Objective Syndromic Phenotyping:  
The Potential of Three-Dimensional Facial Morphometrics

Stefanie Kung B.Sc. (Hons)

The thesis is presented for the degree of Doctor of Philosophy of The University of Western Australia

2015
DECLARATION FOR THESES CONTAINING PUBLISHED WORK AND/OR WORK PREPARED FOR PUBLICATION

The examination of the thesis is an examination of the work of the student. The work must have been substantially conducted by the student during enrolment in the degree.

Where the thesis includes work to which others have contributed, the thesis must include a statement that makes the student’s contribution clear to the examiners. This may be in the form of a description of the precise contribution of the student to the work presented for examination and/or a statement of the percentage of the work that was done by the student.

In addition, in the case of co-authored publications included in the thesis, each author must give their signed permission for the work to be included. If signatures from all the authors cannot be obtained, the statement detailing the student’s contribution to the work must be signed by the coordinating supervisor.

Please sign one of the statements below.

<table>
<thead>
<tr>
<th>1. This thesis <strong>does not contain</strong> work that I have published, nor work under review for publication.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student Signature ..........................................................................................................................................................</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. This thesis contains <strong>only sole-authored</strong> work, some of which has been published and/or prepared for publication under sole authorship. The bibliographical details of the work and where it appears in the thesis are outlined below.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student Signature ..........................................................................................................................................................</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. This thesis contains published work and/or work prepared for publication, <strong>some of which has been co-authored</strong>. The bibliographical details of the work and where it appears in the thesis are outlined below. The student must attach to this declaration a statement for each publication that clarifies the contribution of the student to the work. This may be in the form of a description of the precise contributions of the student to the published work and/or a statement of percent contribution by the student. This statement must be signed by all authors. If signatures from all the authors cannot be obtained, the statement detailing the student’s contribution to the published work must be signed by the coordinating supervisor.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 3 Manuscript: A dysmorphometric analysis to investigate facial phenotypic signatures as a foundation for non-invasive monitoring of lysosomal storage disorders. SK wrote the manuscript with major input and revisions from all other authors, acquired the syndromic facial data, and implemented the assessments. MW and GB were involved in drafting of the manuscript, while PC, JG and PLS provided valuable critical feedback. PC developed the fundamentals behind the anthropometric mask, the statistical face-space, dysmorphometrics, and the normal equivalent, with conceptual input from MW. PC provided facial mapping of the data, constructed the normative facial model of covariance, established normative reference discordancy statistics. GB and JG provided clinical insight into investigated syndromes, rallied support from the MPS Society and fostered international collaborations. All authors read and approved the final manuscript.</td>
</tr>
<tr>
<td>Student Signature ..........................................................................................................................................................</td>
</tr>
</tbody>
</table>
Chapter 4 Manuscript: Monitoring of therapy for mucopolysaccharidosis type I using dysmorphometric facial phenotypic signatures.
SK wrote the manuscript with subsequent revisions and input from MW, GB, JG and PC. GB and JG conceived the study. SK acquired the longitudinal facial data and implemented the facial assessments. PC performed the facial mapping of the data and generated normative reference discordancy statistics. All authors were involved in the interpretation of the results. SB, AM, JG and PLS provided valuable clinical insight into the patient’s treatment regime, and the investigated syndrome.

SK wrote the article with strong input from MW and GB. GB conceived the study. Facial data was obtained through the collective efforts of SK, MW, GB and JC. SK generated the normative reference asymmetry statistics. PC and SK were both involved in constructing and performing data analysis. Conceptual explanations were the result of collaboration between all authors. PLS, JC and JG provided clinical insights and editorial input.

Co-Authors’ Signatures

Gareth Baynam (GB) Signature

Mark Walters (MW) Signature

Peter Claes (PC) Signature

Peter Le Souef (PLS) Signature

Jack Goldblatt (JB) Signature

Andrew Martin (AM) Signature

Shanti Balasubramaniam (SB) Signature

John Clement (JC) Signature

Student (SK) Signature

Coordinating Supervisor Signature
ABSTRACT

Facial patterns play a unique and important role in identifying conditions of abnormal growth and development, including in syndromic disease. A syndrome is a combination of features that collectively indicate a particular condition. Biologists and clinicians continue to strive for objective assessment of facial form; as a result, different schools of thought have emerged, each presenting different approaches with their own strengths and limitations. Facial dysmorphology and asymmetry are particularly of interest when studying syndromic facial form. Craniofacial abnormalities, including abnormalities of form or asymmetry, are found in many syndromes, and objective approaches are required to supplement subjective clinical assessment; the methodological approach needs to be tailored to the problem at hand. In the field of syndromic facial phenotyping, this objective approach should ideally be able to distinguish between normal and abnormal facial patterns; it should be robust enough to detect wide spectrum of abnormalities (e.g. subtle to severe presentations) and be able to quantify facial abnormalities based both on individual features (regionally) and as a holistic entity (globally).

Our aim was to illustrate how novel techniques can be used as a 3D morphometric toolkit for investigating syndromic facial phenotypes. This was achieved through the use of objective approaches that can (1) analyze syndromic facial asymmetry and dysmorphology patterns on both a global and regional level, and (2) to provide biological insights that might facilitate the diagnoses and treatment monitoring of these conditions. This thereby effectively arms the non-expert with a developing toolkit that translates expert knowledge for availability to a broader base of end-users. In this thesis, we used two main geometric morphometric concepts for application to syndrome analysis, (1) the framework of dysmorphometrics, and (2) the framework for decomposing fluctuating and directional asymmetry in spatially dense landmark configurations. Both are novel recent techniques that, before the tenure of this thesis, had not been applied to syndromic cohorts.

Syndrome discrimination and treatment monitoring applications were demonstrated through the assessment of facial discordancy in Mucopolysaccharidosis Type I (MPS I). Without any prior clinical assumptions or knowledge of the condition, the dysmorphometric approach successfully detected, isolated and quantified MPS I facial
characteristics, and attributed facial discordance severity scores, that correlated with clinical severity across three affected individuals with differing dysmorphology. The application of dysmorphometrics as a non-invasive treatment monitoring tool, was then investigated by longitudinally scoring facial discordancy in an MPS I child receiving enzyme replacement therapy and bone marrow transplantation. The pattern of progressive lessening of facial dysmorphology that was objectively and precisely determined, in both the short and intermediate term, supports its potential as a rapid and non-invasive means of assessing treatment response. The exploratory applications of the complementary two-factor ANOVA decomposition and dysmorphometric approaches were also demonstrated, by investigation of facial symmetry and asymmetry patterns in the unique causally homogenous condition Achondroplasia; insights into the disordered craniofacial growth and developmental stability of the condition were attained. Collectively, through novel applications of a 3D morphometric toolkit for assessments of asymmetry and dysmorphology, we have explored disease biology, determined facial signatures of syndromic phenotypes and their severity, monitored disorder specific medical treatment. This work provides a framework from which to approach refinements in syndromic facial diagnostics and treatment assessment.
ACKNOWLEDGEMENTS

It has been a great privilege to work with my supervisors, Mark Walters, Gareth Baynam, Peter Claes, and Peter LeSouef. Their continued encouragement and support has seen me through these past four years, and the boundless passion and enthusiasm they have for their work never ceases to amaze me. It has definitely been a rewarding experience learning from these knowledgeable individuals, and I now feel better equipped to meet whatever challenges the world decides to throw my way.

I would also like to thank the other PhD students at SPACH, especially Alex. Together, we have laugh, cried, stressed, and struggled our way through this grueling PhD, which has been by far the hardest thing I have ever done. The camaraderie we share made all the seemingly insane moments less so, and I am ever so grateful. I wish you all the best in your future endeavours.

Much appreciation also goes to the dedicated and hardworking women of the CMF research team, Miranda, Megan, and Lyn. Thank you so much for all the help and training I received, you have no idea how invaluable you have been.

Special thanks goes out to the wonderful Michelle, who pointed me in the right direction and provided me with this opportunity in first place. None of this would have been possible without you. Finally, I am so thankful for the continued love and support of Matt. I would not have gotten through the last leg without you!
CONTENTS

ABSTRACT .............................................................................................................................. iv
ACKNOWLEDGEMENTS ........................................................................................................... vi
CONTENTS .......................................................................................................................... vii
LIST OF ABBREVIATIONS .................................................................................................... x
LIST OF FIGURES & TABLES ................................................................................................. xi

1. Unifying Introduction ........................................................................................................ 1
   1.1 Problem Field ........................................................................................................... 1
   1.2 Thesis Outline ........................................................................................................... 2
   1.3 Importance of Facial Morphology in Syndromic Phenotyping ............................... 3
   1.4 The Evolution of Morphometrics ............................................................................ 3
   1.5 Progression of Geometric Morphometrics into the 21st Century ......................... 9
   1.6 Symmetry and Asymmetry in Geometric Morphometrics ................................. 10
   1.7 Establishing a 3D Facial Morphometric Toolkit for Syndromic Phenotyping 11

2. Thesis Scope .................................................................................................................. 15

Part One Preface: .............................................................................................................. 18

   3.1 Abstract ................................................................................................................... 19
   3.2 Introduction ............................................................................................................. 20
   3.3 Methods .................................................................................................................. 22
      3.3.1 Ethics Approvals ............................................................................................ 22
      3.3.2 Participants ..................................................................................................... 22
      3.3.3 3D Image Acquisition .................................................................................... 22
      3.3.4 Anthropometric Masks & Facial Mapping ..................................................... 22
      3.3.5 Statistical Face-Space ...................................................................................... 23
3.3.6 Dysmorphometrics and Normal Equivalents ........................................ 23
3.3.7 Scoring, Analysis & Visualisation of Facial Variants ......................... 24
3.3.8 Normative Population Reference Statistics ....................................... 25
3.4 Results ........................................................................................................ 25
3.5 Discussion .................................................................................................. 28
3.6 Conclusion .................................................................................................. 31

4. Treatment Monitoring Application: Dysmorphometrics for Facial Discordancy ... 33
4.1 Abstract ...................................................................................................... 33
4.2 Introduction ............................................................................................... 33
4.3 Methods ...................................................................................................... 35
  4.3.1 Participants ........................................................................................... 35
  4.3.2 3D Image Acquisition, Data Preparation .............................................. 35
  4.3.3 Anthropometric Masks & Facial Mapping ........................................... 35
  4.3.4 Reference Face Space ........................................................................... 36
  4.3.5 Dysmorphometrics ............................................................................... 36
  4.3.6 Normal Equivalent Facial Assessments ............................................... 37
4.4 Results ........................................................................................................ 38
4.5 Discussion .................................................................................................. 42
4.6 Conclusion .................................................................................................. 44
4.7 Acknowledgments ....................................................................................... 44

Part Two Preface: ............................................................................................... 46

5. Exploratory Applications: Dysmorphometrics and Two-Factor ANOVA Decomposition of Facial Asymmetry ................................................................. 47
  5.1 ABSTRACT ............................................................................................... 47
  5.2 INTRODUCTION ....................................................................................... 48
  5.3 Study Design ............................................................................................. 50
  5.4 METHODS ............................................................................................... 50
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>MPS IH</td>
<td>Mucopolysaccharidosis Type I – Hurler Syndrome</td>
</tr>
<tr>
<td>MPS IHS</td>
<td>Mucopolysaccharidosis Type I – Hurler-Scheie Syndrome</td>
</tr>
<tr>
<td>MPS IS</td>
<td>Mucopolysaccharidosis Type I – Scheie Syndrome</td>
</tr>
<tr>
<td>ERT</td>
<td>Enzyme replacement therapy</td>
</tr>
<tr>
<td>BMT</td>
<td>Bone marrow transplant</td>
</tr>
<tr>
<td>HSCT</td>
<td>Hematopoietic stem cell transplantation</td>
</tr>
<tr>
<td>LSD</td>
<td>Lysosomal storage disorder</td>
</tr>
<tr>
<td>GvHD</td>
<td>Graft-versus-host disease</td>
</tr>
<tr>
<td>AM</td>
<td>Anthropometric mask</td>
</tr>
<tr>
<td>NE</td>
<td>Normal equivalent</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>PCs</td>
<td>Principal components</td>
</tr>
<tr>
<td>RMSE</td>
<td>Root-mean-squared error</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative significant discordance</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>GLS</td>
<td>Generalized Least Squares Procrustes Superimposition</td>
</tr>
<tr>
<td>GPA</td>
<td>Generalized Procrustes Analysis</td>
</tr>
<tr>
<td>EDMA</td>
<td>Euclidean Distance Matrix Analysis</td>
</tr>
<tr>
<td>ML</td>
<td>Maximum Likelihood</td>
</tr>
<tr>
<td>DSM</td>
<td>Dense Surface Models</td>
</tr>
<tr>
<td>ML</td>
<td>Maximum Likelihood</td>
</tr>
</tbody>
</table>
LIST OF FIGURES & TABLES

Figure 2.1  Thesis methodology workflow………………………………………………17
Figure 3.1  Comparison of the Normal Equivalent (NE) versus an average case-matched control………………………………………………………………………24
Figure 3.2  Discordance severity in MPS I clinical subtypes…………………………26
Figure 3.3  Individual III MPS I affected vector map…………………………………27
Figure 4.1  Methodology workflow used for the MPS I treatment monitoring process………………………………………………………………………37
Figure 4.2  MPS I longitudinal treatment monitoring over eight time points……….39
Figure 4.3  Progression of normalized facial discordance proportions along the MPS I treatment course……………………………………………………..40
Figure 4.4  Progression of normalized facial severity scores along the MPS I treatment course……………………………………………………………41
Figure 5.1  Z-RSA and Z-RMSE distributions of the Achondroplastic cohort……….55
Figure 5.2  Asymmetry signature maps for the Achondroplastic cohort…………….57
Figure 5.3  Facial asymmetry shape partitioning in Achondroplasia………………….58
Figure 5.4  Achondroplastic asymmetry model depicting the first three principal components (PC)…………………………………………………………60
Figure 5.5  Achondroplastic symmetry model depicting the first three principal components (PC)…………………………………………………………61
Figure 6.1  The Big Picture: Translation of the 3D facial morphometric toolkit applications……………………………………………………………68
Table 3.1  Overall NE assessment and Z-scores for three MPS I individuals in relation to summary reference statistics………………………………………25
1. Unifying Introduction

“With increasing interest in congenital malformation syndromes fervor spreading through the medical specialty, it is difficult for editors and medical journal readers to separate spurious from durable and meaningful syndromes. Thus there is a need for judicious sifting of organization and census for the plethora of syndromic literature into meaningful patterns.” – Gorlin’s Foreword, Syndromes of the Head and Neck, 1964.

1.1 Problem Field

Facial patterns play a unique and important role in identifying syndromic conditions characterized by abnormal form (dysmorphology); they are also indicative of the developing brain, heart, lungs and limbs in normal development (Hammond and Suttie, 2012, Winter, 1996). The means to assess the face objectively for the purposes of defining facial dysmorphology, inclusive of facial asymmetry, is a challenging and an active field of translational research. A range of theoretical and technical approaches has been explored to transform raw facial form data to a set of discrete and precise facial variables that can provide signatures of dysmorphology. Achieving these aims will contribute to the elucidation of the biological processes of syndromic conditions and establish a framework to improve clinical syndromic phenotyping in diagnosis, treatment assessment, and establishing genotype-phenotype relationships.

Features of craniofacial abnormalities, inclusive of facial asymmetry, have been described for numerous syndromic conditions (Gorlin et al., 1990). To supplement subjective qualitative descriptions, advances in technology, in particular computers that can perform iterative tasks, are facilitating the application of algorithms to process large amounts of information to provide objective quantitative approaches. With respect to objective syndromic facial phenotyping, an algorithm approach needs to distinguish between normal and abnormal facial variation, as well as attribute a degree of severity to disease presentations, and ideally provide identification and quantification of facial features (regional) and as a holistic entity (global).
This thesis explores and illustrates the application of a novel 3-Dimensional (3D) geometric morphometric toolkit for the objective quantification of syndromic facial phenotypes. Specifically, it demonstrates the transformation of raw 3D facial images obtained from optical range finding scanners into spatially dense numerical descriptors of facial form variances in example syndromic cohorts. These data transformations are inclusive of systematic modeling of facial asymmetry and the application of modified geometric morphometric workflows (D; (Claes et al., 2012a), to identify global and regional facial form anomalies to provide biological insights that may facilitate diagnoses and treatment monitoring of syndromic conditions. The realization of the potential of these approaches aims to equip the non-expert with a toolkit that can translate expert implicit knowledge.

1.2 Thesis Outline

This thesis is organized as a series of papers; Chapter 1 presents a case for objective syndromic phenotyping and a review of the evolution of 3D facial morphometric toolkits. Chapter 2 presents an overall scope of the thesis, along with a methodological workflow of the novel techniques applied to the selected syndromic cohorts. Chapters 3, 4 and 5 are manuscripts that detail the outcomes of these investigations that form the main body of this work, and collectively present a framework for the application of these techniques to various syndromic analyses, inclusive of syndrome discrimination (Chapter 3), monitoring of therapy (Chapter 4) and driving innovative exploratory biological research (Chapter 5). Chapter 6 collectively discusses the main findings of this work, their significance in the field of syndromic facial phenotyping, the potential translation of the proposed 3D facial morphometric toolkit into other clinical and research settings, as well as the caveats and future directions of this emergent technology.
1.3 Importance of Facial Morphology in Syndromic Phenotyping

Clinical dysmorphology and abnormal facial asymmetry patterns play an important role in providing diagnostic clues to syndromes and rare diseases. Investigating these aspects in human dysmorphic syndromes has also been increasingly important in the discovery of developmental genes (Winter, 1996). The human face is uniquely reflective of the craniofacial primordia (Baynam et al., 2013a, Helms et al., 2005), and through the migration of neural crest cells, facial development becomes intricately linked to that of the developing brain, heart, lungs and limbs (Hammond and Suttie, 2012, Winter, 1996). Accordingly, craniofacial abnormalities are seen in conditions with protean manifestations across organ systems. Electronic databases such as the Online Mendelian Inheritance In Man (OMIM) and the London Dysmorphology Database (LDDB), and published texts like Gorlin’s Syndromes of the Head and Neck (Gorlin et al., 1990), are fundamental resources that support the clinical application of dysmorphology; however, imprecise and inconsistent facial descriptions are sometimes used, making comparisons difficult and subjective (Hammond, 2007). In response to the need for standardized and objective phenotypic description, an expert group of dysmorphologists collaborated to develop a domain specific lexicon, the elements of morphology (Allanson et al., 2009, Carey et al., 2009, Hall et al., 2009, Hennekam et al., 2009). This standardized language promotes the investigation of phenotype-phenotype and phenotype-genotype correlations, provides a basis for machine readability, and is synergistic with deeply precise 3D image capture and analysis.

1.4 The Evolution of Morphometrics

Morphometrics is the quantitative analysis of form and size differences. Traditional morphometrics mainly involved measures of dimensions (lengths, angles and mass) of biological forms. These measures however are often highly correlated, where shape information is difficult to separate from size (Zelditch et al., 2004). Research questions relating to form variances have required a modified approach to measure differences in shape, independent of size. This challenge required a capacity to align and superimpose representations of forms to measure shape discordances. To achieve this required an
assumption of structural homology, where homologous features or landmarks could be defined. Shape variation and covariation are then investigated as a function of residual differences in landmark locations of aligned representations of form (Dryden and Mardia, 1998, Bookstein, 1997).

Alongside the maturation of mathematical descriptors of shape has been the evolutions of data capture techniques and technologies. Traditional objective measurements of the facial form, required for supplementing subjective clinical assessment, involved direct anthropometry. These techniques involved measures of inter-landmark distances, angles and ratios, taken directly from the patient and compared to reference data. The clinical application of these approaches was facilitated by the pioneering work of Lesley Farkas (1981) and colleagues. However, these techniques were both time consuming and required operator expertise. To overcome these challenges, indirect methods utilizing photogrammetry techniques were explored. Initially, these involved capturing 2-dimensional images in controlled conditions; however, persistent lighting and pose artifacts presented their own difficulties. More recently, with the advent of personal computing, 3D surface scanning technology (e.g. laser scanning and stereophotogrammetry) emerged. Commercial 3D stereophotogrammetry systems, in particular, are increasingly available with fast capture times and spatially dense point cloud range finding data that can be rendered on a computer to establish a polygon shell that accurately and precisely represents a subject’s face. These developments are fueling a resurgence of facial anthropometrics in clinical applications, in particular, studies of syndromic craniofacial morphology (Loos et al., 2003, Allanson, 1997, Allanson et al., 1993, Allanson et al., 1985).

