level, informed-decision making and uptake rates.

6) Participants' experience with a State-wide carrier-screening program (Figure 2)
   - Tools include: participant pre-result and post-result telephone interview.
      - This qualitative study will evaluate participant experience by measuring their knowledge of the implications of the test, considerations, attitudes, whether they have deliberated before deciding, and reproductive autonomy.

Methods of analysis

All participants with available data will be included in the study analyses.

A mixed method strategy will be employed. Findings from interviews and surveys will guide the analysis to provide statistical information on the association between a particular behaviour and opinions or attitudes about the program. Quotes from participants will be used to reinforce the correlation.

Descriptive statistics will be based on frequency distributions for categorical data and means, standard deviations, confidence intervals (CI) and ranges, or medians, interquartile range, and ranges for continuous data, depending on normality. Univariate analysis will include $\chi 2$ and Fisher exact tests, as appropriate, for

categorical comparisons between groups, and t-tests and non-parametric Mann-Whitney U test for continuous outcomes. Categorical variables may be recoded into binary indicators if appropriate. Data will be analysed using a statistical software package such as SAS or IBM SPSS.

Thematic analysis will be used for interviews. The data generated will be managed using Nvivo.

Probability values of <0.05 will be considered statistically significant.

ETHICS AND DISSEMINATION

This study, protocol and all instruments including the informed consent document have been approved by the Women and Newborn Health Service Human Research Ethics Committee (HREC) at King Edward Memorial Hospital for Women (Approval number: RGS0000000946) and the University of Western Australia Human Research Ethics Committee (Approval number: RA/4/20/4258).

Withdrawal & handling of withdrawals

Participants (health professional or couples) may choose to withdraw at any time for any reason. Withdrawal will in no way affect participating couple's current or future medical care. If participants (health professional/couples) withdraw, any of their data or samples that were collected will be kept, unless specifically requested to be destroyed.

If samples or data have been anonymised, it may not be possible to destroy them. Participants can notify the Chief Investigator, in writing, of their wishes in relation to the samples and data already collected. Withdrawn samples will be discarded in a timely manner.

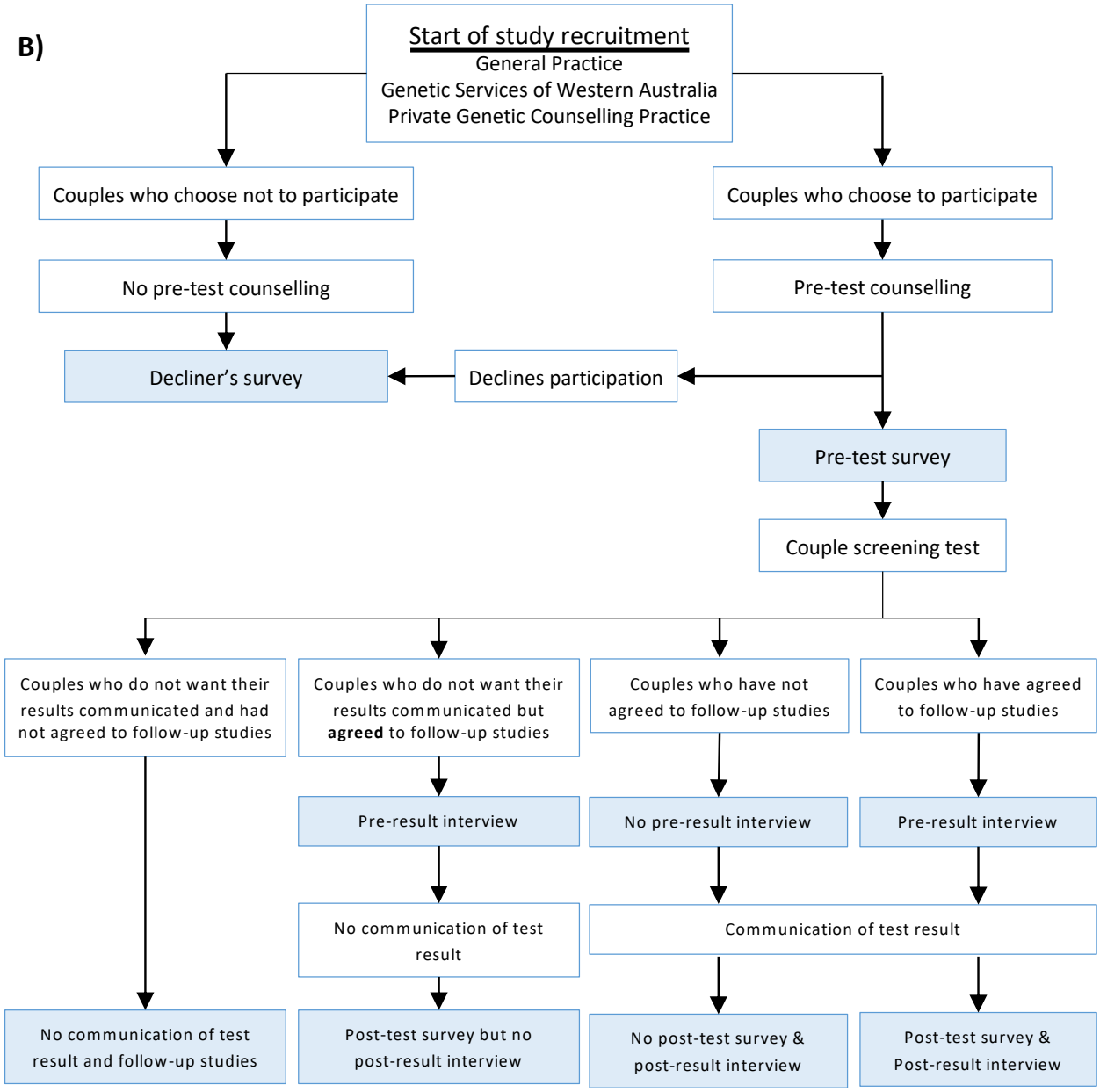


Figure 2: All recruiters will provide pre-test counselling to couples interested in the carrier-screening test. Couples who are interested in participating in the study will complete a pre-test survey. Otherwise, the couple will complete a decliner's survey if they wish. Couples can choose to participate in follow-up studies and will receive a post-test survey, as well as participate in either a pre-result or post-result interview. Mixed-methods longitudinal study will be conducted, in which assessments are made at three time points through either questionnaires and/or semistructured interviews, with both participating couples and recruiters.

Assessment of Safety

The risks to the participants are those normally associated with drawing blood. It is possible participants may feel some discomfort during the blood test and that there may be some bruising, swelling or bleeding where the needle enters the skin. Some people can feel a little light-headed when blood is taken.

Issues may arise which are associated with a couple, or family, knowing the potential cause of a disorder, as opposed to the difficulties of not knowing the cause. All couples that receive a "high-risk" result will be referred for genetic counselling.

Residual risk

Residual risk will be addressed during pre-test counselling and within the study information. Residual risks will exist, as some mutations may be undetected within the limitations of the test. A low-risk result does not mean that the couple has no chance of having a child with one of the screened disorders.

The carrier-screening test also does not screen for all recessive genetic disorders, nor does it include dominantly inherited genetic disorders. This test also does not screen for chromosomal number or structural abnormalities or other health issues that may be identified in future offspring. Therefore, the risk of a couple having a child with these possibilities is not altered by carrier-screening.

All participants will be able to contact the study genetic counsellor for any clarification.

Study closure

The study will continue until target numbers have been recruited at each site.

When the study is concluded, all records from the study will be stored in a secure setting by the Principal Investigator Professor Nigel Laing for a period of 10 years and then destroyed.

Data Collection

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Source Data

Source data will include DNA sequencing data and study specific forms completed by the treating clinician or genetic counsellor.

Data Capture Methods

Sequencing data will be captured and processed within the Department of Diagnostic Genomics, PathWest according to NATA-accredited protocols.

All study specific forms will have patient identifying details, but when entered into the study database, the patient will be identified only by the re-identifiable, study-specific unique identifier number (UIN) assigned to each patient at enrolment. Evaluative data will be captured in both paper and electronic forms. Processed evaluative data will be transferred into a secure electronic database.

Data Storage

All evaluation data will be de-identified and stored in a password-protected database in the University of Western Australia (UWA). In addition, all hard copy data collected (such as patient specific forms), will be kept in a locked cabinet within a secure building. All electronic data will be stored in a password protected, backed up, location in the UWA Institutional Research Data Store (IRDS).

DNA sequencing data will be stored by the Department of Diagnostic Genomics, PathWest according to NATA protocols. The PathWest data will only be available to PathWest staff.

Data and Record Retention

All hard copy data (including consent forms and hard copy evaluation forms) will be stored in locked filing cabinets during the study. Data will be kept for a period of 10 years.

Dissemination

All results from the evaluative studies will be published.

All supporting documents such as quantitative and qualitative instruments, education syllabus, information leaflet for health professionals and couples and advertising materials are provided as supplementary information after references.

Supplementary information for this Chapter is available on page 255 in the Appendix section.

PRESENTATIONS AND AWARDS:

This publication was curated and chosen by the Centre for Disease Control staff for entry into the Public Health Genomics and Precision Health Knowledge Base (PHGKB) (v6.0). (https://phgkb.cdc.gov/PHGKB/translationClip.action?action=archive&date=06/27/2019)

The CDC PHGKB is an online, continuously updated, searchable database of published scientific literature, CDC resources, and other materials that address the translation of genomics and precision health discoveries into improved health care and disease prevention. The Knowledge Base is curated by CDC staff and is regularly updated to reflect ongoing developments in the field.

REFERENCE

1. Ong R, Edwards S, Howting D, Kamien B, Harrop K, Ravenscroft G, Davis M, Fietz M, Pachter N, Beilby J, Laing N. Study protocol of a multicentre cohort pilot study implementing an expanded preconception carrier-screening programme in metropolitan and regional Western Australia. BMJ Open 2019;**9**(6):e028209 doi: 10.1136/bmjopen-2018-028209published
2. Bell CJ, Dinwiddie DL, Miller NA, Hateley SL, Ganusova EE, Mudge J, Langley RJ, Zhang L, Lee CC, Schilkey FD, Sheth V, Woodward JE, Peckham HE, Schroth GP, Kim RW, Kingsmore SF. Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med 2011;**3**(65):65ra4 doi: 10.1126/scitranslmed.3001756published
3. Nazareth SB, Lazarin GA, Goldberg JD. Changing trends in carrier screening for genetic disease in the United States. Prenat Diagn 2015;**35**(10):931-5 doi: 10.1002/pd.4647published
4. Matar A, Kihlbom U, Hoglund AT. Swedish healthcare providers' perceptions of preconception expanded carrier screening (ECS)-a qualitative study. J Community Genet 2016;**7**(3):203-14 doi: 10.1007/s12687-016-0268-2published
5. Lazarin GA, Goldberg JD. Current controversies in traditional and expanded carrier screening. Curr Opin Obstet Gynecol 2016;**28**(2):136-41 doi: 10.1097/GCO.0000000000000256published
6. Plantinga M, Birnie E, Abbott KM, Sinke RJ, Lucassen AM, Schuurmans J, Kaplan S, Verkerk MA, Ranchor AV, van Langen IM. Population-based preconception carrier screening: how potential users from the general population view a test for 50 serious diseases. Eur J Hum Genet 2016;**24**(10):1417-23 doi: 10.1038/ejhg.2016.43published
7. Abuli A, Boada M, Rodriguez-Santiago B, Coroleu B, Veiga A, Armengol L, Barri PN, Perez-Jurado LA, Estivill X. NGS-Based Assay for the Identification of Individuals Carrying Recessive Genetic Mutations in Reproductive Medicine. Hum Mutat 2016;**37**(6):516-23 doi: 10.1002/humu.22989published
8. Wilfond BS, Kauffman TL, Jarvik GP, Reiss JA, Richards CS, McMullen C, Gilmore M, Himes P, Kraft SA, Porter KM, Schneider JL, Punj S, Leo MC, Dickerson JF, Lynch FL, Clarke E, Rope AF, Lutz K, Goddard KAB. Lessons Learned From A Study Of Genomics-Based Carrier Screening For Reproductive Decision Making. Health Aff (Millwood) 2018;**37**(5):809-16 doi: 10.1377/hlthaff.2017.1578published
9. Lau TK. Obstetricians should get ready for expanded carrier screening. BJOG 2016;**123 Suppl 3**:36-8 doi: 10.1111/1471-0528.14196published
10. Chokoshvili D, Janssens S, Vears D, Borry P. Designing expanded carrier screening panels: results of a qualitative study with European geneticists. Per Med 2016;**13**(6):553-62 doi: 10.2217/pme-2016-0018published
11. Korngiebel DM, McMullen CK, Amendola LM, Berg JS, Davis JV, Gilmore MJ, Harding CO, Himes P, Jarvik GP, Kauffman TL, Kennedy KA, Simpson DK, Leo MC, Lynch FL, Quigley DI, Reiss JA, Richards CS, Rope AF, Schneider JL, Goddard KA, Wilfond BS. Generating a taxonomy for genetic conditions relevant to reproductive planning. Am J Med Genet A 2016;**170**(3):565-73 doi: 10.1002/ajmg.a.37513published
12. Henneman L, Borry P, Chokoshvili D, Cornel MC, van El CG, Forzano F, Hall A, Howard HC, Janssens S, Kayserili H, Lakeman P, Lucassen A, Metcalfe SA, Vidmar L, de Wert G, Dondorp WJ, Peterlin B. Responsible implementation of expanded carrier screening. Eur J Hum Genet 2016;**24**(6):e1-e12 doi: 10.1038/ejhg.2015.271published
13. American College of Obstetricians and Gynecologists. Committee Opinion No. 690: Carrier Screening in the Age of Genomic Medicine. Obstet Gynecol 2017;**129**(3):e35-e40 doi: 10.1097/AOG.0000000000001951published

14. Edwards JG, Feldman G, Goldberg J, Gregg AR, Norton ME, Rose NC, Schneider A, Stoll K, Wapner R, Watson MS. Expanded carrier screening in reproductive medicine-points to consider: a joint statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine. Obstet Gynecol 2015;**125**(3):653-62 doi: 10.1097/AOG.0000000000000666published
15. Benn P, Chapman AR, Erickson K, Defrancesco MS, Wilkins-Haug L, Egan JF, Schulkin J. Obstetricians and gynecologists' practice and opinions of expanded carrier testing and noninvasive prenatal testing. Prenat Diagn 2014;**34**(2):145-52 doi: 10.1002/pd.4272published
16. Lazarin GA, Hawthorne F, Collins NS, Platt EA, Evans EA, Haque IS. Systematic Classification of Disease Severity for Evaluation of Expanded Carrier Screening Panels. PLoS One 2014;**9**(12):e114391 doi: 10.1371/journal.pone.0114391published
17. Lazarin GA, Detweiler S, Nazareth SB, Ashkinadze E. Genetic Counselors' Perspectives and Practices Regarding Expanded Carrier Screening after Initial Clinical Availability. J Genet Couns 2016;**25**(2):395-404 doi: 10.1007/s10897-015-9881-1published
18. Holtkamp KC, van Maarle MC, Schouten MJ, Dondorp WJ, Lakeman P, Henneman L. Do people from the Jewish community prefer ancestry-based or pan-ethnic expanded carrier screening? Eur J Hum Genet 2016;**24**(2):171-7 doi: 10.1038/ejhg.2015.97published
19. Molster CM, Lister, K., Metternick-Jones, S., Baynam, G., Clarke, A. J., Straub, V., Dawkins, H. J. S., and Laing, N. Outcomes of an International Workshop on Preconception Expanded Carrier Screening: Some Considerations for Governments. . Frontiers Public Health doi: https://doi.org/10.3389/fpubh.2017.00025published
20. Ben-Shachar R, Svenson A, Goldberg JD, Muzzey D. A data-driven evaluation of the size and content of expanded carrier screening panels. Genet Med 2019;**21**(9):1931-39 doi: 10.1038/s41436-019-0466-5published
21. Chang F, Li MM. Clinical application of amplicon-based next-generation sequencing in cancer. Cancer Genet 2013;**206**(12):413-9 doi: 10.1016/j.cancergen.2013.10.003published
22. Massie J, Curnow L, Gaffney L, Carlin J, Francis I. Declining prevalence of cystic fibrosis since the introduction of newborn screening. Arch Dis Child 2010;**95**(7):531-3 doi: 10.1136/adc.2009.172916published
23. Smith M, Calabro V, Chong B, Gardiner N, Cowie S, du Sart D. Population screening and cascade testing for carriers of SMA. Eur J Hum Genet 2007;**15**(7):759-66 doi: 10.1038/sj.ejhg.5201821published
24. Archibald A, Smith M, Burgess T, Amor D. Reproductive Genetic Carrier Screening for Cystic Fibrosis, Fragile X Syndrome and Spinal Muscular Atrophy in Australia: Outcomes of 12,000 Tests. Australian & New Zealand Journal of Obstetrics & Gynaecology 2017;**57**:12-12
25. Todd EJ, Yau KS, Ong R, Slee J, McGillivray G, Barnett CP, Haliloglu G, Talim B, Akcoren Z, Kariminejad A, Cairns A, Clarke NF, Freckmann ML, Romero NB, Williams D, Sewry CA, Colley A, Ryan MM, Kiraly-Borri C, Sivadorai P, Allcock RJ, Beeson D, Maxwell S, Davis MR, Laing NG, Ravenscroft G. Next generation sequencing in a large cohort of patients presenting with neuromuscular disease before or at birth. Orphanet J Rare Dis 2015;**10**:148 doi: 10.1186/s13023-015-0364-0published
26. Beecroft SJ, Yau KS, Allcock RJN, Mina K, Gooding R, Faiz F, Atkinson VJ, Wise C, Sivadorai P, Trajanoski D, Kresoje N, Ong R, Duff RM, Cabrera-Serrano M, Nowak KJ, Pachter N, Ravenscroft G, Lamont PJ, Davis MR, Laing NG. Targeted gene panel use in 2249 neuromuscular patients: the Australasian referral center experience. Ann Clin Transl Neurol 2020;**7**(3):353-62 doi: 10.1002/l3.51002published

27. WHO. International Classification of Functioning, Disability and Health (ICF). 2003. Date Accessed www.who.int/classifications/icf/training/icfchecklist.pdf)
28. Illumina Inc. Illumina Professional Product Services brochure. Date Accessed 11/04/20. https://www.illumina.com/content/dam/illumina-marketing/documents/services/professional-product-services-handout-070-2016-019-web.pdf.
29. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;**17**(5):405-24 doi: 10.1038/gim.2015.30published
30. Feng Y, Ge X, Meng L, Scull J, Li J, Tian X, Zhang T, Jin W, Cheng H, Wang X, Tokita M, Liu P, Mei H, Wang Y, Li F, Schmitt ES, Zhang WV, Muzny D, Wen S, Chen Z, Yang Y, Beaudet AL, Liu X, Eng CM, Xia F, Wong LJ, Zhang J. The next generation of population-based spinal muscular atrophy carrier screening: comprehensive pan-ethnic SMN1 copy-number and sequence variant analysis by massively parallel sequencing. Genet Med 2017;**19**(8):936-44 doi: 10.1038/gim.2016.215published
31. Biancalana V, Glaeser D, McQuaid S, Steinbach P. EMQN best practice guidelines for the molecular genetic testing and reporting of fragile X syndrome and other fragile X-associated disorders. Eur J Hum Genet 2015;**23**(4):417-25 doi: 10.1038/ejhg.2014.185published
32. Henneman L, Borry P, Chokoshvili D, Cornel MC, van El CG, Forzano F, Hall A, Howard HC, Janssens S, Kayserili H, Lakeman P, Lucassen A, Metcalfe SA, Vidmar L, de Wert G, Dondorp WJ, Peterlin B. Responsible implementation of expanded carrier screening. Eur J Hum Genet 2017;**25**(11):1291 doi: 10.1038/ejhg.2017.159published
33. Azimi M, Schmaus K, Greger V, Neitzel D, Rochelle R, Dinh T. Carrier screening by next-generation sequencing: health benefits and cost effectiveness. Mol Genet Genomic Med 2016;**4**(3):292-302 doi: 10.1002/mgg3.204published
34. Schofield D, Alam K, Douglas L, Shrestha R, MacArthur DG, Davis M, Laing NG, Clarke NF, Burns J, Cooper ST, North KN, Sandaradura SA, O'Grady GL. Cost-effectiveness of massively parallel sequencing for diagnosis of paediatric muscle diseases. NPJ Genom Med 2017;**2** doi: 10.1038/s41525-017-0006-7published
35. Prior TW. Next-generation carrier screening: are we ready? Genome Med 2014;**6**(8):62 doi: 10.1186/s13073-014-0062-xpublished
36. Laing N. What is the role of preconception carrier screening in neurology? Advances in Clinical Neuroscience and Rehabilitation 2016(16):2
37. SUPERIOR HEALTH COUNCIL BELGIUM. Expanded carrier screening in a reproductive context. Towards a responsible implementation in the healthcare system. In: Superior Health Council, ed. Belgium, 2017.
38. Ong R, Howting D, Rea A, Christian H, Charman P, Molster C, Ravenscroft G, Laing NG. Measuring the impact of genetic knowledge on intentions and attitudes of the community towards expanded preconception carrier screening. J Med Genet 2018;**55**(11):744-52 doi: 10.1136/jmedgenet-2018-105362published
39. BPMRI. Busselton Population Medical Research Institute - History. Date Accessed 11/04. http://bpmri.org.au/about-us/history.html#item-3.

CHAPTER 3 SUMMARY DISCUSSION

This chapter has discussed our screening methodology including site selection, recruitment and reporting strategies. I have also discussed our evaluation methods and the various time points in which data will be collected for further investigations. Results from the evaluations should inform us if our current approach will work within the WA health system and hopefully identify bottlenecks and ways of improving the delivery along the way.

This chapter has also identified and discussed at length, the optimised workflow in order to maximise data we obtain per run, including probe volume used and the bioinformatic pipeline we plan to employ.

Preparations for the pilot study was therefore completed at this point and the pilot study was ready to begin recruitment.

Another PhD student, genetic counsellor Ms Samantha Edwards, will continue the evaluations not completed within my PhD study, focusing on the service delivery and challenges experienced by general practitioners and genetic counsellors. In addition, Samantha will evaluate the efficacy of the education materials developed for this study and the long-term clinical utility of this test for high-risk couples, which goes beyond the timeline available for my PhD studies.

The pilot study implementation and results will be presented in Chapter 4.

CHAPTER 4

Results from offering an expanded carrier-screening test in Western Australia

CHAPTER 4 PREFACE

This chapter details the key findings from offering an expanded carrier-screening test in Western Australia. This is the first pilot study that explored the best way to implement a carrier-screening program through components of an Australian state health system. I performed all the laboratory work including preparing and sequencing the libraries as well as variant analyses.

I show that it is feasible to offer carrier-screening with little disruption to the current PathWest infrastructure and workflow. I also show that 1 in 32 couples screened have an increased chance of having a child affected by one of the recessive disorders screened for. This had major implications for resource requirements when considering offering a carrier-screening program in the health system.

This work has been prepared for submission and is therefore formatted in a manuscript style. As a result, certain sections, for example "Disorder and gene selection for the targeted gene panel", described in this manuscript are replicated from Chapter 3.

CHAPTER 4

Results from offering an expanded carrier-screening test in Western Australia

Prepared manuscript

Royston Ong
Mark Davis
Richard Allcock
Grace Androga
Samantha Edwards
Benjamin Kamien
Karen Harrop
Gianina Ravenscroft
Michael Fietz
Nicholas Pachter
John Beilby
Nigel Laing

INTRODUCTION

Carrier-screening involves screening individuals or couples[1,2] for recessive disorders. Since carriers of recessive disorders are usually unaffected, children with recessive disorders are most often born into families with no history of the disorder[3]. There are currently more than 1,800 autosomal and X-linked recessive disorders described[3]. A significant proportion of these are severe paediatric disorders[4].

Recent studies show that the risk of having a child with these severe recessive disorders is higher than[5] or equal to[6] the birth prevalence of children with Down syndrome[7]. Other studies have shown that genetic disorders are responsible for a significant proportion of infant morbidity and mortality[8] and that the burden of genetic disorders on patients, families and society in terms of suffering is therefore large[3]. A study published in 2016 by the Western Australian Office of Population Health Genomics, indicated that about 30% of the rare disorder patient study cohort had to wait between 5 to 20 years for an accurate diagnosis and that 50% had received at least one misdiagnosis[9]. This lengthy diagnostic period is known as a "diagnostic odyssey". An early genetic diagnosis in children of high-risk couples should therefore avoid the diagnostic odyssey and reduce anxiety and psychological stress in parents and allow for more reproductive options.

Couples who are both carriers of the same recessive disorder can make preparations to conceive an unaffected child through preimplantation genetic diagnosis, prenatal testing, gamete donation, adoption, or looking into means of ameliorating the disorder severity through medications and lifestyle changes[10-13]. Carrier-screening therefore provides the greatest benefit before

pregnancy[10,14,15] and has been suggested to empower couples to make informed decisions about their future pregnancies[10,16,17]. Recognising these benefits, some European countries such as The Netherlands, Sweden and Belgium, have started exploring ways to make carrier-screening testing more readily available[1,18-20].

In Australia, however, only commercial entities[21,22] offer carrier-screening tests for a limited number of recessive disorders including Tay-Sachs disease (TSD) (MIM 272800), cystic fibrosis (CF) (MIM 219700) and thalassemia. For example, the Victorian Clinical Genetic Services (VCGS) has been offering a user-paid carrier-screening service, PrePair™, screening for Fragile X syndrome (MIM 300624), CF and spinal muscular atrophy (MIM 253300) since 2012[23]. TSD screening programs are performed in New South Wales and Victoria and no TSD-affected children have been born to Australian parents who have been screened[24]. However, such targeted TSD carrier-screening is not routinely available in other States.

There are no surveillance or monitoring mechanisms in Australia to record and report on the collective impact of rare disorders. However, a study by the Western Australian Office of Population Health Genomics of 467 rare disorders indicated that although only 2% of the Western Australian population were affected by these disorders, the disorders accounted for 10.5% of the 2010 annual Western Australian hospitalisation expenditure. This amounted to a total of $395 million dollars[25]. Since Western Australia has only 10% of the Australian population, extrapolating across Australia would suggest an annual hospitalisation expenditure for these disorders of $3.95 billion dollars.

Next generation sequencing (NGS) provides the means to sequence multiple genes and multiple samples at once. Studies have also shown that NGS can be used to detect carriers of pathogenic variants effectively and affordably[26-28].

This study reports the findings of a carrier-screening pilot study using an expanded gene panel and implemented within the Western Australian public healthcare system.

METHODS

Study protocol

A protocol paper detailing the study design was published previously[29]. It uses a couples-based approach[1]. Briefly, couples were recruited from three sites in metropolitan Perth, the capital of Western Australia, and four general practice clinics in the town of Busselton, in the South-West region of Western Australia. The inclusion criteria for couples were that both members of the couple should be over 18 years of age and the couple should not knowingly be pregnant at the time of recruitment.

The initial aim was to recruit 250 couples over a two-year period.

Potential participants meeting the study criteria were offered, during a regular consultation with their healthcare provider, a study information pack to take home. Interested couples returned for couples-based pre-test counselling by their health care provider. All participating general practitioners had received upskilling in appropriate genetic counselling.

Couples participating in the study then signed the consent form and were provided with a study collection form. Using this form, couples had their blood samples taken

at any of the Western Australian Department of Health PathWest Laboratory Medicine (PathWest) specimen collection centres located throughout the State. Samples collected at these collection centres were delivered to the PathWest Department of Diagnostic Genomics at the QEII Medical Centre in Perth, through the statewide PathWest courier system.

DNA was extracted at PathWest from the blood samples for analysis using a QIASymphony. The DNA was analysed using a NGS targeted gene panel of 474 genes. In addition, spinal muscular atrophy (*SMN1)* and Fragile X testing were performed as per standard diagnostic practice in PathWest Diagnostic Genomics using qPCR[30] and repeat-primed PCR[31] respectively.

Library preparation

All the laboratory work for this study was performed in the West Australian Department of Health, PathWest, Department of Diagnostic Genomics. Libraries were prepared using Illumina Flex reagent kits (Nextera DNA Flex Library Prep, Catalogue# 20018705) according to the manufacturer's protocol. Libraries were sequenced using the NextSeq™ 550 Sequencing System. Reads were aligned and variant calling performed using BaseSpace Software (Illumina Inc). A variant call format (vcf) file was generated for each sample. Each vcf file was annotated, filtered and analysed in Alissa Interpret (Agilent Technologies) as used routinely in the Department of Diagnostic Genomics.