3D facial scanning fully preserves the spatial information of the facial form, it is non-invasive, is clinically more applicable to the capture of the young and less cooperative, and is technically suited for objective and precise phenotyping of syndromes and rare diseases. The past decade has seen a general change in the focus of facial studies of syndromic disease with an increasing emphasis on computational models of facial shape variation for discriminating facial morphological differences, and less emphasis on the delineation of characteristic individual facial features (Hammond et al., 2004). Facial morphometrics has now become a quantitative study of facial shape variation, with a numerical descriptive component that enables objective comparisons between facial forms.
Chapter 1: Unifying Introduction

Several approaches exist to (1) represent the shape of the face, (2) to superimpose and align two or more facial representations, and finally (3) extract and analyze facial shape variation.

Representation of facial shape using sparse landmarks employs manual indication of homologous or corresponding landmarks on different forms for comparative purposes, and are used extensively in physical anthropology (Slice, 2005, de Menezes et al., 2009, Ferrario et al., 1996) and studies of dysmorphologic conditions (Heuze et al., 2012, Weinberg et al., 2008, Starbuck et al., 2013, Dellavia et al., 2010, Sforza et al., 2012, Sforza et al., 2009). Without the need for strong computing power or highly specialized software, measurement of linear distances, ratios, and angles using this landmark-based strategy has its clear advantages. However, as datasets grow larger, manual indication of corresponding landmarks becomes more labor-intensive and prone to error. Additionally, some salient facial features (e.g. cheek and forehead regions) can be underrepresented through the lack of landmarks that have distinct corresponding anatomical locations, and yet shape aspects of these same regions can contain useful biological information (Hammond et al., 2004). Landmarks should ideally provide a sufficiently comprehensive representation of facial shape such that variables of biological significance can be discovered (Zelditch et al., 2004).

An example of an alternative strategy of non-landmark facial representations is the extraction of facial profiles and outline data from the facial surface data (Samir et al., 2006). These curve-based techniques (e.g. Fourier analysis) were developed to address the sparsity issues of landmark-based strategies, but this solution could not reliably establish anatomical correspondence between extracted curves (Smeets et al., 2010). Extracting sparse landmark/curve data from 3D surface data can inadvertently lose important information; the 3D facial surface should be analyzed in its entirety and as an entity instead. This is important as all of the facial topography is retained, and analyses based on the entire 3D point cloud coordinate data (~250,000 data points) can localize patterns of variation in the facial form. Full surface-based approaches using spatially dense landmarks have been explored by Hammond et al. (2004), Hutton et al. (2003), Claes et al. (2011a), Claes (2007), Smeets et al. (2010).
Once facial shape has been adequately represented, different strategies exist for alignment of these landmark configurations to account for pose and orientation differences, an essential requirement before the comparison of facial forms. This alignment is termed as ‘superimposition’ because the landmark configurations are placed on top of one another by mathematical operations that do not alter shape (i.e. translation, scaling and rotation of landmark coordinates) (Zelditch et al., 2004). Several superimposition methods are available, and these include; (1) Generalized least squares Procrustes superimposition that superimposes landmark configurations into a common frame of reference (Rohlf and Slice, 1990a, Goodall, 1991); and (2) approaches using deformational fields and partial warps to describe one facial form in terms of the another such as thin-plate spline deformations (Farkas, 1981, Richtsmeier et al., 2002). An alternative strategy is to use alignment-invariant approaches such as Euclidean Distance Matrix Analysis (EDMA) that uses pairwise linear distances between landmarks, as opposed to its landmark coordinates, to describe shape variance and does not require superimposition (Lele, 1991, Lele and Richtsmeier, 1991, Richtsmeier et al., 2002).

Generalized least squares Procrustes superimposition (GLS), in particular, is the most commonly used superimposition method. Procrustes superimpositions minimize differences between landmark configurations, and rely on mathematical operations to remove all information unrelated to shape (Zelditch et al., 2004). The distinction of GLS is the minimization of ‘Procrustes distances’ (the summed squared distances between corresponding landmarks) that makes it ideal for Procrustes superimposition. The alignment of more than two facial forms using the GLS method is an iterative process called generalized Procrustes analysis (GPA). It requires, (1) each landmark configuration to be centered at the origin by translation, (2) selection of one landmark configuration to be used as a reference from which all other configurations are rotated to minimize the summed squared distances between corresponding landmarks, and (3) an average shape to be calculated and used as the new reference where all configurations are rotated to optimal alignment. Finally this process is iteratively repeated until there is no difference between the newest and previous reference (Zelditch et al., 2004). It is also notable, with correct adjustments for degrees of freedom, the use of partial warps from the deformational thin-plate spline technique will produce precisely the same results as those obtained from the coordinates of the GLS method (Zelditch et al., 2004).
Both of these approaches have been employed in various studies of 3D facial morphometrics, most notably in the dense surface models (DSM) of Hammond et al. (2004) and Hutton et al. (2003), and the quasilandmark mapping of Claes (2007) and (Claes et al., 2012b); all of which employ automated algorithms for mapping dense 3D point cloud data into known coordinate systems. In order to build a DSM, Hammond et al. (2004) and Hutton et al. (2003) firstly used the Procrustes algorithm to compute mean landmarks, and then achieved alignment using thin-plate splines to warp facial surfaces to the mean landmarks, enabling dense correspondence of tens of thousands of points to be established across all their facial forms. Claes (2007) achieved alignment and established dense correspondence between facial surfaces through a similar but improved mapping strategy involving anthropometric masks (Claes et al., 2012b). An anthropometric mask (AM) is analogous to the landmark definitions used in traditional anthropometrics, and AM mapping is distinct in its introduction of quasilandmarks (~10,000), which are spatially dense one-to-one correspondences (Claes et al., 2012b) that are mapped onto 3D facial surfaces in a non-rigid manner using a thin-plate spline based deformational model (Claes, 2007). This mapping allows the AM to be aligned and deformed accordingly to the anatomically corresponding features of the underlying facial form, iteratively allowing more flexibility to accommodate for subtler differences, and finally results in a dense set of corresponding quasilandmark configurations (Claes et al., 2012b).

In a different study, the GPA approach was also employed to successfully align mapped facial surfaces and their reflected images for a database of faces (Claes et al., 2012c). Reflected images were constructed simply by inverting x-coordinate signs of the original landmark configurations. Using pooled samples of original and reflected configurations, the AM was non-rigidly mapped onto all faces, resulting in a homologous set spatially dense quasilandmark configurations. The GPA approach was then performed to remove differences in position, orientation and scale, thereby allowing the alignment of a database of densely corresponded facial configurations.

True and accurate facial comparisons require standardization, maintenance of anatomical correspondence between landmark configurations, and then once dense correspondence has been established any number of parameters can be compared and analyzed using various statistical analyses. Statistical shape analysis is defined as the
geometrical analysis of shape data in which statistics are used to describe common geometrical properties (Dryden and Mardia, 1998), the outcome of which is a multidimensional statistical space representative of typical form variation. In the 1980s, this statistical theory of shape along with the advent of coordinate landmark-based techniques greatly contributed to the morphometric revolution (Mitteroecker and Gunz, 2009, Slice, 2005). Geometric morphometrics, in particular, is the statistical shape analysis that is based on coordinates of landmarks, and is accomplished through the Procrustes paradigm (Mitteroecker and Gunz, 2009), where aligned Procrustes shape coordinates are projected into a tangent shape space (i.e. Kendall’s space), in which ordination methods, such as principal component analysis, and/or multivariate statistical methods can be performed. These analyses test for shape differences among groups, or for patterns of covariation between shape and other continuous variables (Adams et al., 2013).

Principal component analysis (PCA) is a popular ordination technique that describes the maximum shape variation with the minimum number of independent variables or principal components (PC) (Cleall et al., 1979, Shlens, 2009). PCA is a tool for simplifying descriptions of variation among individuals, and not only produces new sets of independent variables (PCs) that are linear combinations of the original variables, but also produces scores on those variables for individuals, which can then be plotted and used visually to inspect patterns (Zelditch et al., 2004). By modeling residual shape variances from a GPA, an ordination method such as PCA can provide valuable insight into data patterns, and may even reveal patterns for addressing biological questions. The advantage of PCA is that most of the variation in the sample can usually be described with only a few PCs, meaning that as little as three PCs can describe 90% of the total variation. PCA can also combines both shape and size components to describe morphological changes, allowing faces to be represented as a single point in a multidimensional statistical shape space, in which distances between points can be used to measure variation (Aeria et al., 2010), and has been successfully used in studies of facial variation (Claes, 2007, Hammond et al., 2004, Hennessy et al., 2004). However, PCA is constrained by its inability to control the type of variation extracted by each PC, where orthogonal axes are rotated to describe the largest proportion of variance in a descending order. Therefore, PCA is dependent on the distribution of the dataset being studied, and may pose a problem when the specific variation of interest is not reflected (Smeets et al., 2010). On the plus side, the great utility
of PCA lies in the fact that many of the facial variables measured will exhibit covariances because of interactions and influences by common processes (Zelditch et al., 2004).

The inability to target specific, property-related variation (e.g. age, BMI, gender, ethnicity) within the PCA face space was resolved through the use of property pathways (Aeria et al., 2010). Aeria (2008) introduced a logical solution, by extending the use of Euclidean distances (simple linear distances) as measures of similarity within a PCA space with reference to specific variations of interest and incorporating property pathways, which are defined as the combination of PCs that cumulatively describe the direction of variation caused by the property. Using the direction of a property pathway, distances can be decomposed into property dependent and independent components, which in turn, allows the targeting of specific variation. These decomposed components presented a novel method for quantifying facial similarities (Aeria et al., 2010).

1.5 Progression of Geometric Morphometrics into the 21st Century

The measurement of shape has rapidly evolved into the rigorous and quantitative discipline of modern morphometrics. While morphometric analyses were mostly accomplished through traditional methods for much of the 20th century, over time their shortcomings have greatly limited their utility for biological interpretations. In the 21st century, there has been a greater understanding of the various morphometric methodologies, and the emphasis of these approaches are seen in many studies of craniofacial dysmorphology and asymmetry (Mutsvangwa and Douglas, 2007, Badawi-Fayad and Cabanis, 2007), as well as primary anthropological studies (Badawi-Fayad and Cabanis, 2007). Yet despite these achievements, theoretical progress continues in the discipline, particularly in the development of more specialized extensions and applications to address specific biological questions (Adams et al., 2013).

For example, extension of geometric morphometric analyses to account for missing landmarks. Incomplete forms are usually eliminated from analyses, as morphometric analyses require all forms to have the same set of landmarks. A solution would be estimation of these landmark locations so that partial forms can be included. In cases of bilateral symmetry, a simple approach is to estimate missing landmark locations by
reflecting their corresponding landmarks across the midline of the form; however, this can only be implemented for symmetrical forms. An alternative extends the logic of semilandmarks to the estimation of missing landmarks (Mitteroecker and Gunz, 2009). This approach determines missing landmark locations using the thin-plate spline to minimize shape differences between the incomplete and reference forms. While this strategy is currently based on minimizing bending energy, a similar method minimizing Procrustes distances would also be applicable. In combination with landmark and semilandmark approaches, these methods collectively provide a more comprehensive quantitative analysis of biological shape variation and greatly expand the utility of the Procrustes paradigm.

Other examples of advancements within geometric morphometrics include, quantification of phenotypic trajectories within statistical shape spaces (Collyer and Adams, 2013, Aeria et al., 2010), applications in quantitative genetics (Klingenberg and Monteiro, 2005, Adams et al., 2011), and identifying modular components in morphometric data (Mitteroecker and Gunz, 2009, Klingenberg, 2013a), all of which can provide new avenues for the field of facial morphometrics.

1.6 Symmetry and Asymmetry in Geometric Morphometrics

Studying symmetry deviations at a population level, such as aspects of fluctuating and directional asymmetry, is another area in which traditional morphometric methods have been applied (Klingenberg et al., 2002, Klingenberg and McIntyre, 1998). A mathematical decomposition of shape variation into its symmetry components for bilaterally symmetric shapes was later developed (Mardia et al., 2000) and recent work has led to extension of this approach for other types of symmetry (Savriama and Klingenberg, 2011). Analysis of symmetry and asymmetry are now frequently examined in geometric morphometric studies (Adams et al., 2013).
1.7 Establishing a 3D Facial Morphometric Toolkit for Syndromic Phenotyping

A variety of morphometric approaches has evolved (Mitteroecker and Gunz, 2009, Richtsmeier et al., 2002, Slice, 2007, Zelditch et al., 2004). Despite being particularly relevant to the aims of syndromic facial phenotyping, none of these approaches was suited to dealing with ‘abnormalities’ (or unusual facial form differences) as well as the relative inability of these techniques to separate abnormal facial form differences from typical variations. That is, syndromic dysmorphologies are often associated with a level of facial disharmony that can present as an extensive and highly localized difference in the facial form (Claes et al., 2012a).

Claes et al. (2012a) illustrated the challenge of studying form abnormalities with geometric morphometrics illustrated by the Pinocchio effect, whereby a mathematical bias is created by a substantial and localized form difference (i.e. large changes limited to a few landmarks). This Pinocchio effect can have an adverse impact on superimposition, especially when GLS is used to align landmark configurations. The least squares criterion distributes the displacement of those few landmarks across all other landmarks, and thus the Pinocchio effect appears to be “smeared out” over all landmarks. False covariances could also be induced as a consequence of these influential landmarks (Zelditch et al., 2004). In syndromic facial phenotyping, this Pinocchio effect can be considered as representative of a facial abnormality, where the highly dysmorphic region could bias the analysis and influence the Procrustes superimposition with the smearing effect of affected landmarks across its neighboring unaffected landmarks. As a result, residual differences after a least squares Procrustes superimposition would not reflect true form differences. In order to resolve this superimposition problem, Claes et al. (2012a) introduced a novel extension to the geometric morphometrics approach, namely dysmorphometrics (Claes et al., 2012a).

Dysmorphometrics is the quantitative measurement of atypical form differences, which are modeled as outliers in comparison to an appropriate reference range (i.e. norm) (Claes et al., 2012a). While morphometric studies generally focus on typical variation and covariation patterns over entire landmark configurations in a population; dysmorphometrics, in contrast, focuses on individual localized differences and the variation
patterns of that difference over a group of individuals. In the scenario of Pinocchio, it determines the exact feature in his facial form that makes him different. The essence of dysmorphometrics is the detection of atypical variation, whilst simultaneously accounting for typical variations that are considered as confounding factors. Morphological abnormalities exist within what is typically considered ‘normal’ and are mostly arbitrarily defined, the concept of modeling facial form difference as outliers in relation to an inlier normative reference was conceived by Claes et al. (2012a). This requires (1) atypical form differences to be significantly different from the norm, (2) a ‘robust’ superimposition to accommodate this, and (3) determination of an appropriate normative reference. A ‘robust’ approach to superimposition is required to mitigate the Pinocchio effect, and in statistics, a ‘robust’ method implies relative insensitivity to outliers in the data, as it would with large localized form differences. Like GLS, robust fitting superimposition methods aim to minimize differences between corresponding landmark configurations, however, they do not use the Procrustes distance metric for their superimpositions (Zelditch et al., 2004).

There are different superimposition strategies that are robust against outliers, however, not all allow for meaningful and adaptive outlier detection (Claes et al., 2012a). An alternative robust approach is based on identifying and excluding highly variable landmarks, followed by superimposition of the remaining landmarks. This is provided through the extension of a Procrustes Maximum Likelihood (ML) estimator with outlier processes, which models inliers and outliers as separate variables, and the result is a superimposition augmented in such a way that detection of significant outliers occurs simultaneously as landmarks are being aligned/superimposed (Claes et al., 2012a). In syndromic facial phenotyping, this extended Procrustes ML-estimator can be used to superimpose the landmark configuration of a given dysmorphic facial scan onto all of the landmark configurations that make up the normative reference population. The detection of outliers that occurs during the alignment is an iterative process that continues until no further outliers are detected.

The utility of the dysmorphometrics approach in the field of syndromic facial phenotyping is also dependent on the localization of facial discordancy with respect to a normative reference; but how do we first define what is normal. Until recently, the common morphometric approach to this problem was the construction of facial averages or
archetypes from normal and dysmorphic population databases, with a closed-ended classification of individuals (Hammond et al., 2005, Shaweesh et al., 2006). This archetypal approach, however, loses its power to individualize and is limited, as average faces often do not resemble typical faces in a population.

Recently, Claes et al. (2013) proposed an innovative strategy for the establishment of ‘normalized’ faces that can be tailored specifically to each patient, and that was accomplished without the need for large compilations of abnormal population data. They combined the dysmorphometrics approach with a normative statistical face space, which is essentially an extension of facial archetypes that not only captures the average facial form but encompasses differences and covariance within a normative reference population as well, thus describing the boundaries of normality in all its complexity. The strength of this statistical face space lies in its ability to construct synthetic faces within the range of harmonic/concordant facial variations (Claes et al., 2013).

The dysmorphometrics approach involves the following data transformations: (1) automated mapping using an anthropometric mask (Claes et al., 2012b) is performed on all facial landmark configurations to establish spatially dense anatomical correspondence between all facial scans; (2) the landmark configuration of a given dysmorphic facial scan is aligned onto normative reference landmark configurations using the robust-fitting extended Procrustes ML-estimator; (3) this robust superimposition creates a ‘normal equivalent’ (NE) facial configuration that is essentially a patient-specific normalized reference built upon the harmonic components from the patient’s facial form. The normative reference population is then represented as a point distribution model using PCA around the average configuration. The benefit of this is that within-population variances (i.e. typical variations) are compensated for during superimposition, and treated as other confounding variables would.

This approach to detect facial anomaly shows great promise for application to syndromic facial phenotyping. It allows for patient-specific facial assessments and their visualization; the approach has an open classification allowing the assessment of unknown facial discordancy without any prior knowledge. Dysmorphometrics is comparatively less restrictive than morphometric analyses and has a greater range of applications.
Dysmorphometrics for facial discordancy, for instance, objectively quantifies the magnitude and direction of facial shape changes and visualizes this graphically through dysmorphograms (e.g. distance maps, outlier maps and vector maps). Dysmorphometrics can also be further extended for assessing facial asymmetry through the use of reflected landmark configurations. When robust superimpositions are performed; in this case, inlier landmarks are located in symmetrical regions while outlier landmarks occupy asymmetrical regions.

During the tenure of this PhD thesis, the dysmorphometrics approach; along with its associated extensions of normative face spaces and innovative construction of norms were used in applications for various complementary areas of biological and clinical research. Wei et al. (2011) demonstrated the techniques of AM mapping and robust superimpositions in the augmentation of linear anthropometrics in orthodontics, by automating the extraction of facial midlines and facial convexity angle measurements, and correlating these with commonly used anthropometric parameters to assess surface-based facial harmonic relationships. Walters et al. (2013) studied the potential of dysmorphometrics in discriminating between cases of condylar hyperplasia using spatially dense assessments of facial asymmetry. Baynam et al. (2013b) demonstrated the potential of this approach as a tool for monitoring treatment response of mTOR inhibitor therapy.

In addition to the dysmorphometrics approach, Claes et al. (2012c) also explored the use of a two-factor ANOVA partitioning to discover facial asymmetry aspects and this was applied in a study of sexual dimorphism. Bilateral symmetry is an important facial characteristic, and it is possible to investigate aspects of symmetry and asymmetry separately when examining facial shape. Previous facial asymmetry studies typically use sparse facial landmark to represent their facial forms, where the final choice in landmark locations could ultimately lead to conflicting results. Within the framework of geometric morphometrics, Claes et al. (2012c) partitioned facial shape that was represented in a spatially dense manner into patterns of symmetry and asymmetry following a two-factor ANOVA design. This framework invoked a high number of variables due to the spatially dense data, and thus required the implementation of permutational and computationally feasible statistics.
2. Thesis Scope

Our aim was to illustrate how novel techniques in the proposed 3D facial morphometric toolkit can be utilized in applications for investigating syndromic facial phenotypes. In this thesis, we used two geometric morphometric concepts for application to syndromic analysis, (1) the framework of dysmorphometrics, and (2) the decomposition of fluctuating and directional components to facial asymmetry in spatially dense landmark configurations. Both are novel techniques that, before the tenure of this thesis, had not been applied to syndromic cohorts. The overall objectives were to (1) analyze syndromic facial asymmetry and dysmorphology patterns on both a global and regional level, and (2) to provide biological insights that might facilitate the diagnoses and treatment monitoring of these conditions.

Part One demonstrates the syndrome discrimination and treatment monitoring applications of the dysmorphometrics approach in Chapters 3 and 4 respectively, through the assessment of facial discordancy in Mucopolysaccharidosis Type I (MPS I). We firstly investigated the potential of this approach to distinguish between three MPS I affected individuals with differing degrees of clinical severity (Chapter 3), and secondly, investigated the potential of this approach as a non-invasive treatment monitoring tool by longitudinally scoring facial discordancy in an MPS I child receiving enzyme replacement therapy and bone marrow transplantation (Chapter 4). MPS disorders are rare, require timely and expensive treatments, and the characteristic coarsening of facial features, caused by the accumulation of stored product in this condition, makes it an obvious cohort for demonstrating the ability of an approach to detect and attribute severity. Additionally, the existence of established registries, which are already coordinated at international levels, allows for realistic translation of these applications, and is a further reason for selection of this particular syndrome group. In these chapters, our objectives were to resolve the following research questions:

- Can the dysmorphometrics approach objectively detect MPS I facial dysmorphologies?
- Can the approach localize and quantify the detected facial discordances?
- Do the discordances reflect known dysmorphologies of the MPS I facial phenotype?
• How can this approach discriminate between inherent overlap in facial phenotype within this disease spectrum?
• How can this approach be further extended to monitoring of treatment therapies?

Part Two demonstrates the exploratory applications of the 3D morphometric toolkit for investigating facial symmetry and asymmetry patterns in the unique causally homogenous condition, Achondroplasia and employs two different but complementary approaches to test this (Chapter 5). The first-tiered analysis uses the dysmorphometrics approach to assess total facial asymmetry at an individual level, while the second-tiered analysis employs a more complex two-factor ANOVA approach to partition total facial asymmetry at the population level, into its fluctuating and directional components. Achondroplasia has characteristic craniofacial dysmorphology (e.g. prominent forehead and depressed nasal bridge) owing to major craniofacial growth constraints. The condition is also unique in that almost all affected individuals have the same point mutation, thus providing an ideal and refined window for these investigations. In addition, the accessibility to a relatively large sample of affected individuals (n > 30) allowed these investigations to be performed at a population level. In this chapter, our objectives were to resolve the following questions:

• Can the Dysmorphometric approach objectively localize and quantify facial asymmetry of Achondroplastic individuals?
• What is the facial asymmetry signature of the Achondroplastic cohort?
• Can the two-factor ANOVA partitioning approach successfully decompose fluctuating and directional components?
• What biological information do these components provide?
• How can these approaches complement each other?

The following figure (Figure 2.1) depicts the methodological workflow of the novel techniques used in this study. Further information regarding facial scan acquisition, preparation and processing, as well as demographics of the normative reference range (Perth-WA Face Space) used in the subsequent results chapters can be found in the Appendix section (A1-A4).
Figure 2.1 Thesis Methodology Workflow. Diagram of the 3D facial morphometric toolkit components employed in thesis Chapter 3 (yellow), Chapter 4 (blue) and Chapter 5 (green & purple) depicting the novel techniques and data transformations used in the mapping, alignment and superimpositions of full 3D facial surfaces, and finally their corresponding facial assessment outcomes.
Part One Preface:

FACIAL DISCORDANCY

Chapter 3 investigates the application of the dysmorphometrics approach for investigating facial discordancy and its potential in using the syndromic facial phenotype to discriminate between subtypes within a disease spectrum. This was performed cross-sectionally on three individuals affected with MPS I, where a continuum of disease and facial severity exists. During the tenure of this thesis, this chapter was published as a research report in the Journal of Inherited Metabolic Diseases in mid-2012.

Chapter 4 similarly employs the dysmorphometrics approach, through the assessment of facial discordancy, and demonstrates its application on a longitudinal series of facial images acquired over 18 months of a treated MPS I child, who has undergone both enzyme replacement therapy and bone marrow transplantation. In this chapter, we investigate the treatment-monitoring potential of this approach, and its utility in ‘precision medicine’. This chapter builds on the previous chapter, and was recently submitted to the Journal of Inherited Metabolic Diseases for review in June 2014, where a decision is still pending.
3. Syndrome Discrimination Application: Dysmorphometrics for Facial Discordancy

Article Title: “A dysmorphicometric analysis to investigate facial phenotypic signatures as a foundation for non-invasive monitoring of Lysosomal Storage Disorders”
[Submitted: Jan 2012/ Revised: April 2012/Accepted: May 2012/Published: June 2012]

3.1 Abstract

Some Lysosomal Storage Disorders (LSDs), including Mucopolysaccharidosis type 1 (MPSI), are associated with characteristic facies. Methods such as three-dimensional (3D) facial scanning and geometric morphometric techniques can potentially generate detailed objective descriptions of these facial phenotypes. This approach can facilitate discriminating the inherent overlap in facial phenotypes within these disease spectra, and the non-invasive monitoring of disease progression and treatment.

3D facial images of three MPS I-affected individuals and 400 reference subjects (aged 5-25 years) were obtained using a 3dMD camera. (Atlanta, Georgia). Images were fitted with an anthropometric mask, comprising a set of spatially dense quasi-landmarks. A statistical face-space was constructed from the reference image set and the MPS I-affected individuals were compared to this face-space utilizing an emerging methodology known as dysmorphometrics. This facilitated simultaneous identification of harmonic and discordant facial regions. A relative significant discordance (RSD) score quantified proportional facial discordance for a given individual, whilst a root-mean-squared-error (RMSE) score measured the degree of facial discordance providing a severity measure.

A consistent facial pattern, with differential severities, primarily affecting the frontal, nasal, infraorbital and cheek regions, was detected in all three individuals. As expected, there was greater discordance (RMSE, RSD) with clinically severe MPS I when compared to attenuated disease.
Objective detection and localization of MPS I facial characteristics was achieved, and severity scores were attributed. This spatially dense dysmorphometric facial phenotyping technique has the potential to be used for non-invasive treatment monitoring and as a discriminatory tool.

3.2 Introduction

Lysosomal storage disorders (LSDs) are a group of inborn metabolic disorders, associated with disrupted lysosomal function that causes widespread lysosomal accumulation of undegraded macromolecules (Aldenhoven et al., 2009). Untreated, they inevitably result in progressive multisystem disease. With the advent of disease modifying treatments, timely diagnosis and monitoring is increasingly pivotal to improving prognosis of affected individuals including, but not limited to, some mucopolysaccharidoses (e.g. MPS I) and Fabry disease. MPS affected individuals are described as having characteristic “coarse” facies. However, the detection of LSDs based on facial features can be challenging owing to overlapping facial phenotypes (Clarke, 1997), clinical inexperience and attenuated disease. Supplementary approaches to discriminate between and within disorders and monitor treatment in a definitive manner are, therefore, required.

There have been promising, but limited; attempts toward objective definition of LSD-associated facial phenotypes and advances in imaging technology are facilitating extension of these preliminary investigations. A two-dimensional (2D) photogrammetric study by Boehringer et al. (Boehringer et al., 2006) discriminated MPS III from a number of non-LSD syndromic conditions, and a proof-of-principle study by Cox-Brinkman et al. (Cox-Brinkman et al., 2007) utilized three-dimensional (3D) face shape analysis to characterize the face of Fabry disease. The latter study used spatially dense 3D surface modeling and morphometric analysis, to quantify differences between male and female Fabry individuals, as well as in comparison to healthy controls, with classification specificities of 85% and 67% respectively (Cox-Brinkman et al., 2007).

Quantitative morphometric techniques have demonstrated great potential in detecting facial dysmorphology and variation. Hammond (2007) established facial archetypes for a small number of syndromic conditions. However, this methodology did not discriminate the impact of the condition from normal facial variation, and only visualised
the facial dysmorphology expressed as net population difference. It was also based on a closed classification setup, with the individual always attributed to either one of the defined populations, even when they did not belong to any of them. Statistically, this setup is equivalent to an unpaired analysis, which requires the appropriate sample number to obtain sufficient statistical power. These limitations have in part, been recognised by Hammond et al. Hammond et al. (2012) and this has similarly been addressed by Claes et al. (Claes et al., 2012a) with, an alternate dysmorphometrics strategy that makes comparisons to population based ‘averages’. This was previously applied to measure changes in facial shape as a result of surgical intervention (Claes et al., 2012b) and asymmetric abnormalities (Claes et al., 2011b).

This chapter explores the use of dysmorphometrics (Claes et al., 2010, Claes et al., 2012a) to discriminate subtle facial characteristics of MPS I. Normal variation can be learned from a reference dataset of individuals without pathology and obtained with relative ease. However, the collection of facial data from individuals with rare diseases is more challenging. A Dysmorphometric approach does not require comprehensive datasets of patient groups to generate a facial anomaly map. This facilitates investigations into facial phenotypic signatures in rare conditions. In difference to archetype analysis, an open-classification is performed, using only the normal variation from an appropriate reference dataset, which is encoded into a statistical face-space (Aeria et al., 2010, Wei et al., 2011, Claes et al., 2012a). This facilitates an individual-specific assessment and visualization of a problem or hypothesis, through the construction of a normal-equivalent or case-specific control (Claes et al., 2010, Claes et al., 2012a).

This approach facilitates investigations into syndromic phenotypic signatures of rare conditions and a process to address the inherent overlap in facial phenotypes within disease spectra. It can also be applied to the non-invasive monitoring of disease progression and evaluation of therapeutic effects. From this exploratory study of three MPS I affected individuals, we describe regional discrepancy scores that can be employed to establish discordance signatures in combination with a continuous severity index, to investigate aspects of metabolic and other rare diseases.
Chapter 3: Syndrome Discrimination

3.3 Methods

3.3.1 Ethics Approvals

Ethics approvals (PMHHEC: 1801/EP, 1443/EP and 1488/EP) were granted by the Princess Margaret Hospital for Children Ethics Committee in Perth, Australia.

3.3.2 Participants

The normative reference cohort (Perth face-space project) consisted of four hundred (400) healthy individuals aged 5-25 years. Subjects completed a questionnaire on relevant medical history and informed consent. Subjects with prior craniofacial surgery or a suspected syndromic condition with craniofacial manifestations were excluded.

Three participants clinically diagnosed with MPS I (severe or attenuated) and of Western European ancestry, were recruited at the 11th International Symposium on MPS and related diseases (Adelaide, SA, Australia; July 2010). Individual I was a 21-year-old female diagnosed with Hurler-Scheie syndrome (MPS IHS, OMIM #607015); Individual II was a 23-year-old female diagnosed with Scheie syndrome (MPS IS, OMIM #607016); and Individual III was a 14-year-old male diagnosed with Hurler syndrome (MPS IH, OMIM #607014).

3.3.3 3D Image Acquisition

3D images were captured using a 3dMDFacial™ stereophotogrammetric system (3dMD Inc., Atlanta, Georgia, USA) with sub-millimeter precision (Aldridge et al., 2005). Facial shape was represented as a point cloud consisting of approximately 200,000 points defined in a 3D coordinate space.

3.3.4 Anthropometric Masks & Facial Mapping

An anthropometric mask (AM) (Claes et al., 2012b) consisting of a spatially-dense ~10,000 quasi-landmark configuration, was non-rigidly mapped onto 3D facial scans of affected and normative reference individuals (403 3D images). This mapping process is equivalent to the indication of traditional anthropometric landmarks (Claes et al., 2011b, Claes et al., 2012b) and establishes homology among the 3D faces, thus allowing image data from different individuals to be standardized and analysed in a spatially-dense manner.
3.3.5 Statistical Face-Space

A statistical face-space, constructed from normative reference individuals, describes facial variations and covariances (harmonic interrelationships) present within the general population (Aeria et al., 2010, Wei et al., 2011, Claes et al., 2012a). A generalised Procrustes fit (Rohlf and Slice, 1990b) rotates, translates and scales the quasi-landmark configurations into the same coordinate space. Shape variation is then described by Procrustes distance residuals. Subsequently, the extent and modes of facial variation are calculated using Principal Component Analysis (PCA) to elucidate complex harmonic interrelationships found in facial form variations. This ‘normative’ statistical face-space was created to define the boundaries or statistical limits of typical facial variation found in the reference population (Claes et al., 2012a).

3.3.6 Dysmorphometrics and Normal Equivalents

Dysmorphometrics identifies abnormal facial morphology, as outliers in comparison to a given normative reference (Claes et al., 2012a). In this scenario, normative references are encoded within the face-space and the outliers reflect discordancy in facial form. Dysmorphometrics involves a robust superimposition of the reference face-space onto the patient’s facial scan, where each of the 10,000 quasi-landmarks is assigned a confidence value. This reflects the confidence of such a point being harmonic (value closer to 1) or discordant (= outlier, value closer to 0) against a p-value of 0.05.

This superimposition establishes the best description of a given face only in terms of harmonious facial variation, known as the “Normal Equivalent” (NE). It eliminates confounding variables like position and orientation differences, and considers typical within-population differences (e.g. variances induced by gender, age, BMI, ethnicity) as confounders as well. This allows for construction of case-matched controls, more specifically, generation of a patient-specific and population-based matched reference (see Figure 3.1 for comparison to averaged age-gender matched control), as long as the variation is described by the face-space. Thus, providing a more sensitive and specific analysis on the syndromic facial phenotype. We used a face-space comprised predominantly of Western European ethnic variance. Technical details can be found in (Claes et al., 2012a).
Chapter 3: Syndrome Discrimination

3.3.7 Scoring, Analysis & Visualisation of Facial Variants

When the NE is superimposed on to the patient scan, differences between corresponding landmarks of the two configurations provides the means to measure magnitude (distance) and directions (vector) of facial discordances. Distance maps were summarised by a root-mean-squared-error (RMSE) score, which takes into account both variance and possible bias, as an error in millimetres (mm). This RMSE score was applied to measure severity for the observed discordancy. Confidence maps were, summarised by relative significant discordance (RSD) percentages. This localized the discrepancy and provided an overall proportion of dysmorphology for a given individual. Finally, vector maps provide directional information on the observed facial discordance. The distance, outlier and vector maps collectively provide a facial discordance signature specific to the MPS I affected individual.
3.3.8 Normative Population Reference Statistics

Some discordance is to be expected in the ‘normative’ reference range as a consequence of scan/mapping artefacts, and/or the reduction of total variance modelled to 98%. Typical facial values in the reference population were established using a leave-one-out approach to compute discordance scores for the 400 healthy individuals. As a means of reference, distributions of overall RMSE and RSD scores for the ‘normative’ cohort were used to express patient values as Z-scores.

3.4 Results

Reference cohort distributions were characterized by mean of 10.6% RSD (1.8% SD) and 0.91mm RMSE (0.22 mm SD), summary statistics are provided in Table 3.1. These summary statistics provided the data for calculation of Z-scores for the MPS I subjects.

<table>
<thead>
<tr>
<th></th>
<th>Dysmorphology (RSD in %)</th>
<th>Relative Severity (RMSE in mm)</th>
<th>Z-RSD (%)</th>
<th>Z-RMSE (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference (mean)</td>
<td>10.6</td>
<td>0.91</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reference (SD)</td>
<td>1.8</td>
<td>0.22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Individual I (MPS IHS)</td>
<td>9.08</td>
<td>0.95</td>
<td>-0.84</td>
<td>+0.18</td>
</tr>
<tr>
<td>Individual II (MPS IS)</td>
<td>10.31</td>
<td>1.19</td>
<td>-0.16</td>
<td>+1.27</td>
</tr>
<tr>
<td>Individual III (MPS IH)</td>
<td>12.84</td>
<td>1.46</td>
<td>+1.24</td>
<td>+2.50</td>
</tr>
</tbody>
</table>

Table 3.1 Overall NE Assessment & Z-scores for three MPS I individuals in relation to summary reference statistics. % Dysmorphology scores were based on RSD values of the outlier map; quantified the extent of the discordance and depicted the “affected area”. The RMSE (mean ± SD) score quantified the overall degree of discordance in mm; provided a measure of severity of the “affected area”. Z-scores described the extent of difference to reference distributions (reference range). MPS IHS (Hurler-Scheie Syndrome), MPS IS (Scheie Syndrome) and MPS IH (Hurler Syndrome).
Figure 3.2 Discordance Severity in MPS I clinical subtypes. Dysmorphometric facial assessment depicting the distance and outlier map components of each MPS I affected individual’s discordance signature. The distance maps (top row) of Individuals I, II and III respectively (A, B, and C), illustrates in mm the magnitude of the facial discordance observed where regions of discordance above a threshold of 3mm (0 on the scale) are visualised using a colour bar. The corresponding images D, E, and F (bottom row) are outlier maps that depict statistically significant areas of facial discordance. Distance map and scale bar in 0-5mm (0 blue, 5mm red), while outlier map and scale bar in 0-0.3 (0 white, 0.3 black).

Discordance signatures (i.e. outlier and distance maps) for the three MPS I individuals are depicted in Figure 3.2. A consistent facial discordance with varying severity was observed for all three MPS I individuals (Figure 3.2; Table 3.1). Individual I exhibited facial variants in the infraorbital region, nasal tip, and nasio/mento-labial sulci (Figure 3.2A, D). A similar discordance pattern was observed in Individual II with an additional variant in the frontal region (Figure 3.2B, E). All facial variants detected in Individuals I and II were observed in Individual III, with the exception of the nasal tip (Figure 3.2C, F). Among all discordant facial regions observed in Individuals I, II and III, two out of the three affected individuals were found to be discordant at the naso/mento-labial sulci, as well as the frontal, nasal and infraorbital regions.

Sorting the cases on RMSE and RSD values provides a severity differential, with Individual I presenting discordance scores of 0.95mm RMSE and 9.08% RSD, these were
elevated in Individual II (RMSE 1.19mm; RSD 10.31%), and Individual III had the most severe discordance scores of 1.46mm RMSE and 12.84% RSD (Table 3.1). The facial dysmorphology for both Individual I and II was subtle with discordance scores that were within the reference range (Z-RSD: -0.84% and Z-RSD: -0.16% respectively). The severity of the observed facial discordance was likely to be mediated as all patients were treated (e.g. ERT/BMT). This indicates the sensitivity of the technique to detect subtle differences.

![Figure 3.3 Individual III MPS I Affected Vector Map. Dysmorphometric vector map of Individual III depicting vectors associated with substrate accumulation resulting in a “ballooning” effect (A) of facial features and tethering in the nasolabial folds. A close up view (B) and in profile view (C) are also shown, illustrating specific vector movements at the brow ridges, nasal bridge/tip, upper lip, and infraorbital, nasolabial and mentolabial regions.](image)

The vector map provided information on magnitude and direction of the facial discordance. In Individual III the vector map revealed an overall “ballooning effect” (Sandubray et al., 2012) of the facial tissues, a close up of this in profile view, showed specific vector displacements at the brow ridges, glabella/frontal region, infraorbital region, upper lip and both nasolabial and mentolabial sulci (Figure 3.3). Expansive displacements
were observed at the brow ridges and upper lip, different to the glabella, nasolabial and mentolabial sulci, and infraorbital regions that were depressed.

3.5 Discussion

Lysosomal Storage Disorders (LSDs) can manifest a broad phenotypic spectrum. MPS I affected individuals have been classified into: Hurler, Hurler-Scheie, or Scheie syndrome. Previously this classification was thought to reflect separate entities; however, they are now recognized as part of a continuum and affected individuals can be classed on a spectrum of severe or attenuated MPS I (Muenzer 2004). We objectively quantified a facial phenotype of three individuals affected with MPS I and sorted them on a severity scale using summary discordance values (Figure 3.2). This severity scale reflected established criteria (severe-attenuated) and found a consistent pattern of facial discordance indicative of a specific signature.

This signature was detectable in Individuals I and II, with attenuated forms, despite discordance scores that fell within the limits of the normative reference. It is not unexpected that phenotypic facial signatures, particularly of attenuated MPS I, can exist within normative ranges. However, a consistent pattern of facial discordance was found, indicating that detection of subtle phenotypes was achievable. This signature was similarly seen in Individual III who was diagnosed with a more severe form of MPS I. He presented with the greatest facial variation, as reflected in the dysmorphometry scores that were outside the reference range (Z-RSD; 1.24; and Z-RSME; 2.50).