Bioinformatic pipeline for couple-based analysis of NGS data

The bioinformatic pipeline for analysis of the NGS data on a couple basis is shown in the flow diagram in Chapter 3, Section 1 (Page 122). Data for each sample was first compared to a list of previously observed pathogenic variants in the "*Managed*

Variant List" (MVL) in Alissa, generated over time by PathWest. Known pathogenic variants in any carrier-screening genes identified in the MVL were piped into the final variant list. All potential loss of function variants, canonical splice site variants (±2bps of an exon), variants ±5bp from any donor or acceptor splice sites and missense variants of less than 0.5% allele frequency in population databases, were retained in the individual final variant list. The individual final variant lists for both partners were then analysed together to identify candidate variants in the same gene(s) in each couple.

Variant classification and definition of high-risk couples

A suite of software and databases was used in the classification of variants, such as the Alissa Interpret "variant review function". In particular, the Clinical Variation Database (ClinVar) and Human Genetic Mutation Database Professional (HGMD Professional) were used to provide reports of rare variants previously listed as pathogenic. ACMG guidelines were used to curate variants in the same gene in each couple for pathogenicity[32] (Table S4). Only when likely pathogenic or pathogenic (Class4 or Class5) variants were identified in the same gene in both members of a couple, or identified on the X chromosome in the female partner, were the couple considered as "high-risk" and reported as such.

Fragile X syndrome

Following PathWest Diagnostic Genomics standard practice, a couple was defined as at high-risk if the female partner had a CGG repeat expansion between 55 and 200 repeats in one allele. A result of ≤54 repeats would be reported as "low risk" for having a child with Fragile X syndrome. Gray zone alleles (45–54 CGG repeats) were

reported as "low risk" because women with gray zone alleles are not at risk of having a child with Fragile X syndrome[6].

Spinal muscular atrophy type 1

Following PathWest Diagnostic Genomics standard practice, a couple was defined as at high-risk if both of members of a couple were identified as carriers of SMN1 exon 7 deletion using qPCR method[30].

High risk couples

All high-risk couples were referred to Genetic Services WA (GSWA), the clinical genetics arm of the Western Australian Department of Health, and treated as a standard high-risk couple referral. Counselling of these high-risk couples was provided at no additional cost to the couple.

Disorder and gene selection for the targeted gene panel

Only disorders in OMIM with a known molecular basis were reviewed for inclusion in the screening panel (Table S1). Large insertion-deletions, expansions and chromosomal aberrations were excluded due to the technological limitations of next generation sequencing to detect such variants. Disorders were also excluded if they had reduced penetrance or were risk-factors for a disorder.

The remaining Mendelian disorders were grouped into either dominantly or recessively inherited disorders. Only recessive disorders were further curated. To determine disorder severity, the OMIM application-programming interface (API) was used to interrogate the OMIM database for specific terms such as "Death", "Mortality", and "Dead" under the headings of "Description", "Clinical Synopsis" and "Clinical Features". All recessive disorders not identified by API were then

manually categorised based on the severity criteria using information from "Clinical Synopsis", "Description" and "Clinical Features". Duplicates were then removed and the remaining recessive disorders were categorised as Category 1, 2 or 3 (Table S2).

Briefly, Category 1 recessive disorders cause infant and childhood mortality while Category 2 recessive disorders are chronic and severely reduce the quality of life for the patient. Category 3 recessive disorders are those that do not meet the criteria for Category 1 or 2.

Designing the carrier-screening panel

A total of 403 genes associated with 451 recessive disorders met the criteria for Category 1 (Table S3). These genes were the core of the panel design. In late 2017, we collaborated with the Victorian Clinical Genetic Services (VCGS) and merged their expanded carrier-screening gene list of 117 genes (personal communication with Professor Martin Delatycki), with the 403 genes, in a bid to produce a semi-standardised carrier-screening screening panel in at least two Australian states. As a result of that collaboration, the final carrier-screening panel consisted of 474 autosomal recessive and X-linked genes associated with mainly infant and childhood lethal and debilitating disorders. The list of disorders screened also included three common recessive disorders: spinal muscular atrophy (MIM 253300), fragile X (MIM 300624) and cystic fibrosis (MIM 219700); with a few Category 2 and 3 genes included from the VCGS expanded carrier-screening gene list. Genomic start and end positions of each exon in the carrier-screening panel were then generated using UCSC's table browser function. The genomic locations were submitted for probe production by Illumina.

Ethics

Ethics approvals for the project were obtained from the University of Western Australia Human Research Ethics Committee (RA/4/1/8847) and the ethics committee of the Western Australian Department of Health (RGS0000000946) which covered both PathWest Laboratory Services and Genetic Services WA.

RESULTS

Demographics

The initial aim for the study was to recruit 250 couples over a two-year period starting in September 2018. A total of 462 participants (231 couples) were recruited by the end of January 2020 (17 months) when recruiting was halted.

Six couples withdrew (2.6%) from the study after recruitment, therefore, 225 couples were analysed in the study (Table 1). Of the four couples who withdrew, one couple split up after recruitment while two couples decided that it was not the right time to do the test and the last couple could not agree on whether to continue with testing. 89% of couples were recruited from metropolitan sites and 11% from regional sites. The average age of the participants was 34years (21yo – 53yo) for males and 32years (20yo – 46yo) for females. Two thirds of couples (68%) were of Caucasian ethnicity (Non-Finnish European), while a fifth were of mixed ethnicities (Table 1).

Table 1: Demographics, logistics of offering carrier-screening test to and variant analysis of study participants

Demographics	n	%
Metropolitan couples participated	200	88.9%
Regional couples participated	25	11.1%
Couple Ethnicity: Ashkenazi Jewish (ASH)	4	1.9%
Couple Ethnicity: African (AFR)	1	0.5%
Couple Ethnicity: East Asian (EAS)	8	3.8%
Couple Ethnicity: Middle Eastern (ME)	7	3.3%
Couple Ethnicity: Mixed couple (MIX)	41	19.3%
Couple Ethnicity: Non-Finnish European (NFE)	144	67.9%
Couple Ethnicity: South Asian (SAS)	7	3.3%
Average male participant's age	34 yo (21 – 53)	
Average female participant's age	32 yo (20 – 46)	
Number of couples who withdraw from the study	6	2.6%
Logistics		
Couples that became pregnant after sample provision	14	6.2%
Samples collected in: Week 1	297	66.0%
Samples collected in: Week 2	47	10.4%
Samples collected in: Week 3	23	5.1%
Samples collected in: Week 4	25	5.6%
Samples collected in: Week 5	11	2.4%
Samples collected in: Week 6	9	2.0%
Samples collected in: Week 7	5	1.1%
Samples collected in: Week 8	7	1.6%
Samples collected in: Week 9	2	0.4%
Samples collected in: After 10 weeks	24	5.3%
More than 8-week TAT to report	327	72.7%
Less than 8-week TAT to report	123	27.3%
Variant Analysis		
Total rare coding variants	5,393	
Number of X-linked variants	94	
Total Class4/5 pathogenic variants	520	
Known Class4/5 pathogenic variants	409	
Novel Loss of Function variants	111	
Average pathogenic variants / couple	2.01 (0-7)	
Novel likely pathogenic/pathogenic X-linked variants	6	
Average time to analyse each couple	15min (0:30sec-84mins)	
Novel high-risk couples identified	7 (3.5%)	

Logistics

All DNA samples were obtained from blood even though saliva collection was an option. More than 65% (n=297) of samples were collected within the first week following recruitment with a further 10% (n=47) in week two. 24 couples took more than 10 weeks to send their samples in, including one couple who took 15 weeks to decide to participate (Figure 1A). The times taken to receive samples were similar between sites (Figure 1B) and between genders in the cohort (Figure 1C). 14 couples fell pregnant (6.2%) after sample provision (Table 1). The study had a projected 4 to 8-week turnaround time from the receipt of samples at PathWest to reporting results back to couples, however 70% of couples received their reports more than 8 weeks after sample receipt. The reason for this number of couples receiving results after the projected turnaround time were almost exclusively due to issues in reagent supplies.

NGS Variant analysis

Analysis of couple NGS data

The analysis pathway followed for the NGS data for each couple is shown in Figure S1.

188 of the 225 couples (83.5%) had variants in the same gene. On average, each couple had two genes for which they had variants in the same genes. Ninety-four of the 225 female participants had at least one rare coding X-linked variant (41.7%) (Table 1).

The average time to analyse each couple was approximately 15 minutes. It took only around 30 seconds to analyse couples without any variants in the same gene and up to 84 minutes to analyse a couple who both had candidate pathogenic

variants in multiple genes. The amount of time taken depended largely on the availability of evidence for the pathogenicity of the variants in question (Table 1).

Analysis of positive control couples

Couples with a known family history of a recessive disorder, who wished to know if they were at risk for other severe recessive disorders, were recruited if they met the inclusion criteria[29]. These "known carrier couples" acted as positive controls to validate the analysis pipeline: their carrier status was blinded during analysis. A total of 12 positive control couples were recruited during the study. Of these couples, eight positive control couples were correctly identified as at high-risk for the disorders they were known to be carriers for. Nine variants were identified in these positive control couples. Eight of the nine variants were loss of function variants, which included a complete deletion of *MTM1*. There was also a single pathogenic missense variant in *DMD* (Table 2A).

Three of the four positive control couples not correctly identified had variants with insufficient evidence available to support pathogenicity according to ACMG guidelines. Couple 15806 had a homozygous variant (c.733C>T p.Arg245Cys) in the gene *SGSH.* Variants in *SGSH* are associated with Mucopolysaccharidosis 3A (MIM 252900). The novel variant was absent in population databases such as gnomAD. The variant was a novel missense change at a residue where a different pathogenic missense change had been seen previously. In addition, multiple *in-silico* algorithms predicted this variant as pathogenic. Therefore, according to the ACMG guidelines, this variant was classified as a Variant of Unknown Significance (VOUS), as there was insufficient evidence available to support pathogenicity at that stage (Table 2B).

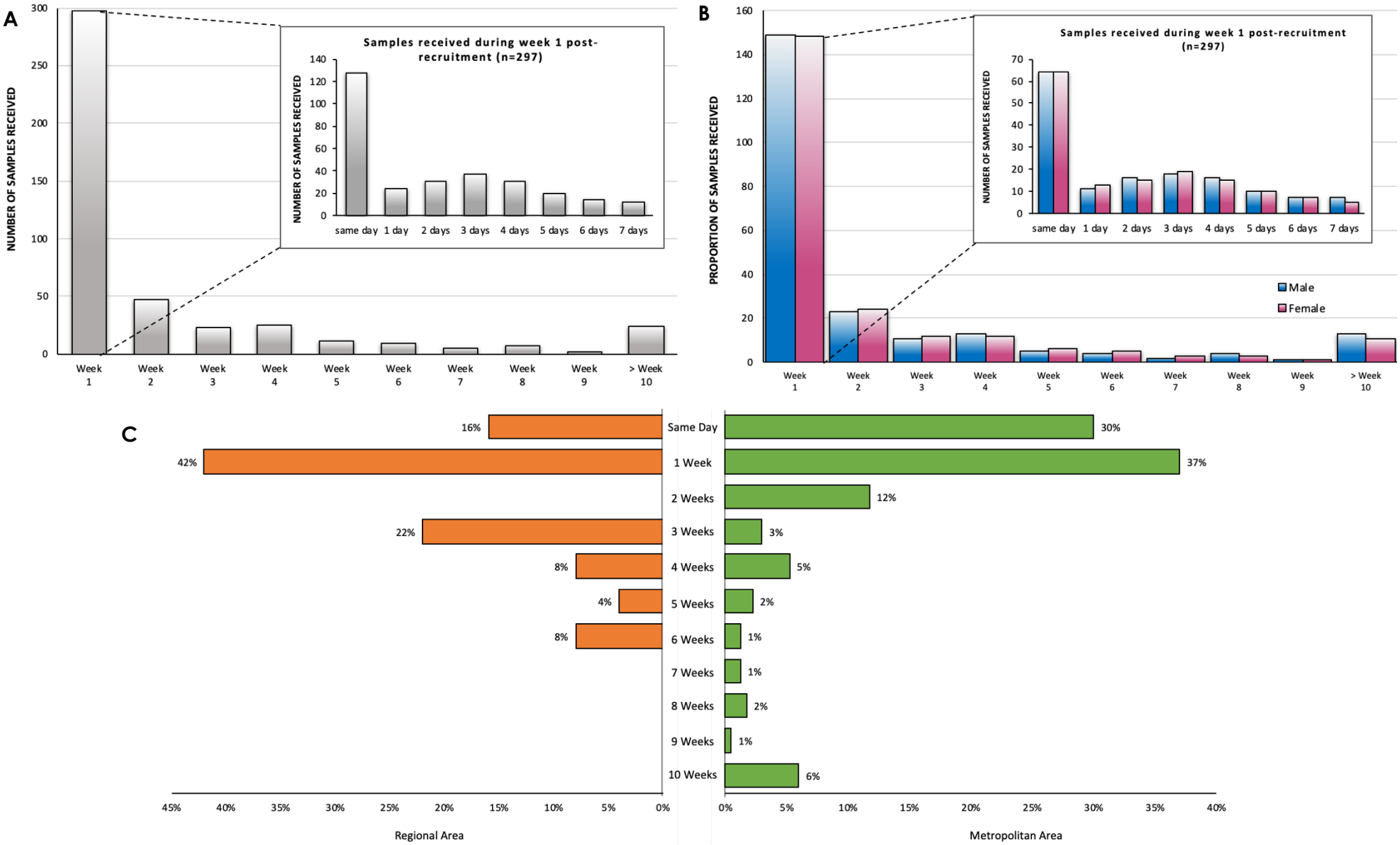


Figure 1: Weeks taken to receive samples

A: Distribution of the number of weeks it took for samples to arrive at the laboratory.
B: Distribution of the weeks taken by each gender to provide blood samples. Blue represents male, purple presents female.
C: Distribution of the weeks taken to receive blood samples in the laboratory separated by location (Orange: metropolitan vs Green: regional)

Each insert represents the distribution of samples within the first week.

Couple 15672 had a novel deletion and a novel missense variant identified in the gene *HSD17B4* known to be associated with D-bifunctional protein deficiency (MIM 261515). Small deletions in this gene were known to be associated with the disorder. In addition, this deletion was absent from population databases and predicted by multiple *in-silico* algorithm as pathogenic. In light of these pieces of evidence, this novel deletion was classified as a Pathogenic variant (Class 5). In contrast, the novel missense variant was not observed in population databases but had not been reported in ClinVar or HGMD Professional. The in-silico predictions of pathogenicity were conflicting. This missense variant was therefore classified as a VOUS. As a result of this lack of evidence for the novel missense variant and a Class 5 novel deletion, the known carrier Couple 15672 was therefore classified as "low-risk" (Table 2B) for that disorder.

Couple 17875 had a well published pathogenic variant in ATP7B causing Wilson disorder (MIM 277900) and a novel missense variant. The novel missense variant had a relatively common frequency of 0.1% in gnomAD. Although multiple in-silico evidence supports pathogenicity, ultimately the conflicting interpretation of this variant in ClinVar and a general lack of functional evidence led to the classification of this variant as a VOUS. Accordingly, the variant was classified as "low-risk" (Table 2B).

The last positive control couple had a *SMN1* (2+0) genotype which cannot be detected using the study methods (Table 2B).

Novel high-risk couples

Seven novel high-risk couples were identified in this study (Table 2D). Amongst these, three couples had Fragile X CGG repeats within the premutation range of 55 to 200

repeats in *FMR1*. Another couple were both carriers of the common exon 7 deletion in *SMN1*. Three other couples were at high-risk of having an affected child with a very rare disorder (Table 2D). Of the novel high-risk couples, 85.7% (6/7) had variants in the X chromosome.

The overall incidence of novel high-risk couples in the screened cohort is 1 in 32 couples (3.1%) (Table 2D).

Overall cohort data

Of the 5,393 rare coding variants collated from the data for all individuals in the study, 409 were listed as pathogenic and likely pathogenic in variant databases such as ClinVar and/or HGMD Professional. A further 111 rare coding variants were novel loss of function (LOF) variants, in genes in which LOF was associated with a disorder. This therefore resulted in a total of 520 Class4/Class5 variants in the 450 individuals in the study. At least one Class4/Class5 variant was identified in 217 out of the 474 genes in the targeted panel (45.8%).

A total of 94 X-linked variants were identified in our female participants, nine X-linked variants were classified as "likely pathogenic/pathogenic". Of these nine likely pathogenic/pathogenic X-linked variants, six were identified in novel high-risk couples while three were identified in positive control couples.

Table 2A: 8 positive controls correctly identified in our study

Couple	Ethnicity	Gene	Variant	Disorder	MIM #	Class	Comments	Evidence
15838	NFE	*CFTR*	c.3293G>A (p.Trp1098*)	Cystic fibrosis	602421	5#	well published variant	- Predicted null variant in a gene where LOF is a known mechanism of disorder (V.S) - Absent in population database (M) - Reputable source reports pathogenicity (Su)
15859	ME	*SEPN1*	c.1446delC (p.Asn483Thrfs*11)	Rigid spine muscular dystrophy	602771	5#	novel variant	- Predicted null variant in a gene where LOF is a known mechanism of disorder (V.S) - Absent in population database (M) - Multiple in-silico predicts pathogenicity (Su)
15951	NFE	*PEX7*	c.875T>A (p.Leu292*)	Rhizomelic chondrodysplasia punctata type 1	215100	4*	novel variant	- Predicted null variant in a gene where LOF is a known mechanism of disorder (V.S) - Observe at low frequency in population database (M) - Multiple in-silico predicts pathogenicity (Su)
		PEX7	c.120C>G (p.Tyr40*)			5#	well published variant	- Predicted null variant in a gene where LOF is a known mechanism of disorder (V.S) - Well published variant (S) - Observe at low frequency in population database (M) - Multiple in-silico predicts pathogenicity (Su)
16506	ME	*HBB*	c.315+1G>A	Beta thalassemia	613985	5#	well published variant	- Predicted null variant in a gene where LOF is a known mechanism of disorder (V.S) - Well published variant with multiple patient case (S) - Observed at low frequency in population databases (M) - Reputable sources report variant as pathogenic (Su)
16622	SAS	***MTM1***	whole gene deletion of MTM1	X-linked myotubular myopathy	310400	5#	well published variant	- Variant is null variant in a gene where LOF is a known mechanism of disorder (V.S) - Reported previously in multiple affected families – VCV000658991.1, PMID 20434914, PMID 9305655 (M)
16858	NFE	***DMD***	c.2176G>T (p.Val726Phe)	Becker muscular dystrophy	300376	4*	novel variant	- This variant was observed in multiple families with affected children in the PathWest database (S) - This missense variant was absent in population databases (gnomAD) (M)
17042	SAS	***IDS***	c.1393C>T (p.Gln465*)	Mucopoly-saccharidosis II	309900	5#	Well published variant	- Predicted null variant in a gene where LOF is a known mechanism of disorder (V.S) - Variant is absent in gnomAD (M) - Located in a mutational hot spot (M) - Previously reported in a publication (PMID: 8830188) and reported in ClinVar as pathogenic (221202) (M) - Multiple in-silico predicts pathogenicity (Su)
17750	EAS	*SMN1*	1 copy of *SMN1* allele	Spinal muscular atrophy type 1	253300	5#	Well published variant	- Well-established disorder-causing variant (V.S)

^ ACMG Class 3: Variant of unknown significance (VOUS); * ACMG Class 4: Likely pathogenic; # ACMG Class 5: Pathogenic
V.S: Very strong evidence; S: Strong evidence; M: Moderate evidence; Su: Supporting evidence
NFE: Non-Finnish European descent; ME: Middle Eastern descent; SAS: South Asian descent; AFR: African descent
Genes in bold indicate genes on the X-chromosome

Table 2B: 4 known carrier couples not or incorrectly identified in our cohort

Couple	Ethnicity	Gene	Variant	Disorder	MIM #	Class	Comments	Evidence
15806	ME	*SGSH*	c.733C>T; (p.Arg245Cys)	Mucopolysaccharidosis 3A	252900	3^	novel variant	- Absent in population databases. (M) - Novel missense change at a residue where a different pathogenic missense change has been seen previously (M) - Multiple in-silico predicts pathogenicity (Su)
15672	NFE	*HSD17B4*	c.1132G>A; (p.Gly378Arg)	D-bifunctional protein deficiency	261515	3^	novel variant	- Absent in population database (M)
		HSD17B4	c.1717_1718del			5#	novel variant	- Absent in population databases. (M) - Predicted null variant in a gene where LOF is a known mechanism of disorder (V.S) - Multiple in-silico predicts pathogenicity (Su)
17875	NFE	*ATP7B*	c.3955C>T; (p.Arg1319*)	Wilson's disease	277900	5#	Well published variant	- Variant is present at very low frequency in gnomAD (Su) - Variant is also located in a region where multiple pathogenic variants reside (M) - Known pathogenic variant in multiple publications identified in HGMD and ClinVar (35728) (V.S). - The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls (S)
			c.2972C>T; p.Thr991Met			3^	novel variant	- Variant is observed at a moderately common frequency of 0.1% in gnomAD (Su) - Located in a region where multiple pathogenic variants were previously reported (S) - Multiple lines of computational evidence support a deleterious effect on the gene product (Su) - HGMD reports this as disorder causing mutation however ClinVar has conflicting interpretation of this variant (35712)
15632	AFR	*SMN1*	1 copy of SMN1	Spinal muscular atrophy	253300	N/A	1+0 genotype	- N/A
		SMN1	2 copies of SMN1				2+0 genotype	- genotype that cannot be detected with NGS

Table 2C: 1 high-risk couple with a previous family history provided with molecular diagnosis

Couple	Ethnicity	Gene	Variant	Disorder	MIM #	Class	Comments	- Evidence
16418	ME	*CEP290*	c.5649dupA (p.Leu1884Thrfs*23)	Joubert syndrome	610188	5#	published variant	- Null variant in a gene where LOF is a known mechanism of disorder (V.S) - Well established functional studies show deleterious effect (S) - Absent from population databases (M) - Previously seen in three cases in a compound heterozygous manner and all have been diagnosed with Joubert syndrome (M) - Multiple in-silico predicts pathogenicity (Su)

^ ACMG Class 3: Variant of unknown significance (VOUS); * ACMG Class 4: Likely pathogenic; # ACMG Class 5: Pathogenic; N/A: not applicable
V.S: Very strong evidence; S: Strong evidence; M: Moderate evidence; Su: Supporting evidence
NFE: Non-Finnish European descent; ME: Middle Eastern descent; SAS: South Asian descent; AFR: African descent

Table 2D: 7 novel high-risk couples identified in our study

Couple	Ethnicity	Gene	Variant	Disorder	MIM #	Class	Comments	ACMG Evidence
15533	NFE	***FMR1***	55 repeats	Fragile X	300624	5#	well published variant	premutation range
16243	NFE	***FMR1***	55 repeats	Fragile X	300624	5#	well published variant	premutation range
17215	NFE	***FMR1***	80 repeats	Fragile X	300624	5#	well published variant	premutation range
16851	NFE	*SMN1*	1 copy of SMN1 allele	Spinal muscular atrophy	253300	5#	well published variant	- Predicted null variant in a gene where LOF is a known mechanism of disorder (V.S) - Well published variant (S)
16421	NFE	***MTM1***	c.1238G>A (p.Ser413Asn)	X-linked myotubular myopathy	310400	4*	novel variant	- Variant is absent from population databases (M) - Novel missense change at a residue where a different pathogenic missense change has been seen before (M) - Variant is in a mutation hotspot (M) - Multiple in-silico predicts pathogenicity (Su) - Missense variant in a gene for which missense variants are known to cause disorder (Su)
16228	NFE	***RS1***	c.305G>A (p.Arg102Gln)	X-linked Juvenile retinoschisis	312700	5#	well published variant	- Increased segregation in at least 5 publications with multiple cases (S) - Observed at very low frequency in population databases (M) - Located in a mutational hot spot and critical and well-established functional domain (M) - Missense variant in a gene in which missense variants are a common mechanism of disorder (Su) - Multiple in-silico predicts pathogenicity (Su) - Reputable sources report variant as pathogenic (Su)
16174	NFE	***RS1***	c.307C>A (p.Leu103Ile)	X-linked Juvenile retinoschisis	312700	4*	novel variant	- Absent in population databases (M) - Located in a mutational hot spot and critical and well-established functional domain (M) - Novel missense change at a residue where a different pathogenic missense change has been seen before (M) - Missense variant in a gene in which missense variants are a common mechanism of disorder (Su) - Multiple in-silico predicts pathogenicity (Su)

* ACMG Class 4: Likely pathogenic; # ACMG Class 5: Pathogenic
V.S: Very strong evidence; S: Strong evidence; M: Moderate evidence; Su: Supporting evidence
NFE: Non-Finnish European descent
Genes in bold indicate genes on the X-chromosome

Frequency of common pathogenic variants

The number of Class4/Class5 variants in each participant varied from zero (n=138) to five (n=3) with the average being 1.2. Thus, 312 of the 450 participants (69.3%) were carriers for at least one Class4/Class5 variant in the 474 genes screened.

The most common single pathogenic variant in the cohort was deletion of exon 7 of *SMN1* which was observed in 13 individuals. This was followed by p.Phe508del in *CFTR* observed in eight individuals. The variants p.Glu292Val in *ABCA3* and c.-32-13T>G in *GAA* were identified in seven individuals (Table 3A).

One in 20 individuals were *CFTR* carriers, followed by one in 35 who carried the deletion of exon 7 in *SMN1* and one in 40 individuals were *GAA* carriers (Table 3B).

Novel variants identified

Of the seven novel high-risk couples identified, two couples had a novel X-linked pathogenic variant. A novel missense variant in *MTM1* (c.1238G>A p.Ser413Asn) was identified in Couple 16421. This novel missense variant was absent from population databases and was a residue where a different pathogenic missense change has been seen before. In addition, this locus had a high rate of pathogenic missense variants that was previously described in the literature and previously observed in PathWest data, indicating functional importance to the protein. These three pieces of evidence were counted as "Moderate" each. Furthermore, multiple *in-silico* algorithms predicted this variant to be pathogenic, and missense variants in this gene are known to cause the disorder. Both these latter pieces of evidence constituted "Supporting Evidence". According to ACMG, variants with two moderates and two supporting pieces of evidence are classified as "Likely Pathogenic" (Class4)(Table 2D).

Another novel missense variant was identified in the X-linked gene *RS1* (c.307C>A p.Leu103Ile) in Couple 16174. This novel missense variant was absent in population databases and was located in a hotspot where a number of pathogenic variants were previously described. In addition, this novel missense variant was at a codon where a different pathogenic missense change has been seen before and multiple *in-silico* algorithms predicted pathogenicity. Taken together, this variant was classified as a "Likely Pathogenic" (Class4) variant.

Molecular diagnosis of Family 16418 with a previous history of an undiagnosed disorder

Family 16418 had previously lost a child with multiple fetal abnormalities which were thought to result from a ciliopathy. This couple was recruited by GSWA for carrier-screening, to rule out other recessive disorders screened by the targeted gene panel. A variant in the gene *CEP290 (*c.5649dupA p.Leu1884Thrfs*23) was identified in both partners. This variant was absent in gnomAD and was in a gene where LOF is a known mechanism of the disorder. In addition, knockout functional studies in zebrafish had shown a phenotype consistent with the disorder[33]. Finally, this variant had been identified previously in three cases with Joubert syndrome 5 (MIM 610188)[34]. Accordingly, this variant was classified as a "Pathogenic" (Class5) variant (Table 2C).

Joubert syndrome is a recessive ciliopathy that presents with multiple co-morbidities such as psychomotor delay, ataxia and neonatal breathing abnormalities. Therefore, it was considered that this pathogenic variant is the molecular cause for the recessive disorder in this couple's deceased child.