Tethering of the Submuscular Aponeurotic System (SMAS) (Thaller et al 1990) may delineate a pattern of accumulation of extracellular substrates in facial compartments. The pattern of facial variation was consistent with this proposition, manifesting a flattened frontal region in relation to prominently raised brow ridges, depressed infraorbital regions, and a prominent nasal tip in relation to a flattened nasal bridge and expansive alae nasi. The hypothesized tethering was, in particular, evident in the depressed nasolabial and mentolabial sulci; this is in accordance with the overall facial ballooning (Figure 3A) or ‘puffiness’ characteristic of this condition (Sandubray et al 2012).
The potential of dysmorphometrics to provide an objective facial discordance signature and attribute severity on a continuous scale was demonstrated in a small cohort of MPS I individuals. Facial histograms provide data of the overall pattern of dysmorphology as well as patterns most frequently encountered, facilitating detection of subtle presentations. This is made possible by using NE controls that effectively remove confounding normal facial variations associated with gender, age and population affinity (Claes et al 2012a). This approach, when combined with a large normative dataset, can potentially address sample size limitations inherent in the study of rare disorders. Additionally, in contrast to other morphometric analyses (Hammond et al 2005, Shaweesh et al 2006), it allows for open classifications and individual-specific assessments, which facilitates a progression from descriptive dysmorphology to quantitative dysmorphometrics. Our findings supported the possibility that, using these techniques on an expanded cohort of individuals, a consistent pattern of facial variation may be revealed in both severe and attenuated MPS I. Given the rarity of these conditions; further investigations will optimally be approached by multicenter collaborations.

In theory, dysmorphometrics allows the use of a mixed control group (e.g. in gender, age and ethnicity). The face-space is a global shape model of covariance requiring all facial areas and features to be consistent, and in harmony, with each other for construction of the NE. It is therefore impossible for the face-space to randomly combine facial features from different subgroups that do not match. The reference face-space is inclusive in nature, enabling the same face-space to generate different age-, sex-, and ethnicity- matched controls for answering distinct biological questions, as long as the variation is within the face-space. If an affected individual has facial features that show some degree of overlap with a subgroup of individuals of another gender or ethnicity, it is possible to make comparisons to a subspace constructed from subjects of the overlapping gender/ethnicity. However, this may only be required when the overlap extends to the majority of the face (e.g. > 40% of the facial area). Limits of the applicability of subspace reference will require further testing.

There are considerable difficulties in objectively monitoring LSD treatment and establishing optimal dosages, particularly for presymptomatic patients in the absence of overt clinical features or suitable biomarkers (Fuller et al 2004, Langford-Smith et al 2010).
Given the finding of apparent gradation of facial phenotype, we propose further study on the potential utility of 3D facial analysis for non-invasive disease monitoring and assessment of treatment response.

The development of new therapeutics (Wraith et al 2005, Sifuentes et al 2007, Munoz-Rojas et al 2008, Bijarnia et al 2009, Clarke et al 2009, Cox-Brinkman et al 2010, Lachmann 2010, Langford-Smith et al 2010, Schiffmann 2010) provides an impetus for timely LSD diagnosis, as the efficacies of these treatments are dependent on initiating therapy prior to the development of irreversible complications (Meikle et al 2004, Meikle 2007). The challenge of achieving a definitive LSD diagnosis is complicated by overlapping phenotypic spectra (Meikle et al 2004, Meikle 2007). Therefore, investigations have been developed to allow simultaneous analysis for a number of these related conditions (e.g. urine metabolic screens and enzyme analysis panels) (Nielsen et al 2010). However, with current biochemical investigations, false negatives may occur, particularly with attenuated subtypes, (Meikle et al 2004, Meikle 2007) hence alternative approaches deserve investigation. We suggest that detection of subtle facial features can be objectively quantified and may facilitate MPS screening and diagnosis.

Development of effective LSD therapies is hindered by disease rarity (Muro 2010), and some current therapeutic strategies remain suboptimal and costly. Insights from specific LSD registries have significantly contributed to knowledge of disease spectra and natural history, but incomplete data on long-term outcomes and cost-effectiveness has raised concerns of the utility of this approach (Hollak et al 2011). These factors argue for studies with multicentre coordination that cross boundaries of individual therapies. 3D facial analysis lends itself to such studies as hardware costs are modest, there is minimal consumable cost, data can be attained non-invasively from multiple sites, and centralised analysis for existing registries is possible (Pastores et al 2007). Lifelong treatments for some LSDs necessitates longitudinal outcome-monitoring regimes, hence the need for innovative monitoring strategies that are cost-effective and preferably non-invasive. Additionally, the following factors support facial analysis as particularly suitable for very young individuals: (1) it poses no health risk, (2) capture time is fast in comparison to other medical imaging, and (3) if required, image capture can be easily repeated until a suitable image is obtained.
3D facial analysis may be useful for investigating disease biology to identify factors underlying phenotypic expression and to trace causal links between genotypes, environmental factors, and phenotypes. Genotype-phenotype correlations in individuals with MPS I are poorly understood and are only partly related to the frequency of private IDUA gene mutations (Terlato and Cox 2003). Therefore, analysis of spatially dense facial phenotypes may provide a novel avenue for resolving these genotype-phenotype relationships.

Phenomics, defined as the acquisition of high-dimensional phenotypic data that inherently spans multiple levels of an organism, has been suggested for exploring pathogenesis (Houle et al 2010). When coupled with other scientific methods, 3D facial analysis has yielded insights into rare disease biology (Tobin et al 2008). According to Houle et al. (2010), the three key elements that foster phenomic developments are technological development, statistical and analytical capabilities, and integration incentives. Our approach satisfies the first two criteria and the rarity of LSDs provides an impetus for the last. We argue that quantitative phenotyping approaches, such as dysmorphometrics, (Claes et al 2012a) can augment the repertoire of scientific protocols traditionally applied to disease studies (Houle et al 2010, Klingenberg 2002). Ultimately, the unique combination of the multisystem nature of LSDs, disorder specific treatments and high-resolution multidimensional LSD facial data, may provide a platform for new insights into facial and systems biology.

### 3.6 Conclusion

This exploratory study using dysmorphometrics supported the validity of non-invasive 3D quantification of facial profiles of individuals with MPS I. Accordingly, objective high-resolution determination of patterns of facial variants may facilitate delineation of the MPS I disease spectrum, including in those with subtle facial phenotypes. This variance-based approach, which is uniquely suitable to multicenter applications, provides the means to quantify LSD facial dysmorphology as a holistic entity. Discrete phenotypes hereby defined can support further investigations including correlations with other LSD-related endpoints, such as disorder-related complications, which may facilitate treatment monitoring. Furthermore, when combined with molecular approaches, it may allow for
novel explorations of pathogenic processes in other metabolic and genetic syndromic conditions.
4. Treatment Monitoring Application: 
Dysmorphometrics for Facial Discordancy

Entitled: “Monitoring of Therapy for Mucopolysaccharidosis Type I using Dysmorphometric Facial Phenotypic Signatures” [Submitted: June 2014/ Accepted: Jan 2015/ Published: March 2015]

4.1 Abstract
There is a pattern of progressive facial dysmorphology in Mucopolysaccharidosis type I (MPS I). Advances in 3D facial imaging have facilitated the development of tools, including dysmorphometrics, for objective and precise detection of these facial phenotypes. Therefore, we investigated the application of dysmorphometrics as a non-invasive therapy-monitoring tool, by longitudinally scoring facial dysmorphology in a child with MPS I receiving enzyme replacement therapy (ERT) and bone marrow transplantation (BMT). Both dysmorphometric measures showed a decreasing trend, and the greatest differences were found in the severity of facial discordance (Z-RMSE), displaying scores >3 SD higher than the mean at their peak, in comparison to Z-RSD scores that mostly fell within the normative range (maximum; 1.5 SD from the mean). In addition to the general trend of reduced facial dysmorphology with treatment, initial fluctuations were also evident that may have related to transient subcutaneous facial fluctuations, in the context of conditioning for bone marrow transplant. These findings support the potential of our approach as a sensitive, non-invasive, and rapid means of assessing treatment response or failure in clinical trials, and for established therapies, and would be applicable for other inherited disorders of metabolism

4.2 Introduction
Lysosomal storage disorders (LSD), which include Mucopolysaccharidosis type I (MPS I), are a heterogeneous group of genetic disorders caused by a deficiency of one or more degradation enzymes essential for normal cell metabolism (Muhlstein et al., 2013). The lack of α-L-iduronidase leads to multisystemic accumulation of its substrates within the lysosomes. This causes a variably expressed systemic disorder, which can be clinically
classified into severe or attenuated forms; this distinction influences therapeutic options (Clarke and Heppner, 2011).

The development of MPS I-targeted treatments, including enzyme replacement therapy (ERT) and allogeneic hematopoietic stem cell transplantation (HSCT), has dramatically changed prognosis. However, limitations of current treatments and cost of therapy are motivating the development of novel approaches (van Gelder et al., 2012). This requires the means to assess treatment response in an objective and recurrent manner. These assessments will preferentially be non-invasive, deeply precise, relatively inexpensive, and portable. Existing monitoring modalities are limited in their ability to objectively document responses to therapy following short-term clinical trials due to the variability of the phenotypes, the irreversibility of some complications, and the invasive nature of some investigations. Assessing response to therapy for MPS I has generally included physical/mobility tests to examine joint function, the 6-minute walking test (6MWT), lung function, changes in hepato-splenomegaly, and biochemical assays of glycosaminoglycan (GAG) substrate (Church et al., 2007).

MPS I has a pattern of progressive facial dysmorphology, particularly in untreated cases. Advances in 3D facial imaging have facilitated the development of anthropometric tools, including dysmorphometrics, to objectively detect these facial phenotypes (Claes et al., 2012a, Claes et al., 2012b, Claes et al., 2013, Hammond and Suttie, 2012). Previously, dysmorphometrics was used to cross-sectionally detect and localize MPS I-associated facial dysmorphologies and, thereby, establish an objective facial phenotype. These individual signatures were attributed severity scores to discriminate between individuals clinically diagnosed with MPS I subtypes (Kung et al., 2012). In this study, we investigate the application of dysmorphometrics as a non-invasive treatment-monitoring tool, by longitudinally scoring facial dysmorphology in a treated MPS I-affected child.
4.3 Methods

4.3.1 Participants

A normative reference cohort of approximately 1000 individuals was obtained from the Perth Face-Space Project, aged 1mth – 25 years and of self-reported ancestry. Participants completed a questionnaire on relevant medical history, and those with prior craniofacial surgery or suspected syndromic conditions with craniofacial manifestations were excluded from this cohort. This reference cohort was imperative for the construction of patient-specific controls. Ethics approvals (PMHEC: 1801/EP, 1443/EP, and 1488/EP) were granted by Princess Margaret Hospital for Children Ethics Committee in Perth, Western Australia.

The child with severe MPS I was recruited from the Princess Margaret Hospital for Children in Perth, Western Australia. He initially presented to the Emergency Department at 6 months of age with a cough, and a chest X-ray revealed paddle-shaped ribbed, suggestive of a mucopolysaccharidosis. Weekly ERT infusions began at 10 months of age, followed by bone marrow transplantation at 12 months of age, with a further 3 months of ERT post-BMT. 3D facial scans were ascertained at eight time points.

4.3.2 3D Image Acquisition, Data Preparation

3D facial scans were captured using a 3dMDFacial™ stereophotogrammetric camera system, and facial shape was expressed as a point cloud of approximately 200,000 points in a 3D coordinate space, the reliability and precision of which have been validated (Aldridge et al., 2005). These facial scans or point cloud data were brought into closer alignment by manual indication of twelve anatomical landmarks (right/left exocanthion\(^1,4\), right/left endocanthion\(^2,3\), pronasale\(^6\), lateral nasal ala corners\(^5,7\), right/left cheilion\(^8,10\), upper lip tubercle\(^9\), vermilion border\(^11\), and chin point\(^12\)), in preparation for the facial mapping process (see Appendices; A1). This decreases computational time and provides a basis for surface registration during facial mapping.

4.3.3 Anthropometric Masks & Facial Mapping

A spatially dense indicated set of 10,000 quasi-landmarks landmarks was obtained using a non-rigid surface registration (mapping) technique of a predefined facial template
(anthropometric mask) (Claes et al., 2012b). This fully automated facial mapping process required re-sampling of the raw point cloud data into a more comparable format and was performed across all faces in the dataset. A reference scan, known as an anthropometric mask and representing the standard of connectivity for all scans, was then created through an iterative ‘bootstrapping’ method, as described by Claes (2007). Once facial scans were mapped, dense anatomical correspondence was achieved and allowed for biologically valid comparisons to be performed.

4.3.4 Reference Face Space

To define the statistical limits of typical facial variation in a normative reference population, a statistical face space was constructed from 1000 individuals in our reference range cohort. A generalized Procrustes fit rotated, translated, and scaled the quasi-landmark configurations into the same coordinate space, where shape variation was described by Procrustes distance residuals. This statistical face space, via principal component analysis (PCA), describes variations in facial form and elucidates complex harmonic interrelationships between these variations.

4.3.5 Dysmorphometrics

Using the dysmorphometric approach (Claes et al., 2012a), normative references were encoded within the face space, and outliers in comparison to this normative reference reflected discordancy in the facial form (i.e. facial dysmorphology). This process involves the robust superimposition of the reference face space onto the patient’s facial scan, where each of the 10,000 quasi-landmarks is assigned a confidence value against a p-value of 0.05. A value closer to one reflects the tendency of that point being harmonic (inlier), while a value, closer to zero reflects its tendency of being discordant (outlier). Through this superimposition, patient-specific and population-based matched references called ‘Normal Equivalents’ (NE) were generated. The NE (Claes et al., 2013) describes any given face in terms of harmonious facial variation (i.e. patient’s facial scan without the dysmorphology), and its construction was performed without any a-priori knowledge of the condition itself. NE facial scans were generated at the eight assessment time points during the treatment course.
4.3.6 Normal Equivalent Facial Assessments

To analyze the facial discordancy at each time point, each NE was superimposed onto its chronologically corresponding patient scan. The differences between corresponding landmarks of each NE-patient scan pair provided the means to measure both the magnitude and vectors (direction) of the observed facial discordances. Global scores RSD (relative significant discordance; %) and RMSE (root mean squared error; mm) provide an overall measure of discordant facial proportions and discordance severity, respectively. The NE assessment also outputs two dysmorphograms that enable visualization of discordances on a facial manifold, namely, (i) distance and (ii) outlier facial maps. Distance facial maps highlight localized regions of RMSE, which take into account both variance and possible bias, as an error in millimeters (mm). Outlier (confidence) maps highlight localized regions of RSD on the facial surface, while vector maps provide directional information on the observed facial discordance. Collectively, these distance, outlier, and vector maps provide an individualized dysmorphic signature. The method workflow presented in this study is summarised in the Figure 4.1. Normalised Z-scores (Z-RSD, Z-RMSE) were also generated from reference summary NE statistics obtained from the normative population, RSD (mean 10.6 and SD 1.8) and RMSE (mean 0.91 and SD 0.22).

Figure 4.1 Method workflow used for the MPS I treatment-monitoring process.
4.4 Results

The objective measures showed similar trends for both global RSD and RMSE discordance scores over the treatment course; their regional differences are highlighted in facial outlier and distance maps, respectively (Figure 4.2). The greatest amount of change was seen at the lower two-thirds of the face; in particular, facial discordance at the nasal, perioral/ labial, cheek, and mandibular regions diminished over the treatment course. The fullness of the upper lip, though reduced, was relatively persistent.

Both discordance outcomes showed some fluctuations over the first few months, before a steady decline. This pattern is visually most notable in the graphs of normalized Z-scores presented in Figure 4.3 (Z-RSD) and Figure 4.4 (Z-RMSE), respectively. Within the first month (T1-2), ERT had a greater apparent impact on the severity of the facial discordance, compared to the proportion of discordance. Z-RSD and Z-RMSE scores both increased rapidly after BMT conditioning (T3-5) and peaked at around four months after BMT (T5, T6), during the BMT/ERT/cyclosporin phase. Progressive lessening of facial severity and discordance becomes apparent after 4-5 months (T5, T6), which corresponded to the BMT/ERT/cyclosporin treatment phase. This reduction continued until the final assessment time point. Overall, both Z-scores showed a decreasing trend, which was more pronounced in the Z-RMSE scores; Z-RMSE displayed larger scores (>3 SD higher than the mean) at its peak, in comparison to the Z-RSD scores that mostly fell within the normative range (max point: 1.5 SD from the mean).
Figure 4.2 MPS I longitudinal treatment monitoring over eight time points.
Figure 4.3 Progression of normalized facial discordance proportions along the MPS I treatment course. Z-relative significant discordance (Z-RSD; %; red points) scores were computed to enable standardization against the normative reference population.
Figure 4.4 Progression of normalized facial severity scores along the MPS I treatment course. Z-root mean squared error (Z-RMSE; mm; *purple points*) scores were computed to enable standardization against the normative reference population.
4.5 Discussion

For the first time we objectively, non-invasively, and dynamically assessed the changing 3-dimensional facial dysmorphology in a child undergoing disorder-specific treatment for a systemic metabolic disorder, namely, MPS I. This deeply precise dysmorphometric assessment demonstrated a reduction in facial dysmorphology which was in accordance with expectations from clinical experience and with that based on an exploratory cross-sectional study, demonstrating the correlation of the severity of facial and clinical MPS I phenotype (Kung et al., 2012) (see Chapter 3). Additionally, it builds upon previous 3D facial analysis-based treatment monitoring of a localized facial pathology (Baynam et al., 2013b) and of a dysmorphic non-metabolic disorder (de Souza et al., 2013). This study extends the findings of the aforementioned investigations, and they support that deep facial phenotyping may have applications for the development of treatment response biomarkers.

The greatest differences were found in the severity of facial discordance (root mean square error, RMSE). Additionally, overall, the longitudinal pattern of changes in the proportion of facial discordance (relative significant discordance, RSD) was in accordance with the pattern of the RMSE. This supports that approaches that provide multiple facial outcome measures may act to (i) corroborate each other, (ii) provide complementary approaches to summarise variations in facial form, and (iii) provide contrasting windows through which to consider the implications of found facial variation.

As treatments, including ERT and BMT, may ameliorate, but not reverse or prevent all MPS I manifestations (Wynn, 2011), the expectation is of an initial period of improvement followed by a period of stabilization. Therefore, it is possible that there will be some persistence of residual facial dysmorphology and/or its partial recurrence. Our study’s findings are in accordance with anticipated improvement/ stabilization; however, there may be persisting residual/ recurrent facial dysmorphology over time.
The fluctuations in the global discordance scores detected during the first few months of therapy are notable. There was an initial reduction in facial severity during the initiation of ERT. Subsequently there was an overall ballooning facial effect, which may be related to transient subcutaneous facial fluctuations that were the result of the effects of conditioning and events in the initial post-BMT period. Should the documented variations be correlating with acute treatment events, this might then indicate our approach could be both a sensitive and rapid means of assessing treatment response or failure for established therapies, in drug development and clinical trials.

It is important to note that the measures (RSD, RMSE) used in this study are, by definition, relative scores. The purpose of the NE is to remove confounding factors like within-population variances (e.g. age, gender, ethnicity, and body mass index), which provides a more individualized assessment. It is the harmonic regions of the patient facial scan that drives the construction of the corresponding NE facial scan. Longitudinal changes, such as ageing, alter the patient’s facial configuration at each time point, which then alters the harmonic interrelationships within that patient scan. This, in turn, changes the NE with each new time point. Hence, in this study we see eight similar yet different NE facial scans of the same patient. Therefore, direct comparisons of normal equivalents between time points require consideration within this context. For instance, the fullness of the labial region, on a background of the typical fuller face of infancy, might be more “harmonic” and, therefore less discordant when compared to labial fullness in an older child. Interestingly, this labial fullness is seen in the 14-year-old MPS IH individual reported by Kung et al. (2012) (see Chapter 3), where lip prominence was detected as a discordant feature 13 years after BMT. This unmasking of present, but challenging to discern without objective support, and age-dependent dysmorphology could partly explain challenges in unaided clinical facial assessment. However, they may be identified with objective and precise approaches such as the one described herein.

This study was limited to the assessment of one individual, and other treatment endpoints were not available for comparison. Further studies will be required to investigate
additional individuals and importantly to relate their facial phenotype to existing outcome measures. Given the individual rarity of these conditions, this will require multi-institutional assessments, e.g., urinary glycosaminoglycans and measures of respiratory function and endurance. Fortunately, the wide geographic dispersion of currently available robust and precise imaging equipment can facilitate this in the immediate term. Also, rapid advances in the development of increasingly portable and less costly 3D imaging equipment will make this increasingly feasible in the intermediate term including for point of care or ultimately in-home image capture.

This approach could also be expanded to other metabolic and non-metabolic disorders with known (or as yet unappreciated) facial phenotypes with current, and emerging, disorder-specific therapies.

4.6 Conclusion

This longitudinal study demonstrated objective and deeply precise changes in facial morphology in a child with treated MPS I. If corroborated by further studies, including correlation with other objective treatment outcomes, this supports the use of dysmorphometric 3D facial analysis as a non-invasive and relatively inexpensive treatment biomarker to rapidly assess therapeutic response in this condition and possibly other disorders with facial dysmorphology. Additionally, our longitudinal findings in a young child, when coupled with previous cross-sectional studies (Kung et al. 2012, Chapter 3), suggest that this approach could aid early clinical classification.

4.7 Acknowledgments

We are indebted to the child with MPS I and his family for participation in this study and permission for image use. We would like to thank Genzyme Australia for providing an unrestricted educational grant. The Princess Margaret Hospital Foundation in Perth, Western Australia, also provided support for this study. RD-Connect, the associated
National Health and Medical Research Council of Australia, the Raine Clinician Research Fellowship supported GB’s contribution. All authors declare that they have no competing interests. Genzyme provided an unrestricted educational grant, and had no role in the interpretation/analysis of the data, or the decision to submit the manuscript for publication. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000(5). Informed consent was obtained from all participants included in the study.
Chapter 5 demonstrates the exploratory applications of the Dysmorphometric and two-factor ANOVA decomposition approaches, through the investigation of facial symmetry and asymmetry patterns in the short-statured syndromic condition, Achondroplasia. As a consequence of the novel techniques applied in this chapter, insights into the disordered craniofacial growth and developmental stability of the condition were attained. The dysmorphometrics approach is once again employed but here, it is utilized to assess facial asymmetry. In contrast, a two-factor ANOVA approach is also used to assess facial asymmetry, but takes it one step further than the dysmorphometrics approach, by partitioning the total variation in Achondroplastic facial asymmetry into its directional and fluctuating components, and reveals some thought-provoking facial patterns. This study is the first of its kind, and was recently submitted to the Journal of Anatomy in April 2014, where it is currently under review.
5. Exploratory Applications: Dysmorphometrics and Two-Factor ANOVA Decomposition of Facial Asymmetry

Manuscript Title: “Asymmetry and Symmetry in Syndromic Growth: Three-Dimensional Facial Analysis in Achondroplasia” [Submitted: April 2014]

5.1 ABSTRACT

As facial asymmetry is common in individuals with and without craniofacial syndromes, investigation of symmetric and asymmetric variation provides windows into biological processes, and studies of symmetric/asymmetric variation are context dependent and complementary. At the population level, with the appropriate statistical modeling, it is possible to distinguish between directional and fluctuating components of facial asymmetry. This decomposition allows for targeted phenotypic assessments that can be employed for various investigations. Achondroplasia is an ideal and refined window for investigating syndromic asymmetry phenotypes as it has characteristic craniofacial dysmorphology and unique causal genetic homogeneity, with almost all cases associated with the same point mutation. Here we apply a two-factor ANOVA extension to spatially dense facial asymmetry assessments, to distinguish directional and fluctuating asymmetry in a cohort of Achondroplastic patients. We additionally investigate symmetric variation in these individuals. Partitioning the 3D facial shape variation into symmetry and directional and fluctuating asymmetry components revealed that the majority of the phenotypic variance was accounted for by symmetrical variation and significant levels of both directional and fluctuating asymmetry were also demonstrated. Furthermore, the patterns found were consistent with known disease biology. We suggest that our approach to the decomposition of facial variation into symmetric, and asymmetric, components can be applied to other cohorts of individuals with disordered craniofacial development to provide unique insights into disease biology and, by refining facial signatures, support the development of facial diagnostics.
5.2 INTRODUCTION

Minor asymmetries are common during craniofacial development (Cohen, 1995) and a degree of facial asymmetry is expected in the general population. Facial asymmetry is also a diagnostic feature of some craniofacial syndromes and some conditions affecting the face are known to have preferential laterality. For example, the left-sidedness of cleft lip (Cohen, 2006), and the right-sided displacement of mid-facial features in fetal alcohol syndrome (Klingenberg et al., 2010). Whilst severe asymmetries are readily recognizable, subtle presentations are more difficult to discern and discrete patterns of syndromic asymmetry largely remain to be determined. However, novel assessments, such as robust and spatially-dense facial asymmetry assessments (Claes et al., 2011a, Claes et al., 2012c), have the potential to systematically detect and define facial asymmetries in conditions with known (Walters et al., 2013), and as yet undescribed, asymmetry.

The variance in population (cohort) level asymmetry can be decomposed into directional asymmetry (DA), fluctuating asymmetry (FA), and antisymmetry (Tomkins and Kotiaho, 2001). Antisymmetry, which is the bias in asymmetry that occurs with equal frequency on both sides (Palmer and Strobeck, 1992), goes beyond our scope and will not be addressed in this thesis. From here on, we focus solely on directional and fluctuating asymmetry. DA is the consistent unilateral bias in asymmetry on a population level, and it is considered a feature of normal development (Graham et al., 1993, Palmer and Strobeck, 1992). In contrast, FA has no unilateral bias and its quantitative determination is thought to measure random deviations from the ideal state of symmetry that are reflective of the level of genetic and environmental stress experienced by individuals or populations during development (Tomkins and Kotiaho, 2001). Decomposition of asymmetry into directional and fluctuating components may provide for complementary investigations of the normal and disordered facial phenotypes. Specifically, the DA component may provide insights to primary developmental processes and measurement of FA may provide an index of adaptation and co-adaptation (Graham et al., 2010). FA is thought to arise from presumed random variations in developmental processes, and it is considered to be primarily of non-genetic origin, although genetic factors may modulate its expression (Takahashi et al. 2011). It is a component of within-individual variation that provides an opportunity to investigate the developmental origin of phenotypic variation (Klingenberg, 2013b,
Klingenberg et al., 2010). Non-directional differences in bilateral development can be considered as the outcome of two opposing forces, developmental noise, and developmental stability. Developmental stability is conceptualized as internal developmental buffering mechanisms that can modify genotype-phenotype relationships to provide an individual with the ability to overcome random developmental noise (Kimmerle and Jantz, 2005). Whilst investigations into buffering mechanisms may lead to a greater understanding of observed variation, its precise measurement is challenging. Developmental stability and noise cannot be observed independently, nor have their variation components been separated with ease; fluctuating asymmetry becomes a useful surrogate measurement for both (Lens et al., 2002). Although developmental stability cannot be readily estimated, fluctuating asymmetries are estimable (Moller and Manning, 2003).

There are methods, such as two-factor analysis of variance (ANOVA) models, to test whether significant levels of DA and FA are present at the population level (Palmer and Strobeck, 1992). The two-factor ANOVA design used in this study, involves the decomposition of bilateral shape variation into its symmetrical and asymmetric components. This design was originally derived by Mardia et al. (2000), extended by Klingenberg and McIntyre (1998), Klingenberg et al. (2002) to include FA and measurement error, and further modified by Claes et al. (2012c) for application to spatially dense 3D faces, where original and reflected landmark superimpositions are used in place of traditional right-minus-left |R-L| measurements to measure asymmetry. This morphometric approach was recently used to analyze facial symmetry and asymmetry in a population unselected for craniofacial disorders (Claes et al., 2012c). These modifications provide the ability to separate and objectively study symmetric and asymmetric components of facial shape, which can now be applied to investigations of disordered growth that in turn may provide novel insights into facial biology and development in health and disease. As Achondroplasia is a clinically well-defined entity and it has unique causal genetic homogeneity, with almost all cases due to the same point mutation, it provides a refined window of disease biology. Therefore, we chose to initiate partitioned investigations of facial variation in disordered growth by analysis of a cohort of Achondroplastic individuals.
5.3 Study Design

Achondroplasia has proven to be a useful model for studying effects of abnormal endochondral bone formation on craniofacial development (Cohen et al., 1985), as the associated short cranial base creates an unstable growth platform which might promote asymmetry, and it may also be a uniquely parametised model for investigating symmetry and asymmetry. In this study, we present a two-tiered analysis investigating facial asymmetry/symmetry patterns in Achondroplasia. The first tier of facial asymmetry analysis (i.e. spatially-dense asymmetry assessment) was performed on the Achondroplastic cohort at an individual level and was statistically summarised with Z-scores. The second tier of facial asymmetry analysis (i.e. two-factor ANOVA partitioning of DA and FA) was performed at a population level on the achondroplastic cohort. As part of this second analysis, a multivariate approach, principal component analysis (PCA), was also employed to extract and analyze patterns of covariation to interrogate facial signatures of this condition that may provide insight into the developmental basis of syndromic asymmetries. We hypothesized that whilst the condition may visually appear relatively symmetric, the partitioned objective assessments would identify patterns of asymmetry.

5.4 METHODS

5.4.1 Participants

To establish overall normative reference asymmetry statistics, data were obtained from a sample of 913 unaffected individuals (aged 1mth-55yrs) from a reference dataset of 3D facial images (Perth Face Space Project). A cohort of 33 individuals with Achondroplasia (ACH) was recruited from the Short Statured Peoples’ Association (SSPA) Convention in Perth, Western Australia 2011, and from the Royal Children’s Hospital in Melbourne. They were aged between 1-48 years and of self-reported European ancestry. Ethics approvals (PMHEC: 1801/EP, 1443/EP, 1488/EP) were granted by the Princess Margaret Hospital for Children Ethics Committee in Perth, Australia.
5.4.2 Facial Mapping and Reflecting

3D facial data of participants were captured using a 3dMDFacial™ stereophotogrammetric system (3dMD Inc., USA), the precision and repeatability of which was previously tested (Aldridge et al., 2005). An automated facial mapping process (Claes, 2007) was performed by the non-rigid mapping of an anthropometric mask (Claes et al., 2012b) on all 3D facial scans, where each resultant facial configuration consisted of ~10,000 unpaired and non-symmetrical points (Claes et al., 2011a), known as quasi-landmarks. This facial mapping process, therefore, establishes a homologous dataset of facial configurations, and allows asymmetry assessments from different individuals to be standardized in a spatially dense manner (Claes et al., 2011a). Reflected facial images were constructed by inverting the x-coordinate (Klingenberg and McIntyre, 1998) of the original facial configuration quasi-landmarks. In doing so, these reflected configurations lose compatibility and require re-mapping with their original configurations to re-establish homology. This re-indication step is akin to, and replaces, the relabeling step in Klingenberg’s protocol for object symmetry (Klingenberg et al., 2002). From this point forward, different techniques were employed for the two tiers of facial asymmetry analyses and these are described below.

5.4.3 Spatially-Dense Facial Asymmetry Assessment

This spatial assessment of facial asymmetry (Claes et al., 2011a) was based on an automated and robust facial asymmetry analysis, using a weighted least squares superimposition protocol for comparison of a facial scan with its reflected image.

5.4.3.1 Robust Superimposition

In order to accommodate detection of a wide range of asymmetries (i.e. from mild to severe), a two-step robust superimposition was needed (Claes et al., 2011a). Based on the concept of inliers and outliers, quasi-landmarks located in asymmetrical facial regions were considered as outliers, while inliers were located in symmetrical regions. A confidence value was assigned to each quasi-landmark, reflecting the confidence of that point being an inlier or an outlier. These values were then used in the two-step optimization procedure as weights to iteratively update the superimposition in a weighted least-squares manner until no further change was observed. For this analysis, the atypicality value (i.e. one minus the confidence value) was used for the presence of asymmetry.
5.4.3.2 Scoring, Analysis and Visualization of Facial Asymmetries

A root mean squared error (RMSE; mm), and relative significant asymmetry (RSA; %), score (Claes et al., 2011a) was calculated for the cohort of Achondroplastic individuals. Total asymmetry scores per individual provided information on the severity of the total asymmetry in millimeters (RMSE), as well as the proportion of the face affected by this total asymmetry (RSA). This was similarly performed on the unaffected reference individuals, generating overall normative reference summary asymmetry statistics (see Appendix; A3), thus enabling Z-scores to be computed for each Achondroplastic individual. An average distance facial map was constructed by averaging the homologous quasi-landmark RMSE values, and projected onto the average consensus configuration. The average distance map is useful as an asymmetry signature, reflecting the average magnitude of facial asymmetry observed in Achondroplasia, where regions of asymmetry above a threshold of 3mm are visualized using a colour bar. In addition, an Achondroplastic occurrence map was also constructed (Figure 5.2;D). This was performed by placing a global threshold of 0.2 on the continuous range of atypicality values (0 → 1), thereby creating discrete values (0 or 1) to depict the occurrences of significant asymmetry at each landmark within the Achondroplastic cohort.

5.4.4 Two-Factor Procrustes ANOVA Analysis

While the first tier of asymmetry assessments is useful for analyzing total facial asymmetry in Achondroplasia, it cannot partition DA and FA. The second tier of facial asymmetry assessments approaches this by implementation of the modified two-factor Procrustes ANOVA analysis previously described (Claes et al., 2012c). This analysis was performed on the Achondroplastic cohort at a population level.

5.4.4.1 Procrustes Superimposition

Once Achondroplastic facial scans were mapped and reflected, according to the aforementioned processes, they were subjected to a generalized least-squares Procrustes (GLS) superimposition method (Rohlf and Slice, 1990a), where original facial configurations and their reflected images were superimposed onto each other. This approach not only eliminates confounding factors like pose, orientation and scale differences from the dataset, but also constructs a Kendall shape space that is centered on the overall consensus configuration, and where each facial configuration is then represented.
as Procrustes shape coordinates (Claes et al., 2012a, Mitteroecker and Gunz, 2009). The resultant mean configuration (consensus) produced is completely symmetric, and it is possible to decompose the variation around this consensus into its bilaterally symmetric and asymmetric components (Mardia et al., 2000, Savriama and Klingenberg, 2011).

5.4.4.2 Partitioning of Facial Shape Variation

In order to extract these components of symmetry and asymmetry, a partitioning of the variation around the mean facial shape was performed (Klingenberg et al., 2002). The symmetry component was computed from the average of the original configuration and its reflected configuration. Symmetric variation is the variation of these averages among individuals in the dataset. Asymmetry, on the other hand, was measured from the differences between the original and reflected configurations. This was further partitioned into two separate components of asymmetry, where DA was the average asymmetry and FA was the variation of the individual asymmetries (Savriama and Klingenberg, 2011). This partitioning of facial shape variation was performed and reported using the full two-factor ANOVA design (Klingenberg et al., 2002), where components were decomposed into effects due to individuals and reflections, the individual X reflection interaction, and lastly measurement error.

The main effect called ‘individuals’ represents the inter-individual variation within a cohort, and it is the variation among symmetry components that have been corrected for the effects of asymmetry (Claes et al., 2012c). The main effect of ‘reflections’ is interpreted as a measure of DA, where mean (R – L) traditional trait values have a normal distribution with a mean value deviating from zero (Palmer and Strobeck, 1992). The ‘individual x sides’ interaction term is the failure of the effect of individuals to be the same from side to side, and hence represents FA (Graham et al., 2010), which is the measure of random deviations from a normal distribution of right minus left values with a mean value of zero (Palmer and Strobeck, 1992). To aid biological interpretations, these main effects and interaction terms were respectively renamed as symmetry variation, DA and FA (row headings) in Figure 5.3.

Replicate measurements were not performed for this analysis, i.e. single quasi-landmark facial configuration for each individual and only one facial mapping process was
performed. As a result, the measurement error term had to be computed using a combination of (1) noise injection and (2) additive main and multiplicative interaction (AMMI) modeling, the technical details of which have been previously reported (Claes et al., 2012c).

5.4.4.3 Statistical Tests of Significance

Statistical significance of two-factor ANOVA effects was assessed through the use of permutation tests, where each test used 1000 random permutations of the observations, and a P-value generated based on the number of times the permuted values were greater or equal to the observed values. Effect-size statistics approximate the size and thus the practical importance of the observed differences. An effect that is large in absolute magnitude (MS) could also be unimportant in relative terms ($F$-ratio) (Claes et al., 2012c). For this reason, F-statistics were performed on all components of variation for the Achondroplastic cohort under study.

5.4.4.4 PCA Modeling of Symmetry and Asymmetry Patterns

Property pathways (Aeria et al., 2010), phenotypic trajectories or motion paths (Collyer and Adams, 2013), in the multivariate shape space can be used to describe changes in facial shape. For a more detailed investigation of the patterns of facial symmetry and asymmetry, we employed the multivariate approach, PCA. As with most applications of PCA, the first few principal components (PCs) account for the majority of the total variation in the dataset, thereby effectively reducing its dimensionality. In addition, PCA also allows us to analyze and visualize patterns of variation so that they can be interpreted biologically (Klingenberg and McIntyre, 1998). Here, we model the symmetry and asymmetry patterns and illustrate facial variation changes at the positive and negative limits according to the first three principal modes of variation in asymmetry (Figure 5.4) and symmetry (Figure 5.5).
5.5 RESULTS

![Z-RSA Distribution of the Achondroplastic Cohort](image1)

![Z-RMSE Distribution of the Achondroplastic Cohort](image2)

Figure 5.1. Z-RSA and Z-RMSE Distributions of the Achondroplasia Cohort. Data points highlighted in dark blue depict normal asymmetry with Z-scores falling within range of (-1SD ≤ x ≤ 1SD), light blue data points reflect the outer ranges of normal asymmetry (-2SD ≤ x ≤ 2SD), while data points in orange and red indicate abnormal asymmetry (x > 2SD or x < -2SD).

Descriptive summary asymmetry statistics for the normative reference range are given in the Appendices (A3, Table 1), where we employed age-matched grouping to enable better estimation of asymmetry changes during the developmental stages of an individual from infant to adult. The Wilcoxon rank sum test showed that RSA scores for
males (median = 11.52) differed significantly from females of the 12-17 year old cohort (median = 10.31; \( W = 12077, z = -3.275, P < 0.001, r = -0.22 \)). This was also seen in the 18-30 year old cohort, where male RSA scores (median = 11.38) were significantly different from that of females (median = 10.88; \( W = 35517, z = -3.055, P < 0.002, r = -0.16 \)). Likewise, the Wilcoxon rank sum test for RMSE scores for both 12-17 year old (median = 1.33) and 18-30 year old males (median = 1.41) showed significant differences from their 12-17 year old female (median = 1.16; \( W = 11880, z = -3.709, P < 0.001, r = -0.25 \)) and 18 – 30 year old female counterparts (median = 1.24; \( W = 34708, z = -3.966, P < 0.001, r = -0.21 \)). From the Wilcoxon tests, it was decided that RMSE and RSA scores had to be calculated separately for males and females, for the 12-17 and 18-30 year old age ranges of the normative reference population, while the genders for the other age ranges could be merged. The means and standard deviations of these normative reference RMSE and RSA scores (Appendix A3, Table 1) were then used to generate the Z-RSA and Z-RMSE scores of the achondroplastic cohort (Figure 5.1).

Spatially dense facial asymmetry assessment of individual Achondroplastic individuals was performed as the first tier of investigation. The relative proportion of facial asymmetry (Z-RSA scores) for most of the Achondroplastic individuals was outside the limits of normal asymmetry. Fifteen of the 33 (45%) clearly displayed asymmetry proportions that were 3 – 4 SD from the mean, while a third of the Achondroplastic individuals (11 out of the 33) displayed less than normative asymmetry proportions (Figure 1; top). In contrast, based on Z-RMSE scores, the magnitude (i.e. severity) of asymmetry was within the normative range for the majority of the Achondroplastic individuals (94%). Only one individual displayed an asymmetry magnitude that was comparatively greater than the normative range with Z-scores of 3 SD from the mean, while one other showed a less than normative asymmetry magnitude (<2 SD) (Figure 5.1; bottom). Biologically, these scores are of relevance as the Z-score between zero and one indicates normal asymmetry, a Z-score between one and two indicates asymmetry closer to the outer ranges of normal asymmetry, and a Z-score of more than two indicates abnormal asymmetry (Claes et al., 2013). The average distance facial map for the Achondroplastic cohort, depicted in Figure 5.2C, highlighted localized regions of RMSE at the forehead, orbits, lateral mid-face and angle of the mandible. The Achondroplastic occurrency map (Figure 5.2D), on the other
hand, showed the presence of significant asymmetry at the upper and bilateral forehead, perioral, orbital, and nasal regions, and the corners of the mouth, in 100% of the Achondroplastic individuals.