Table 3A: Distribution of common pathogenic/likely pathogenic variants

Gene	Pathogenic variant	*n*=	ClinVar/HGMD	Study Frequency	gnomAD Frequency
SMN1	Exon 7 deletion	13	Pathogenic/DM	3.2%	-
CFTR	p.Phe508del	8	Pathogenic/DM	2.0%	0.8%
GAA	c.-32-13T>G	7	Pathogenic/DM	1.7%	0.3%
ABCA3	p.Glu292Val	7	Pathogenic/DM	1.7%	0.3%
PMM2	p.Arg141His	7	Pathogenic/DM	1.5%	0.4%

DM: Disease-causing Mutation

Table 3B: Carrier frequency of top 5 most common genes

Gene	Disorder	MIM #	# of individuals with pathogenic variants	Carrier Freq (1 in)	Published Carrier Freq (1 in)
CFTR	Cystic Fibrosis	602421	23	22.3	33
SMN1	Spinal muscular atrophy	253300	13	30.9	50^
GAA	Glycogen storage disease II	232300	11	36.5	71
ABCC6	Pseudoxanthoma elasticum	264800	10	45.0	80*
ATP7B	Wilson's disease	277900	10	45.0	240

Note: non-annotated carrier frequencies were obtained from: https://cdn1.sema4.com/wp-content/uploads/Sema4_Carrier-Screen-Residual-Risk_v1-Enhanced.pdf; ^ PMID: 21364876; * PMID: 28486967

Reliability of variants reported in variant databases

A total of three pathogenic variants (21.4%) identified in novel high-risk or positive control couples had discordant descriptions between ClinVar and HGMD Professional. Two pathogenic variants were not listed in ClinVar but listed in HGMD Professional, while the third variant was listed as associated with different recessive disorders in ClinVar and HGMD Professional.

The variant that was said to be associated with different disorders in the database was the homozygous *CEP290* variant identified in Couple 16418. The variant was listed in ClinVar as being associated with retinitis pigmentosa (ClinVar ID: 99860). Meanwhile HGMD Professional (HGMD Accession#: CI062251) had three cited publications[33,35,36] that demonstrated that this variant was in fact associated with Joubert syndrome (MIM 610188), a severe recessive infantile disorder (Table S4).

DISCUSSION

In this study we have successfully implemented a pilot carrier-screening program using existing components of the Western Australian public healthcare system, in both metropolitan Perth and the rural town of Busselton in the South-West of Western Australia. Collection of blood samples using the existing PathWest collection centres and courier infrastructure was shown to be effective.

Implementation of the pilot study indicated that there is an appetite for carrier-screening in Western Australia. We aimed initially to recruit 250 couples in a two-year period, but recruited 225 couples in 17 months, when we had to stop recruiting. The 225 couples were recruited from only seven sites in the whole state. More than 70% of blood samples were received by the laboratory within two weeks post recruitment. The low rate of withdrawal further suggested that there was high

interest in using the test and study couples were keen to participate and receive their results in a timely manner.

Fourteen of the study couples (6.2%) became pregnant after recruitment. Pregnant couples have limited time to consider their reproductive options and have fewer options if they are identified to be at high-risk of having an affected child. It becomes crucial to report screening results back to all pregnant couples as quickly as possible. Samples from couples who told the study team that they had become pregnant were given priority in the sequencing queue. An increase in the number of pregnant couples tested would apply additional strain to laboratory staff to report results back quickly. The potential for a higher number of pregnant couples being tested must be taken into consideration when offering a carrier-screening test as part of routine healthcare.

We found that four of the seven novel high-risk couples (57.1%) identified were at risk of having a child affected with Fragile X or spinal muscular atrophy, two recessive disorders commonly screened for in Australia[6]. The remaining three high-risk couples were at risk of having an affected child for very rare disorders such X-linked myotubular myopathy (OMIM 310400) which have reported prevalence of 1 in 50,000 newborn males respectively[37]. The identification of the molecular cause for Family 16418 who had a child affected by Joubert syndrome was fortuitous. Again, this finding highlights the potential for a carrier-screening program to identify carriers for very rare recessive disorders. These rare disorders identified in our study are not typically associated with a particular ethnic group.

Taken together, our findings support previous data that carrier-screening programs will be effective at identifying high-risk couples in recessive disorders that are not commonly screened for, when offered community wide[10,17,38].

We found that bioinformatic analysis of variants on a couple's basis for carrier-screening, in a diagnostic laboratory already using NGS, such as the PathWest laboratory, is relatively straightforward. It was possible to modify existing bioinformatic pipelines to analyse variants on a couples' basis with confidence. Identifying known or well-defined pathogenic variants is usually very straightforward, especially when they are loss of function variants. Classifying novel potential pathogenic missense variants in unaffected individuals on the other hand, has been[39] and will continue to be, more difficult because there is no phenotype. This lack of phenotype precludes the use of some evidence for variant classification such as segregation data (Table S5).

We find that almost a quarter of high-risk couples identified in our study had variants that were either not in ClinVar but in HGMD Professional, or listed as being associated with different disorders in the two variant databases. The availability of variant databases providing consistent and accurate phenotype-genotype information for rare and private variants is therefore pivotal for any future accurate implementation of carrier-screening. This is important since information provided from carrier-screening has life-changing ramifications for couples making important reproductive decisions, e.g., deciding whether to go through *in-vitro* fertilisation, based on the results they receive. The issues include variants that have been seen and classified as "pathogenic" in diagnostic laboratories but have not been uploaded to public variant databases such as ClinVar, due to lack of resources or

other reasons. To that end, the Australian national research project "Australian Genomics" has developed a variant sharing tool to automatically collate and exchange key information about clinically curated variants between diagnostic laboratories and clinical services[40].

Recent studies had estimated the proportion of high-risk couples in the general population to be between 0.5% to about 4.5%[4,5,41,42]. Almost all of these studies, have a sample size between 23,000 to 346,000 individuals and screened between 50 to 415 recessive disorders. The one[5] that did not have a large cohort, randomly calculated their carrier couple incidence by selecting data from 100 males and 100 females. Therefore, while our sample size is relatively small, the proportion of high-risk couples identified in our study is not unexpected but towards the higher end of previous estimations.

The considerable proportion of novel carrier couples identified in our study however, has implications for the resource requirements if carrier-screening is implemented into the Australian public health system. As discussed by others[1,20], screening couples and the disclosure of only couple-based results, streamlines the whole workflow and also decreases workload.

Our study has also identified key considerations that may affect a couple's experience when implementing a carrier-screening program through the health system. Although the study proposed a 4-8 weeks turnaround time, meeting this timeframe was an issue for two thirds of the couples (Table 1). The delays were caused by supply chain issues in part due to the remoteness of Western Australia. Another issue, at times, was waiting for samples to fill a run. However, this latter will likely not be an issue if carrier-screening is implemented on a larger scale.

In conclusion, we have shown one in 32 couples participating in our study were at high-risk of having a child with a severe recessive disorder. We have also shown that the provision of a carrier-screening program can work within an Australian public health system. In Australia, a $20m research project, Mackenzie's Mission, has now been established to investigate how carrier-screening could be made available free to any couple that wants to access carrier-screening across this vast country[43]. Mackenzie's Mission partly arose because of this pilot study, which therefore has been invaluable in paving the way for the much bigger, nationwide project. Starting to recruit couples for Mackenzie's Mission was the reason we had to stop recruiting couples for the pilot study. We could not recruit couples to the two projects simultaneously.

ACKNOWLEDGMENTS

We acknowledge Professor Martin Delatycki for sharing VCGS's expanded carrier-screening panel gene list during the development of the targeted panel used in this study. The study was supported by the Zac Pearson Legacy and a Perpetual Trustees Impact Philanthropy grant from the Helen Leech Endowment. NL is supported by NHMRC Fellowship APP1117510, GR by NHMRC Fellowship APP1122952. The funding agencies had no involvement in the design, completion or writing of this study.

PRESENTATIONS AND AWARDS:

An abstract for this study was chosen for an oral presentation and as being amongst the top 15 posters at the 2019 Human Genetics Society of Australasia conference in Wellington, New Zealand.

REFERENCES

1. Plantinga M, Birnie E, Schuurmans J, Buitenhuis AH, Boersma E, Lucassen AM, Verkerk MA, van Langen IM, Ranchor AV. Expanded carrier screening for autosomal recessive conditions in health care: Arguments for a couple-based approach and examination of couples' views. Prenat Diagn 2019;**39**(5):369-78 doi: 10.1002/pd.5437published Online First: 2019/02/14].
2. Nigel Laing GR, Royston Ong. Explainer: what is pre-pregnancy carrier screening and should potential parents consider it? 2017. https://theconversation.com/explainer-what-is-pre-pregnancy-carrier-screening-and-should-potential-parents-consider-it-79184.
3. Antonarakis SE. Carrier screening for recessive disorders. Nat Rev Genet 2019;**20**(9):549-61 doi: 10.1038/s41576-019-0134-2published Online First: 2019/05/31].
4. Guo MH, Gregg AR. Estimating yields of prenatal carrier screening and implications for design of expanded carrier screening panels. Genet Med 2019;**21**(9):1940-47 doi: 10.1038/s41436-019-0472-7published Online First: 2019/03/09].
5. Plantinga M, Birnie E, Abbott KM, Sinke RJ, Lucassen AM, Schuurmans J, Kaplan S, Verkerk MA, Ranchor AV, van Langen IM. Population-based preconception carrier screening: how potential users from the general population view a test for 50 serious diseases. Eur J Hum Genet 2016;**24**(10):1417-23 doi: 10.1038/ejhg.2016.43published Online First.
6. Archibald AD, Smith MJ, Burgess T, Scarff KL, Elliott J, Hunt CE, Barns-Jenkins C, Holt C, Sandoval K, Siva Kumar V, Ward L, Allen EC, Collis SV, Cowie S, Francis D, Delatycki MB, Yiu EM, Massie RJ, Pertile MD, du Sart D, Bruno D, Amor DJ. Reproductive genetic carrier screening for cystic fibrosis, fragile X syndrome, and spinal muscular atrophy in Australia: outcomes of 12,000 tests. 2017 Online First.
7. World Health Organisation. Births with Down's syndrome per 100 000 live births. 2017. Date Accessed 1/11. https://gateway.euro.who.int/en/indicators/hfa_603-7120-births-with-downs-syndrome-per-100-000-live-births/.
8. Kingsmore SF, Lantos JD, Dinwiddie DL, Miller NA, Soden SE, Farrow EG, Saunders CJ. Next-generation community genetics for low- and middle-income countries. Genome Med 2012;**4**(3):25 doi: 10.1186/gm324published Online First.
9. Molster C, Urwin D, Di Pietro L, Fookes M, Petrie D, van der Laan S, Dawkins H. Survey of healthcare experiences of Australian adults living with rare diseases. Orphanet J Rare Dis 2016;**11**:30 doi: 10.1186/s13023-016-0409-zpublished Online First.
10. Edwards JG, Feldman G, Goldberg J, Gregg AR, Norton ME, Rose NC, Schneider A, Stoll K, Wapner R, Watson MS. Expanded carrier screening in reproductive medicine-points to consider: a joint statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine. Obstet Gynecol 2015;**125**(3):653-62 doi: 10.1097/AOG.0000000000000666published Online First.
11. Franasiak JM, Olcha M, Bergh PA, Hong KH, Werner MD, Forman EJ, Zimmerman RS, Scott RT, Jr. Expanded carrier screening in an infertile population: how often is clinical decision making affected? Genet Med 2016;**18**(11):1097-101 doi: 10.1038/gim.2016.8published Online First: 2016/11/01].
12. Wienke S, Brown K, Farmer M, Strange C. Expanded carrier screening panels-does bigger mean better? J Community Genet 2014;**5**(2):191-8 doi: 10.1007/s12687-013-0169-6published Online First: 2013/09/26].
13. Kihlbom U. Ethical issues in preconception genetic carrier screening. Ups J Med Sci 2016:1-4 doi: 10.1080/03009734.2016.1189470published Online First.
14. Holtkamp KC, van Maarle MC, Schouten MJ, Dondorp WJ, Lakeman P, Henneman L. Do people from the Jewish community prefer ancestry-based or pan-ethnic expanded carrier

screening? Eur J Hum Genet 2016;**24**(2):171-7 doi: 10.1038/ejhg.2015.97published Online First: 2015/05/15].
15. Chokoshvili D, Vears DF, Borry P. Reproductive autonomy in expanded carrier screening: more than meets the eye? Expert Rev Mol Diagn 2018;**18**(12):993-94 doi: 10.1080/14737159.2018.1544496published Online First: 2018/11/06].
16. Rose NC. Expanded carrier screening: too much of a good thing? Prenat Diagn 2015;**35**(10):936-7 doi: 10.1002/pd.4638published Online First: 2015/08/15].
17. Nazareth SB, Lazarin GA, Goldberg JD. Changing trends in carrier screening for genetic disease in the United States. Prenat Diagn 2015;**35**(10):931-5 doi: 10.1002/pd.4647published Online First.
18. Matar A, Hansson MG, Hoglund AT. "A perfect society"- Swedish policymakers' ethical and social views on preconception expanded carrier screening. J Community Genet 2019;**10**(2):267-80 doi: 10.1007/s12687-018-0389-xpublished Online First: 2018/09/28].
19. SUPERIOR HEALTH COUNCIL BELGIUM. Expanded carrier screening in a reproductive context. Towards a responsible implementation in the healthcare system. In: Superior Health Council, ed. Belgium, 2017.
20. Schuurmans J, Birnie E, van den Heuvel LM, Plantinga M, Lucassen A, van der Kolk DM, Abbott KM, Ranchor AV, Diemers AD, van Langen IM. Feasibility of couple-based expanded carrier screening offered by general practitioners. Eur J Hum Genet 2019;**27**(5):691-700 doi: 10.1038/s41431-019-0351-3published Online First: 2019/02/12].
21. Sonic Genetics. Beacon Expanded Carrier Screen. Date Accessed 21/04/2019. https://www.sonicgenetics.com.au/rcs/beacon/.
22. Victorian Clinical Genetic Services. Prepair Genetic Carrier Screening. 2019. Date Accessed 22/04/2019. https://www.vcgs.org.au/tests/prepair.
23. Nigel Laing GR, Michelle Farrar, Royston Ong. What prospective parents need to know about gene tests such as 'prepair'. Analysis 2017. https://theconversation.com/what-prospective-parents-need-to-know-about-gene-tests-such-as-prepair-87083.
24. Lew RM, Proos AL, Burnett L, Delatycki M, Bankier A, Fietz MJ. Tay Sachs disease in Australia: reduced disease incidence despite stable carrier frequency in Australian Jews. Med J Aust 2012;**197**(11):652-4 doi: 10.5694/mja12.11010published Online First: 2012/12/13].
25. Walker CE MT, Davis G, Miller LJ, Girschik J, Brameld K, Sun W, Rath A, Aymé S, Zubrick SR, Baynam GS, Molster C, Dawkins HJ, Weeramanthri TS. The collective impact of rare diseases in Western Australia: an estimate using a population-based cohort. Genetic Medicine doi: doi: 10.1038/gim.2016.143.published Online First.
26. Bell CJ, Dinwiddie DL, Miller NA, Hateley SL, Ganusova EE, Mudge J, Langley RJ, Zhang L, Lee CC, Schilkey FD, Sheth V, Woodward JE, Peckham HE, Schroth GP, Kim RW, Kingsmore SF. Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med 2011;**3**(65):65ra4 doi: 10.1126/scitranslmed.3001756published Online First.
27. Punj S, Akkari Y, Huang J, Yang F, Creason A, Pak C, Potter A, Dorschner MO, Nickerson DA, Robertson PD, Jarvik GP, Amendola LM, Schleit J, Simpson DK, Rope AF, Reiss J, Kauffman T, Gilmore MJ, Himes P, Wilfond B, Goddard KAB, Richards CS. Preconception Carrier Screening by Genome Sequencing: Results from the Clinical Laboratory. Am J Hum Genet 2018;**102**(6):1078-89 doi: 10.1016/j.ajhg.2018.04.004published Online First: 2018/05/15].
28. Ceyhan-Birsoy O, Murry JB, Machini K, Lebo MS, Yu TW, Fayer S, Genetti CA, Schwartz TS, Agrawal PB, Parad RB, Holm IA, McGuire AL, Green RC, Rehm HL, Beggs AH, BabySeq Project T. Interpretation of Genomic Sequencing Results in Healthy and Ill Newborns: Results from the BabySeq Project. Am J Hum Genet 2019;**104**(1):76-93 doi: 10.1016/j.ajhg.2018.11.016published Online First: 2019/01/05].

29. Ong R, Edwards S, Howting D, Kamien B, Harrop K, Ravenscroft G, Davis M, Fietz M, Pachter N, Beilby J, Laing N. Study protocol of a multicentre cohort pilot study implementing an expanded preconception carrier-screening programme in metropolitan and regional Western Australia. BMJ Open 2019;**9**(6):e028209 doi: 10.1136/bmjopen-2018-028209published Online First: 2019/06/19].
30. Feng Y, Ge X, Meng L, Scull J, Li J, Tian X, Zhang T, Jin W, Cheng H, Wang X, Tokita M, Liu P, Mei H, Wang Y, Li F, Schmitt ES, Zhang WV, Muzny D, Wen S, Chen Z, Yang Y, Beaudet AL, Liu X, Eng CM, Xia F, Wong LJ, Zhang J. The next generation of population-based spinal muscular atrophy carrier screening: comprehensive pan-ethnic SMN1 copy-number and sequence variant analysis by massively parallel sequencing. Genet Med 2017;**19**(8):936-44 doi: 10.1038/gim.2016.215published Online First.
31. Biancalana V, Glaeser D, McQuaid S, Steinbach P. EMQN best practice guidelines for the molecular genetic testing and reporting of fragile X syndrome and other fragile X-associated disorders. Eur J Hum Genet 2015;**23**(4):417-25 doi: 10.1038/ejhg.2014.185published Online First: 2014/09/18].
32. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;**17**(5):405-24 doi: 10.1038/gim.2015.30published Online First.
33. Sayer JA, Otto EA, O'Toole JF, Nurnberg G, Kennedy MA, Becker C, Hennies HC, Helou J, Attanasio M, Fausett BV, Utsch B, Khanna H, Liu Y, Drummond I, Kawakami I, Kusakabe T, Tsuda M, Ma L, Lee H, Larson RG, Allen SJ, Wilkinson CJ, Nigg EA, Shou C, Lillo C, Williams DS, Hoppe B, Kemper MJ, Neuhaus T, Parisi MA, Glass IA, Petry M, Kispert A, Gloy J, Ganner A, Walz G, Zhu X, Goldman D, Nurnberg P, Swaroop A, Leroux MR, Hildebrandt F. The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. Nat Genet 2006;**38**(6):674-81 doi: 10.1038/ng1786published Online First: 2006/05/10].
34. Chaki M, Hoefele J, Allen SJ, Ramaswami G, Janssen S, Bergmann C, Heckenlively JR, Otto EA, Hildebrandt F. Genotype-phenotype correlation in 440 patients with NPHP-related ciliopathies. Kidney Int 2011;**80**(11):1239-45 doi: 10.1038/ki.2011.284published Online First: 2011/08/26].
35. Tory K, Lacoste T, Burglen L, Moriniere V, Boddaert N, Macher MA, Llanas B, Nivet H, Bensman A, Niaudet P, Antignac C, Salomon R, Saunier S. High NPHP1 and NPHP6 mutation rate in patients with Joubert syndrome and nephronophthisis: potential epistatic effect of NPHP6 and AHI1 mutations in patients with NPHP1 mutations. J Am Soc Nephrol 2007;**18**(5):1566-75 doi: 10.1681/ASN.2006101164published Online First: 2007/04/06].
36. Baala L, Audollent S, Martinovic J, Ozilou C, Babron MC, Sivanandamoorthy S, Saunier S, Salomon R, Gonzales M, Rattenberry E, Esculpavit C, Toutain A, Moraine C, Parent P, Marcorelles P, Dauge MC, Roume J, Le Merrer M, Meiner V, Meir K, Menez F, Beaufrere AM, Francannet C, Tantau J, Sinico M, Dumez Y, MacDonald F, Munnich A, Lyonnet S, Gubler MC, Genin E, Johnson CA, Vekemans M, Encha-Razavi F, Attie-Bitach T. Pleiotropic effects of CEP290 (NPHP6) mutations extend to Meckel syndrome. Am J Hum Genet 2007;**81**(1):170-9 doi: 10.1086/519494published Online First: 2007/06/15].
37. US National Library of Medicine. X-linked myotubular myopathy. 2019. Date Accessed https://ghr.nlm.nih.gov/condition/x-linked-myotubular-myopathy#statistics.
38. Langlois S, Benn P, Wilkins-Haug L. Current controversies in prenatal diagnosis 4: pre-conception expanded carrier screening should replace all current prenatal screening for

specific single gene disorders. Prenat Diagn 2015;**35**(1):23-8 doi: 10.1002/pd.4532published Online First.
39. Scheps KG, Hasenahuer MA, Parisi G, Targovnik HM, Fornasari MS. Curating the gnomAD database: Report of novel variants in the globin-coding genes and bioinformatics analysis. Hum Mutat 2020;**41**(1):81-102 doi: 10.1002/humu.23925published Online First: 2019/09/26].
40. Australian Genomics Health Alliance. SHARIANT. 2020. Date Accessed 15th January. https://www.australiangenomics.org.au/resources/tools/shariant/.
41. Rowe CA, Wright CF. Expanded universal carrier screening and its implementation within a publicly funded healthcare service. J Commun Genet 2020;**11**(1):21-38 doi: 10.1007/s12687-019-00443-6published Online First.
42. Ropers HH. On the future of genetic risk assessment. J Community Genet 2012;**3**(3):229-36 doi: 10.1007/s12687-012-0092-2published Online First: 2012/04/03].
43. Delatycki MB, Laing NG, Moore SJ, Emery J, Archibald AD, Massie J, Kirk EP. Preconception and antenatal carrier screening for genetic conditions: The critical role of general practitioners. Aust J Gen Pract 2019;**48**(3):106-10 doi: 10.31128/AJGP-10-18-4725published Online First: 2019/07/01].

CHAPTER 5

Final discussion and future directions

FINAL DISCUSSION AND FUTURE DIRECTIONS

There are currently 2,248 known recessive human disorders (Chapter 3). Many couples with children affected by recessive disorders do not have any family history of the disorder[1]. These couples therefore did not know they had a high risk of having an affected child. Carrier-screening is effective at informing individuals with no previous family history of their carrier status[2,3]. Thus, carrier-screening affords many couples who are at high risk of having a child affected by a recessive disorder a chance to consider their reproductive options. However, carrier-screening using traditional methods of analysis is expensive to deploy on a population level and can only screen for a handful of genes at a time.

At the turn of the decade, technological advancement in multiple parallel sequencing methods made screening hundreds of genes simultaneously affordable. The seminal study by Bell *et al.* in 2011[4] and later that by Haque *et al.*[5] demonstrated that this new technology could detect carrier status for more individuals affordably, and more disorders than screening then in place.

In 2017 The American College of Obstetricians & Gynaecologists recommended that all patients should be offered preconception carrier-screening, according to their values, including expanded carrier-screening for multiple genetic disorders[6].

Interest in offering carrier-screening as part of healthcare increased in Europe, specifically in Belgium, Sweden and The Netherlands[7-9]. However, Israel stands out globally, in having the most comprehensive publicly funded carrier-screening program. This program screens more than 50 genes and has provided more than 900,000 carrier-screening tests to Israel's population of 9 million people between 2015 and 2017[10].

The questions at the start of my PhD were (1) should Western Australia consider offering a population-wide carrier-screening test and (2) how should Western Australia go about offering such a test?

Australian couples who wish to access a carrier-screening test can do so through user-paid services. However, there is no publicly funded carrier-screening testing in any of the Australian health systems for families without a family history. This then begs the questions whether (1) there is interest in and (2) whether it is feasible to implement, a carrier-screening program embedded in an Australian healthcare system.

My PhD project was therefore to determine the interest in, feasibility and requirements of implementing a carrier-screening test in the Western Australian health system.

To reflect on the findings of my PhD project, it is useful to return to the objectives of my project and address them individually:

1. **To understand the preferences and attitudes of the Western Australian community and health professionals about carrier-screening in Western Australia**

   The two projects conducted in Chapter 2 were important to inform the overall study about key implementation considerations and potential barriers to offering or using a carrier-screening test in Western Australia.

   Key implementation considerations

   My studies have shown that there is significant interest within both the community and health professionals in Western Australia about using carrier-

screening if it was available. More than two-thirds of the WA community and health professionals expressed interest in using the test. In fact, this level of interest is amongst the highest in recent publications detailed in the systematic review by Van Steijvoort *et al.*[11].

Further, most of the community and health professionals preferred screening for recessive disorders that affected lifespan of children or infants. The interest to screen for severe life-limiting disorders is consistent with current literature and screening recommendations[12-14]. Matching the study by Lazarin *et al.*[13], almost all the health professionals preferred to screen for recessive disorders that severely impact quality of life.

The predominant choice to access the test for both the community and health professionals was through General Practitioners (GP). This is not surprising given that most couples visit their GP more frequently than any other medical specialists. However, twice as many health professionals compared to the community wanted additional access through other health services such as gynaecological/obstetric clinics and genetic counselling clinics. This suggests that health professionals were more aware of the complexities of carrier-screening and that accessing the test through a specialist may more adequately address any potential issues (Chapter 2, Section 2).

A similar observation was made in sub-populations of the community with a higher genetic knowledge. These sub-groups also expressed similar concerns to health professionals about discrimination, confidentiality and privacy (Chapter 2, Section 1).

Potential barriers

Tailored approaches may be required for different sub-populations in Western Australia to address specific concerns that may otherwise pose potential barriers to uptake of carrier-screening[15]. For example, in populations where genetic knowledge is considered "Low" to "Good", the focus might have to be on educating members of the community about the principles of carrier-screening. My study of the community demonstrated that a substantial proportion answered incorrectly questions that tested their understanding of carrier-screening. As a result, poor genetic literacy may affect decision-making processes around carrier-screening.

On the other hand, in sub-populations where there is "High" genetic knowledge, the emphasis should perhaps be on reassuring them about the different policies in place in Australia, such as the Moratorium on Genetic Tests in Life Insurance[16] introduced by the Financial Services Council (FSC). The Moratorium is to ensure the community can access a level of life insurance without being asked about the result of a previously taken genetic test[16]. The FSC is the leading peak body which sets mandatory standards and develops policy for member companies in Australia's financial services sector. Open dialogues about the Moratorium and other key issues will help address, reduce and reassure this particular sub-population about issues regarding discrimination, confidentiality and privacy.

My studies and others have shown that a lower level of education is associated with lower genetic knowledge and reduced comprehension of the test[17]. The level of genetic comprehension within the community is

termed genetic literacy. However, genetic literacy cannot be transformed overnight. Increasing genetic literacy will require a consistent and holistic approach focussed on engaging the community as a whole including Australians who speak English as their second language. It is particularly important to cater to non-native speakers or indigenous communities as they are less likely to access healthcare services which leads to poorer health outcomes[18]. As an example, the apparent lack of information on Covid-19 in enough languages other than English during the current pandemic raised concerns about exposing these migrant communities to the deadly virus with misinformed practices[19].

Another potential barrier identified in this study is the need to upskill current health professionals, especially GPs and specialist genetic service providers. Many GPs are currently unfamiliar with current carrier-screening technologies, and the tests they provide or their limitations. It is therefore important that provisions are made for GPs to be comfortable offering these tests. Such provisions can exist in the form of continuous education program (CEP) points[20] or website where they can easily and readily access the necessary information[21]. Additionally, these information pathways also provide an avenue to increase awareness about more common rare disorders amongst our population and what to look out for. For example, The Royal Australian College of General Practice (RACGP), supported by the Federal Health Department, launched the Beware the Rare education campaign in 2020[20]. It offers both CEP points and a source of information about rare disorder GPs can readily access.

In addition, access to medical scientists to clarify diagnostic laboratory results and support from genetic counsellors are particularly important for any health professionals offering the test. This year, 2020, The Royal Australian College of General Practitioners launched a new education campaign for GPs to increase awareness of rare recessive genetic disorders and promote genetic carrier-screening[21].

Continuous data collection on the types of questions raised during education sessions in different settings and sections of the community will identify concerns and even deployment issues, early on in the program. For example, my study has collected pre- and post-education survey and interview data from healthcare professionals. Additionally, pre-test and post-test surveys as well as interview data has also been collected from participating couples. The original aim 5 of my PhD was "To evaluate the effectiveness of the tools developed for health professionals during the study.". I was unable in the time available to evaluate the tools that were developed further than demonstrating that their use in the study was effective in that the whole protocol worked. In depth analysis of the data collected will be completed through the PhD studies of the study genetic counsellor, Ms. Samantha Edwards. Findings from her PhD should help provide opportunities to develop viable long-term solutions for any perceived issues.

2. Determine and develop the panel of genes to screen.

The criteria used for designing the panel of genes to screen was based on two carrier-screening workshops conducted in 2015[22] and 2016. Outcomes from the workshops were considered together with the then current screening

recommendations in published literature and the survey results in Chapter 2. Amalgamating this information, I decided to focus on recessive disorders affecting infant and childhood lifespan.

I then categorised the entire recessive disorder list of more than 2,248 entries in the Online Mendelian in Man database. The final gene panel consisted of 474 genes associated with mainly childhood and infant lethal recessive disorders.

Genomic coordinates of the start and end positions of each exon of the 474 genes included in the carrier-screening panel were generated using UCSC's table browser function. Gene regions with lower capture efficiency in other testing were then identified. Additional probes were added to these low-capture regions to improve coverage across these regions. The gene list had a total capture area or "footprint" of the panel of 1.23Mbp. Spinal muscular atrophy (*SMN1*) and Fragile X testing were performed as per standard diagnostic practice in PathWest Diagnostic Genomics using qPCR and repeat-primed PCR respectively.

3. **Developing an end-to-end protocol for a carrier-screening program leveraging on components of the WA health system.**

Mirroring the expectations of the community identified in my survey in Chapter 2, I developed and published a detailed study protocol[23] that leveraged existing research and health infrastructures. This study protocol includes all resources the study team designed for implementation of the carrier-screening pilot project, including education resources for health professionals and interested couples, patient information and consent forms.

Recruiting sites and recruiters

The study team invited the Genetic Services of WA (GSWA), the Busselton Population Medical Research Institute, GP obstetricians in Busselton and a GP practice in metropolitan Perth, to participate in the study. In addition, the study team also invited a private genetic counselling clinic to recruit couples who wished to access a carrier-screening test and did not have a family history of a genetic disorder. Participation of these sites allowed the study to better understand implementation requirements in different regions of WA and of different settings, e.g. genetic counsellor vs. GP.

From these sites, GPs, clinical geneticists, and genetic counsellors recruited couples into the study. The protocol included details of plans to evaluate recruiters' and couples' experiences after the pilot study completed. This participation will shed light on specific challenges faced by each profession during different time points in the study. In addition, long term follow-up studies with couples will determine the clinical utility of carrier-screening including couple's experiences around taking the screening test and having results returned. These future studies will also allow the study to evaluate tools developed as part of this PhD project.

Sample collection, sequencing and analytical methods

I proposed using the sample collection and sequencing methodology employed by the PathWest Division of Diagnostic Genomics. Blood samples would be collected at PathWest collection centres. DNA would be extracted from blood using the PathWest standard DNA extraction protocol. Sequencing would be performed with Illumina Next Generation Sequencing

(NGS) Platforms in The PathWest Neurogenetic Unit. The PathWest Neurogenetic Unit was an early adopter of NGS in the diagnostic setting, beginning such testing in 2013 and now uses the Illumina NGS Platform exclusively[24]. The years of experience of the PathWest Neurogenetic Unit in designing, running and reporting data from targeted panels was invaluable in informing and facilitating the implementation of the study.

Current PathWest bioinformatic pipelines were modified so that variants in the same genes in each couple was presented in the final variant list for curation. The American College of Medical Genetics (ACMG) guidelines were used to curate variants present in the same genes in each couple. Only pathogenic and likely pathogenic variants were reported as "High-risk". Variants of unknown significance and benign variants were reported as "Low-risk". "High-risk" couples were provided post-test counselling through Genetic Services Western Australia, following standard procedures for families with a family history of a genetic disorder.

Evaluating the sample collection and sequencing workflow shed light on the challenges faced by laboratories in providing a short turnaround time (TAT) for reports for couples. A short TAT is especially important to couples who became pregnant after providing their blood samples or are pregnant at the time of recruitment. A short TAT provides couples with additional time they may need to make informed reproductive decisions.

4. Performing a pilot study of carrier-screening.

There were two phases to performing the pilot study. The first phase consisted of optimising the NGS sequencing protocol and optimising the bioinformatics

pipeline. This included sequencing and analysis of positive controls. The second phase was to run the pilot study including recruiting of potential couples, sequencing and analysis of samples, through to providing laboratory reports to couples who participated and counselling the couples about their results.

Optimisation phase

A standard PathWest protocol was first used to determine the baseline values of the carrier-screening gene panel. The PathWest typical acceptable quality metrics of a run are having an average read depth of more than 100-fold, having a uniformity of more than 98% and having an average 20-fold (20x) coverage at more than 99% of the targeted regions. Probe volumes were adjusted until optimal efficiency was achieved.

The final probe volume used, 6.6µl was a variation on the manufacturer's instructions for the volume of probes to use. The average read depth using this probe volume was 165-fold coverage, with a uniformity of 97% and having a 20-fold coverage of 99%. The parameters allowed me to maximise the amount of data received. The parameters also allowed me to have sufficient coverage and quality of data to be confident that the genes of interest were sequenced reliably.

Pilot study results

Key Findings

I have shown that provision of a carrier-screening program can work within an Australian public health system. The pilot study commenced in August 2018 and concluded in March 2020. It recruited a total of 231couples across two

WA regions and seven sites. Of the 231 couples who were recruited, only six withdrew from the study. This high participation rate is contrary to previous studies in a recent literature review[11]. This possibly reflects the level of interest in carrier-screening in Western Australia highlighted in the surveys in Chapter 2.

A total of seven novel high-risk couples were identified indicating an incidence of 1 in 32 high-risk couples (3.1%) in the cohort. As discussed in Chapter 4, the proportion of high-risk couples identified in our study is not unexpected, but towards the higher end of what recent studies had shown[25-28].

Of the seven novel high-risk couples identified, four were at risk of having an affected child with two recessive disorders commonly screened for in commercial testing in Australia – Fragile X and spinal muscular atrophy[29]. The remaining three high-risk couples were at risk of having an affected child for very rare disorders such X-linked myotubular myopathy with a reported prevalence of 1 in 50,000 newborn males[30]. Given the outbred population in Australia, this incidence of high-risk couples is likely to be similar in other parts of the world and so has implications for carrier-screening world-wide.

Our findings support previous data that expanded carrier-screening programs will be more effective at identifying high-risk couples for recessive disorders than commonly offered screening programs[5].

The considerable proportion of novel carrier couples identified in our study has implications for resource planning if carrier-screening is implemented into the Australian public health system. However, as discussed by others[31,32],

the disclosure of only couple-based results we have used, streamlines the whole workflow and decreases workload.

Logistics

Collection of blood samples using the existing PathWest collection centres and courier infrastructure was shown to be effective. About 75% of the study samples were received at the PathWest laboratory within two weeks after recruitment. Turnaround time (TAT) for 30% of the cohort was within the expected 8-week period. Fourteen couples (6.2%) became pregnant after providing their samples. As discussed earlier, TAT is especially crucial for pregnant couples in order for them to make timely informed reproductive decisions. In our study, the main issues with TAT appear to be reagent and supply chain issues. This may be unique to Australia's geographical isolation, especially Western Australia's geographic isolation, and may not necessarily apply to other countries closer to suppliers. Another issue pertaining to TAT was at times waiting for samples to fill a sequencing run. This will likely not be an issue if carrier-screening is implemented on a larger scale as a steady flow of samples will be likely.

Sequencing and analytical methods

The modified bioinformatics pipeline worked well to extract variants in the same gene in each couple for analysis. I was able to identify seven novel high-risk couples and eight positive controls in my study.

In more than 80% of couples, both partners in a couple had rare coding variants in the same gene, careful curation of these genes and variants were required to assign pathogenicity. Whilst it took on average 15 minutes to analyse the data for each couple in my study, screening for a larger number of recessive disorders will undoubtedly increase analysis time. Rowe *et al.* have shown that there is a positive correlation between the number of recessive disorders screened for and the number of couples who are carriers of at least one recessive disorder[33]. Invariably this will put a strain on diagnostic laboratory workforce. This suggests considerable implications for workforce management and scalability for health systems wanting to introduce a carrier-screening program. However, Kaseniit *et al.* [34] showed that it is possible to perform variant interpretation at scale if a set of common pathogenic variants in the gene panels were pre-interpreted. These authors also suggested that laboratories can better predict labour requirements as more testing is done.

The insufficiency of current ACMG guidelines and the issue of inconsistency in variant databases described in Chapter 4 present ongoing issues for any carrier-screening program[34,35]. This may even develop into a potential barrier to introducing carrier-screening in health systems. However, a modified set of ACMG guidelines for carrier-screening tests and automated algorithms

to capture previously classified "pathogenic" variants in diagnostic laboratories should improve false negative rates[36]. Having clear guidelines recommended by an international collaborative consortium, tailored for carrier-screening could standardise the interpretive methodology to facilitate increased adoption of carrier-screening into health systems.

Further considerations

The clinical utility of carrier-screening has been called into question previously[37,38] however Johansen Taber *et al.*[39] showed recently that 60% of high-risk couples took some form of action upon learning of their high-risk status. These authors also demonstrated that 86% of high-risk couples who took action were carriers for either a profound or severe recessive disorder.

This finding was recapitulated in a systematic review by Cannon *et al*.[40] in which they highlighted that most high-risk couples chose to prevent the birth of an affected child. Pregnant high-risk couples would often choose to pursue prenatal diagnosis, followed by elective termination of the affected pregnancy.

Indeed, during the course of this study, one identified high-risk couple received approval by the West Australian Reproductive Technology Council (RTC) for a fertility clinic to proceed with pre-implantation genetic diagnosis. The Reproductive Technology Council in Western Australia ensures that testing of embryos is only carried out when there is a high risk of a serious genetic disorder. More importantly, the Reproductive Technology Council also ensures that patients have received appropriate genetic counselling[41]. In addition, another couple recently had prenatal diagnosis, while a low-risk

couple decided that they would pause trying to have children temporarily while they resolve other health issues. These findings imply that both high-risk and low-risk couples are using their results to make reproductive decisions. There are plans for long term studies to understand the clinical utility and impact of the outcomes of the study to the participants' reproductive decision-making processes through the PhD of the study genetic counsellor, Ms. Samantha Edwards.

Future Directions

Molster *et al.* have previously suggested that all requirements for an "end-to-end" service should be available prior to implementing any carrier-screening programs[22]. Provision of such components include information and education packs for the public and/or target population. In addition, Molster *et al.* and others have emphasized the importance of upskilling health professionals and clear pathways and support for couples identified as high-risk [11,22,27,42]. These pathways would, for example, ideally include publicly-funded IVF-PGD cycles and prenatal screening support for high-risk couples which is available for example in Israel[43] but not yet in Australia. Ultimately, an "end-to-end" service should aim to support any couple's reproductive decision including support for affected children through mediating and managing severity with lifestyle changes.

To that end, the efficacy of the tools developed for the study, such as the education materials, will also be investigated by the study genetic counsellor, Ms. Samantha Edwards during her PhD. Her PhD will also evaluate the experiences and challenges faced by the recruiting healthcare professionals as well as personnel requirements. The findings from these studies will provide a more complete picture of the requirements for implementing carrier-screening in relation to the West Australian health system infrastructure.

Mackenzie's' Mission

During my PhD studies, an Australian national reproductive carrier-screening project called Mackenzie's Mission was launched.

Mackenzie's Mission is a three-year pilot study funded by the Australian Federal health department Medical Research Future Fund to screen 10,000 couples from

across all Australian States and territories for more than 750 recessive disorders. Mackenzie's Mission is scheduled to run between 2019 and 2021[44]. This comprehensive nationwide study aims to evaluate the resources required to make reproductive carrier-screening work nationally in the public health systems in all States and Territories. It will evaluate methods of delivering carrier-screening at scale for Australia's population of ~25 million people. It will also evaluate different sequencing, data analysis and reporting methods. It aims to also explore the ethical issues faced by couples, the psychosocial impact on couples, the clinical utility of the screening; and the full health economic impact of carrier-screening[45]. The aim of Mackenzie's Mission is to investigate how to make carrier-screening freely available for every couple in Australia who wish to use carrier-screening.

The tools developed during my study, its findings, as well as the lessons learnt, have been instrumental in paving the way for Mackenzie's Mission.

Towards the end of my PhD, I was invited, because of my experience in developing the gene panel for our WA PCS study, to participate in developing the gene panel for Mackenzie's Mission. This resulted in the second author publication Kirk *et al.* 2020 describing how the 1300 genes to be screened in Mackenzie's Mission were chosen[46].

As Mackenzie's Mission winds down towards the end of 2021, considerations will need to be given as to how reproductive carrier-screening can be funded in Australia long term to ensure equity of access. Should reproductive carrier-screening be funded through a program similar to the Australian Newborn Screening (NBS) program[47], which is funded by the State Health Departments and therefore available at no out of pocket expense? Or should funds be provided centrally

through the Federal Health Department rebate system[48] by which private and public pathology requests can be reimbursed?

The critical outcome of Mackenzie's Mission is equity of access to carrier-screening for all Australians. It was shown that disabilities and poverty were intricately linked[49]. Banks *et al.* identified in their systematic review that more than 80% of the literature they reviewed found a positive association between disability and a poverty marker, especially for families with someone with a disability[49]. For Australia, it was recently estimated around 1 in 6 people with disability also live in poverty, compared to 1 in 10 Australians without disability[50]. Ultimately, the need to provide a comprehensive and equitable reproductive healthcare system may include adoption of popular reproductive tests. This could include increasing the number of disorders included in newborn screening programs and providing a subsidy for non-invasive prenatal testing[51] and all other first trimester testing[52]. In October 2020, fragile X syndrome (FXS), spinal muscular atrophy (SMA) and cystic fibrosis (CF) was recommended to be listed on the Federally funded Medicare Benefits Schedule (MBS)[53]. This recommendation takes the goal of having a Federally funded carrier-screening test one step closer to reality.

Mackenzie's Mission came about as a result of effective lobbying by patient groups, families and healthcare professionals. We are in an unprecedented period where patient groups have significant influence over health practice, policy and outcomes. As an example, the recently accelerated approval granted by the Food and Drug Administration's (FDA) Advisory Committee to Exondys 51 for treatment of Duchenne muscular dystrophy (MIM# 310200) was due to an "intense and near-

incessant pressure from a large public audience" during the committee meeting which was packed with patients and advocates[54].

When engaged respectfully, a study like Mackenzie's Mission offers various key stakeholders a platform to provide their input – an opportunity that is hard to come by. It allows the wider community to have a voice for their preferences and what options they want provided to them.

As Wapner *et al*.[42] mentioned in their commentary,

> **"In this modern era of medicine, as we strive to be more patient-centric, instead of asking medical professionals and professional societies to define what is an appropriate carrier-screening panel, should we be asking our patients about their values and priorities with regard to genetic screening?"**

As a concluding remark, I would like to comment that pilot studies have a very important role to play. Findings from other studies in different health systems in other countries may provide information about healthcare programs but ultimately, it is through local pilot studies that both a system's unique strengths can be maximised and its flaws identified and mitigated.

My PhD study has achieved its overall aim of showing it was feasible to successfully implement an end-to-end carrier-screening program that can integrate into the Western Australian health system. The study team has provided results to all couples and had the pleasure of receiving news that some high-risk couples have made reproductive decisions based on our findings. This was a pleasure because this confirmed that the proposed protocol worked in practice from end to end. Couples were able to use the pilot study to make informed reproductive choices, and thus

achieve reproductive autonomy, that fitted with their norms and values. This clearly demonstrated the utility of the program; that the program worked, was effective and turned out to be feasible.

More research studies have been planned. The study team and I will continue contributing to this growing field in the years to come. We are also looking forward to Australia being a leader in the population-wide carrier-screening space, reducing genetic roulette[55] and the burden of severe recessive disorders on families.

References

1. Henneman L, Borry P, Chokoshvili D, Cornel MC, van El CG, Forzano F, Hall A, Howard HC, Janssens S, Kayserili H, Lakeman P, Lucassen A, Metcalfe SA, Vidmar L, de Wert G, Dondorp WJ, Peterlin B. Responsible implementation of expanded carrier screening. Eur J Hum Genet 2016;**24**(6):e1-e12 doi: 10.1038/ejhg.2015.271published
2. Archibald AD, Smith MJ, Burgess T, Scarff KL, Elliott J, Hunt CE, Barns-Jenkins C, Holt C, Sandoval K, Siva Kumar V, Ward L, Allen EC, Collis SV, Cowie S, Francis D, Delatycki MB, Yiu EM, Massie RJ, Pertile MD, du Sart D, Bruno D, Amor DJ. Reproductive genetic carrier screening for cystic fibrosis, fragile X syndrome, and spinal muscular atrophy in Australia: outcomes of 12,000 tests. 2017
3. Kaback M, Lim-Steele J, Dabholkar D, Brown D, Levy N, Zeiger K. Tay-Sachs disease--carrier screening, prenatal diagnosis, and the molecular era. An international perspective, 1970 to 1993. The International TSD Data Collection Network. JAMA 1993;**270**(19):2307-15
4. Bell CJ, Dinwiddie DL, Miller NA, Hateley SL, Ganusova EE, Mudge J, Langley RJ, Zhang L, Lee CC, Schilkey FD, Sheth V, Woodward JE, Peckham HE, Schroth GP, Kim RW, Kingsmore SF. Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med 2011;**3**(65):65ra4 doi: 10.1126/scitranslmed.3001756published
5. Haque IS, Lazarin GA, Kang HP, Evans EA, Goldberg JD, Wapner RJ. Modeled Fetal Risk of Genetic Diseases Identified by Expanded Carrier Screening. JAMA 2016;**316**(7):734-42 doi: 10.1001/jama.2016.11139published
6. American College of Obstetricians and Gynecologists. Committee Opinion No. 690: Carrier Screening in the Age of Genomic Medicine. Obstet Gynecol 2017;**129**(3):e35-e40 doi: 10.1097/AOG.0000000000001951published
7. SUPERIOR HEALTH COUNCIL BELGIUM. Expanded carrier screening in a reproductive context. Towards a responsible implementation in the healthcare system. In: Superior Health Council, ed. Belgium, 2017.
8. Matar A, Hansson MG, Hoglund AT. "A perfect society"- Swedish policymakers' ethical and social views on preconception expanded carrier screening. J Community Genet 2019;**10**(2):267-80 doi: 10.1007/s12687-018-0389-xpublished
9. Schuurmans J, Birnie E, Ranchor AV, Abbott KM, Fenwick A, Lucassen A, Berger MY, Verkerk M, van Langen IM, Plantinga M. GP-provided couple-based expanded preconception carrier screening in the Dutch general population: who accepts the test-offer and why? Eur J Hum Genet 2019 doi: 10.1038/s41431-019-0516-0published
10. Singer A, Sagi-Dain L. Impact of a national genetic carrier-screening program for reproductive purposes. Acta Obstet Gynecol Scand 2020 doi: 10.1111/aogs.13858published
11. Van Steijvoort E, Chokoshvili D, J WC, Peeters H, Peeraer K, Matthijs G, Borry P. Interest in expanded carrier screening among individuals and couples in the general population: systematic review of the literature. Hum Reprod Update 2020;**26**(3):335-55 doi: 10.1093/humupd/dmaa001published
12. Edwards JG, Feldman G, Goldberg J, Gregg AR, Norton ME, Rose NC, Schneider A, Stoll K, Wapner R, Watson MS. Expanded carrier screening in reproductive medicine-points to consider: a joint statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine. Obstet Gynecol 2015;**125**(3):653-62 doi: 10.1097/AOG.0000000000000666published

13. Lazarin GA, Detweiler S, Nazareth SB, Ashkinadze E. Genetic Counselors' Perspectives and Practices Regarding Expanded Carrier Screening after Initial Clinical Availability. J Genet Couns 2016;**25**(2):395-404 doi: 10.1007/s10897-015-9881-1published
14. Korngiebel DM, McMullen CK, Amendola LM, Berg JS, Davis JV, Gilmore MJ, Harding CO, Himes P, Jarvik GP, Kauffman TL, Kennedy KA, Simpson DK, Leo MC, Lynch FL, Quigley DI, Reiss JA, Richards CS, Rope AF, Schneider JL, Goddard KA, Wilfond BS. Generating a taxonomy for genetic conditions relevant to reproductive planning. Am J Med Genet A 2016;**170**(3):565-73 doi: 10.1002/ajmg.a.37513published
15. Kraft SA, Duenas D, Wilfond BS, Goddard KAB. The evolving landscape of expanded carrier screening: challenges and opportunities. Genet Med 2019;**21**(4):790-97 doi: 10.1038/s41436-018-0273-4published
16. Financial Services Council. FSC Standard No. 11: Moratorium on Genetic Tests in Life Insurance, 2019.
17. Gilmore MJ, Schneider J, Davis JV, Kauffman TL, Leo MC, Bergen K, Reiss JA, Himes P, Morris E, Young C, McMullen C, Wilfond BS, Goddard KAB. Reasons for Declining Preconception Expanded Carrier Screening Using Genome Sequencing. J Genet Couns 2017;**26**(5):971-79 doi: 10.1007/s10897-017-0074-ypublished
18. Zhou Q. Accessing disability services by people from culturally and linguistically diverse backgrounds in Australia. Disabil Rehabil 2016;**38**(9):844-52 doi: 10.3109/09638288.2015.1062925published
19. Wibawa T. Push for multilingual COVID-19 resources to help elderly people who don't speak English. *ABC* 2020 Saturday 21 March.
20. The Royal Australian College of General Practitioners. Beware the Rare. 2020. Date Accessed 06/02/2021. https://bewaretherare.com.au/carrier-screening/.
21. The Royal Australian College of General Practitioners (RACGP). RACGP offers new training for GPs on deadly rare hereditary disease, June 2020.
22. Molster CM, Lister, K., Metternick-Jones, S., Baynam, G., Clarke, A. J., Straub, V., Dawkins, H. J. S., and Laing, N. Outcomes of an International Workshop on Preconception Expanded Carrier Screening: Some Considerations for Governments. . Frontiers Public Health doi: https://doi.org/10.3389/fpubh.2017.00025published
23. Ong R, Edwards S, Howting D, Kamien B, Harrop K, Ravenscroft G, Davis M, Fietz M, Pachter N, Beilby J, Laing N. Study protocol of a multicentre cohort pilot study implementing an expanded preconception carrier-screening programme in metropolitan and regional Western Australia. BMJ Open 2019;**9**(6):e028209 doi: 10.1136/bmjopen-2018-028209published
24. Beecroft SJ, Yau KS, Allcock RJN, Mina K, Gooding R, Faiz F, Atkinson VJ, Wise C, Sivadorai P, Trajanoski D, Kresoje N, Ong R, Duff RM, Cabrera-Serrano M, Nowak KJ, Pachter N, Ravenscroft G, Lamont PJ, Davis MR, Laing NG. Targeted gene panel use in 2249 neuromuscular patients: the Australasian referral center experience. Ann Clin Transl Neurol 2020;**7**(3):353-62 doi: 10.1002/acn3.51002published
25. Guo MH, Gregg AR. Estimating yields of prenatal carrier screening and implications for design of expanded carrier screening panels. Genet Med 2019;**21**(9):1940-47 doi: 10.1038/s41436-019-0472-7published
26. Plantinga M, Birnie E, Abbott KM, Sinke RJ, Lucassen AM, Schuurmans J, Kaplan S, Verkerk MA, Ranchor AV, van Langen IM. Population-based preconception carrier screening: how potential users from the general population view a test for 50 serious diseases. Eur J Hum Genet 2016;**24**(10):1417-23 doi: 10.1038/ejhg.2016.43published

27. Rowe CA, Wright CF. Expanded universal carrier screening and its implementation within a publicly funded healthcare service. J Commun Genet 2020;**11**(1):21-38 doi: 10.1007/s12687-019-00443-6published
28. Ropers HH. On the future of genetic risk assessment. J Community Genet 2012;**3**(3):229-36 doi: 10.1007/s12687-012-0092-2published
29. Archibald A, Smith M, Burgess T, Amor D. Reproductive Genetic Carrier Screening for Cystic Fibrosis, Fragile X Syndrome and Spinal Muscular Atrophy in Australia: Outcomes of 12,000 Tests. Australian & New Zealand Journal of Obstetrics & Gynaecology 2017;**57**:12-12
30. US National Library of Medicine. X-linked myotubular myopathy. 2019. Date Accessed https://ghr.nlm.nih.gov/condition/x-linked-myotubular-myopathy#statistics.
31. Schuurmans J, Birnie E, van den Heuvel LM, Plantinga M, Lucassen A, van der Kolk DM, Abbott KM, Ranchor AV, Diemers AD, van Langen IM. Feasibility of couple-based expanded carrier screening offered by general practitioners. Eur J Hum Genet 2019;**27**(5):691-700 doi: 10.1038/s41431-019-0351-3published
32. Plantinga M, Birnie E, Schuurmans J, Buitenhuis AH, Boersma E, Lucassen AM, Verkerk MA, van Langen IM, Ranchor AV. Expanded carrier screening for autosomal recessive conditions in health care: Arguments for a couple-based approach and examination of couples' views. Prenat Diagn 2019;**39**(5):369-78 doi: 10.1002/pd.5437published
33. Rowe CA, Wright CF. Expanded universal carrier screening and its implementation within a publicly funded healthcare service. J Community Genet 2020;**11**(1):21-38 doi: 10.1007/s12687-019-00443-6published
34. Kaseniit KE, Collins E, Lo C, Moyer K, Mar-Heyming R, Kang HP, Muzzey D. Inter-lab concordance of variant classifications establishes clinical validity of expanded carrier screening. Clin Genet 2019;**96**(3):236-45 doi: 10.1111/cge.13582published
35. Ceyhan-Birsoy O, Murry JB, Machini K, Lebo MS, Yu TW, Fayer S, Genetti CA, Schwartz TS, Agrawal PB, Parad RB, Holm IA, McGuire AL, Green RC, Rehm HL, Beggs AH, BabySeq Project T. Interpretation of Genomic Sequencing Results in Healthy and Ill Newborns: Results from the BabySeq Project. Am J Hum Genet 2019;**104**(1):76-93 doi: 10.1016/j.ajhg.2018.11.016published
36. Antonarakis SE. Carrier screening for recessive disorders. Nat Rev Genet 2019;**20**(9):549-61 doi: 10.1038/s41576-019-0134-2published
37. Rose NC. Expanded carrier screening: too much of a good thing? Prenat Diagn 2015;**35**(10):936-7 doi: 10.1002/pd.4638published
38. Langlois S, Benn P, Wilkins-Haug L. Current controversies in prenatal diagnosis 4: pre-conception expanded carrier screening should replace all current prenatal screening for specific single gene disorders. Prenat Diagn 2015;**35**(1):23-8 doi: 10.1002/pd.4532published
39. Johansen Taber KA, Beauchamp KA, Lazarin GA, Muzzey D, Arjunan A, Goldberg JD. Clinical utility of expanded carrier screening: results-guided actionability and outcomes. Genet Med 2019;**21**(5):1041-48 doi: 10.1038/s41436-018-0321-0published
40. Cannon J, Van Steijvoort E, Borry P, Chokoshvili D. How does carrier status for recessive disorders influence reproductive decisions? A systematic review of the literature. Expert Rev Mol Diagn 2019;**19**(12):1117-29 doi: 10.1080/14737159.2020.1690456published
41. West Australian Reproductive Technology Council. Consumer Information. Date Accessed 8/05/2020. https://www.rtc.org.au/consumer-information/.
42. Wapner RJ, Biggio JR, Jr. Commentary: Expanded carrier screening: how much is too much? Genet Med 2019;**21**(9):1927-30 doi: 10.1038/s41436-019-0514-1published