<table>
<thead>
<tr>
<th>A: Achondroplasia Symmetric Group Average</th>
<th>B: Achondroplasia Directional Asymmetry</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Symmetric Group Average" /></td>
<td><img src="image2" alt="Directional Asymmetry" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C: Achondroplasia Average Distance Map</th>
<th>D: Achondroplasia Occurrency Map</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3" alt="Distance Map" /></td>
<td><img src="image4" alt="Occurrency Map" /></td>
</tr>
</tbody>
</table>

Figure 5.2. Asymmetry signature maps for the Achondroplastic cohort. (A) The symmetric group average, (B) Achondroplastic directional asymmetry, differences between mean original and reflected configurations amplified three times and visualized onto (A), (C) the average distance map and (D) asymmetry occurrence map of the Achondroplastic cohort.
<table>
<thead>
<tr>
<th>EFFECT</th>
<th>MS</th>
<th>F-ratio</th>
<th>P&lt;sub&gt;1000&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual Variation</td>
<td>3.90E-03</td>
<td>15.84</td>
<td>**</td>
</tr>
<tr>
<td>Directional Asymmetry</td>
<td>6.51E-04</td>
<td>2.64</td>
<td>**</td>
</tr>
<tr>
<td>Fluctuating Asymmetry</td>
<td>2.47E-04</td>
<td>92.5</td>
<td>**</td>
</tr>
<tr>
<td>Error</td>
<td>2.67E-06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 5.3. Facial asymmetry shape partitioning in Achondroplasia.** Two-factor ANOVA decomposition of Achondroplastic facial shape variation using an isotropic model. P1000 Column: P values using 1000 permutations with * corresponding to p<0.05 (light green), ** corresponding to p<0.001 (yellow), and dark green corresponding to non-significant areas (p≥0.05). MS (mean square) is the sum of squares divided by the appropriate degrees of freedom, reflecting the magnitude of effect. F (F-ratio) is the MS divided by an appropriate error MS, reflecting the relative magnitude or strength of the effect. The interaction term is used as error term for the main effects of individuals and sides, and the actual error term is used for the interaction term.
Two-factor ANOVA partitioning of symmetry and asymmetry, at the cohort level, was performed as the second tier of analysis. The facial shape decomposition into its symmetry, DA, and FA components is illustrated in Figure 3. The mean squares (MS) reflect absolute effect magnitude, while the $F$-ratios reflect relative effect magnitude. Overall, all three components were significant ($P < 0.05$), with the effect of the symmetry and FA components being highly significant ($P < 0.001$).

The symmetric component accounted for most of the total variance; it was focused at the forehead, nose, mid-face, philtrum, lips, and chin region (Figure 5.3; top row). Achondroplasia is not known to be associated with marked clinically apparent facial asymmetries, thus high symmetric variation is in accordance with expectation. A consistent pattern of asymmetry was also detected in the Achondroplastic cohort, as indicated by the significant levels of DA ($P < 0.05$). This was localized to the forehead, orbits, zygoma, lateral mid-face and at the angle of the mandible (Figure 5.3; second row); i.e. similar regions to those of the average distance map from the first-tiered asymmetry assessment (Figure 5.2C). The facial FA pattern was similar to that revealed in the occurrence map from the first-tiered robust asymmetry assessment. Significant FA encompassed the majority of the facial region, albeit with relative sparing of the mid-face and central forehead regions (Figure 5.3; third row). In contrast, the error term was evenly scattered over the entire face, consistent with the expected noise in the data (Figure 5.3; fourth row).
Figure 5.4. Achondroplastic asymmetry model depicting the first three principal components (PC). Coloured facial maps (left column) highlight regions of the face associated with the first, second and third modes of asymmetry variation respectively. The positive and negative morphs (right columns) shown here are static images at PC value = ±3σ of each individual PC axis. Moving along this axis provides information on the vectorial relationship of the changing facial regions.

The first three PCs of the Achondroplastic asymmetry model of variance are depicted in Figure 5.4. The first PC represents the strongest mode of asymmetry variation and accounts for (33.7%) of the total asymmetry variation, involving mostly the upper two-thirds of the face and the angle of the mandible. The second and third PCs explain 14.4% and 10.3% of the total asymmetry variance respectively, both of which involve movement of the upper facial quadrants in relation to the chin point. Facial regions associated with motion paths along each PC are depicted with coloured facial maps (Figure 5.4; effect column). Regions highlighted in red indicate those regions with the greatest magnitude of change, in comparison to dark blue areas where no change is observed. For visualization purposes, positive (PC value = 3σ) and negative morphs (PC value = -3σ) along the individual PC axis were shown as static images (Figure 5.4). Moving along each PC axis provides information on the vector relationships of the changing facial regions.
Figure 5.5. Achondroplastic symmetry model depicting the first three principal components (PC). Coloured facial maps (middle column) highlighting facial regions associated with the first, second and third modes of symmetric variation respectively. The positive and negative morphs shown here in both frontal (left column) and profile (right column) views are static images at PC value $\pm 3\sigma$ of each individual PC axis.

Similarly, the Achondroplastic symmetry model of variance (Figure 5.5) depicts the effects, positive and negative morphs of the first 3 PCs, explaining 29.4%, 20.8%, and 11.8% of the total symmetric variance respectively. Consistent with the age distribution of the cohort, the first PC appears to capture age-related changes, namely the increase in facial height, an increase in facial width and nasal prominence. Notably, the second and third PCs appeared to be related to shape changes characteristic of the Achondroplastic facies. The second PC displayed frontal bossing with retrusion of the perioral region including at the premaxilla. While the third PC displayed a short nose with an upturned nasal tip and chin prominence.

5.6 DISCUSSION

In this study, for the first time, we applied a new spatially dense asymmetry assessment to a well defined and genetically homogenous condition characterized by disordered craniofacial growth, namely Achondroplasia. Additionally, we partitioned facial symmetric and asymmetric variance and decomposed asymmetry into directional and fluctuating components. Initially, we compared affected individuals against their corresponding reflected images and these outcomes were contrasted against normative population
summary statistics to produce Z-scores. The majority of Achondroplastic individuals had a facial asymmetry magnitude (Z-RMSE) within the normative range, and approximately half of them had relative proportions of facial asymmetry (Z-RSA) that were abnormal when compared to the normative cohort. Subsequently, a full two-factor ANOVA analysis (Claes et al., 2012c) was employed to provide the simultaneous estimation and decomposition of the total phenotypic variance into its components (Klingenberg et al., 2002, Claes et al., 2012c, Klingenberg and McIntyre, 1998). The model partitioned facial shape into three different components of variation: symmetry, DA and FA. Significant variations in all asymmetry components were identified. As expected, our analyses indicated that the symmetric component accounted for the majority of the total shape variation, which is consistent with expected variation between each Achondroplastic individual in (non-FGFR3) genetic and environmental background, and with facial variation in the general population. Within asymmetric components, variation in FA was more marked than DA, and visually their patterns differed.

Cumulatively, from our two differing assessment approaches, we discovered DA in the average distance map, and a demonstrable overlap between the occurrency map and FA. The highlighted facial regions show a one-to-one relationship between DA and the average distance map. This is in accordance with expectation as DA is defined as being an average of differences between left and right sides or, in this case, between original scans and their reflected images. Similarly, the occurrency map gives us insight to the FA through the same exhibited pattern of facial asymmetry, albeit with a difference in colour schemes as the colors in each of these facial maps have different meanings. The generation of similar results by complementary approaches that are agnostic of each other, and that are keeping with expectation, supports the validity of our approach.

The localization of the DA facial signature to particular regions of the face might be reflective of compensatory reactions to altered biomechanics arising in the context of known primary craniofacial disturbances in Achondroplasia. The Achondroplastic craniofacial phenotype is primarily a perturbation of endochondral osteogenesis (Cohen, 2006). Restricted growth at the cranial base, in conjunction with relatively preserved mandibular growth, creates the characteristic midfacial deficiency with mandibular pseudoprognathism (Barone et al., 1994). Occlusal instability inherent in this anatomical
context can create upper midfacial asymmetries along with mandibular displacements. In the setting of altered occlusal planes, and in order to obtain biomechanical function (e.g. for occlusion and mastication), we speculate that compensatory bone growth occurs along the processes of the retrusive maxilla, at sites of muscle-to-bone attachments (entheses), to enable muscle action in the plane of mechanical advantage. The result of primary disruption in craniofacial development and secondary compensatory reactions might be anticipated to result in asymmetries including at those regions highlighted by our study, namely the zygomatic, malar, and mandibular regions. It is also possible that there may be systematic primary disruptions of the establishment of symmetry/asymmetry in Achondroplasia. If so, compensatory events may also occur in a milieu of systematically altered patterning of symmetry/asymmetry, which at the population level would result in DA. Contrasting the finding of significant FA across the majority of the face might represent less uniform, with respect to laterality and facial region, and more individualized and fine grained adaptations. Even though the restricted cranial base is distant to the facial surface, the interrelatedness of craniofacial growth means that altered cranial base development can ultimately and indirectly impact the facial surface level which could manifest as widespread minor compensatory adjustments (FA).

Our approach to partitioning symmetry and asymmetry, and decomposition of asymmetric types, may provide novel avenues for objective and partitioned assessments of the facial dysmorphology of individual conditions. In accordance with this proposition, the symmetry models display findings consistent with the clinical facial phenotype. Whilst, as anticipated, the first mode of variation (PC) demonstrated variation apparently consistent with age, the second and third modes of variation were consistent with Achondroplastic facial dysmorphology. In Achondroplasia, perturbed growth at cranial base synchondroses, normally sites of active growth, impairs the ability of the cranial floor to accommodate the expanding brain (Enlow and Hans, 2008) and frontal bossing develops (Cohen et al., 1985). This frontal bossing was reflected in the second PC (Figure 5.5), along with the retrusion of the premaxilla. The third PC demonstrated a prominent chin likely reflecting the mandibular pseudoprognathism described in this condition. Additionally, other craniofacial dysmorphism noted in Achondroplasia were seen and they included a shortened nose, depressed nasal bridge, and an upturned nasal tip. Our objective and partitioned approach may have applications for studying other causes of disordered craniofacial growth.
Additionally, the generation of symmetric and asymmetric facial signatures of individual conditions may refine facial diagnostics.

FA is regarded as a surrogate measure for developmental instability (DI) (Van Dongen et al., 2005). Under given environmental and genetic conditions, DI reflects the inability of an individual to produce a consistent phenotype, also known as the tendency to produce a morphological change in response to developmental perturbations. These perturbations cause deviations from the normal (regular) phenotype, while processes such as regulatory feedback, may restore the growth trajectory back towards the regular phenotype (Moller and Manning, 2003). Additionally, these developmental perturbations may also occur within a developmental constraint, defined as a “bias on the production of variant phenotypes or limitation on phenotypic variability” (Maynard Smith, 1985). Investigating DI and its regulatory milieu is biologically important and challenging. In our study, we observed higher than normative values of FA when compared to that described in a normal population (Claes et al., 2012c). Given this, Achondroplastic affected individuals can be considered to have greater DI when compared to a population unselected for craniofacial anomaly. Other studies have used alternative approaches to investigate the relationship between FA and DI, including that of Starbuck et al. (2013) who recently investigated cohorts of Trisomy 21 (Down syndrome) patients and Trisomy 21-unaffected individuals. They showed differing amounts of FA between these groups, and a Trisomy 21 facial pattern of FA that was consistent with the healthy controls from Claes et al. (2011a) study of facial asymmetry. In contrast to our study, the investigation of FA in Trisomy 21 means that despite having an extra number 21 chromosome present in all affected individuals, they will still individually differ at multiple loci (multiple alleles) on this extra chromosome, as compared to the genetic homogeneity of a group of individuals whose disorder is caused by the same single nucleotide change. Similarly, their use of Euclidean distance matrix analysis (EDMA) on a sparse number of facial landmarks constrains its use as a fine-grained analysis, and they did not correct for DA on the basis of an a priori assumption of an absence of DA in the studied faces. To our knowledge, facial DA and FA have not been previously investigated in a partitioned manner using spatially dense landmarks and a genetically homogenous condition. Our study provides a framework to objectively determine and partition asymmetry to provide novel approaches to DI and related phenomena. Analysis of a single genetically homogenous condition provided a
targeted approach to commence this journey. However, further studies, including of this and other fibroblast growth factor receptor related conditions and other syndromes, are required to explore our findings.

A final consideration is that the two-factor ANOVA framework for partitioning and analyses of asymmetry and symmetry used in this paper, and founded in an approach in Claes et al. (2012b), could be extended to investigation of other aspects of shape variation. Of particular interest is the potential application of the proposed framework to dysmorphometrics (Claes et al., 2012a) and discordancy spaces, where the partitioning of facial shape into its variation components is based on facial dysmorphology (Claes et al., 2013). Such an expansion may facilitate analysis of syndrome clusters, discovery of novel subgroups within existing syndrome cohorts, and promote refined genotype-phenotype correlations. This technology is continually evolving and its emerging techniques show great promise for biological applications, especially in elucidating biological pathways and patterns.

5.7 CONCLUSION

Decomposing components of facial variation into symmetric and asymmetric components and asymmetry types allows for novel investigations of disordered and syndromic craniofacial growth that can be utilized to explore disease biology and to refine facial diagnostics.

5.8 ACKNOWLEDGEMENTS

We would like to thank all participants, everyone involved with the 2011 Short Statured Peoples’ Association (SSPA) Convention in Perth, Western Australia, and Genzyme Australia. This work is supported by the Princess Margaret Hospital Foundation in Perth, Western Australia, and RD-Connect, an FP7 programme, and has been approved by the PMH Ethics Committee (PMHEC: 1801/EP, 1443/EP and 1488/EP). All authors declare that they have no competing interests. Genzyme provided an unrestricted educational grant and had no role in the interpretation/analysis of the data, or in the preparation of the manuscript, nor in the decision to submit the manuscript for publication.
6. General Discussion

The principal aim of these linked studies was to illustrate the applicability of a novel 3D facial morphometric toolkit for the objective investigation of syndromic facial phenotypes. Specifically, this included two geometric morphometric techniques that had not previously been applied to syndrome cohorts; dysmorphometrics and the two-factor ANOVA framework for decomposing asymmetry applied to spatially dense landmark configurations. Through the objective analysis of syndromic facial asymmetry and dysmorphology, global and regional facial patterns were detected, and we were able to provide new windows for biological insights. Collectively, this might facilitate the diagnoses and treatment monitoring of conditions displaying facial dysmorphology.

In this thesis, we demonstrated the syndrome discrimination and treatment monitoring applications of the dysmorphometrics approach, through the assessment of facial discordancy in MPS I. Without any prior clinical assumptions or knowledge of the condition, this dysmorphometric approach achieved successful detection, isolation and quantification of MPS I facial characteristics. By attributing facial discordancy severity scores, we were also able to objectively assess the severity of the facial phenotype, which correlated with clinical severity (subtype) within this MPS I disease spectrum (Chapter 3). This approach was extended for investigation of application to the non-invasive monitoring of medical treatments by longitudinally scoring facial discordancy in an MPS I affected child receiving ERT and BMT. The potential of this approach was supported by the objective and precise determination of MPS I facial dysmorphology, which showed a progressive lessening in both the short and intermediate terms (Chapter 4) and supported that it has the capability to ascertain deep and granular phenotypic information; deep phenotyping. While not explored in this thesis, longitudinal Dysmorphometric assessments could be investigated as an objective means of documenting natural history. The 3D facial morphometric toolkit, through dysmorphometrics and two-factor ANOVA decomposition, were also applied to investigation of facial asymmetry and symmetry patterns in Achondroplasia (Chapter 5). The combination of facial dysmorphology and locus and allelic homogeneity in this cohort, provided a refined window to commence assessment of portioned syndromic asymmetry and symmetry, to support refinement of facial diagnostic signatures, and to explore patterns of disordered craniofacial growth.
Cumulatively, we have explored disease biology, determined facial signatures of syndromic phenotypes and their severity, as well as objective monitoring of disorder-specific medical treatment (Figure 6.1). This work allows the elucidation of meaningful facial patterns, providing the framework from which to approach refinements in syndromic facial diagnostics and treatment monitoring. During the tenure of this thesis, our research group and its collaborators used components of the 3D facial morphometric toolkit for investigations that complement those completed within this thesis. Namely, the (1) use of automated facial anthropometrics (Wei et al., 2011), (2) the use of dysmorphometrics for facial asymmetry for syndrome discrimination (Walters et al., 2013), (3) monitoring of mTOR inhibitor therapy (Baynam et al., 2013b), and (4) use of the two-factor ANOVA to explore facial asymmetry in sexual dimorphism (Claes et al., 2012c).

The developing 3D facial morphometric toolkit translates complex statistical data into expert knowledge and meaningful indices, thus enabling its availability to a broader base of end-users. In particular, its use as a first-tier diagnosis for the non-dysmorphologist is envisioned and will likely be possible with time and continued developments in this area of research. In the clinical setting, the 3D facial morphometric toolkit may also assist in the clinical training of recognizing syndromic facial phenotypes, and provides a toolkit for deep phenotyping for precision medicine, including enabling genotype-phenotype correlations. The application of differentiating within disorders of genetic heterogeneity is another extension that warrants further interest, and could be applied to other spectra or syndrome datasets, for example, its use on other Mucopolysaccharidoses types, skeletal dysplasias, and the RASopathies (Noonan syndrome, Leopard syndrome, Legius syndrome, Costello syndrome, CFC syndrome, Neurofibromatosis type I). Further applications of the 3D facial morphometric toolkit and future directions that are in process or planned are presented in Figure 6.1.
Figure 6.1. The Big Picture: Translation of the 3D Facial Morphometric Toolkit Applications. In a clock-wise direction from the bottom, topics discussed include: (1) WA Face Space, (2) syndrome support groups, (3) recent applications, (4) the potential applications in the clinical setting, (5) familial morphometrics, (6) future research directions from this work, and lastly (7) the applications completed in this PhD thesis.
The research work presented in this thesis also provides the foundation for further analytic expansion, such as (1) extension of the two-factor ANOVA partitioning methodology for the exploration of facial discordancy patterns in syndrome populations, which will ultimately be dependent on the normative reference and mapping strategy use, and (2) application of the two-factor ANOVA partitioning of facial asymmetry on syndromic conditions with complex spectra, for example the Oculo-Auriculo-Vertebral Syndrome (OAVS) spectrum of disorders. Facial asymmetries in individual syndromes within this spectrum vary significantly and it is suspected that this may be due to causal heterogeneity. The strategic use of a two-factor ANOVA partitioning could aid where traditional clustering analysis has failed; by discovering previously unknown patterns of facial asymmetry at a population level that may facilitate the discrimination of as yet occult OAVS subtypes. With further expansion of existing syndromic datasets, it is likely that syndrome-specific discordancy statistical face spaces could be developed in future studies. This presents exciting possibilities, particularly when property pathways within these discordancy face spaces are explored.

The dysmorphometrics approach is supported by the appropriate determination of a normative reference dataset that can be defined by a statistical face space, and the strength of the statistical face space lies in its ability to construct a wide range of synthetic faces, which is entirely dependent on a sufficient amount of underlying data (Claes et al., 2013). This means that as many different individuals of different age, ethnicity and BMI as possible, are, incorporated into the normative reference cohort from which sufficient typical facial variation is later inferred. The lack of sufficient individuals for a certain age or ethnic group is sometimes a source for contention in other comparative studies. In this thesis, the advantage of our approach is that metadata (e.g. age, ethnicity, and BMI) is associated with each facial scan, and these attributes can be interpolated from the statistical face space, thus enabling normal equivalent faces to be constructed for different patients using a single face space. Additionally, while a reduced number of individuals may exist for some age groups (Appendix, Figure A2), this is not as influential to the results of these studies as one might assume. It is important to remember that the key to our approach is variance, where the establishment of enough variance creates the hypothetical boundary of typicality of the statistical face space. Further attainment of a large sample of similar individuals merely populates and fills in this space; it does not expand the existing boundary of normal facial
variation, meaning that this sample is unlikely to add any new information. Of course, there are always advantages of having a large sample size, particularly, the accessibility to a greater number of statistical and analytical tools. In a clinical setting, it is difficult to attain large sample numbers for a particular condition, especially for rare diseases. The novel approach used in this study addresses this limitation head on by not being bound by sample numbers. One caveat of the dysmorphometrics approach to bear in mind is the fact that facial profiling using this approach provides a summation of facial anomaly. This means that until the emergence of expanded datasets, the discrimination between underlying disease state, impacts of BMT and immunosuppressant influences of corticosteroids (e.g. Prednisone), will remain elusive. The emergence of the means to quantify facial changes as a function of shape anomaly will enable these investigations.