43. Zlotogora J. Genetics and genomic medicine in Israel. Mol Genet Genomic Med 2014;**2**(2):85-94 doi: 10.1002/mgg3.73published
44. Delatycki MB, Laing NG, Moore SJ, Emery J, Archibald AD, Massie J, Kirk EP. Preconception and antenatal carrier screening for genetic conditions: The critical role of general practitioners. Aust J Gen Pract 2019;**48**(3):106-10
45. Mackenzie's Mission. What is Mackenzies Mission. 2020. Date Accessed 08/05/2020. https://www.mackenziesmission.org.au/what-is-mackenzies-mission/.
46. Kirk EP, Ong R, Boggs K, Hardy T, Righetti S, Kamien B, Roscioli T, Amor DJ, Bakshi M, Chung CWT, Colley A, Jamieson RV, Liebelt J, Ma A, Pachter N, Rajagopalan S, Ravine A, Wilson M, Caruana J, Casella R, Davis M, Edwards S, Archibald A, McGaughran J, Newson AJ, Laing NG, Delatycki MB. Gene selection for the Australian Reproductive Genetic Carrier Screening Project ("Mackenzie's Mission"). Eur J Hum Genet 2020 doi: 10.1038/s41431-020-0685-xpublished
47. The Royal Australian College of General Practitioners (RACGP). Genomics in general practice. 2019. Date Accessed 08/05/2020. https://www.racgp.org.au/clinical-resources/clinical-guidelines/key-racgp-guidelines/view-all-racgp-guidelines/genomics-in-general-practice/newborn-screening.
48. Australian Federal Department of Health. Pathology under Medicare. 2020. Date Accessed 08/06/2020. https://www1.health.gov.au/internet/main/publishing.nsf/Content/health-pathology-aboutus-index.htm.
49. Banks LM, Kuper H, Polack S. Poverty and disability in low- and middle-income countries: A systematic review. PLoS One 2017;**12**(12):e0189996 doi: 10.1371/journal.pone.0189996published
50. Davidson P, Saunders, P., Bradbury, B. and Wong, M.,. Poverty in Australia. ACOSS/UNSW Poverty and Inequality Partnership Report No Sydney: ACOSS, 2018.
51. Gadsbøll K, Petersen OB, Gatinois V, Strange H, Jacobsson B, Wapner R, Vermeesch JR, Group TN-mS, Vogel I. Current use of noninvasive prenatal testing in Europe, Australia and the USA: A graphical presentation. Acta Obstetricia et Gynecologica Scandinavica 2020;**99**(6):722-30 doi: 10.1111/aogs.13841published
52. Department of Health Western Australia. Screening tests in the first 3 months of pregnancy. Date Accessed 16 July. https://healthywa.wa.gov.au/Articles/S_T/Screening-tests-in-the-first-3-months-of-pregnancy.
53. The Royal College of Pathologists of Australasia. Reproductive screening for fragile X syndrome, spinal muscular atrophy and cystic fibrosis recommended for funding by MSAC, 2020.
54. Railroading at the FDA. Nat Biotechnol 2016;**34**(11):1078 doi: 10.1038/nbt.3733published
55. Laing NG. What is the role of preconception carrier screening in neurology. Advances in Clinical Neuroscience and Rehabilitation 2016;**16**(1):2

APPENDIX

CHAPTER 2:

Section 1

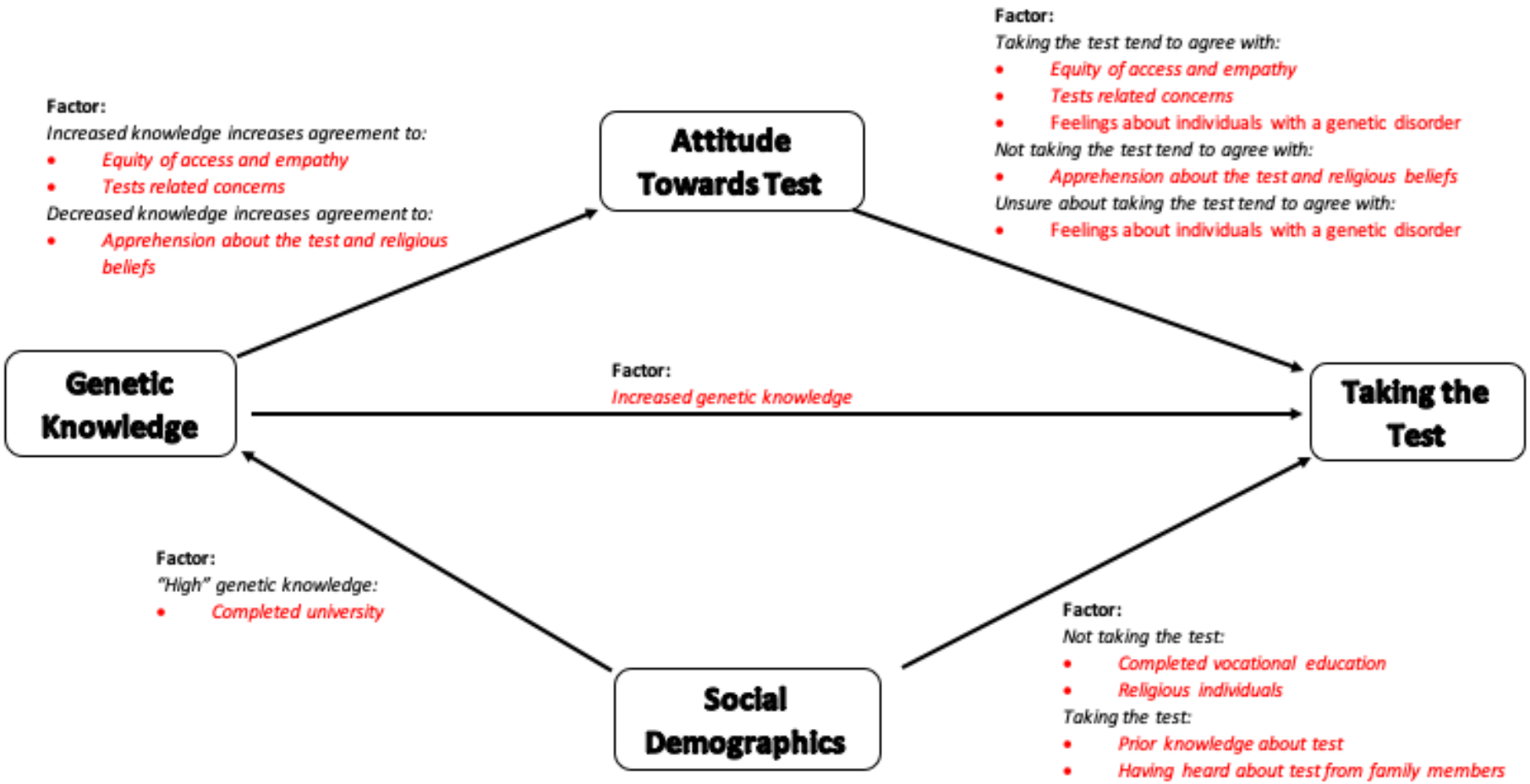
Measuring the impact of genetic knowledge on intentions and attitudes of the community towards a carrier-screening test

Published

DOI: 10.1136/bmjopen-2018-028209

Supplementary information

Chapter 3, Section 1 Supplementary Figure 1



Supplementary table 1: Factor analysis - Principal Component Analysis method

Attitude statements

Items	Component			
	Factor1 *Apprehension about the test and religious beliefs*	**Factor2** *Equity of access and empathy*	**Factor3** *Feelings about individuals with a genetic disorder*	**Factor4** *Tests related concerns*
Will do more harm than good	0.855			
Is morally unacceptable	0.849			
Will not improve people's lives	0.824			
Will lead to discrimination of people with recessive diseases	0.745			
I will be discriminated against if I am identified as a carrier.	0.666			
Raising a child with a severe recessive disease would be rewarding for me.	0.662			
Preconception carrier screening should only be offered to people with a family history.	0.652			
I think that children with severe recessive diseases are ''manifestations of God's will''.	0.645			
If I had a preconception carrier screening test, I would worry that the results might not remain confidential.	0.621			
My religion would be important in my decision to participate in preconception carrier screening.	0.568			
Will reduce the reason to find a cure and/or improve treatments for severe recessive diseases	0.501			
Should be offered in Western Australia		0.85		
Should be made available for all couples planning a pregnancy		0.808		
Provides couples with reproductive choices		0.789		
Will reduce suffering associated with recessive diseases		0.74		
I think that being screened for severe recessive diseases before pregnancy is the right thing to do.		0.458		
It would be better if no one with a severe recessive disease had been born.			0.813	
I think it is wrong to knowingly bring a child with a severe recessive disease into the world.			0.779	
It is difficult for a person with a severe recessive disease to have a very good life.			0.638	
A post-test consultation with a genetic counsellor would be essential.				0.637
Preconception carrier screening may result in an increase in my insurance rates.				0.601
If the costs were the same, I would prefer to be tested for a larger number of recessive diseases rather than for a smaller number.				0.472
Percentage variance accounted for	33.70%	16.40%	6.10%	4.80%
Cumulative percentage of variance	33.70%	50.10%	56.20%	61.00%

Supplementary table 2: Genetic questions and correct answers

Questions	True	False	Don't Know	Correct answer	Incorrect answer
An individual with a genetic mutation for a recessive disorder is known as a carrier	542	61	229	542(65%)	290(35%)
A carrier of a genetic disorder carries a mutation for that disorder but does not have the disease	548	84	200	548(66%)	284(34%)
If both my partner and I test negative for a specific disorder, our baby will definitely not have that disorder	207	400	225	400(48%)	432(52%)
I can be a carrier for a genetic disorder even though there is no history of the disorder in my family	555	86	191	555(67%)	277(33%)
Individuals in certain ethnic groups have an increased risk of being carriers of certain abnormal genes	609	42	181	609(73%)	223(27%)
If both my parents are carriers, I have a 75% chance of becoming a carrier	423	111	298	111(13%)	721(87%)
Healthy parents can have a child with an inherited disease	658	43	131	658(79%)	174(21%)
If a person is the carrier of a disease gene, it means that they have the disease	99	581	152	581(70%)	251(30%)
Half your genes come from your mother and half from your father	518	154	160	518(62%)	314(38%)
A gene is part of a chromosome	505	81	246	505(61%)	327(39%)
Genes are segments of DNA that encode information critical for development	645	32	155	645(78%)	187(22%)
Genetic mutations may either harm or have little to no effect on an organism	542	77	213	542(65%)	290(35%)
Genetic mutations in the DNA of any cells will be passed on to offspring	231	289	312	289(35%)	543(65%)
Some harmful genetic mutations can be inherited	668	48	116	668(80%)	164(20%)
You cannot develop harmful genetic mutations from lifestyle choices	274	312	246	312(38%)	520(63%)

	Genetic Mutations	Environmental Factors	Mixture	Don't Know		
Eye color	623	19	102	88	623(75%)	209(25%)
Food poisoning	12	685	55	80	685(82%)	147(18%)
Spina bifida	463	42	144	183	144(17%)	688(83%)
Frost bite	19	704	21	88	704(85%)	128(15%)
Cystic fibrosis	554	29	93	156	554(67%)	278(33%)
Diabetes	94	60	599	79	599(72%)	233(28%)

Note: red words indicate correct answers.

Supplementary table 3: Binary Logistic Regression
Social demographics and genetic knowledge
Note: red values indicate significance of <0.05

Genetic knowledge	Age	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
High[a]	Intercept	.	0.445	.	.	.
	18-44	0.328	0.521	1.388	0.510	3.781
	45-64	0.628	0.260	1.874	0.629	5.584
	Constant[b]	-1.056	0.010	0.348	.	.

a. The reference category is: Other levels of genetic knowledge
b. The reference category is: 65+

Genetic knowledge	Gender	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
High[a]	Female	0.353	0.018	1.423	1.063	1.904
	Constant[b]	-0.864	0.000	0.422	.	.

a. The reference category is: Other levels of genetic knowledge
b. The reference category is: Male

Genetic knowledge	Education level	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
High[a]	Intercept	.	0.000	.	.	.
	Completed university	1.059	0.000	2.885	1.947	4.275
	Completed vocation education	-0.094	0.680	0.911	0.583	1.421
	Currently studying vocation education or university	0.449	0.094	1.566	0.927	2.647
	Constant[b]	-1.112	0.000	0.329	.	.

a. The reference category is: Other levels of genetic knowledge
b. The reference category is: Completed year 12 or equivalent

Genetic knowledge	Religiosity/Spirituality	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
High[a]	Yes I am religious/spiritual	-0.073	0.664	0.930	0.670	1.291
	Constant[b]	-0.659	0.000	0.517	.	.

a. The reference category is: Other levels of genetic knowledge
b. The reference category is: No I am religious/spiritual

Genetic knowledge	Personal Income	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
High[a]	Below average to average income	-0.364	0.207	0.695	0.395	1.224
	Constant[b]	-0.194	0.447	0.824	.	.

a. The reference category is: Other levels of genetic knowledge
b. The reference category is: Above average income

Genetic knowledge	Relationship status	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
High[a]	In a relationship	0.010	0.958	1.010	0.685	1.491
	Constant[b]	-0.706	0.000	0.494	.	.

a. The reference category is: Other levels of genetic knowledge
b. The reference category is: Not in a relationship

Genetic knowledge	Parenthood experience	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
High[a]	Intercept	.	0.035	.	.	.
	Been a parent before	-0.037	0.518	0.964	0.349	2.660
	Have not been a parent before	-0.061	0.518	0.941	0.341	2.600
	Constant[b]	-0.606	0.508	0.545	.	.

a. The reference category is: Other levels of genetic knowledge
b. The reference category is: No we are expecting a child soon

Genetic knowledge	Relationship status	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
High[a]	Yes I want to be a parent	-0.035	0.826	0.965	0.705	1.321
	Constant[b]	-0.633	0.000	0.531	.	.

a. The reference category is: Other levels of genetic knowledge
b. The reference category is: No I don't want to be a parent

Supplementary table 4: Chi-square of independence

Social demographics and genetic knowledge

		Genetic_knowledge_interquartile				Chi-Square of independence		
		High (n=)	Good (n=)	Some (n=)	Low (n=)	Value	df	p-value
Age	<17	0	0	0	0	19.25	9	0.028
	18 – 24	35	42	18	17			
	25 – 44	208	258	79	62			
	45 – 64	33	42	5	2			
	65+	8	19	2	2			
Gender	Male	113	162	62	44	19.2	9	0.07
	Female	168	199	42	39			
Education	Completed university	148	114	30	12	63.39	9	<0.001
	Completed vocation education	53	121	31	25			
	Currently studying vocation education or university	34	40	12	14			
	Completed year 12 or equivalent	49	86	31	32			
Religiosity	Yes	118	149	47	31	4.14	6	0.67
	No	166	212	57	52			
Personal Income	$125,000 and over	28	26	7	1	15.82	12	0.20
	$80,000 - $124,999	61	75	21	13			
	$50,000 - $79,999	70	79	34	21			
	$30,000 -$49,999	35	65	16	15			
	$0 - $29,999	65	82	20	21			
Relationship status	In a relationship	205	263	72	53	3.00	3	0.39
	Not in a relationship	79	98	32	30			
Parenthood experience	Yes	143	188	50	34	28.12	9	0.03
	No	135	169	52	42			
	No, we are expecting a child soon.	6	4	2	5			
Parenthoood intention	Yes	199	248	83	57	5.06	3	0.17
	No	85	113	21	26			

Note: red values indicate significance of <0.05

Supplementary table 5: Multinomial logistic regression

Social demographics and taking the test

Note: red values indicate significance of <0.05

Would you take the test?[a]	Education level	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
No	Intercept	-2.295	0.000	.	.	.
	Completed university	0.197	0.579	1.218	0.607	2.444
	Completed vocational education	0.777	0.027	2.175	1.094	4.322
	Currently studying university or vocational education	0.575	0.179	1.777	0.769	4.110
	Completed year 12 or equivalent	0	.	.	.	.
Unsure	Intercept	-0.834	0.000			
	Completed university	-0.516	0.018	0.597	0.389	0.916
	Completed vocational education	-0.198	0.384	0.820	0.525	1.281
	Currently studying university or vocational education	-0.326	0.273	0.722	0.403	1.292
	Completed year 12 or equivalent	0	.	.	.	.

a. The reference category is: Yes.

b. This parameter is set to zero because it is redundant.

Would you take the test?[a]	Religiosity/Spirituality	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
No	Intercept	-2.603	0.000	.	.	.
	Is a religious/spiritual person	1.117	0.039	3.054	1.057	8.825
	Not a religious/spiritual person	0.344	0.533	1.411	0.478	4.168
	Not sure whether I'm a religious/spiritual person	0	.	.	.	.
Unsure	Intercept	-0.944	0.000	.	.	.
	Is a religious/spiritual person	-0.150	0.606	0.861	0.488	1.520
	Not a religious/spiritual person	-0.204	0.472	0.815	0.467	1.422
	Not sure whether I'm a religious/spiritual person	0	.	.	.	.

a. The reference category is: Yes.

b. This parameter is set to zero because it is redundant.

Would you take the test?[a]	Invidual annual income	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
Yes	Intercept	1.642	0.000	.	.	.
	$125,000 and over	-0.414	0.297	0.661	0.304	1.439
	$80,000 - $124,999	0.820	0.033	2.270	1.067	4.829
	$50,000 - $79,999	0.624	0.082	1.866	0.924	3.769
	$30,000 -$49,999	0.362	0.341	1.435	0.682	3.022
	$0 - $29,999	0	.	.	.	.
Unsure	Intercept	0.511	0.048	.	.	.
	$125,000 and over	-0.799	0.118	0.45	0.165	1.225
	$80,000 - $124,999	0.492	0.260	1.636	0.695	3.853
	$50,000 - $79,999	0.857	0.030	2.357	1.086	5.115
	$30,000 -$49,999	0.405	0.344	1.500	0.648	3.472
	$0 - $29,999	0	.	.	.	.

a. The reference category is: No.

b. This parameter is set to zero because it is redundant.

Chapter 2, Section 1 Supplementary Table 6

Supplementary table 6: Chi-square of independence

Prior knowledge and taking the test

Note: red values indicate significance of <0.05

		Would you take the test?				Chi-Square of independence		
		Yes (n=)	No (n=)	Unsure (n=)	Total (n=)	Value	df	p-value
Have you heard about preconception carrier screening before this survey?	YES	184	25	30	239	18.88	2	<0.001
	NO	378	59	156	593			
Know about it through searches on the internet	NO	153	25	27	205	5.62	2	0.048
	YES	31	0	3	34			
Know about it from other advertising on the internet	NO	178	25	29	232	0.84	2	1.00
	YES	6	0	1	7			
Know about it other forms of advertising	NO	177	24	27	228	2.28	2	0.23
	YES	7	1	3	11			
Know about it through social media	NO	162	24	25	211	2.04	2	0.38
	YES	22	1	5	28			
Know about it from films, television or other media (e.g. newspaper or magazine articles)	NO	135	20	19	174	2.04	2	0.36
	YES	49	5	11	65			
Know about it from friends	NO	132	20	24	176	1.49	2	0.52
	YES	52	5	6	63			
Know about it from family	NO	143	25	27	195	8.89	2	0.01
	YES	41	0	3	44			
Know about it through formal studies	NO	165	23	30	218	3.48	2	0.19
	YES	19	2	0	21			
Know about it from participating in research	NO	179	25	30	234	0.38	2	1.00
	YES	5	0	0	5			
Know about it from a healthcare professional	NO	148	21	28	197	3.01	2	0.24
	YES	36	4	2	42			
Know about it through my work	NO	170	22	27	219	1.13	2	0.60
	YES	14	3	3	20			

Chapter 2, Section 1 Supplementary Table 7

Supplementary table 7: Multinomial logistic regression
Prior knowledge and taking the test
Note: red values indicate significance of <0.05

Would you take the test?[a]	Awareness about test	B	p-value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
Yes	Intercept	0.885	0.000	.	.	.
	Heard about preconception carrier screening before this survey	0.929	0.000	2.531	1.649	3.886
	Did not hear about preconception carrier screening before this survey	0[b]	.	.	.	.
No	Intercept	-0.972	0.000	.	.	.
	Heard about preconception carrier screening before this survey	0.790	0.011	2.203	1.198	4.053
	Did not hear about preconception carrier screening before this survey	0[b]	.	.	.	.

a. The reference category is: Unsure.
b. This parameter is set to zero because it is redundant.

Chapter 2, Section 1 Supplementary Table 8

Supplementary table 8: Chi-square of independence
Genetic knowledge and taking the test
Note: red values indicate significance of <0.05

		Would you take the test?			Chi-Square of independence		
		Yes (n=)	No (n=)	Unsure (n=)	Value	df	p-value
Genetic Knowledge	High	210	31	43	88.62	6	<0.001
	Good	266	27	68			
	Some	65	12	27			
	Low	21	14	48			

Supplementary table 9: Chi-square of independence

Genetic knowledge, taking the test and post-decision concerns

Note: red values indicate more than expected number of individuals who have "high" genetic knowledge and expressed a concern.

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	If we take it, pregnancy becomes less natural	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	4	7	0.002	1	0.968
	% within If we take it, pregnancy becomes less natural	36.4%	63.6%			
	Adjusted residual	0.0	0.3			
	No (n=)	27	46			
	% within If we take it, pregnancy becomes less natural	37.0%	63.0%			
	Adjusted residual	0.0	0.0			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I am not concerned about my privacy regarding my genetic information	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	4	4	0.651	1	0.427
	% within I am not concerned about my privacy regarding my genetic information	50.0%	50.0%			
	Adjusted residual	0.8	-0.8			
	No (n=)	27	49			
	% within I am not concerned about my privacy regarding my genetic information	35.5%	64.5%			
	Adjusted residual	0.8	-0.8			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I don't trust the test results	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	1	7	2.262	1	1.050
	% within I don't trust the test results	12.5%	87.5%			
	Adjusted residual	-1.5	1.5			
	No (n=)	30	46			
	% within I don't trust the test results	39.5%	60.5%			
	Adjusted residual	1.5	-1.5			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I am not interested in finding out my genetic information	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	12	8	0.971	1	0.324
	% within I am not interested in finding out my genetic information	60.0%	40.0%			
	Adjusted residual	1.0	-1.0			
	No (n=)	19	19			
	% within I am not interested in finding out my genetic information	50.0%	50.0%			
	Adjusted residual	-1.0	1.0			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I don't believe it would be useful to me	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	11	13	1.150	1	0.283
	% within I don't believe it would be useful to me	45.8%	54.2%			
	Adjusted residual	1.1	-1.1			
	No (n=)	20	40			
	% within I don't believe it would be useful to me	33.3%	66.7%			
	Adjusted residual	-1.1	1.1			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I am concerned the information will have a negative impact on my life	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	9	9	1.687	1	0.194
	% within I am concerned the information will have a negative impact on my life	50.0%	50.0%			
	Adjusted residual	1.3	-1.3			
	No (n=)	22	44			
	% within I am concerned the information will have a negative impact on my life	33.3%	66.7%			
	Adjusted residual	-1.3	1.3			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I am concerned the information will have a negative impact on my family members	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	9	6	4.183	1	0.410
	% within I am concerned the information will have a negative impact on my family members	60.0%	40.0%			
	Adjusted residual	2.0	-2.0			
	No (n=)	22	47			
	% within I am concerned the information will have a negative impact on my family members	31.9%	68.1%			
	Adjusted residual	-2.0	2.0			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I don't trust the organisations/companies/people offering the test	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	2	2	0.309	1	0.578
	% within I don't trust the organisations/companies/people offering the test	50.0%	50.0%			
	Adjusted residual	0.6	-0.6			
	No (n=)	29	51			
	% within I don't trust the organisations/companies/people offering the test	36.3%	63.8%			
	Adjusted residual	-0.6	0.6			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I am opposed to genetic testing	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	2	4	0.035	1	0.851
	% within I am opposed to genetic testing	33.3%	66.7%			
	Adjusted residual	-0.2	0.2			
	No (n=)	29	49			
	% within I am opposed to genetic testing	37.2%	62.8%			
	Adjusted residual	0.2	-0.2			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I am concerned what other people might do with my genetic information	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	7	2	7.232	1	0.007
	% within I am concerned what other people might do with my genetic information	77.8%	22.2%			
	Adjusted residual	2.7	-2.7			
	No (n=)	24	51			
	% within I am concerned what other people might do with my genetic information	32.0%	68.0%			
	Adjusted residual	-2.7	2.7			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I am concerned I could be discriminated against based on my personal genetic test results	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	7	4	3.884	1	0.049
	% within I am concerned I could be discriminated against based on my personal genetic test results	22.6%	36.4%			
	Adjusted residual	2.0	-2.0			
	No (n=)	24	49			
	% within I am concerned I could be discriminated against based on my personal genetic test results	32.9%	67.1%			
	Adjusted residual	-2.0	2.0			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I am concerned that obtaining my personal genetic information will have negative implications on my ability to obtain health, life and/or disability insurance	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	11	5	8.608	1	0.030
	% within I am concerned that obtaining my personal genetic information will have negative implications on my ability to obtain health, life and/or disability insurance	22.6%	31.3%			
	Adjusted residual	2.9	-2.9			
	No (n=)	20	48			
	% within I am concerned that obtaining my personal genetic information will have negative implications on my ability to obtain health, life and/or disability insurance	29.4%	70.6%			
	Adjusted residual	-2.9	2.9			

Would you take the test?	Variable	Genetic knowledge		Chi-Square of independence		
	I am concerned that my employer could discriminate based on my personal genetic results	High	Others	Value	df	p-value
No (n=84)	Yes (n=)	2	4	0.035	1	0.851
	% within I am concerned that my employer could discriminate based on my personal genetic results	22.6%	66.7%			
	Adjusted residual	-0.2	0.2			
	No (n=)	29	49			
	% within I am concerned that my employer could discriminate based on my personal genetic results	37.2%	62.8%			
	Adjusted residual	0.2	-0.2			

Chapter 2, Section 1 Supplementary Table 10

			Factor1 Mean	Factor2 Mean	Factor3 Mean	Factor4 Mean
If you are offered preconception carrier screening, would you accept the test?	Yes	High Genetic Knowledge	2.15	4.38	3.05	4.03
	No	High Genetic Knowledge	2.93	3.21	1.92	3.76

Chapter 2, Section 1 Supplementary Table 11

Supplementary table 11: Chi-square of independence

Genetic knowledge and correctly answered genetic questions

Red words indicate correct answer. Red numbers highlight statistical significance.

	Genetic Knowledge Level								Chi-Square of independence		
	High		Good		Some		Low				
Genetic questions testing basic concepts	Count (%)	Adjusted Residual	Count (%)	Adjusted Residual	Count (%)	Adjusted Residual	Count (%)	Adjusted Residual	Value	df	p-value
Q1. An individual with a genetic mutation for a recessive disorder is known as a carrier (True)											
True	247 (45.57%)	9.5	253 (46.68%)	2.6	39 (7.2%)	-6.3	3 (0.55%)	-12.4	250	6	<0.001
False	14 (22.95%)	-1.9	21 (34.43%)	-1.5	16 (26.23%)	3.4	10 (16.39%)	1.7			
Don't Know	23 (10.04%)	-9.0	87 (37.99%)	-1.9	49 (21.4%)	4.8	70 (30.57%)	12.2			
Q2. A carrier of a genetic disorder carries a mutation for that disorder but does not have the disease (True)											
True	260 (47.45%)	11.2	242 (44.16%)	0.6	39 (7.12%)	-6.5	7 (1.28%)	-11.6	278.73	6	<0.001
False	17 (3.1%)	-2.8	37 (6.75%)	0.1	21 (3.83%)	3.7	9 (1.64%)	0.2			
Don't Know	7 (1.28%)	-10.5	82 (14.96%)	-0.8	44 (8.03%)	4.7	67 (12.23%)	12.7			
Q5. Individuals in certain ethnic groups have an increased risk of being carriers of certain abnormal genes (True)											
True	268 (44.01%)	9.9	286 (46.96%)	3.4	50 (8.21%)	-6.2	5 (0.82%)	-14.6	332.31	6	<0.001
False	6 (14.29%)	-2.8	15 (35.71%)	-1.0	16 (38.1%)	5.1	5 (11.9%)	0.4			
Don't Know	10 (5.52%)	-9.2	60 (33.15%)	-3.1	38 (20.99%)	3.9	73 (40.33%)	15.4			
Q9. Half your genes come from your mother and half from your father (True)											
True	244 (47.1%)	10.1	212 (40.93%)	-1.8	53 (10.23%)	-2.5	9 (1.74%)	-10.2	285.09	6	<0.001
False	32 (20.78%)	-3.9	90 (58.44%)	4.2	24 (15.58%)	1.3	8 (5.19%)	-2.2			
Don't Know	8 (5%)	-8.6	59 (36.88%)	-1.9	27 (16.88%)	1.9	66 (41.25%)	14.7			
Q7. Healthy parents can have a child with an inherited disease (True)											
True	278 (42.25%)	9.6	311 (47.26%)	4.4	54 (8.21%)	-7.3	15 (2.28%)	-14.4	326.53	6	<0.001

False	3 (6.98%)	-3.9	16 (37.21%)	-0.8	15 (34.88%)	4.6	9 (20.93%)	2.5			
Don't Know	3 (2.29%)	-8.4	34 (25.95%)	-4.4	35 (26.72%)	5.4	59 (45.04%)	14.6			
Q10. A gene is part of a chromosome (True)											
True	244 (48.32%)	10.7	218 (43.17%)	-0.2	31 (6.14%)	-6.9	12 (2.38%)	-9.1	230.79	6	<0.001
False	17 (20.99%)	-2.6	35 (43.21%)	0.0	25 (30.86%)	5.3	4 (4.94%)	-1.6			
Don't Know	23 (9.35%)	-9.8	108 (43.9%)	0.2	48 (19.51%)	4.0	67 (27.24%)	10.8			
Q11. Genes are segments of DNA that encode information critical for development (True)											
True	280 (43.41%)	10.5	313 (48.53%)	5.6	47 (7.29%)	-8.4	5 (0.78%)	-16.4	409.94	6	<0.001
False	2 (6.25%)	-3.4	9 (28.13%)	-1.8	13 (40.63%)	4.9	8 (25%)	2.9			
Don't Know	2 (1.29%)	-9.6	39 (25.16%)	-5.1	44 (28.39%)	6.6	70 (45.16%)	16.2			
Q12. Genetic mutations may either harm or have little to no effect on an organism (True)											
True	271 (50%)	13.2	234 (43.17%)	-0.2	35 (6.46%)	-7.2	2 (0.37%)	-12.6	358.23	6	<0.001
False	7 (9.09%)	-4.9	44 (57.14%)	2.6	21 (27.27%)	4.1	5 (6.49%)	-1.1			
Don't Know	6 (2.82%)	-11.2	83 (38.97%)	-1.5	48 (22.54%)	5.1	76 (35.68%)	14.5			
Q14. Some harmful genetic mutations can be inherited (True)											
True	279 (41.77%)	9.4	325 (48.65%)	6.2	55 (8.23%)	-7.5	9 (1.35%)	-16.8	478.03	6	<0.001
False	5 (10.42%)	-3.6	17 (35.42%)	-1.1	21 (43.75%)	6.7	5 (10.42%)	0.1			
Don't Know	0 (0%)	-8.4	19 (16.38%)	-6.3	28 (24.14%)	4.1	69 (59.48%)	19.2			

	Genetic Knowledge Level								Chi-Square of independence		
	High		Good		Some		Low				
Genetic questions testing understanding	Count (%)	Adjusted Residual	Count (%)	Adjusted Residual	Count (%)	Adjusted Residual	Count (%)	Adjusted Residual	Value	df	p-value
Q3. If both my partner and I test negative for a specific disorder, our baby will definitely not have that disorder (False)											
True	66 (31.88%)	-0.8	108 (52.17%)	2.9	29 (14.01%)	0.8	4 (1.93%)	-4.5	201.22	6	<0.001
False	188 (47%)	7.5	164 (41%)	-1.3	40 (10%)	-2.1	8 (2%)	-7.4			
Don't Know	30 (13.33%)	-7.7	89 (39.56%)	-1.4	35 (15.56%)	1.6	71 (31.56%)	12.6			
Q4. I can be a carrier for a genetic disorder even though there is no history of the disorder in my family (True)											
True	255 (45.95%)	10.2	253 (45.59%)	1.8	42 (7.57%)	-6.1	5 (0.9%)	-12.4	300.56	6	<0.001
False	9 (10.47%)	-4.9	44 (51.16%)	1.5	26 (30.23%)	5.3	7 (8.14%)	-0.6			
Don't Know	20 (10.47%)	-7.9	64 (33.51%)	-3.1	36 (18.85%)	3.0	71 (37.17%)	14.3			
Q6. If both my parents are carriers, I have a 75% chance of becoming a carrier (False)											
True	161 (38.06%)	2.4	209 (49.41%)	3.6	38 (8.98%)	-3.1	15 (3.55%)	-6.3	142.14	6	<0.001
False	65 (58.56%)	5.8	25 (22.52%)	-4.8	21 (18.92%)	2.2	0 (0%)	-3.8			
Don't Know	58 (19.46%)	-6.7	127 (42.62%)	-0.3	45 (15.1%)	1.7	68 (22.82%)	9.2			
Q8. If a person is the carrier of a disease gene, it means that they have the disease (False)											
True	8 (8.08%)	-5.8	51 (51.52%)	1.7	29 (29.29%)	5.4	11 (11.11%)	0.4	380.63	6	<0.001
False	272 (46.82%)	11.7	267 (45.96%)	2.3	36 (6.2%)	-8.4	6 (1.03%)	-13.1			

Don't Know	4 (2.63%)	-9.1	43 (28.29%)	-4.2	39 (25.66%)	5.4	66 (43.42%)	15.2			
Q13. Genetic mutations in the DNA of any cells will be passed on to offspring (False)											
True	60 (25.97%)	-3.1	128 (55.41%)	4.3	38 (16.45%)	2.1	5 (2.16%)	-4.7	248.45	6	<0.001
False	181 (62.63%)	12.6	91 (31.49%)	-5.1	14 (4.84%)	-4.9	3 (1.04%)	-6.3			
Don't Know	43 (13.78%)	-9.6	142 (45.51%)	1.0	52 (16.67%)	2.8	75 (24.04%)	10.5			
Q15. You cannot develop harmful genetic mutations from lifestyle choices (False)											
True	92 (33.58%)	-0.2	146 (53.28%)	4.0	30 (10.95%)	-0.9	6 (2.19%)	-5.3	221.67	6	<0.001
False	162 (51.92%)	8.4	119 (38.14%)	-2.4	27 (8.65%)	-2.6	4 (1.28%)	-6.5			
Don't Know	30 (12.2%)	-8.6	96 (39.02%)	-1.6	47 (19.11%)	3.7	73 (29.67%)	12.3			

	Genetic Knowledge Level								Chi-Square of independence		
	High		Good		Some		Low				
Genetic question testing misconceptions	Count (%)	Adjusted Residual	Count (%)	Adjusted Residual	Count (%)	Adjusted Residual	Count (%)	Adjusted Residual	Value	df	p-value
Q16. Eye colour (Genetic Mutations)											
Genetic Mutations	263 (42.22%)	8.5	294 (47.19%)	3.8	54 (8.67%)	-5.8	12 (1.93%)	-13.4	373.91	9	<0.001
Environmental Factors	0 (0%)	-3.2	4 (21.05%)	-2.0	9 (47.37%)	4.6	6 (31.58%)	3.2			
Mixture	18 (17.65%)	-3.7	50 (49.02%)	1.2	21 (20.59%)	2.6	13 (12.75%)	1.0			
Don't Know	3 (3.41%)	-6.4	13 (14.77%)	-5.7	20 (22.73%)	3.1	52 (59.09%)	16.3			
Q17. Food poisoning (Environmental Factors)											
Genetic Mutations	0 (0%)	-2.5	4 (33.33%)	-0.7	5 (41.67%)	3.1	3 (25%)	1.7	427.13	9	<0.001
Environmental Factors	269 (39.27%)	6.7	333 (48.61%)	6.6	69 (10.07%)	-4.6	14 (2.04%)	-16.5			
Mixture	14 (25.45%)	-1.4	19 (34.55%)	-1.4	12 (21.82%)	2.2	10 (18.18%)	2.1			
Don't Know	1 (1.25%)	-6.5	5 (6.25%)	-7.1	18 (22.5%)	2.8	56 (70%)	18.8			
Q18. Spina bifida (Mixture)											
Genetic Mutations	176 (38.01%)	2.6	234 (50.54%)	4.7	46 (9.94%)	-2.5	7 (1.51%)	-9.1	265.69	9	<0.001
Environmental Factors	8 (19.05%)	-2.1	17 (40.48%)	-0.4	12 (28.57%)	3.2	5 (11.9%)	0.4			
Mixture	84 (58.33%)	6.7	46 (31.94%)	-3.0	9 (6.25%)	-2.5	5 (3.47%)	-2.9			
Don't Know	16 (8.74%)	-8.2	64 (34.97%)	-2.6	37 (20.22%)	3.6	66 (36.07%)	13.3			
Q19. Frost bite (Environmental Factors)											
Genetic Mutations	1 (5.26%)	-2.7	6 (31.58%)	-1.1	7 (36.84%)	3.2	5 (26.32%)	2.4	445.62	9	<0.001
Environmental Factors	281 (39.91%)	8.2	337 (47.87%)	6.1	71 (10.09%)	-4.9	15 (2.13%)	-17.7			
Mixture	2 (9.52%)	-2.4	8 (38.1%)	-0.5	8 (38.1%)	3.6	3 (14.29%)	0.7			
Don't Know	0 (0%)	-7.1	10 (11.36%)	-6.4	18 (20.45%)	2.4	60 (68.18%)	19.3			
Q20. Cystic fibrosis (Genetic Mutations)											
Genetic Mutations	252 (45.49%)	9.7	253 (45.67%)	1.9	42 (7.58%)	-6.1	7 (1.26%)	-11.8	334.32	9	<0.001

Environmental Factors	2 (6.9%)	-3.1	15 (51.72%)	0.9	10 (34.48%)	3.6	2 (6.9%)	-0.6			
Mixture	22 (23.66%)	-2.3	47 (50.54%)	1.5	18 (19.35%)	2.1	6 (6.45%)	-1.2			
Don't Know	8 (5.13%)	-8.5	46 (29.49%)	-3.9	34 (21.79%)	3.9	68 (43.59%)	15.5			
Q21. Diabetes (Mixture)											
Genetic Mutations	9 (9.57%)	-5.3	56 (59.57%)	3.4	20 (21.28%)	2.7	9 (9.57%)	-0.1			
Environmental Factors	9 (15%)	-3.2	26 (43.33%)	0.0	20 (33.33%)	5.1	5 (8.33%)	-0.4	460.41	9	<0.001
Mixture	266 (44.41%)	10.0	270 (45.08%)	1.6	50 (8.35%)	-5.8	13 (2.17%)	-12.0			
Don't Know	0 (0%)	-6.7	9 (11.39%)	-6.0	14 (17.72%)	1.5	56 (70.89%)	19.0			

CHAPTER 2:

Section 2

Attitudes towards and knowledge of an expanded carrier-screening test amongst Western Australian health professionals

Prepared manuscript

Royston Ong
Samantha Edwards
Denise Howting
Alethea Rea
Hayley Christian
Gina Ravenscroft
Nigel Laing

Supplementary information

Table S1A: Follow up considerations when participants want to take the test

Will take the test (n=155; 76.4%)		
Statement	**NO**	**YES**
I will do the test if the diseases that are screened affects lifespan of any children or infants.	5 (3%)	150 (97%)
I will do the test if the diseases that are screened is chronic and requires me to be a full-time carer.	5 (3%)	150 (97%)
I will do the test if the diseases that are screened first show symptoms when my child is an adult but still able to look after himself/herself.	41 (26%)	114 (74%)
I would want to access this test through my: General Practitioner (GP)	25 (16%)	130 (84%)
I would want to access this test through my: Midwife	88 (57%)	67 (43%)
I would want to access this test through my: Gynaecologist/Obstetrician	25 (16%)	130 (84%)
I would want to access this test through my: Genetic counsellor	30 (19%)	125 (81%)
I would want to access this test through my: Through mail or online ordering	124 (80%)	31 (20%)

I will do a preconception carrier-screening test if it costs me	Free	< AUD50	AUD50 to AUD200	AUD200 to AUD500	AUD500 to AUD1000	Any amount
	25 (16%)	28 (18%)	49 (32%)	25 (16%)	11 (7%)	17 (11%)

Table S2: Genetic questions and correct answers

Questions		True	False	Don't Know	Correct answer	Wrong answer
1	An individual with a genetic mutation for a recessive disorder is known as a carrier.	**174**	17	12	85.7%	14.3%
2	A carrier of a genetic disorder carries a mutation for that disorder but does not have the disease.	**193**	9	1	95.1%	4.9%
3	If both my partner and I test negative for a specific disorder our baby will definitely not have that disorder.	42	**147**	14	72.4%	27.6%
4	I can be a carrier for a genetic disorder even though there is no history of the disorder in my family.	**185**	5	13	91.1%	8.9%
5	Individuals in certain ethnic groups have an increased risk of being carriers of certain abnormal genes.	**197**	2	4	97.0%	3.0%
6	If both my parents are carriers, I have a 75% chance of becoming a carrier.	66	**109**	28	53.7%	46.3%
7	Healthy parents can have a child with an inherited disease.	**199**	1	3	98.0%	2.0%
8	If a person is the carrier of a disease gene it means that they have the disease.	1	**199**	3	98.0%	2.0%
9	Half your genetic material comes from your mother and half from your father.	**186**	13	4	91.6%	8.4%
10	A gene is part of a chromosome.	**189**	7	7	93.1%	6.9%
11	Genes are segments of DNA that encode information critical for development.	**191**	7	5	94.1%	5.9%
12	Genetic mutations may either harm or have little to no effect on an organism.	**190**	6	7	93.6%	6.4%
13	Genetic mutations in the DNA of any cell will be passed on to offspring.	14	**173**	16	85.2%	14.8%
14	Some harmful genetic mutations can be inherited.	**198**	4	1	97.5%	2.5%

Questions		Genetic Mutations	Environmental Factors	Mixture	Don't Know	Correct answer	Wrong answer
15	eye colour	**182**	0	18	3	89.7%	10.3%
16	food poisoning	0	**189**	13	1	93.1%	6.9%
17	spina bifida	62	19	**117**	5	57.6%	42.4%
18	frostbite	0	**196**	5	2	96.6%	3.4%
19	cystic fibrosis	**188**	0	10	5	92.6%	7.4%
20	diabetes	7	6	**189**	1	93.1%	6.9%

Table S3: Distribution of attitude statements amongst health professionals.

Bars represent proportion of health professionals who either agree (purple) or disagree (pink) to various statements. Grey bars represent proportion of health professionals who show ambivalence to statements.

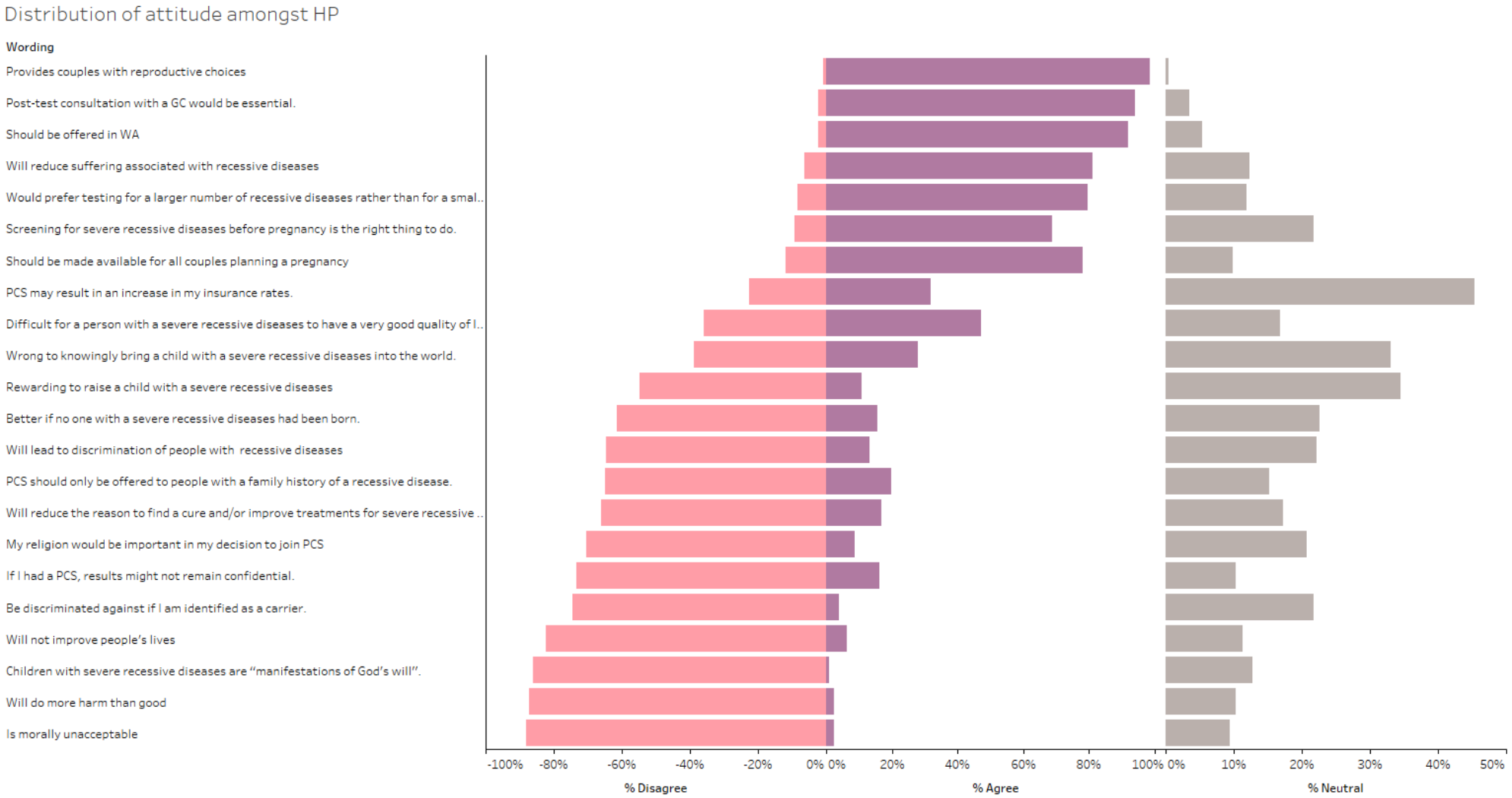


Table S4: Chi-Square test of social demographic factors and intentions to take the test

Prior Awareness/Where did you hear about PCS from?		Would you take the test?				Chi-Square of independence		
		Yes (n=)	No (n=)	Unsure (n=)	Total (n=)	Value	df	p-value
Have you heard about preconception carrier screening before this survey?	Yes	124	14	25	163	0.63	2	0.78
	No	31	2	7	40			
Through searches on the internet	Yes	8	1	2	11	0.09	2	1.00
	No	147	15	30	192			
From other advertising on the internet	Yes	3	0	0	3	0.94	2	0.62
	No	152	16	32	200			
Other forms of advertising	Yes	2	0	0	2	0.63	2	1.00
	No	153	16	32	201			
Through social media	Yes	6	1	4	11	3.88	2	0.12
	No	149	15	28	192			
From films, television or other media (e.g. newspaper or magazine articles)	Yes	6	1	2	9	0.49	2	0.78
	No	149	15	30	194			
Friends	Yes	17	5	7	29	6.66	2	**0.03**
	No	138	11	25	174			
Family	Yes	14	3	1	18	3.25	2	0.20
	No	141	13	31	185			
Formal studies	Yes	32	6	8	46	2.47	2	0.30
	No	123	10	24	157			
From participating in research	Yes	13	1	2	16	0.23	2	0.90
	No	142	15	30	187			
From a healthcare professional	Yes	25	0	1	26	6.57	2	**0.04**
	No	130	16	31	177			
Through my work	Yes	81	7	16	104	0.44	2	0.77
	No	74	9	16	99			

Note: Bold numbers indicate significant association

Table S5: Cross tabulation of social demographic factors and intentions to take the test

profession				would_you_accept_the_test		Total
				Yes	No/Unsure	
Practitioner	Heard about PCS from friends	Yes	Count	9	9	18
			% within Heard about PCS from friends	50.0%	50.0%	100.0%
Non-practitioner	Heard about PCS from friends	Yes	Count	8	3	11
			% within Heard about PCS from friends	72.7%	27.3%	100.0%
Total	Heard about PCS from friends	Yes	Count	17	12	29
			% within Heard about PCS from friends	58.6%	41.4%	100.0%

profession				would_you_accept_the_test		Total
				Yes	Unsure	
Practitioner	Heard about PCS from a healthcare professional	Yes	Count	14	1	15
			% within Heard about PCS from a healthcare professional	93.3%	6.7%	100.0%
Non-practitioner	Heard about PCS from a healthcare professional	Yes	Count	11	-	11
			% within Heard about PCS from a healthcare professional	100.0%	-	100.0%
Total	Heard about PCS from a healthcare professional	Yes	Count	25	1	26
			% within Heard about PCS from a healthcare professional	96.2%	3.8%	100.0%

Table S6A: Attitudes of health professionals and intentions to take the test

Bold numbers represent proportions of health professionals with intentions to use the test

% of HP that agreed to attitude statements supporting use and implementation of PCS in WA and decision (yes, no, unsure)				
Questions	Attitude Statements	No (%)	Unsure (%)	Yes (%)
1	Provides couples with reproductive choices	7.4%	15.3%	**75.9%**
20	A post-test consultation with a genetic counsellor would be essential.	7.4%	14.8%	**71.9%**
2	Should be offered in Western Australia	6.9%	15.3%	**70.0%**
4	Will reduce suffering associated with recessive diseases	5.9%	13.8%	**61.6%**
21	If the costs were the same, I would prefer to be tested for a larger number of recessive diseases rather than for a smaller number.	6.4%	13.8%	**59.6%**
3	Should be made available for all couples planning a pregnancy	5.9%	13.3%	**59.1%**
16	I think that being screened for severe recessive diseases before pregnancy is the right thing to do.	4.9%	12.3%	**51.7%**
10	It is difficult for a person with a severe recessive diseases to have a very good quality of life.	3.9%	6.4%	36.9%
19	Preconception carrier screening may result in an increase in my insurance rates.	2.0%	6.9%	23.2%
13	I think it is wrong to knowingly bring a child with a severe recessive diseases into the world.	2.0%	5.9%	20.2%
22	Preconception carrier screening should only be offered to people with a family history of a recessive disease.	1.5%	3.4%	14.8%
5	Will reduce the reason to find a cure and/or improve treatments for severe recessive diseases	2.5%	3.4%	10.8%
17	If I had a preconception carrier screening test, I would worry that the results might not remain confidential.	1.0%	4.9%	10.3%
11	It would be better if no one with a severe recessive diseases had been born.	1.5%	3.4%	10.8%
6	Will lead to discrimination of people with recessive diseases	1.0%	2.5%	9.9%
12	Raising a child with a severe recessive diseases would be rewarding for me.	0..5%	1.5%	8.9%
18	My religion would be important in my decision to participate in preconception carrier screening.	0.0%	2.0%	6.9%
7	Will not improve people's lives	0.0%	1.0%	5.4%
15	I will be discriminated against if I am identified as a carrier.	0.0%	1.0%	3.0%
9	Is morally unacceptable	0.5%	0.5%	1.5%
8	Will do more harm than good	0.0%	1.0%	1.5%
14	I think that children with severe recessive diseases are ''manifestations of God's will''.	0.0%	0.0%	1.0%

Table S6B: Attitudes of health professionals and intentions to take the test (con't)

Bold numbers represent proportions of health professionals with intentions to use the test

% of HP that disagreed to attitude statements that reflected fears and distrusts in having PCS in WA and decision (yes, no, unsure)				
Questions	Attitude Statements	No (%)	Unsure (%)	Yes (%)
9	Is morally unacceptable	6.9%	13.3%	**68.0%**
8	Will do more harm than good	6.9%	13.3%	**67.0%**
14	I think that children with severe recessive diseases are ‘‘manifestations of God’s will’’.	7.4%	13.3%	**65.5%**
7	Will not improve people’s lives	6.9%	14.3%	**61.1%**
15	I will be discriminated against if I am identified as a carrier.	6.4%	10.3%	**57.6%**
17	If I had a preconception carrier screening test, I would worry that the results might not remain confidential.	5.9%	10.3%	**57.1%**
18	My religion would be important in my decision to participate in preconception carrier screening.	6.9%	11.8%	**51.7%**
5	Will reduce the reason to find a cure and/or improve treatments for severe recessive diseases	3.9%	9.9%	**52.2%**
22	Preconception carrier screening should only be offered to people with a family history of a recessive disease.	4.4%	11.3%	**49.3%**
6	Will lead to discrimination of people with recessive diseases	5.4%	9.4%	**49.8%**
11	It would be better if no one with a severe recessive diseases had been born.	4.4%	9.4%	**47.8%**
12	Raising a child with a severe recessive diseases would be rewarding for me.	3.0%	8.4%	**43.3%**
13	I think it is wrong to knowingly bring a child with a severe recessive diseases into the world.	2.0%	6.4%	30.5%
10	It is difficult for a person with a severe recessive diseases to have a very good quality of life.	3.0%	6.9%	26.1%
19	Preconception carrier screening may result in an increase in my insurance rates.	1.5%	2.0%	19.2%
3	Should be made available for all couples planning a pregnancy	1.5%	1.5%	8.9%
16	I think that being screened for severe recessive diseases before pregnancy is the right thing to do.	0.0%	1.5%	7.9%
21	If the costs were the same, I would prefer to be tested for a larger number of recessive diseases rather than for a smaller number.	1.0%	0.5%	6.9%
4	Will reduce suffering associated with recessive diseases	0.5%	1.5%	4.4%
20	A post-test consultation with a genetic counsellor would be essential.	0.5%	0.5%	1.5%
2	Should be offered in Western Australia	0.5%	0.0%	2.0%
1	Provides couples with reproductive choices	0.5%	0.0%	0.5%

Table S6C: Attitudes of health professionals and intentions to take the test (con't)

Bold numbers represent proportions of health professionals with intentions to use the test

% of HP that were ambivalent to statements and decision (yes, no, unsure)				
Questions	Attitude Statements	No (%)	Unsure (%)	Yes (%)
19	Preconception carrier screening may result in an increase in my insurance rates.	4.4%	6.9%	**34.0%**
12	Raising a child with a severe recessive diseases would be rewarding for me.	4.4%	5.9%	24.1%
13	I think it is wrong to knowingly bring a child with a severe recessive diseases into the world.	3.9%	3.4%	25.6%
11	It would be better if no one with a severe recessive diseases had been born.	2.0%	3.0%	17.7%
6	Will lead to discrimination of people with recessive diseases	1.5%	3.9%	16.7%
16	I think that being screened for severe recessive diseases before pregnancy is the right thing to do.	3.0%	2.0%	16.7%
15	I will be discriminated against if I am identified as a carrier.	1.5%	4.4%	15.8%
18	My religion would be important in my decision to participate in preconception carrier screening.	1.0%	2.0%	17.7%
5	Will reduce the reason to find a cure and/or improve treatments for severe recessive diseases	1.5%	2.5%	13.3%
10	It is difficult for a person with a severe recessive diseases to have a very good quality of life.	1.0%	2.5%	13.3%
22	Preconception carrier screening should only be offered to people with a family history of a recessive disease.	2.0%	1.0%	12.3%
14	I think that children with severe recessive diseases are ‘‘manifestations of God’s will’’.	0.5%	2.5%	9.9%
4	Will reduce suffering associated with recessive diseases	1.5%	0.5%	10.3%
21	If the costs were the same, I would prefer to be tested for a larger number of recessive diseases rather than for a smaller number.	0.5%	1.5%	9.9%
7	Will not improve people’s lives	1.0%	0.5%	9.9%
8	Will do more harm than good	1.0%	1.5%	7.9%
17	If I had a preconception carrier screening test, I would worry that the results might not remain confidential.	1.0%	0.5%	8.9%
3	Should be made available for all couples planning a pregnancy	0.5%	1.0%	8.4%
9	Is morally unacceptable	0.5%	2.0%	6.9%
2	Should be offered in Western Australia	0.5%	0.5%	4.4%
20	A post-test consultation with a genetic counsellor would be essential.	0.0%	0.5%	3.0%
1	Provides couples with reproductive choices	0.0%	0.5%	0.0%

Table S7A: Breakdown of health professionals and attitude to specific statements

Bold numbers represent greater proportions of health professionals supporting statement