A further consideration in constructing the normative face space is deciding whether an individual belongs to the normative reference population, or not. Careful screening of participants’ self-reported medical history is required during the reference recruitment process to reduce the risk of an abnormal facial form falling within the normative range and thus biasing any further analyses. Expansion of existing normative reference datasets is necessary to rigorously test the boundaries of normal facial variation, which could help with this initial decision of normal or not. In addition, the ethnicity representation of individuals from the normative reference population used for the studies within this thesis, though seemingly underrepresented, is reflective of a mixed ethnic group with a Caucasian majority (Appendix, Figure A3), which is reflective of the Western Australian population. Future extension of the normative reference population to be more inclusive of ethnic diversity (e.g. Aboriginal Australians) is also crucial for an accurate assessment of facial dysmorphology and asymmetry in these groups. Expansion of the normative reference range to increasingly account for and define the range of normality will promote the next phase of technological refinements.

Cumulatively, 3D facial analysis may have its greatest clinical utility in rare diseases. There are up to an estimated 8,000 currently named rare diseases and thousands more presumably await discovery and classification (Robinson, 2012); collectively they affect up to 1 in 12 in the population and many have associated facial anomaly. The dysmorphometric and normal equivalent aspect of the proposed 3D facial morphometric
toolkit will especially be useful for investigations in these individually rare conditions. Deep phenotyping is the precise and comprehensive analysis of phenotypic components. Combined with rapid advances in genomic technology, this is showing great potential in accelerating identification of disease subtypes with diagnostic and therapeutic implications, and is driving progress in understanding the biology of human health and disease. A major bottleneck in this endeavor is the development of adequate methods for robustly and objectively capturing and analyzing this phenotypic data (Robinson, 2012), 3D facial shape analysis, particularly dysmorphometrics, is uniquely applicable to this end (Baynam et al., 2013a).

“Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature by the careful investigation of cases of rarer forms of disease. For it has been found in almost all things, that what they contain of useful or of applicable nature, is hardly perceived unless we are deprived of them, or they become deranged in some way”.
William Harvey, MD. London, April 24 1657.
Appendices

A1. 3D Facial Scan Acquisition, Preparation & Processing

Image capture was performed with the 3dMDFacial™ stereophotogrammetric camera system, utilizing two separate camera pods with overlapping viewpoints. Each pod contained a video camera for live positioning, two black-and-white cameras for stereo capture, a colour camera for texture capture, a speckle pattern projector, and a flash unit. Facial geometry is produced from the two stereo camera viewpoints, which eliminates data errors associated with stitching datasets together. An infrared speckle pattern is randomly projected across the subject’s face and a 3D image is automatically constructed by finding corresponding speckles in multiple 2D images. The whole scanning process is extremely rapid and allows image capture to be completed within two milliseconds. The reliability and precision of the 2-pod 3dMDFacial™ system was validated by Aldridge et al. (2005).

Facial shape is represented by the output of the 3dMDFacial™ system; a continuous raw point cloud that consists of approximately 10,000 data points randomly defined in a 3D coordinate space. These data points are connected to form a polygon mesh, and then filled in to form a continuous facial surface. In preparation for facial mapping, these point cloud data (i.e. raw scan output) are manually brought into closer alignment by indication of twelve anatomical landmarks (Figure A1). Although this coarse landmark indication has no significant influence on the final alignment of scans, it does serve the purpose of decreasing computational time and provides a basis for surface registration during the facial mapping process. Additional texture information was a visual aid in the indication of landmarks, even if it was not vital in regards to facial morphological data.

Facial mapping was performed across all faces in the dataset and required re-sampling of the raw point cloud data into a more comparable format in order to establish connectivity between scans. An anthropometric mask, representing the standard of connectivity for all scans, was created through an iterative ‘bootstrapping’ method, as described in Claes (2007). Once facial scans are mapped, dense anatomical correspondence is achieved and allows biologically valid comparisons to be performed.
Figure A1. Sequential Indication of Landmarks; 1) right exocanthion, 2) right endocanthion, 3) left endocanthion, 4) left endocanthion, 5) nasal ala – lateral left corner, 6) pronasale, 7) nasal ala – lateral right corner, 8) right cheilion, 9) upper lip tubercle, 10) left cheilion, 11) vermillion border, and 12) chin point.

00Original: Data folder containing exported 3dMD surface scans; where each scan is stored as a set of three (.obj+.mtl+.bmp file formats).

01Converted: Data folder storing files that have been converted from an obj → mat. format; performed using the Scan Cleaner Tool.

02Cleaned/Prepared: Data folder containing scans that have been cleaned up & indicated with landmarks using the Scan Cleaner Tool. These scans are sent to Peter Claes for further processing.

03Mapped: Data folder containing scans that have been mapped with the Anthropometric Mask (AM), hence all data points on each facial scan have been standardized.

04Mirrored: Data folder containing the mirrored versions of original facial scans. Used for analyzing facial asymmetry.

05NE: Data folder containing patient-specific controls aka Normal Equivalents (NE) based upon a given statistical face space.

Figure A2. Facial data processing pipeline.
A2: WA Normative Face Space Demographics:

The recruitment of normative reference individuals was approved by the Princess Margaret Hospital for Children Ethics Committee (PMHEC) and 3D facial data of approximately 1000 individuals was captured. By documenting specific metadata (e.g. age, BMI and self-reported population affinity), the normative statistical face space can be constrained and tailored to the clinical/biological question at hand. Depending on the approach, in some cases, this constraint can enable comparisons of a more closely matched control group. This reference data is also used to generate descriptive summary statistics for asymmetry or discordancy scores, which in turn, are used to compute standardized Z-scores.

Figure A3. Age distribution of males and females of the WA normative reference range.
Figure A4. Frequency distribution of the WA reference range based on ethnicity and gender.
A3: **Table 1. Normative Reference Asymmetry Summary Statistics.** Descriptive statistics of overall asymmetry scores in a normative reference population with typical growth patterns, with RMSE (root-mean-squared-error) and RSA (relative significant asymmetry) scores calculated separately for males and females for the 12-17 and 18-30 year old reference range cohorts.

<table>
<thead>
<tr>
<th>Age Range</th>
<th>0 - 11yrs</th>
<th>12 - 17yrs</th>
<th>18 – 30yrs</th>
<th>31 - 45yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>RMSE (mm)</td>
<td>RSA (%)</td>
<td>RMSE (mm)</td>
<td>RSA (%)</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.757</td>
<td>3.012</td>
<td>0.435</td>
<td>1.933</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.561</td>
<td>7.092</td>
<td>0.773</td>
<td>8.247</td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>0.844</td>
<td>3.313</td>
<td>0.552</td>
<td>2.592</td>
</tr>
</tbody>
</table>
Facial growth is subject to asymmetry, which may be directional or fluctuating. Directional asymmetry occurs when an anatomical character is systematically greater on one side than the other, as compared to fluctuating asymmetry where there are random deviations in symmetry of character traits (Van Valen, 1962). It is suggested that the degree and/or pattern of asymmetry that may be of most interest and this is considered to be reflective of vitality (Jones, 1987) and/or pathology (DeLeon, 2007).

Twinning and alterations in body patterning such as asymmetry (Boklage, 1987), laterality disturbance (Steinman, 2001) and mirror-image twins (Wszelaki, 1953) are of significant scientific interest and some clinical reports provide evidence for an interwoven genesis of these phenomena (Thacker et al., 2009).

For at least 60 years the relationships between these entities, mainly predicated on data from animal studies undertaken in the first half of the 20th century, have been investigated (Torgersen, 1950). However, the tools to define and quantify these relationships have been limited and, with some exceptions (Brown et al., 1987; Townsend et al., 1986), the majority of these studies have relied on associations with subjectively defined phenotypes. The emerging technologies of 3-dimensional (3D) facial scanning and geometric morphometrics are providing the means to establish objective criteria, including measures of asymmetry, which can be used for phenotypic classification and investigations. Additionally, advances in molecular epigenetics provide new opportunities for novel investigations of mechanisms central to early developmental processes, twinning and related phenotypes. We review the evidence for overlapping etiologies of twinning, asymmetry and selected monogenic and complex diseases, and we suggest that the combination of epigenetic investigations with detailed and objective phenotyping, utilizing 3D facial analysis tools, can reveal insights into the genesis of these phenomena.

**Keywords:** twins, asymmetry, epigenetics, 3-dimensional facial scanning

For decades the relationships of twinning and alterations in body patterning, such as laterality and asymmetry, have been investigated. However, the tools to define and quantify these relationships have been limited and the majority of these studies have relied on associations with subjectively defined phenotypes. The emerging technologies of 3-dimensional (3D) facial scanning and geometric morphometrics are providing the means to establish objective criteria, including measures of asymmetry, which can be used for phenotypic classification and investigations. Additionally, advances in molecular epigenetics provide new opportunities for novel investigations of mechanisms central to early developmental processes, twinning and related phenotypes. We review the evidence for overlapping etiologies of twinning, asymmetry and selected monogenic and complex diseases, and we suggest that the combination of epigenetic investigations with detailed and objective phenotyping, utilizing 3D facial analysis tools, can reveal insights into the genesis of these phenomena.
Advances in molecular genetics have provided new opportunities for novel investigations of mechanisms central to early developmental processes, twinning and related phenotypes. Epigenetics, the study of mitotically or meiotically heritable changes in expression without changes in DNA sequence, is expanding our knowledge of developmental biology and disease (Feinberg, 2010; Groom et al., 2010). One of the major epigenetic mechanisms involves DNA methylation, which can modulate genetic imprinting, the phenomenon by which certain genes are expressed in a parent-of-origin-specific manner (Callinan & Feinberg, 2006). This occurs at imprinting control regions (ICRs), which act as discrete cis-acting DNA elements within clusters of imprinted genes, with methylation state inherited through the germline. Imprinting errors have been recently implicated in developmental asymmetry (Bestor, 2003) and interrelationships of these developmental phenomena with monogenic (Blick et al., 2009) and complex (Feinberg, 2010) diseases have been established.

Complementary to these developments are the emerging technologies of 3-dimensional (3D) facial scanning and geometric morphometrics that are providing the means to establish objective criteria (Smeets et al., 2010), including measures of asymmetry that can be used for phenotypic classification. We review evidence for overlapping etiologies of twinning, asymmetry and selected monogenic and complex diseases. It is suggested that the combination of (epi)genetic investigations with detailed and objective phenotyping, utilizing emerging 3D facial analysis tools, can reveal insights into the pathogenesis of these phenomena.

Before directly addressing observations suggesting the intersection of the above phenomena, a brief overview of factors impacting twinning is appropriate.

**Secular Changes in Rates of Twinning and Implications for Disease**

There has been an approximate doubling of the rate of twin births recorded in the United States over the past two decades (Martin et al., 2006), with similar patterns across the developed world (Blondel & Kaminski, 2002). There are geographic variations in twin rates within the United States (CDC, 1997), but the most significant correlate is with advanced maternal age (AMA; CDC, 1997), which accounts for 1/3 of the increase in twin pregnancies internationally (Blondel & Kaminski, 2002). Another factor is assisted reproduction technology (ART) (Wright et al., 2008). Commensurate with these increased rates are disproportionate morbidity androgenetic anomalies compared to singletons, in particular prematurity (March of Dimes, 2008) and congenital anomalies (Firth et al., 2005). There are implications for the prevalence of monogenic diseases (Blick et al., 2009), and complex phenotypes have also been implicated. The clarification of factors impacting on or associated with twinning is a health imperative.

**Factors Impacting on Twinning**

Mechanisms underlying twinning are both complex and multifactorial with interactions of environmental and genetic factors, which may be mediated via epigenetic mechanisms. They can be linked to zygosity, although there is some overlap. Those associated with dizygotic (DZ) twinning can be considered as maternal factors and include AMA and ART; maternal body mass index and height are also implicated (Firth et al., 2005). There are familial and ethnic relationships with DZ twinning frequency, for example, there are particularly high rates in the Japanese at 1:250 (Leszczynska-Gorzela et al., 2000). The high rate in Nigerians has been postulated to be an environmental influence of elevated estrogen with yam consumption coupled with high dairy intake modulating insulin growth factor 1 (IGF-1) (Steinman, 2006).

Familial (Machin, 2009) and ART factors are also implicated in monozygotic (MZ) twinning. Of particular interest are the (epi)genetic conditions associated with the increased rate of MZ twinning, for example Beckwith-Wiedemann (BWS) (Weksberg et al., 2002). The genetic and environmental factors implicated in twinning may be modified by epigenetic factors. Understanding the factors mediating, or mediated by, the above are important to the understanding of biology and disease.

**The Intersection of Asymmetry and Twinning**

**DISORDERS OF THE CHROMOSOME 11P15 REGION**

Beckwith-Wiedemann (BWS)

BWS is an overgrowth condition that can manifest with asymmetric growth of the soma, paired organs and the face. BWS can result from loss of imprinting at two ICRs within chromosome 11p15.5. ICR1 controls the expression of the IGF2 gene and the non-coding RNA H19 and is methylated on the paternal allele. ICR2 controls the expression of the CDKN1C gene and the antisense RNA KCNQ1OT1 and is methylated on the maternal allele. Generally, twin pairs with ICR2 imprinting defects are discordant, female and the underlying defect is hypomethylation of the maternal allele. The excess of MZ twins among BWS patients with ICR2 hypomethylated is suggestive of a relationship between a methylation defect and the imprinting process. It was first proposed that unequal inner cell mass division, as a consequence of twinning, leads to a differential maintenance of imprinting of the respective twins, where particularly KCNQ1OT1 is vulnerable to a loss of imprinting event (Weksberg et al., 2002). Perhaps more likely is the alternative hypothesis that a lack of maintenance of DNA methylation on the maternal allele of ICR2 at a critical stage of preimplanta-
Russell-Silver Syndrome (RSS)
Russell-Silver Syndrome (RSS) is also possibly phenome-
nologically associated with asymmetry and twinning. Subjects with RSS present with short stature and can also manifest with hemihypotrophy of the soma and/or face. A subset of this condition is associated with disturbances within 11p15.5 ICR1. It can be considered clinically and (epi)genetically as reciprocal to BWS (Eggermann, 2009). MZ twins discordant for RSS have been reported (Begemann et al., 2010; Bergma et al., 1969; Gicquel et al., 2005; Nyhan & Sakati, 1976; Sagot et al., 1996; Sann et al., 1990; Yamasawa et al., 2000) including three sets of twins in which methylation analysis revealed an epimutation in ICR1 only in the affected twin (Begemann et al., 2010). Affected co-twins from two of these MZ pairs also showed maternal hypomethylation at ICR2 (Begemann et al., 2010; Gicquel et al., 2005). RSS was also described in a child conceived by IVF and who had aberrant methylation at multiple imprinted loci (Biek et al., 2009) and aberrant methylation occurs outside the 11p15 region in some BWS individuals conceived by ART (Rossignol et al., 2006).

OCULO-AURICULO- VERTEBRAL SPECTRUM (OAVS), FACIAL ASYMMETRY, TWINNING, ART AND REPRODUCTION ABNORMALITIES.
The most distinctive group of syndromes presenting with facial asymmetries are the disorders that encompass the Oculo-Auriculo-Vertebral Spectrum (OAVS). This spectrum includes conditions with phenotypic overlaps that predominately involve derivatives of the first and second branchial arches. Their manifestations are highly variable and those presenting with the most evident facial asymmetry are individuals with the hemifacial microsomia phenotype. This varied presentation is indicative of a causal heterogeneity where a number of environmental, genetic and multifactorial associations have been reported (Genereviews). As in the previous conditions, there are also associations with (epi)genetic mechanisms, multiple pregnancies and ART.

OAVS has been recurrently associated with multiple gestations, predominantly in discordant MZ twins (reviewed in Baynarm & Goldblatt, 2009). The incidence of multiple pregnancies is almost 10 times more common in association with OAVS than observed in controls (Lawson et al., 2002). There is also an elevated prevalence of twin pregnancies in cases, which is also observed in family members of index cases, when compared to population prevalence (Werler et al., 2004). Furthermore, there is an excess of malformations, including OAVS, in MZ twins and this has led to the conclusion of a common cause for both some malformations and MZ twinning (Schinzel et al., 1979).

The concept of interrelationships of these phenomena is further supported by considering spontaneous fetal loss. At least 12% of natural conceptions involve multiple pregnancies, with a considerable attrition rate with only 2% surviving to term as twins and a further 12% resulting in single births (Boklage, 1990). Most twins are lost very early in pregnancy and at least two thirds of twins evident at 10 weeks of gestation are singleton by the time of birth (Levi, 1976). Theoretically, the remaining singletons are at risk for structural congenital anomalies, including OAVS. Some singletons born with anomalies are likely to have been a sibling of a MZ twin (Hall, 2003). In this context, it is noteworthy that OAVS has been reported in a child whose monozygotic twin died in utero (Paris et al., 1983), and bleeding during the first trimester has been associated with singletons affected with OAVS or microtia (which may be a minimal manifestation of OAVS) (Paris et al., 1983). Mechanically, given shared placental circulations of some MZ twins sharing the same placenta, vascular disturbances such as transient hemodynamic inequalities, embolic phenomena or hemostatic defects have been suggested to render the twin more liable to dysmorphic development (Boles et al., 1987). Therefore, one twin might manifest OAVS and the other be phenotypically normal or one twin might abort and the other manifest OAVS. An alternative or complementary explanation is that OAVS and twinning may share common (epi)genetic origins.
The association of OAVS and MZ twinning with ART has been extensively reviewed (Baynam & Goldblatt, 2009; Wieczorek et al., 2007; Wieczorek et al., 2009). Wieczorek et al. (2007) found associations of reproduction abnormalities and twinning in parents of OAVS affected individuals. Their findings included an increased incidence of OAVS in children conceived by ART and an increased frequency of twins amongst OAVS affected children. They noted the parallel of these associations with BWS (Wieczorek et al., 2007). In accordance with these proposed parallels, KCNQ1OT1 methylation abnormalities have been identified in oocytes from hormonally stimulated cycles (Khoueiry et al., 2008). The cause(s) of MZ twinning in IVF continue to be a topic of intense investigation. Proposed mechanisms include over ripeness ovopathy and intercellular blastocyst discordance. The former postulate is predicated on a frog egg experimental model where an ‘over ripeness’ is positively correlated with monozygotic twinning and associated anomalies (Witschi, 1952). Jongsloot described this phenomena as an over ripeness ovopathy (Jongsloot, 1986) that was conceived of as a ‘delay of either fertilization or ovulation … [that results in a] … continuum of reproductive casualties including dysplasias of one or more developmental fields’. He suggested this as a cause for OAVS in the setting of ‘high risk conceptions’ including in-vitro fertilization (IVF) (Jongsloot, 1987). Given the maturity of oocytes is influenced by the reproductive performance of women and ovarian stimulation procedures (Horshtemke & Ludwig, 2005; Sato et al., 2006), these factors may contribute to MZ twinning and associated phenomena. The latter postulate suggests that discordant cells within the blastocyst, arising from processes including chromosomal anomaly, mutation or epimutation (including methylation disturbance) may identify each other as immunologically foreign resulting in inner cell mass separation (Hall, 2003; James, 2002). Notably, ART is associated with altered methylation (Manipalvitrin, et al., 2009) and there is evidence for epigenetic disturbances of the BAPX1 gene in OAVS (Fischer et al., 2006).