			Practitioner (n=118)					Non-Practitioner (n=85)				Total
			Allied health practitioner	Clinician	Genetic counsellor	Nurse/ Midwife	Mean	Diagnostic lab scientist	Researcher	Other technical positions	Mean	
If the costs were the same, I would prefer to be tested for a larger number of recessive diseases rather than for a smaller number.	Don't know/NA	Count	**8**	3	**4**	3	17.7%	3	3	0	10.6%	24
		% within specific occupation	**22.2%**	7.5%	**30.8%**	10.3%		27.3%	4.5%	0.0%		11.8%
	Disagree	Count	2	7	0	5	10.1%	1	2	0	4.0%	17
		% within specific occupation	5.6%	17.5%	0.0%	17.2%		9.1%	3.0%	0.0%		8.4%
	Agree	Count	26	30	9	21	72.2%	7	61	8	85.4%	162
		% within specific occupation	72.2%	75.0%	69.2%	72.4%		63.6%	92.4%	100.0%		79.8%
Total		Count	36	40	13	29	100.0%	11	66	8	100.0%	203
		% within specific occupation	100.0%	100.0%	100.0%	100.0%		100.0%	100.0%	100.0%		100.0%

			Practitioner (n=118)					Non-Practitioner (n=85)				Total
			Allied health practitioner	Clinician	Genetic counsellor	Nurse/ Midwife	Mean	Diagnostic lab scientist	Researcher	Other technical positions	Mean	
I think that being screened for severe recessive diseases before pregnancy is the right thing to do.	Don't know/NA	Count	**12**	9	**7**	6	32.6%	1	8	1	11.2%	44
		% within specific occupation	**33.3%**	22.5%	**53.8%**	20.7%		9.1%	12.1%	12.5%		21.7%
	Disagree	Count	4	6	0	2	8.3%	0	7	0	3.5%	19
		% within specific occupation	11.1%	15.0%	0.0%	6.9%		0.0%	10.6%	0.0%		9.4%
	Agree	Count	20	25	6	21	59.2%	10	51	7	85.2%	140
		% within specific occupation	55.6%	62.5%	46.2%	72.4%		90.9%	77.3%	87.5%		69.0%
Total		Count	36	40	13	29	100.0%	11	66	8	100.0%	203
		% within specific occupation	100.0%	100.0%	100.0%	100.0%		100.0%	100.0%	100.0%		100.0%

			Practitioner (n=118)					Non-Practitioner (n=85)				Total
			Allied health practitioner	Clinician	Genetic counsellor	Nurse/ Midwife	Mean	Diagnostic lab scientist	Researcher	Other technical positions	Mean	
I will be discriminated against if I am identified as a carrier.	Don't know/NA	Count	6	8	0	5	13.5%	**5**	16	**4**	39.9%	44
		% within specific occupation	16.7%	20.0%	0.0%	17.2%		**45.5%**	24.2%	**50.0%**		21.7%
	Disagree	Count	27	31	12	24	81.9%	6	47	4	58.6%	151
		% within specific occupation	75.0%	77.5%	92.3%	82.8%		54.5%	71.2%	50.0%		74.4%
	Agree	Count	3	1	1	0	4.6%	0	3	0	1.5%	8
		% within specific occupation	8.3%	2.5%	7.7%	0.0%		0.0%	4.5%	0.0%		3.9%
Total		Count	36	40	13	29	100.0%	11	66	8	100.0%	203
		% within specific occupation	100.0%	100.0%	100.0%	100.0%		100.0%	100.0%	100.0%		100.0%

Table S7B: Breakdown of health professionals and attitude to specific statements (con't)

Bold numbers represent greater proportions of health professionals supporting statement

			Practitioner (n=118)					Non-Practitioner (n=85)				
			Allied health practitioner	Clinician	Genetic counsellor	Nurse/ Midwife	Mean	Diagnostic lab scientist	Researcher	Other technical positions	Mean	Total
If I had a preconception carrier screening test, I would worry that the results might not remain confidential	Don't know/NA	Count	4	4	0	2	7.0%	2	8	1	14.3%	21
		% within specific occupation	11.1%	10.0%	0.0%	6.9%		18.2%	12.1%	12.5%		10.3%
	Disagree	Count	27	29	12	25	81.5%	6	43	7	69.1%	149
		% within specific occupation	75.0%	72.5%	92.3%	86.2%		54.5%	65.2%	87.5%		73.4%
	Agree	Count	5	7	1	2	11.5%	**3**	**15**	0	16.7%	33
		% within specific occupation	13.9%	17.5%	7.7%	6.9%		**27.3%**	**22.7%**	0.0%		16.3%
Total		Count	36	40	13	29	100.0%	11	66	8	100.0%	203
		% within specific occupation	100.0%	100.0%	100.0%	100.0%		100.0%	100.0%	100.0%		100.0%

			Practitioner (n=118)					Non-Practitioner (n=85)				
			Allied health practitioner	Clinician	Genetic counsellor	Nurse/ Midwife	Mean	Diagnostic lab scientist	Researcher	Other technical positions	Mean	Total
Will reduce the reason to find a cure and/or improve treatments for severe recessive diseases.	Don't know/NA	Count	**11**	**9**	2	6	20.0%	0	6	1	6.7%	35
		% within specific occupation	**31.4%**	**25.7%**	5.7%	17.1%		0.0%	17.1%	2.9%		17.2%
	Disagree	Count	18	26	10	18	13.4%	10	47	5	15.4%	134
		% within specific occupation	13.4%	19.4%	7.5%	13.4%		7.5%	35.1%	3.7%		66.0%
	Agree	Count	7	5	1	5	13.2%	1	**13**	2	15.7%	34
		% within specific occupation	20.6%	14.7%	2.9%	14.7%		2.9%	**38.2%**	5.9%		16.7%
Total		Count	36	40	13	29	100.0%	11	66	8	100.0%	203
		% within specific occupation	100.0%	100.0%	100.0%	100.0%		100.0%	100.0%	100.0%		100.0%

			Practitioner (n=118)					Non-Practitioner (n=85)				
			Allied health practitioner	Clinician	Genetic counsellor	Nurse/ Midwife	Mean	Diagnostic lab scientist	Researcher	Other technical positions	Mean	Total
It would be better if no one with a severe recessive diseases had been born.	Don't know/NA	Count	9	3	3	4	17.3%	**6**	**19**	2	36.1%	46
		% within specific occupation	25.0%	7.5%	23.1%	13.8%		**54.5%**	**28.8%**	25.0%		22.7%
	Disagree	Count	26	29	10	22	74.4%	3	30	5	45.1%	125
		% within specific occupation	72.2%	72.5%	76.9%	75.9%		27.3%	45.5%	62.5%		61.6%
	Agree	Count	1	8	0	3	8.3%	2	**17**	1	18.8%	32
		% within specific occupation	2.8%	20.0%	0.0%	10.3%		18.2%	**25.8%**	12.5%		15.8%
Total		Count	36	40	13	29	100.0%	11	66	8	100.0%	203
		% within specific occupation	100.0%	100.0%	100.0%	100.0%		100.0%	100.0%	100.0%		100.0%

Table S8: Difference in attitudes towards PCS of health professionals and the community

Agreed to attitude statements		**% Health Professionals**	**% Community**	***% Diff***
Qsn	Attitude Statements			
1	Provides couples with reproductive choices.	99%	77%	*22%*
20	A post-test consultation with a genetic counsellor would be essential.	94%	68%	*27%*
2	Should be offered in Western Australia.	92%	76%	*16%*
4	Will reduce suffering associated with recessive diseases.	81%	67%	*15%*
21	If the costs were the same, I would prefer to be tested for a larger number of recessive diseases rather than for a smaller number.	80%	71%	*9%*
3	Should be made available for all couples planning a pregnancy.	78%	74%	*4%*
16	I think that being screened for severe recessive diseases before pregnancy is the right thing to do.	69%	61%	*8%*
	Mean	85%	71%	*14%*

Note: % reflect proportion with respect to total numbers in a cohort. For example, 203 participants in "Health Professionals" and 832 participants in "Community".

Disagreed to attitude statements		**% Health Professionals**	**% Community**	***% Diff***
Qsn	Attitude Statements			
9	Is morally unacceptable	88%	60%	*28%*
8	Will do more harm than good	87%	59%	*29%*
14	I think that children with severe recessive diseases are ‘‘manifestations of God’s will’’.	86%	55%	*31%*
7	Will not improve people’s lives	82%	55%	*27%*
15	I will be discriminated against if I am identified as a carrier.	74%	47%	*27%*
17	If I had a preconception carrier screening test, I would worry that the results might not remain confidential.	73%	45%	*28%*
18	My religion would be important in my decision to participate in preconception carrier screening.	70%	55%	*16%*
5	Will reduce the reason to find a cure and/or improve treatments for severe recessive diseases	66%	36%	*30%*
22	Preconception carrier screening should only be offered to people with a family history of a recessive disease.	65%	43%	*22%*
6	Will lead to discrimination of people with recessive diseases	65%	41%	*23%*
11	It would be better if no one with a severe recessive disease had been born.	62%	46%	*16%*
12	Raising a child with a severe recessive diseases would be rewarding for me.	55%	40%	*14%*
	Mean	73%	49%	*24%*

Note: % reflect proportion with respect to total numbers in a cohort. For example, 203 participants in "Health Professionals" and 832 participants in "Community".

CHAPTER 3:

Section 1

Designing and optimising a comprehensive carrier-screening gene panel

Royston Ong

Supplementary information

Chapter 3, Section 1 Supplementary Table 1

Gene	Disease name	Pheme #	Chr#	Disease Type	Severity Category
AARS2	Combined oxidative phosphorylation deficiency 8	614096	6p21.1	Mitochondrial	1
ABAT	GABA-transaminase deficiency	613163	16p13.2	Neurodegenerative	1
ABCA12	Ichthyosis autosomal recessive 4B (harlequin)	242500	2q35	Cutaneous	1
ABCA3	Surfactant metabolism dysfunction pulmonary 3	610921	16p13.3	Respiratory	1
ABCC6	Arterial calcification generalized of infancy 2	614473	16p13.11	Cardiovascular	1
ABCD1	Adrenoleukodystrophy	300100	Xq28	Neurodegenerative	1
ACAD9	Mitochondrial complex I deficiency due to ACAD9 deficiency	611126	3q21.3	Mitochondrial	1
ACADS	Short Chain Acyl-CoA Dehydrogenase Deficiency	201470	12q24.31	Metabolic	2
ACADVL	VLCAD deficiency	201475	17p13.1	Metabolic	1
ACAT1	Ketothiolase Deficiency/Beta-ketothiolase deficiency	203750	11q22.3	Metabolic	3
ACE	Renal tubular dysgenesis	267430	17q23.3	Renal	1
ADAMTS2	Ehlers-Danlos syndrome type VIIC	225410	5q35.3	Cutaneous	1
ADAMTSL2	Geleophysic dysplasia 1	231050	9q34.2	Lysosomal	1
AGA	aspartylglucosaminuria	208400	4q34.3	Lysosomal	2
AGK	Sengers syndrome	212350	7q34	Mitochondrial	1
AGL	Glycogen storage disease IIIa	232400	1p21.2	Metabolic	1
AGT	Renal tubular dysgenesis	267430	1q42.2	Renal	1
AGTR1	Renal tubular dysgenesis	267430	3q24	Renal	1
AGXT	Primary Hyperoxaluria Type 1	259900	2q37.3	Metabolic	2
AIRE	Polyglandular Autoimmune Syndrome Type 1	240300	21q22.3	Immunodeficiency	3
AK2	Reticular dysgenesis	267500	1p35.1	Immunodeficiency	1
ALDH3A2	Sjogren-Larsson Syndrome	270200	17p11.2	Developmental	2
ALDH7A1	Epilepsy pyridoxine-dependent	266100	5q23.2	Neurologic	1
ALG1	Congenital disorder of glycosylation type Ik	608540	16p13.3	Metabolic	1
ALG8	Congenital disorder of glycosylation type Ih	608104	11q14.1	Metabolic	1
ALPL	Hypophosphatasia infantile	241500	1p36.12	Skeletal	1
AMT	Glycine encephalopathy	605899	3p21.31	Metabolic	1
ANTXR2	Hyaline fibromatosis syndrome	228600	4q21.21	Cutaneous	1
APOPT1	Mitochondrial complex IV deficiency	220110	14q32.33	Mitochondrial	1
ARHGDIA	Nephrotic syndrome type 8	615244	17q25.3	Renal	1
ARSA	Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)	250100	22q13.33	Lysosomal	2
ARSB	Mucopolysaccharoidosis Type VI/Maroteaux-Lamy (AJ Population)	253200	5q14.1	Lysosomal	2
ARX	Hydranencephaly with abnormal genitalia	300215	Xp21.3	Developmental	1
ASAH1	Farber lipogranulomatosis	228000	8p22	Lysosomal	1
ASL	Argininosuccinic aciduria	207900	7q11.21	Metabolic	1
ASNS	Asparagine synthetase deficiency	615574	7q21.3	Metabolic	1
ASPA	Canavan disease	271900	17p13.2	Metabolic	1

ASS1	Citrullinemia	215700	9q34.11	Metabolic	1
ATM	Ataxia-Telangiectasia	208900	11q22.3	Neurodegenerative	3
ATP7A	Menkes disease	309400	Xq21.1	Metabolic	1
ATP7B	Wilson Disease	277900	13q14.3	Metabolic	2
ATPAF2	Mitochondrial complex V ATP synthase) deficiency nuclear type 1	604273	17p11.2	Mitochondrial	1
B4GAT1	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A	615287	11q13.2	Neuromuscular	1
B9D2	Meckel syndrome 10	614175	19q13.2	Developmental	1
BBS1	Bardet-Biedl Syndrome BBS1-Related	209900	11q13.2	Developmental	2
BBS10	Bardet-Biedl Syndrome BBS10-Related	615987	12q21.2	Developmental	2
BCAP31	Deafness dystonia and cerebral hypomyelination	300475	Xq28	Neurologic	1
BCKDHA	Maple syrup urine disease type Ia	248600	19q13.2	Metabolic	1
BCKDHB	Maple syrup urine disease type Ib	248600	6q14.1	Metabolic	1
BCOR	Microphthalmia syndromic 2	300166	Xp11.4	Developmental	1
BCS1L	GRACILE syndrome	603358	2q35	Metabolic	1
BLM	Bloom Syndrome (AJ Population)	210900	15q26.1	Developmental	2
BMPER	Diaphanospondylodysostosis	608022	7p14.3	Skeletal	1
BOLA3	Multiple mitochondrial dysfunctions syndrome 2	614299	2p13.1	Mitochondrial	1
BRAT1	Rigidity and multifocal seizure syndrome lethal neonatal	614498	7p22.3	Neurodegenerative	1
BSCL2	Encephalopathy progressive with or without lipodystrophy	615924	11q12.3	Neurodegenerative	1
BTD	Biotinidase Deficiency	253260	3p25.1	Metabolic	3
C10orf2	Mitochondrial DNA depletion syndrome 7 (hepatocerebral type)	271245	10q24.31	Mitochondrial	1
CBS	Homocystinuria (B6-responsive and nonresponsive types)	236200	21q22.3	Metabolic	1
CC2D2A	Meckel syndrome 6	612284	4p15.32	Developmental	1
CD27	Lymphoproliferative syndrome 2	615122	12p13.31	Immunodeficiency	1
CD3D	Immunodeficiency 19	615617	11q23.3	Immunodeficiency	1
CD3E	Immunodeficiency 18	615615	11q23.3	Immunodeficiency	1
CD40LG	Immunodeficiency X-linked with hyper-IgM	308230	Xq26.3	Immunodeficiency	1
CEP120	Short-rib thoracic dysplasia 13 with or without polydactyly	616300	5q23.2	Skeletal	1
CEP290	Meckel syndrome 4	611134	12q21.32	Developmental	1
CFL2	Nemaline myopathy 7 autosomal recessive	610687	14q13.1	Neuromuscular	1
CFTR	Cystic Fibrosis	277180	7q31.2	Developmental	3
CHRNA1	Multiple pterygium syndrome lethal type	253290	2q31.1	Neuromuscular	1
CHRND	Multiple pterygium syndrome lethal type	253290	2q37.1	Neuromuscular	1
CHRNG	Escobar syndrome	265000	2q37.1	Developmental	1
CHUK	Cocoon syndrome	613630	10q24.31	Developmental	1
CIITA	Bare lymphocyte syndrome type II complementation group A	209920	16p13.13	Immunodeficiency	1
CLN3	CLN3-Related Neuronal Ceroid Lipofuscinosis/Juvenile Batten Disease	204200	16p11.2	Neurodegenerative	2
CLN5	Ceroid lipofuscinosis neuronal 5	256731	13q22.3	Neurodegenerative	2
CLN6	Ceroid lipofuscinosis neuronal 6	204300	15q23	Neurodegenerative	1
CLN8	Ceroid lipofuscinosis neuronal 8	600143	8p23.3	Neurodegenerative	2
CLPB	3-methylglutaconic aciduria type VII with cataracts neurologic involvement and neutropenia	616271	11q13.4	Metabolic	1

CLRN1	Usher Syndrome Type 3	614180	3q25.1	Ocular	3
CNTNAP1	Lethal congenital contracture syndrome 7	616286	17q21.2	Developmental	1
COA5	Mitochondrial complex IV deficiency	220110	2q11.2	Mitochondrial	1
COG6	Congenital disorder of glycosylation type Iil	614576	13q14.11	Metabolic	1
COG7	Congenital disorder of glycosylation type Iie	608779	16p12.2	Metabolic	1
COL11A1	Fibrochondrogenesis 1	228520	1p21.1	Skeletal	1
COL7A1	EBD inversa	226600	3p21.31	Cutaneous	1
COQ2	Coenzyme Q10 deficiency primary 1	607426	4q21.23	Metabolic	1
COQ4	Coenzyme Q10 deficiency primary 7	616276	9q34.11	Metabolic	1
COQ6	Coenzyme Q10 deficiency primary6	614650	14q24.3	Metabolic	1
COQ9	Coenzyme Q10 deficiency primary 5	614654	16q21	Metabolic	1
COX10	Leigh syndrome due to mitochondrial COX4 deficiency	256000	17p12	Mitochondrial	1
COX15	Cardioencephalomyopathy, fatal infantile due to cytochrome c oxidase deficiency 2	615119	10q24.2	Cardiovascular	1
COX20	Mitochondrial complex IV deficiency	220110	1q44	Mitochondrial	1
CPS1	Carbamoylphosphate synthetase I deficiency	237300	2q34	Metabolic	1
CPT1A	carnitine palmitoyltransferase I (CPT I) deficiency	255120	11q13.3	Metabolic	2
CPT2	CPT II deficiency lethal neonatal	608836	1p32.3	Metabolic	1
CRB2	Ventriculomegaly with cystic kidney disease	219730	9q33.3	Developmental	1
CRLF1	Cold-induced sweating syndrome 1	272430	19p13.11	Neurologic	1
CRTAP	Osteogenesis imperfecta type VII	610682	3p22.3	Skeletal	1
CRYAB	Myopathy myofibrillar fatal infantile hypertrophy alpha-B crystallin-related	613869	11q23.1	Neuromuscular	1
CSPP1	Joubert syndrome 21	615636	8q13.1-q13.2	Neurologic	1
CTNS	Cystinosis	219800	17p13.2	Lysosomal	2
CTSD	Ceroid lipofuscinosis neuronal 10	610127	11p15.5	Neurodegenerative	1
CTSK	Pycnodysostosis	265800	1q21.3	Skeletal	3
CYP11B1	Adrenal hyperplasia congenital due to 11-beta-hydroxylase deficiency	202010	8q24.3	Endocrine	1
CYP1B1	Primary congenital glaucoma	231300	2p22.2	Ocular	3
CYP26B1	Craniosynostosis with radiohumeral fusions and other skeletal and craniofacial anomalies	614416	2p13.2	Skeletal	1
DBT	Maple syrup urine disease type II	248600	1p21.2	Metabolic	1
DCLRE1C	Omenn syndrome	603554	10p13	Immunodeficiency	1
DDR2	Spondylometaepiphyseal dysplasia short limb-hand type	271665	1q23.3	Skeletal	1
DGUOK	Mitochondrial DNA depletion syndrome 3 (hepatocerebral type)	251880	2p13.1	Mitochondrial	1
DHCR7	Smith-Lemli-Opitz Syndrome	270400	11q13.4	Developmental	2
DIS3L2	Perlman syndrome	267000	2q37.1	Developmental	1
DLD	Dihydrolipoamide dehydrogenase deficiency	246900	7q31.1	Metabolic	1
DLL3	Spondylocostal dysostosis 1 autosomal recessive	277300	19q13.2	Skeletal	1
DMD	Duchenne/Becker Muscular Dystrophy	310200	Xp21.2-p21.1	Neuromuscular	2
DMPK	Myotonic Dystrophy 1	160900	19q13.32	Neuromuscular	1
DNAJC19	3-methylglutaconic aciduria type V	610198	3q26.33	Metabolic	1
DNM2	Lethal congenital contracture syndrome 5	615368	19p13.2	Developmental	1
DNMT3B	Immunodeficiency-centromeric instability-facial anomalies syndrome 1	242860	20q11.21	Immunodeficiency	1

DOCK8	Hyper-IgE recurrent infection syndrome autosomal recessive	243700	9p24.3	Immunodeficiency	1
DOLK	Congenital disorder of glycosylation type Im	610768	9q34.11	Metabolic	1
DPAGT1	Congenital disorder of glycosylation type Ij	608093	11q23.3	Metabolic	1
DPM2	Congenital disorder of glycosylation type Iu	615042	9q34.11	Metabolic	1
DPYD	Hereditary Thymine-Uraciluria / Dihydropyrimidine Dehydrogenase Deficiency	274270	1p21.3	Metabolic	2
DSP	Epidermolysis bullosa lethal acantholytic	609638	6p24.3	Cutaneous	1
DYNC2H1	Short-rib thoracic dysplasia 3 with or without polydactyly	613091	11q22.3	Skeletal	1
DYSF	Limb-Girdle Muscular Dystrophy Type 2B	253601	2p13.2	Neuromuscular	2
EDA	Hypohidrotic ectodermal dysplasia (HED) X Linked	305100	Xq13.1	Cutaneous	1
EDNRB	ABCD syndrome	600501	13q22.3	Developmental	1
EFEMP2	Cutis laxa autosomal recessive type IB	614437	11q13.1	Cutaneous	1
EIF2B1	Leukoencephalopathy with vanishing white matter	603896	12q24.31	Neurodegenerative	1
EIF2B2	Leukoencephalopathy with vanishing white matter	603896	14q24.3	Neurodegenerative	1
EIF2B3	Leukoencephalopathy with vanishing white matter	603896	1p34.1	Neurodegenerative	1
EIF2B4	Leukoencephaly with vanishing white matter	603896	2p23.3	Neurodegenerative	1
EIF2B5	Leukoencephalopathy with vanishing white matter	603896	3q27.1	Neurodegenerative	1
ELAC2	Combined oxidative phosphorylation deficiency 17	615440	17p12	Mitochondrial	1
ELOVL4	Ichthyosis spastic quadriplegia and mental retardation	614457	6q14.1	Developmental	1
EMG1	Bowen-Conradi syndrome	211180	12p13.31	Developmental	1
ENPP1	Arterial calcification generalized of infancy 1	208000	6q23.2	Cardiovascular	1
EPG5	Vici syndrome	242840	18q12.3-q21.1	Developmental	1
EPM2A	Epilepsy progressive myoclonic 2A Lafora)	254780	6q24.3	Neurologic	1
ERBB3	Lethal congenital contractural syndrome 2	607598	12q13.2	Developmental	1
ERCC1	Cerebrooculofacioskeletal syndrome 4	610758	19q13.32	Developmental	1
ERCC4	XFE progeroid syndrome	610965	16p13.12	Developmental	1
ESCO2	Roberts syndrome	268300	8p21.1	Developmental	1
ETFA	Glutaric acidemia IIA	231680	15q24.2-q24.3	Metabolic	1
ETFB	Glutaric acidemia IIB	231680	19q13.41	Metabolic	1
ETFDH	Glutaric acidemia IIC	231680	4q32.1	Metabolic	1
ETHE1	Ethylmalonic encephalopathy	602473	19q13.31	Metabolic	1
EXOSC3	Pontocerebellar hypoplasia type 1B	614678	9p13.2	Neurodegenerative	1
EXOSC8	Pontocerebellar hypoplasia type 1C	616081	13q13.3	Neurodegenerative	1
F7	Factor VII deficiency	227500	13q34	Hematologic	1
FADD	Infections recurrent with encephalopathy hepatic dysfunction and cardiovasuclar malformations	613759	11q13.3	Developmental	1
FAH	Tyrosinemia Type I	276700	15q25.1	Metabolic	2
FAM20C	Raine syndrome	259775	7p22.3	Skeletal	1
FANCB	Fanconi anemia complementation group B	300514	Xp22.2	Hematologic	1
FANCC	Fanconi Anemia Type C (AJ Population)	227645	9q22.32	Hematologic	2
FARS2	Combined oxidative phosphorylation deficiency 14	614946	6p25.1	Mitochondrial	1
FBLN5	Cutis laxa autosomal recessive type IA	219100	14q32.12	Cutaneous	1

FBXL4	Mitochondrial DNA depletion syndrome 13 (encephalomyopathic type)	615471	6q16.1	Mitochondrial	1
FGA	Afibrinogenemia congenital	202400	4q31.3	Hematologic	1
FGB	Afibrinogenemia congenital	202400	4q31.3	Hematologic	1
FGFR2	Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis	207410	10q26.13	Developmental	1
FGG	Afibrinogenemia congenital	202400	4q31.3	Hematologic	1
FH	Fumarase deficiency	606812	1q43	Metabolic	1
FIG4	Yunis-Varon syndrome	216340	6q21	Lysosomal	1
FKRP	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A5	613153	19q13.32	Neuromuscular	1
FKTN	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A4	253800	9q31.2	Neuromuscular	1
FLNA	Congenital short bowel syndrome	300048	Xq28	Gastroenterologic	1
FLVCR2	Proliferative vasculopathy and hydraencephaly-hydrocephaly syndrome	225790	14q24.3	Developmental	1
FOXP3	Immunodysregulation(polyendocrinopathy and enteropathy X-linked	304790	Xp11.23	Immunodeficiency	1
FOXRED1	Leigh syndrome due to mitochondrial complex I deficiency	256000	11q24.2	Mitochondrial	1
FRAS1	Fraser syndrome	219000	4q21.21	Developmental	1
FREM2	Fraser syndrome	219000	13q13.3	Developmental	1
FTO	Growth retardation(developmental delay coarse facies and early death	612938	16q12.2	Developmental	1
FUCA1	Fucosidosis	230000	1p36.11	Lysosomal	1
G6PC	Glycogen Storage Disease Type Ia	232200	17q21.31	Metabolic	3
G6PC3	Dursun syndrome	612541	17q21.31	Immunodeficiency	1
GAA	Glycogen storage disease II	232300	17q25.3	Lysosomal	1
GALC	Krabbe disease	245200	14q31.3	Lysosomal	1
GALNS	Mucopolysaccharoidosis Type IVA	253000	16q24.3	Lysosomal	3
GALT	Galactosemia	230400	9p13.3	Metabolic	2
GBA	Gaucher disease (perinatal lethal)	608013	1q22	Metabolic	1
GBE1	Glycogen storage disease IV	232500	3p12.2	Metabolic	1
GCDH	Glutaricaciduria type I	231670	19p13.2	Metabolic	1
GCSH	Glycine encephalopathy	605899	16q23.2	Metabolic	1
GDF1	Right atrial isomerism	208530	19p13.11	Cardiovascular	1
GDF5	Chondrodysplasia Grebe type	200700	20q11.22	Skeletal	1
GFM1	Combined oxidative phosphorylation deficiency 1	609060	3q25.32	Mitochondrial	1
GJA1	Hypoplastic left heart syndrome 1	241550	6q22.31	Cardiovascular	1
GLA	Fabry disease	301500	Xq22.1	Lysosomal	1
GLB1	GM1-gangliosidosis, type I	230500	3p22.3	Lysosomal	1
GLDC	Glycine encephalopathy	605899	9p24.1	Metabolic	1
GLE1	Arthrogryposis, lethal with anterior horn cell disease	611890	9q34.11	Neuromuscular	1
GLUL	Glutamine deficiency congenital	610015	1q25.3	Metabolic	1
GNE	Inclusion Body Myopathy 2	600737	9p13.3	Neuromuscular	2
GNPTAB	Mucolipidosis II alpha/beta	252500	12q23.2	Lysosomal	1
GPHN	Molybdenum cofactor deficiency C	615501	14q23.3	Metabolic	1
GRHPR	Primary Hyperoxaluria Type 2	260000	9p13.2	Metabolic	3
GRIP1	Fraser syndrome	219000	12q14.3	Developmental	1

GSS	Glutathione synthetase deficiency	266130	20q11.22	Metabolic	1
GUSB	Mucopolysaccharoidosis Type VII	253220	7q11.21	Lysosomal	2
HADH	3-hydroxyacyl-CoA dehydrogenase deficiency	231530	4q25	Metabolic	1
HADHA	Fatty liver acute of pregnancy	609016	2p23.3	Metabolic	1
HADHB	Trifunctional protein deficiency	609015	2p23.3	Mitochondrial	1
HADHSC	Hyperinsulinemic hypoglycemia familial4	609975	4q25	Metabolic	1
HBB	Beta Thalassemia	603903	11p15.4	Hematologic	2
HEXA	GM2-gangliosidosis several forms	272800	15q23	Lysosomal	1
HEXB	Sandhoff disease(infantile juvenile and adult forms	268800	5q13.3	Lysosomal	1
HGSNAT	Mucopolysaccharidosis type IIIC Sanfilippo C)	252930	8p11.21	Lysosomal	1
HMGCL	HMG-CoA lyase deficiency	246450	1p36.11	Metabolic	1
HMOX1	Heme oxygenase 1 deficiency	614034	22q12.3	Hematologic	3
HSD17B4	D-bifunctional protein deficiency	261515	5q23.1	Metabolic	1
HSD3B2	3-beta-hydroxysteroid dehydrogenase type II deficiency	201810	1p12	Endocrine	1
HSPG2	Dyssegmental dysplasia Silverman-Handmaker type	224410	1p36.12	Skeletal	1
HYLS1	Hydrolethalus syndrome	236680	11q24.2	Developmental	1
ICK	Endocrine-cerebroosteodysplasia	612651	6p12.2-p12.1	Developmental	1
IDS	Mucoploysaccaridosis type II	309900	Xq28	Lysosomal	2
IDUA	Mucopolysaccharidosis Ih	607014	4p16.3	Lysosomal	1
IER3IP1	Microcephaly epilepsy and diabetes syndrome	614231	18q21.1	Developmental	1
IFNGR1	Immunodeficiency 27A mycobacteriosis AR	209950	6q23.3	Immunodeficiency	1
IFNGR2	Immunodeficiency 28 mycobacteriosis	614889	21q22.11	Immunodeficiency	1
IGHMBP2	Neuronopathy distal hereditary motor type VI	604320	11q13.3	Neuromuscular	1
IKBKAP	Familial Dysautonomia (AJ Population)	223900	9q31.3	Neuromuscular	3
IKBKB	Immunodeficiency 15	615592	8p11.21	Immunodeficiency	1
IKBKG	Ectodermal(dysplasia anhidrotic lymphedema and immunodeficiency	300301	Xq28	Cutaneous	1
IL1RN	Interleukin 1 receptor antagonist deficiency	612852	2q13	Developmental	1
IL2RG	Severe combined immunodeficiency X-linked	300400	Xq13.1	Immunodeficiency	1
IL7R	Severe combined immunodeficiency T-cell negative B-cell/natural killer cell-positive type	608971	5p13.2	Immunodeficiency	1
INSR	Leprechaunism	246200	19p13.2	Metabolic	1
INVS	Nephronophthisis 2 infantile	602088	9q31.1	Renal	1
ISCA2	Multiple mitochondrial dysfunctions syndrome 4	616370	14q24.3	Mitochondrial	1
ISPD	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A7	614643	7p21.2	Neuromuscular	1
ITCH	Autoimmune disease multisystem with facial dysmorphism	613385	20q11.22	Immunodeficiency	1
ITGA6	Epidermolysis bullosa junctional with pyloric stenosis	226730	2q31.1	Cutaneous	1
ITGA8	Renal hypodysplasia/aplasia 1	191830	10p13	Renal	1
ITGB4	Epidermolysis bullosa junctional with pyloric atresia	226730	17q25.1	Cutaneous	1
ITK	Lymphoproliferative syndrome 1	613011	5q33.3	Immunodeficiency	1
IVD	Isovaleric acidemia	243500	15q15.1	Metabolic	1
JAM3	Hemorrhagic destruction of the brain subependymal calcification and cataracts	613730	11q25	Neurologic	1
KCNQ1	Jervell and Lange-Nielsen syndrome	220400	11p15.5-p15.4	Developmental	1

KCTD7	Epilepsy progressive myoclonic 3 with or without intracellular inclusions	611726	7q11.21	Neurologic	1
KIAA0586	Joubert syndrome 23	616490	14q23.1	Neurologic	2
KIF7	Hydrolethalus syndrome 2	614120	15q26.1	Developmental	1
KLHL40	Nemaline myopathy 8 autosomal recessive	615348	3p22.1	Neuromuscular	1
KLHL41	Nemaline myopathy 9	615731	2q31.1	Neuromuscular	1
L1CAM	Hydrocephalus due to aqueductal stenosis	307000	Xq28	Neurologic	1
LAMA3	Epidermolysis bullosa junctional Herlitz type	226700	18q11.2	Cutaneous	1
LAMB2	Pierson syndrome	609049	3p21.31	Developmental	1
LAMB3	Epidermolysis bullosa junctional Herlitz type	226700	1q32.2	Cutaneous	1
LAMC2	Epidermolysis bullosa junctional Herlitz type	226700	1q25.3	Cutaneous	1
LARGE	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A6	613154	22q12.3	Neuromuscular	1
LBR	Greenberg skeletal dysplasia	215140	1q42.12	Skeletal	1
LIAS	Pyruvate dehydrogenase lipoic acid synthetase deficiency	614462	4p14	Metabolic	1
LIFR	Stuve-Wiedemann syndrome/Schwartz-Jampel type 2 syndrome	601559	5p13.1	Skeletal	1
LIPA	Cholesteryl ester storage disease	278000	10q23.31	Metabolic	1
LMNA	Restrictive dermopathy lethal	275210	1q22	Cutaneous	1
LMOD3	Nemaline myopathy 10	616165	3p14.1	Neuromuscular	1
LRPPRC	Leigh syndrome French-Canadian type	220111	2p21	Mitochondrial	1
LTBP4	Cutis laxa autosomal recessive type IC	613177	19q13.2	Cutaneous	1
MALT1	Immunodeficiency 12	615468	18q21.32	Immunodeficiency	1
MAN2B1	Mannosidosis alpha- types I and II	248500	19p13.2	Lysosomal	1
MARS	Interstitial lung and liver disease	615486	12q13.3	Developmental	1
MCOLN1	Familial Mediterranean Fever	252650	19p13.2	Lysosomal	2
MECP2	Encephalopathy neonatal severe	300673	Xq28	Neurodegenerative	1
MESP2	Spondylocostal dysostosis 2 autosomal recessive	608681	15q26.1	Skeletal	1
MKS1	Meckel syndrome 1	249000	17q22	Developmental	1
MLC1	Megalencephalic Leukoencephalopathy with Subcortical Cysts	604004	22q13.33	Neurodegenerative	2
MMACHC	Methylmalonic aciduria and homocystinuria cblC type	277400	1p34.1	Metabolic	1
MOCS1	Molybdenum cofactor deficiency A	252150	6p21.2	Metabolic	1
MOCS2	Molybdenum cofactor deficiency B	252160	5q11.2	Metabolic	1
MOGS	Congenital disorder of glycosylation type IIb	606056	2p13.1	Metabolic	1
MPDU1	Congenital disorder of glycosylation type If	609180	17p13.1	Metabolic	1
MPDZ	Hydrocephalus nonsyndromic autosomal recessive 2	615219	9p23	Neurologic	1
MPI	Congenital disorder of glycosylation type Ib	602579	15q24.1	Metabolic	1
MPV17	Mitochondrial DNA depletion syndrome 6 (hepatocerebral type)	256810	2p23.3	Mitochondrial	1
MRPL3	Combined oxidative phosphorylation deficiency 9	614582	3q22.1	Mitochondrial	1
MRPS16	Combined oxidative phosphorylation deficiency 2	610498	10q22.2	Mitochondrial	1
MRPS22	Combined oxidative phosphorylation deficiency 5	611719	3q23	Mitochondrial	1
MTHFR	Homocystinuria due to MTHFR deficiency	236250	1p36.22	Metabolic	1
MTM1	Myotubular myopathy X-linked	310400	Xq28	Neuromuscular	1
MUT	Methylmalonic aciduria mut0) type	251000	6p12.3	Metabolic	1

MVK	Mevalonic aciduria	610377	12q24.11	Metabolic	1
MYBPC1	Lethal congenital contracture syndrome 4	614915	12q23.2	Developmental	1
MYD88	Pyogenic bacterial infections recurrent due to MYD88 deficiency	612260	3p22.2	Immunodeficiency	1
MYO5A	Griscelli syndrome type 1	214450	15q21.2	Developmental	1
MYO5B	Microvillus inclusion disease	251850	18q21.1	Gastroenterologic	1
NAA10	N-terminal acetyltransferase deficiency	300855	Xq28	Developmental	1
NAGLU	Mucopolysaccharoidosis Type IIIB	252920	17q21.2	Lysosomal	2
NBN	Nijmegen breakage syndrome	251260	8q21.3	Developmental	1
NDUFA12	Leigh syndrome due to mitochondrial complex 1 deficiency	256000	12q22	Mitochondrial	1
NDUFA2	Leigh syndrome due to mitochondrial complex I deficiency	256000	5q31.3	Mitochondrial	1
NDUFA9	Leigh syndrome due to mitochondrial complex I deficiency	256000	12p13.32	Mitochondrial	1
NDUFAF2	Leigh syndrome	256000	5q12.1	Mitochondrial	1
NDUFAF6	Leigh syndrome due to mitochondrial complex I deficiency	256000	8q22.1	Mitochondrial	1
NDUFS3	Leigh syndrome due to mitochondrial complex I deficiency	256000	11p11.2	Mitochondrial	1
NDUFS4	Leigh syndrome	256000	5q11.2	Mitochondrial	1
NDUFS7	Leigh syndrome	256000	19p13.3	Mitochondrial	1
NDUFS8	Leigh syndrome due to mitochondrial complex I deficiency	256000	11q13.2	Mitochondrial	1
NEB	Nemaline myopathy 2 autosomal recessive	256030	2q23.3	Neuromuscular	1
NEU1	Sialidosis type I	256550	6p21.33	Lysosomal	1
NFU1	Multiple mitochondrial dysfunctions syndrome 1	605711	2p13.3	Mitochondrial	1
NHEJ1	Severe combined immunodeficiency with microcephaly growth retardation and sensitivity to ionizing radiation	611291	2q35	Immunodeficiency	1
NHLRC1	Epilepsy progressive myoclonic 2B Lafora)	254780	6p22.3	Neurologic	1
NPC1	Niemann-Pick disease type C1	257220	18q11.2	Metabolic	1
NPC2	Niemann-pick disease type C2	607625	14q24.3	Metabolic	1
NPHP1	Joubert syndrome	256100	2q13	Renal	2
NPHP3	Meckel syndrome 7	267010	3q22.1	Developmental	1
NPHS1	Nephrotic syndrome type 1	256300	19q13.12	Renal	1
NPHS2	Steroid-Resistant Nephrotic Syndrom	600995	1q25.2	Renal	3
OCLN	Band-like calcification with simplified gyration and polymicrogyria	251290	5q13.2	Neurologic	1
OFD1	Simpson-Golabi-Behmel syndrome type 2	300209	Xp22.2	Developmental	1
OPA3	Costeff Optic Atrophy Syndrome	258501	19q13.32	Metabolic	3
ORAI1	Immunodeficiency 9	612782	12q24.31	Immunodeficiency	1
OSTM1	Osteopetrosis autosomal recessive 5	259720	6q21	Skeletal	1
OTC	Ornithine transcarbamylase deficiency	311250	Xp11.4	Metabolic	1
OXCT1	Succinyl CoA:3-oxoacid CoA transferase deficiency	245050	5p13.1	Metabolic	1
P3H1	Osteogenesis imperfecta type VIII	610915	1p34.2	Skeletal	1
PANK2	Pantothenate Kinase-Associated Neurodegeneration	234200	20p13	Neurodegenerative	2
PAX2	Renal hypoplasia isolated	191830	10q24.31	Renal	1
PC	Pyruvate carboxylase deficiency	266150	11q13.2	Metabolic	1
PCDH15	Usher Syndrome Type 1F	609533	10q21.1	Deafness	3

PDHB	Pyruvate dehydrogenase E1-beta deficiency	614111	3p14.3	Metabolic	1
PDSS2	Coenzyme Q10 deficiency primary3	614652	6q21	Metabolic	1
PET100	Mitochondrial complex IV deficiency	220110	19p13.2	Mitochondrial	1
PEX1	Peroxisome biogenesis disorder 1A (Zellweger)	214100	7q21.2	Developmental	1
PEX10	Peroxisome biogenesis disorder 6A (Zellweger)	614870	1p36.32	Developmental	1
PEX12	Peroxisome biogenesis disorder 3A (Zellweger)	614859	17q12	Developmental	1
PEX13	Peroxisome biogenesis disorder 11A (Zellweger)	614883	2p16.1	Developmental	1
PEX14	Peroxisome biogenesis disorder 13A (Zellweger)	614887	1p36.22	Developmental	1
PEX16	Peroxisome biogenesis disorder 8A (Zellweger)	614876	11p11.2	Developmental	1
PEX19	Peroxisome biogenesis disorder 12A (Zellweger)	614886	1q23.2	Developmental	1
PEX2	Zellweger spectrum disorder	614866	8q21.11	Developmental	2
PEX3	Peroxisome biogenesis disorder 10A (Zellweger)	614882	6q24.2	Developmental	1
PEX5	Peroxisome biogenesis disorder 2A (Zellweger)	214110	12p13.31	Developmental	1
PEX6	Peroxisome biogenesis disorder 4A (Zellweger)	614862	6p21.1	Developmental	1
PEX7	Chondrodysplasia punctata rhizomelic type 1	215100	6q23.3	Skeletal	1
PHGDH	Neu-Laxova syndrome1	256520	1p12	Developmental	1
PIGA	Multiple congenital anomalies-hypotonia-seizures syndrome 2	300868	Xp22.2	Developmental	1
PIGN	Multiple congenital anomalies-hypotonia-seizures syndrome 1	614080	18q21.33	Developmental	1
PIP5K1C	Lethal congenital contractural syndrome 3	611369	19p13.3	Developmental	1
PKHD1	Polycystic kidney and hepatic disease	263200	6p12.3-p12.2	Developmental	1
PLA2G6	Infantile neuroaxonal dystrophy 1	256600	22q13.1	Neurodegenerative	1
PLEC	Epidermolysis bullosa simplex with pyloric atresia	612138	8q24.3	Cutaneous	1
PLP1	Pelizaeus-Merzbacher disease	312080	Xq22.2	Neurodegenerative	1
PMM2	Congenital disorder of glycosylation type Ia	212065	16p13.2	Metabolic	1
POLG	Mitochondrial DNA depletion syndrome 4A Alpers type)	203700	15q26.1	Mitochondrial	1
POMGNT1	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A3	253280	1p34.1	Neuromuscular	1
POMGNT2	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies type A8	614830	3p22.1	Neuromuscular	1
POMK	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A12	615249	8p11.21	Neuromuscular	1
POMT1	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A1	236670	9q34.13	Neuromuscular	1
POMT2	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A2	613150	14q24.3	Neuromuscular	1
POR	Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis	201750	7q11.23	Developmental	1
PPT1	Neuronal Ceroid Lipofuscinosis PPT1-Related	256730	1p34.2	Neurodegenerative	2
PRPS1	Arts syndrome	301835	Xq22.3	Neurologic	1
PSAP	Combined SAP deficiency	611721	10q22.1	Lysosomal	1
PSAT1	Neu-Laxova syndrome 2	616038	9q21.2	Developmental	1
PTEN	VATER association with macrocephaly and ventriculomegaly	276950	10q23.31	Developmental	1
PTF1A	Pancreatic and cerebellar agenesis	609069	10p12.2	Gastroenterologic	1
PTH1R	Chondrodysplasia Blomstrand type	215045	3p21.31	Skeletal	1
PTPRC	Severe combined immunodeficiency T cell-negative B-cell/natural killer-cell positive	608971	1q31.3-q32.1	Immunodeficiency	1
PYGM	Glycogen Storage Disease Type V	232600	11q13.1	Metabolic	3
RAB27A	Griscelli syndrome type 2	607624	15q21.3	Developmental	1

RAG1	Omenn syndrome	603554	11p12	Immunodeficiency	1
RAG2	Omenn syndrome	603554	11p12	Immunodeficiency	1
RAPSN	Fetal akinesia deformation sequence	208150	11p11.2	Neuromuscular	1
RARS2	Pontocerebellar hypoplasia type 6	611523	6q15	Neurodegenerative	1
REN	Renal tubular dysgenesis	267430	1q32.1	Renal	1
RET	Renal agenesis	191830	10q11.21	Renal	1
RFX5	Bare lymphocyte syndrome type II complementation group C	209920	1q21.3	Immunodeficiency	1
RFX6	Mitchell-Riley syndrome	615710	6q22.1	Gastroenterologic	1
RFXANK	MHC class II deficiency complementation group B	209920	19p13.11	Immunodeficiency	1
RFXAP	Bare lymphocyte syndrome type II complementation group D	209920	13q13.3	Immunodeficiency	1
RIPK4	Popliteal pterygium syndrome 2 lethal type	263650	21q22.3	Developmental	1
RNASEH2A	Aicardi-Goutieres syndrome 4	610333	19p13.2	Neurodegenerative	1
RNASEH2B	Aicardi-Goutieres syndrome 2	610181	13q14.3	Neurodegenerative	1
RNASEH2C	Aicardi-Goutieres syndrome 3	610329	11q13.1	Neurodegenerative	1
RNU4ATAC	Microcephalic osteodysplastic primordial dwarfism type I	210710	2q14.2	Skeletal	1
RPGRIP1L	Meckel syndrome 5	611561	16q12.2	Developmental	1
RRM2B	Mitochondrial DNA depletion syndrome 8A (encephalomyopathic type with renal tubulopathy)	612075	8q22.3	Mitochondrial	1
RS1	X-Linked Juvenile Retinoschisis	312700	Xp22.13	Ocular	3
SAMHD1	Aicardi-Goutieres syndrome 5	612952	20q11.23	Neurodegenerative	1
SARS2	Hyperuricemia(pulmonary hypertension renal failure and alkalosis	613845	19q13.2	Developmental	1
SCNN1A	Pseudohypoaldosteronism type I	264350	12p13.31	Renal	1
SCNN1B	Pseudohypoaldosteronism type I	264350	16p12.2	Renal	1
SCNN1G	Pseudohypoaldosteronism type I	264350	16p12.2	Renal	1
SCO1	Mitochondrial complex IV deficiency	220110	17p13.1	Mitochondrial	1
SCO2	Cardioencephalomyopathy fatal infantile due to cytochrome c oxidase deficiency 1	604377	22q13.33	Cardiovascular	1
SDHA	Leigh syndrome	256000	5p15.33	Mitochondrial	1
SEPN1	Muscular dystrophy rigid spine1	602771	1p36.11	Neuromuscular	1
SFTPB	Surfactant metabolism dysfunction pulmonary1	265120	2p11.2	Respiratory	1
SGCA	Limb-Girdle Muscular Dystrophy Type 2D	608099	17q21.33	Neuromuscular	2
SGCB	Limb-Girdle Muscular Dystrophy Type 2E	604286	4q12	Neuromuscular	2
SGCG	Limb-Girdle Muscular Dystrophy Type 2C	253700	13q12.12	Neuromuscular	2
SGSH	Mucopolysaccharoidosis Type IIIA	252900	17q25.3	Lysosomal	2
SH2D1A	Lymphoproliferative syndrome X-linked1	308240	Xq25	Immunodeficiency	1
SKIV2L	trichohepatoenteric syndrome	614602	6p21.33	Gastroenterologic	2
SLC12A6	Andermann Syndrome	218000	15q14	Neurodegenerative	2
SLC17A5	Sialic acid storage disorder infantile	269920	6q13	Lysosomal	1
SLC19A2	thiamine-responsive megaloblastic anemia syndrome	249270	1q24.2	Metabolic	3
SLC1A4	congenital hyperinsulinism	616657	2p14	Developmental	2
SLC22A5	Primary carnitine deficiency	212140	5q31.1	Metabolic	3
SLC25A1	Combined D-2- and L-2-hydroxyglutaric aciduria	615182	22q11.21	Metabolic	1

SLC25A15	Hyperornithinemia-hyperammonemia-homocitrullinemia syndrome	238970	13q14.11	Metabolic	1
SLC25A19	Microcephaly Amish type	607196	17q25.1	Developmental	1
SLC25A20	Carnitine-acylcarnitine translocase deficiency	212138	3p21.31	Metabolic	1
SLC25A22	Epileptic encephalopathy early infantile3	609304	11p15.5	Neurologic	1
SLC25A3	Mitochondrial phosphate carrier deficiency	610773	12q23.1	Mitochondrial	1
SLC26A2	Achondrogenesis Ib	600972	5q32	Skeletal	1
SLC26A4	Pendred Syndrome	600791	7q22.3	Deafness	3
SLC33A1	Congenital cataracts hearing loss and neurodegeneration	614482	3q25.31	Neuromuscular	1
SLC35A1	Congenital disorder of glycosylation type IIf	603585	6q15	Metabolic	1
SLC35D1	Schneckenbecken dysplasia	269250	1p31.3	Developmental	1
SLC37A4	Glycogen Storage Disease Type Ib	232220	11q23.3	Metabolic	2
SLC52A2	Brown-Vialetto-Van Laere syndrome 2	614707	8q24.3	Neurologic	1
SLC52A3	Brown-Vialetto-Van Laere syndrome 1	211530	20p13	Neurologic	1
SLC5A1	Glucose/galactose malabsorption	606824	22q12.3	Gastroenterologic	1
SLC6A3	Parkinsonism-dystonia infantile	613135	5p15.33	Neurodegenerative	1
SMN1	Spinal muscular atrophy-1	253300	5q13.2	Neuromuscular	1
SMN2	Spinal muscular atrophy-1 (disease severity regulator)	253400	5q13.2	Neuromuscular	1
SMPD1	Niemann-Pick disease type A	257200	11p15.4	Lysosomal	1
SOX3	Mental retardation X-linked with isolated growth hormone deficiency	312000	Xq27.1	Endocrine	3
SP110	Hepatic venoocclusive disease with immunodeficiency	235550	2q37.1	Developmental	1
SPEG	Centronuclear myopathy 5	615959	2q35	Neuromuscular	1
STAR	Lipoid adrenal hyperplasia	201710	8p11.23	Endocrine	1
STAT1	Immunodeficiency 31B mycobacterial and viral infections autosomal recessive	613796	2q32.2	Immunodeficiency	1
STRA6	Microphthalmia isolated with coloboma 8	601186	15q24.1	Developmental	1
STX11	Hemophagocytic lymphohistiocytosis familial4	603552	6q24.2	Immunodeficiency	1
SUCLG1	Mitochondrial DNA depletion syndrome 9 (encephalomyopathic type with methylmalonic aciduria)	245400	2p11.2	Mitochondrial	1
SUMF1	Multiple sulfatase deficiency	272200	3p26.1	Metabolic	1
SUOX	Sulfite oxidase deficiency	272300	12q13.2	Metabolic	1
SURF1	Leigh syndrome due to COX deficiency	256000	9q34.2	Mitochondrial	1
T	Sacral agenesis with vertebral anomalies	615709	6q27	Skeletal	1
TACO1	Mitochondrial complex IV deficiency	220110	17q23.3	Mitochondrial	1
TBC1D24	Epileptic encephalopathy early infantile16	615338	16p13.3	Neurologic	1
TK2	Mitochondrial DNA depletion syndrome 2 (myopathic type)	609560	16q21	Mitochondrial	1
TMEM216	Meckel syndrome 2	603194	11q12.2	Developmental	1
TMEM231	Meckel syndrome 11	615397	16q23.1	Developmental	1
TMEM237	Joubert syndrome 14	614424	2q33.1	Neurologic	1
TMEM5	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies) type A10	615041	12q14.2	Neuromuscular	1
TMEM67	Meckel syndrome 3	607361	8q22.1	Developmental	1
TNNT1	Nemaline myopathy 5 Amish type	605355	19q13.42	Neuromuscular	1
TPI1	Hemolytic anemia due to triosephosphate isomerase deficiency	615512	12p13.31	Metabolic	1

TPP1	Neuronal Ceroid Lipofuscinosis TPP1-Related	204500	11p15.4	Neurodegenerative	2
TRIP11	Achondrogenesis type IA	200600	14q32.12	Skeletal	1
TSEN2	Pontocerebellar hypoplasia type 2B	612389	3p25.2	Neurodegenerative	1
TSEN54	Pontocerebellar hypoplasia type 4	225753	17q25.1	Neurodegenerative	1
TSFM	Combined oxidative phosphorylation deficiency 3	610505	12q14.1	Mitochondrial	1
TSPYL1	Sudden infant death with dysgenesis of the testes syndrome	608800	6q22.1	Developmental	1
TTC19	Mitochondrial complex III deficiency nuclear type 2	615157	17p12	Mitochondrial	1
TTC21B	Nephronophthisis 12	613820	2q24.3	Renal	1
TTC37	Trichohepatoenteric syndrome 1	222470	5q15	Gastroenterologic	1
TTC7A	Gastrointestinal defects and immunodeficiency syndrome	243150	2p21	Gastroenterologic	1
TTN	Myopathy early-onset with fatal cardiomyopathy	611705	2q31.2	Neuromuscular	1
TTPA	Ataxia with Vitamin E Deficiency	277460	8q12.3	Neurodegenerative	3
TUFM	Combined oxidative phosphorylation deficiency 4	610678	16p11.2	Mitochondrial	1
TYR	Oculocutaneous albinism	203100	11q14.3	Metabolic	3
UBA1	Spinal muscular atrophy X-linked 2 infantile	301830	Xp11.23	Neuromuscular	1
UBR1	Johanson-Blizzard syndrome	243800	15q15.2	Developmental	1
UCP2	congenital hyperinsulinism	-	-	Metabolic	3
UGT1A1	Hyperbilirubinemia familial transient neonatal	237900	2q37.1	Metabolic	1
VIPAS39	Arthrogryposis renal dysfunction and cholestasis 2	613404	14q24.3	Neuromuscular	1
VPS13B	Cohen Syndrome	216550	8q22.2	Developmental	2
VPS33B	Arthrogryposis renal dysfunction and cholestasis 1	208085	15q26.1	Neuromuscular	1
VRK1	Pontocerebellar hypoplasia type 1A	607596	14q32.2	Neurodegenerative	1
VSX2	Micropthalmia/anopthalmia	610092	14q24.3	Ocular	3
WDR34	Short-rib thoracic dysplasia 11 with or without polydactyly	615633	9q34.11	Skeletal	1
WDR60	Short-rib thoracic dysplasia 8 with or without polydactyly	615503	7q36.3	Skeletal	1
WDR73	Galloway-Mowat syndrome	251300	15q25.2	Developmental	1
WNT7A	Fuhrmann syndrome	228930	3p25.1	Skeletal	1
WRN	Werner Syndrome	277700	8p12	Developmental	2
WT1	Meacham syndrome	608978	11p13	Developmental	1
ZIC3	Congenital heart defects nonsyndromic 1 X-linked	306955	Xq26.3	Cardiovascular	1
ZMPSTE24	Restrictive dermopathy lethal	275210	1p34.2	Cutaneous	1

CHAPTER 3:

Section 2

Study protocol of a multicentre cohort pilot study implementing an expanded carrier-screening programme in metropolitan and regional Western Australia

Ong R, Edwards S, Howting D, Kamien B, Harrop K, Ravenscroft G, Davis M, Fietz M, Pachter N, Beilby J, Laing N. Study protocol of a multicentre cohort pilot study implementing an expanded preconception carrier-screening programme in metropolitan and regional Western Australia. BMJ Open 2019;9(6):e028209

doi: 10.1136/bmjopen-2018-028209

Royston Ong

Samantha Edwards

Denise Howting

Ben Kamien

Karren Harrop

Gina Ravenscroft

Mark Davis
Michael Fietz

Nicholas Pachter

John Beilby

Nigel Laing

Supplementary information