TURNER SYNDROME, TWINNING, MOSAICISM AND CHIMERISM

Turner’s original publication described a child with Turner syndrome (TS) who had a normal twin sister (Turner, 1938), and subsequently there have been many reports of this condition among twin pairs (Nance & Uchida, 1964) and an increased incidence of twins is supported by some studies (Carothers et al., 1980). Furthermore TS, like OAVS, is another condition in which MZ twinning has been found to be more frequent in family members of index cases (Nance & Uchida, 1964). The majority of TS cases have a 45, X chromosomal constitution with a related set of mosaic karyotypes including 45X/46XX mosaic (Gardner & Sutherland, 2004). A minority of TS individuals are mosaic for a Y chromosomal cell line (karyotypically this is 45X/46,XY mosaicism; Mendes et al., 1999). There is a wide spectrum of phenotypes associated with 45X/46,XY mosaicism including TS, mixed gonadal dysgenesis and apparently normal males (Telvi et al., 1999). Chromosomal mosaicism is the presence, within the one conceptus, of two or more cell lines that are genetically identical except for the chromosomal difference between them. It is distinguished from chimeraism which is the coexistence of more than one cell line in an individual that is due to the union of two originally separate conceptions (Gardner & Sutherland, 2004); distinguishing mosaicism from chimeraism can be challenging. A number of pairs of MZ twins in which one or both twins has 45X/46,XY mosaicism have been reported (reviewed in (Boles, et al., 1987; Costa et al., 1998)). Additionally, there are reports of MZ twins discordant for 45X/46,XY mosaicism that were discordant for phenotypic sex (Gantt et al., 1980), and chimeric MZ twins with a 45X/46,XY karyotype have also been identified (Gonsoulin et al., 1990).

Other aspects of mosaicism of interest include that a child with Turner syndrome, somatic asymmetry and 45X/46,XX mosaicism was described to have an RSS phenotype, and a further individual with a female phenotype had a male peripheral blood karyotype (46,XY) and an epimutation at 11p15 in her lymphocytes (Bartholdi et al., 2009). Factors relating to chromosomal mosaicism may also be of importance to epigenetic regulation. Somatic and facial asymmetry are phenotypic hallmarks of chromosomal mosaicism (Possum_dysmorphology_database); however, to the best of the authors’ knowledge, asymmetry has not been systematically investigated in Turner syndrome.

3D Facial Analysis: Objective Phenotyping

The capacity to discriminate facial anomalies is critical in the identification of abnormal form associated with many conditions with craniofacial manifestations. Currently this is reliant on subjective assessments that are partly dependent on operator experience and open to inter-operator variability. Furthermore, the molecular cause for many conditions remains to be elucidated and therefore accurate definition of phenotype remains crucial to diagnostics and the understanding of the biology affecting facial morphogenesis.

Phenomics (reviewed in (Houle et al., 2010)) is the large scale high-dimensional phenotyping that is a natural complement to genetic, and epigenetic, technologies to facilitate advances in biology. Phenomics can be used to investigate explanations at phenotypic and (epi)genotypic levels. Imaging modalities are one of the promising technologies in this field (Houle et al., 2010). Emerging techniques utilizing 3-dimensional (3D) facial scanning and geometric morphometric analysis of scan data are providing objective and automated means to identify dysmorphism and asymmetries. Additionally, studies employing 3D facial analysis are facilitating examination of the cell biology underpinning facial dysmorphism (Tobin et al., 2008). A number of approaches are being investigated (Adams et al., 2004; Cox-Brinkman et al., 2007; Deleon & Richtsmeier, 2009; Hammond et al., 2008;
Hammond et al., 2001; Hammond et al., 2005; Richtsmeier et al., 2002; Weinberg et al., 2008) and all utilize methodology dependent on the identification of homologous facial components. They can differ in their means of measuring form differences; however, they are universal in defining form anomaly as either a statistical difference to a normative archetype or differences clustered within an identified area of dysmorphology (Hammond, 2007). However, these approaches do not account for ‘normal’ facial variations that can impact on their discriminatory power.

Recently, Claes et al. have developed a novel approach that uses an ‘expanded’ archetype that encompasses normal population facial variations to detect facial anomalies (Claes et al., 2011). These tools utilize a standardized anthropometric mask (AM) that is fitted to 3D facial scans. This mask provides a mapped high density set of corresponding quasi-landmark data that facilitates statistical approaches to detect harmonic and/or disharmonic covariance in spatial relationships within the form of the face (Figure 1). These techniques can be used to generate a normal equivalent (NE) that can be defined as a patient-specific normalized reference and is considered as the harmonious counterpart of the dysmorphic/asymmetric face.

These strategies, with some modification, can also be employed to quantify facial asymmetries (Figure 2). A robust superimposition is performed with a remapped ‘mirror’ to assess facial asymmetry, where dysmorphology is assessed with superimposition of the synthesized NE (Claes et al., in press).

To provide a measure of discrepancy in form, the degree, distribution and locality of quasi-landmark discordances can be calculated and visualized by color histogram mapping of the patient scans (Figures 1 and 2). A summary statistic reports the overall relative significant discordance (RSD) as a measure of percentage of the face affected, while the root mean square error (RMSE) of the distribution of quasi-landmark data that facilitates statistical approaches to detect harmonic and/or disharmonic covariance in spatial relationships within the form of the face (Table 1). These measures can be employed to investigate asymmetry in twins and singletons.

### Table 1

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Subject</th>
<th>RSD or RSA (%)</th>
<th>RMSE (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>Reference</td>
<td>10.6 (1.8 SD)</td>
<td>0.91 (0.22 SD)</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>Reference females</td>
<td>9.53 (0.21 SD)</td>
<td>0.94 (0.23 SD)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Subject</th>
<th>Z score (SD)</th>
<th>RMSE (mm)</th>
<th>Z-score (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>OAVS twin</td>
<td>10.17</td>
<td>-0.24</td>
<td>1.12</td>
</tr>
<tr>
<td>NE</td>
<td>Unaffected twin</td>
<td>9.36</td>
<td>-0.16</td>
<td>0.82</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>OAVS twin</td>
<td>13.06</td>
<td>+2.92</td>
<td>2.56</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>Unaffected twin</td>
<td>11.66</td>
<td>+1.76</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Note: RMSE scores the overall degree of discordance (e.g., facial disharmony, or asymmetry) in mm, while the RSD/RSA score quantifies the extent of the discordance as a percentage of the face affected. Z scores describe the extent of the difference to reference distributions (i.e., reference range).

1 Oculo-auriculo-vertebral spectrum. 2 Normal equivalent. Root mean square error. Relative significant discordance.

**Asymmetry and Syndromic and Complex Diseases**

A syndrome is a group of signs and symptoms that collectively indicate a particular condition. Facial asymmetry is a recurring theme in a large number of syndromic conditions, for example a search of the Possum dysmorphology database© using the term facial asymmetry yields 239 conditions (Possum_dysmorphology_database). Some of these conditions are known to be associated with epigenetic disorders and others involve genes, such as FGFR2, TWIST, and those of the RAS-MAPK network, that are in pathways associated with developmental processes including cell lineage determination, growth and differentiation (Genecards). These pathways may be subject to epigenetic regulation and therefore their phenotypes may overlap with disorders known to result from epigenetic disturbance.

Facial and somatic asymmetries have also been implicated in evolutionary processes and evolution has been suggested to influence the pathogenesis of complex diseases (Le Souef et al., 2000). Phenomics has been suggested as a tool to investigate the (epi)genetic basis of complex diseases (Houle et al., 2010). Therefore, accurate determination of facial asymmetry may provide for novel applications for investigation of complex phenotypes. Notably, facial asymmetries have been implicated in mate selection preferences and mate selection processes influence evolution. In this context, symmetry in women has been established as an attractive trait as rated by men (Lie et al., 2008). A biological underpinning for this observation may be the association of somatic symmetry and female fertility (Jasienska, 2006). From a twin perspective, asymmetry, including facial asymmetry, has been demonstrated in MZ twins (Burke & Healy, 1993; Yager, 1984). Additionally, in subjective comparisons of monozygotic twin pairs, the twin with perceived facial symmetry was rated as the more attractive. This perceived attractiveness was directly related to the magnitude of the asymmetry (Mealey et al., 1999). It is noteworthy that the association of Interleukin-4 (IL-4) gene, amongst other immunoregulatory genes, with complex phenotypes has been suggested.
to be influenced by evolutionary processes (Baynam et al., 2007; Le Souef, et al., 2000) and epigenetic factors are fundamental to regulation of expression of these genes (Sanders, 2006). Studies of cord blood T cells identified that the most outstanding functional group of IL-4 regulated genes included components of the RAS-MAPK pathway (Lund et al., 2007). Mutations in genes in this pathway occur in Noonan syndrome and related condi-
Appendices

Monogenic and Complex Diseases and a Role for 3D Facial Analysis

FIGURE 2
Facial asymmetry assessments of a female monozygotic twin pair discordant for OAVS: affected twin (left column), unaffected twin (right column). Outlier maps based on standard deviations measures of the distribution of distances set at 2SD (A,B) highlight areas with the most variance, the distance maps (C,D) quantify the amount of divergence in mm, and the vector maps (E,F) provide additional directional information.

...tions (Genereviews) and facial asymmetry is one of the principal modes of facial variance of this condition (Hammond et al., 2004). Finally, a relationship between schizophrenia, asymmetry (of the brain) and twinning has been postulated (Boklage, 1977). These factors suggest interacting networks underlying asymmetries, that have been influenced by evolution, are partly epigenetically regulated and that this may have relevance for complex
Appendices

Gareth Baynam, Peter Claes, Jeffrey M. Craig, Jack Goldblatt, Stefanie Kung, Peter Le Souef, and Mark Walters

disease. Given the relationships between asymmetry and twinning, these networks may have further relevance to twin studies.

In conclusion, tools for 3D facial analysis may provide the resolution to identify and quantify phenotypic variations, including degrees of facial asymmetry, to facilitate novel investigations of twinning, syndromic and complex diseases. Ultimately, investigations of the networks mediating intersections of these phenomena will require phenomic assessments to be coupled with (epi)genetic studies.

References


Weksberg, R., Shuman, C., Caluseriu, O., Smith, A. C., Fei, Y. L., Nishikawa, I., Stockley, T. L., Best, L., Chitayat, D., Olney, A., Ives, E., Schneider, A., Bestor, T. H., Li, M., Sadowski, P., & Squire, J. (2002). Discordant KCNQ1OT1...
imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. Human Molecular Genetics, 11, 1317–1325.


The Facial Evolution: Looking Backward and Moving Forward

Gareth Baynam1,2, Mark Walters3, Peter Claes4, Stefanie Kung2, Peter LeSouef2, Hugh Dawkins5–7, David Gillett3, and Jack Goldblatt1,2

1Genetic Services of Western Australia, Princess Margaret and King Edward Memorial Hospitals, Perth, Australia; 2School of Paediatrics and Child Health, University of Western Australia, Perth, Australia; 3Cranio-Maxillo-Facial Unit, Princess Margaret Hospital for Children, Perth, Australia; 4K.U. Leuven, Medical Imaging Research Centre, Faculty of Engineering, Leuven, Belgium; 5Office of Population Health Genomics, Department of Health, Perth, Australia; 6Centre for Population Health Research, Curtin Health Innovation Research Institute, Curtin University of Technology, Perth, Australia; 7School of Pathology and Laboratory Medicine, University of Western Australia, Perth, Australia

Communicated by Peter N. Robinson
Received 1 June 2012; accepted revised manuscript 30 August 2012.
Published online 3 October 2012 in Wiley Online Library (www.wiley.com/humanmutation). DOI: 10.1002/humu.22219

ABSTRACT: Three-dimensional (3D) facial analysis is ideal for high-resolution, nonionizing, noninvasive objective, high-throughput phenotypic, and phenomic studies. It is a natural complement to (epi)genetic technologies to facilitate advances in the understanding of rare and common diseases. The face is uniquely reflective of the primordial tissues, and there is evidence supporting the application of 3D facial analysis to the investigation of variation and disease including studies showing that the face can reflect systemic health, provides diagnostic clues to disorders, and that facial variation reflects biological pathways. In addition, facial variation has been related to evolutionary factors. The purpose of this review is to look backward to suggest that knowledge of human evolution supports, and may instruct, the application and interpretation of studies of facial morphology for documentation of human variation and investigation of its relationships with health and disease. Furthermore, in the context of advances of deep phenotyping and data integration, to look forward to suggest approaches to scalable implementation of facial analysis, and to suggest avenues for future research and clinical application of this technology. Hum Mutat 34:14–22, 2013. © 2012 Wiley Periodicals, Inc.

KEY WORDS: three dimensional; facial analysis; phenotypes; rare diseases; dysmorphology; vaccines; evolution

Introduction

Imaging is ideal for phenomic studies, which involve the acquisition of high-dimensional phenotypic data on an organism-wide scale, as it spans molecular to whole organism spatial registers [Walter et al., 2010]. Phenomic studies are the natural complement to (ep)genetic technologies to facilitate advances in biology as phenomic data are required to understand which genomic variants affect phenotypes, to investigate pleiotropy, and to unravel complex phenomena including health and evolutionary fitness [Houle et al., 2010]. Houle et al. (2010) suggested that a coalition of factors support the timeliness of consideration of phenomic technologies. These include the increasing availability and rapid emergence of analytical and bioinformatic approaches; progress in the dynamic integration of phenomena at multiple levels from genes through to whole organisms; and that, in many cases, phenotypic data are the most powerful predictors of important biological outcomes. In addition, the complexity of genetic causation supports the integration of phenomics with other -omics technologies to investigate causal networks.

The face is uniquely reflective of the craniofacial primordia (see Fig. 1) and it can reflect systemic health, provide diagnostic clues to disorders, and there is evidence supporting the application of three-dimensional (3D) facial analysis to the study of variation and disease [Baynam et al., 2011, 2012; Hammond et al., 2012]. Some known and prospective roles for large-scale objective assessment of facial morphology have recently been elegantly described [Hammond and Suttie, 2012] as part of a special issue on deep phenotyping in this journal. The purpose of this review is, first, to look backward to suggest that knowledge of human evolution supports, and may instruct, the application of objective high-resolution studies of facial morphology for documentation of human variation and investigation of its relationships with health and disease; vaccine responses will be used for illustrative purposes. Second, this review will look forward to suggest approaches to scalable implementation of facial analysis, and to propose some avenues for future research that ultimately may have clinical implications.

Evolutionary Influences: Looking Backward

Vaccine Responses as an Example of Evolutionary Influences on Phenotype

Vaccination is unequivocally important in prevention of infectious diseases and there are increasing applications to noninfectious diseases. Vaccination responses are complex immunogenetically mediated phenotypes, which may be representative of phenomena relevant to other immunologically mediated phenotypes.
A nexus of factors support interrelationships between vaccine responses, evolution, and facial phenotypes; and that these can now be assessed with robust, sensitive, and scalable methods. Notably: (1) associations between candidate genes selected on an evolutionary basis and vaccine responses have been described [Baynam, 2008; Baynam, et al., 2007a, 2008]; (2) genetic diversity of key immunoregulatory loci are revealed in human faces [Lae et al., 2008]; (3) facial phenotypes have been implicated in mate selection preferences [Lae et al., 2008] and these processes influence evolution; (4) the face is uniquely reflective of all the primordial tissues; (5) 3D facial analysis has been successfully employed to elucidate biological pathways [Tobin et al., 2008]; and (6) advances in imaging systems and algorithms continue to improve the ability of 3D facial analysis to explore facial variation [Claes et al., 2010a,b, 2011b, 2012b].

There are a number of factors mediating vaccine responses and their ontogeny. These include, but are not limited to, genetic factors, gender effects, gene–gender effects, and epigenetics.

**Genetics**

The importance of genetic influences is suggested by differences in vaccine responses between individuals and ethnic groups [Poland and Jacobson, 1998], high heritability of specific antibody and Th2 cytokine responses to vaccines [Hohler et al., 2002; Ovsyannikova et al., 2004; Tan et al., 2001], and genetic association studies (reviewed in [Blackwell et al., 2009; Yucesoy et al., 2009]). Notably, associations of cytokine gene variations with known relationships with atopy, which were selected within an evolutionary framework [Le Souef et al., 2000] and that interact within biological pathways [Wiertsema et al., 2007], have been shown with vaccine responses [Baynam et al., 2007a,b, 2008; Wiertsema et al., 2007].

**Gender**

Gender is an important influence on disease and vaccination. Gender differences in measles vaccine antibody responses have been shown in adults and children [Benn et al., 1997; Green et al., 1994]. In addition, gender-dependent postmeasles vaccination differences in T-cell proliferation [Leon et al., 1993], side effects [Shohat et al., 2000], and mortality from various diseases have been reported [Aaby et al., 1993; Holt et al., 1993; Weiss, 1992]. Gender-dependent variation extends outside measles vaccination; for example, a herpes simplex virus vaccine was more efficacious in women than in men [Zhang et al., 2008].
Gene–gender interactions

Gender influences gene expression at X-chromosome and autosomal loci [Delongchamp et al., 2005; Kim et al., 2006; Lyon, 1965; Talebizadeh et al., 2006], gender-dependent genetic effects have been reported for disorders mediated by immunological and inflammatory mechanisms [Han et al., 2008; Rana et al., 2007] and they have been described for vaccine responses [Baynam et al., 2008; Gordeva et al., 2006]. Children of different genders were found to have characteristic HLA DR markers of humoral response to diphtheria toxoid and measles vaccine [Gordeva et al., 2006] and gender-dependent associations of cytokine gene alleles have been described with responses to these vaccines [Baynam et al., 2008]. In the latter study, the cytokine genotypes that were investigated had been previously associated with atopy, were originally selected on an evolutionary basis, and had been associated with altered vaccine responses [Baynam et al., 2007b; Wiertsema et al., 2007].

Epigenetics

Epigenetics is the study of heritable changes in gene expression unrelated to DNA sequence changes. Progress in this field is expanding our knowledge of developmental biology and disease, and epigenetic mechanisms have been proposed to modulate gender-dependent genetic effects [Bottema et al., 2005] and gene–environment interaction effects of vaccine responses [Baynam et al., 2007a].

One of the major epigenetic mechanisms, methylation of CpG dinucleotides, regulates cytokine gene transcription (reviewed in [Wilson et al., 2003]) and it has been shown to have a gender differential [Sarter et al., 2005]. Promoters are enriched for CpG dinucleotides [Gardiner-Garden and Frommer, 1987] and gender-dependent genotype effects have been reported in association with promoter polymorphisms [Bartfai et al., 2003; Cheong et al., 2005; Karlilainen et al., 2002, 2003; Lio et al., 2002; Okayama et al., 2005; Szczeklik et al., 2004]. Methylation is also integral to the prototypical gender-specific genetic process of X-inactivation [Panning and Jänicl, 1996], an example of monoallelic exclusion, that is, the process of expressing only one of two alleles of a gene, which has been implicated in regulation of immune genes, including cytokine genes [Bayley et al., 2003; Matesanz et al., 2008]. Therefore, gender can exert its effects through epigenetic influences, which may be mediated by methylation and monoallelic exclusion. It is also notable that a genome wide association study on multiple tissues suggested that gender influenced methylation of autosomes, as well as sex chromosomes. It identified clusters of autosomal genes methylated differentially by sex; this included genes expected to influence immunological processes. On the basis of these findings, the authors suggested that controlling for gender is important when investigating the effects of methylation and that these effects may be entangled with nongenetic factors [Liu et al., 2010].

Ontogeny

Exposures, and other nongenetic factors, that act in early life are likely to interact with a child’s genotype to modulate responses to other nongenetic factors [Hoffjan et al., 2005]. Accordingly, the probability of detecting genetic effects is increased when analyses account for important nongenetic factors [Baynam et al., 2007a; Colilla et al., 2003; Wiertsema et al., 2006] and when these investigations are pursued within a biological framework of, in this instance, vaccine response ontogeny. The flexibility of cytokine gene expression may be greater earlier in development [Lohning et al., 2002] and therefore relationships between gender, genes, and vaccine responses may be optimally analysed in early childhood [Baynam et al., 2008]. In this context, it is noteworthy that cord blood cytokine responses predict childhood vaccine responses and associate with cytokine genotypes that include those with demonstrated gene–gender interactions [Baynam, 2008].

Although studies in early life may provide the most fertile ground for unmasking interaction effects and their possible epigenetic basis, studies in the elderly may also provide further insights. Accordingly, epigenetic marks may be most divergent in the elderly [Martin, 2005] and gender-dependent effects of IL-10 have been described in an elderly cohort [Caruso et al., 2004].

A Direction for Further study: 3D Facial Analysis

Evolutionary pressures may shape patterns of vaccine responses and phenomic technologies are providing novel avenues to explore these phenomena. The efficacy of (epi)genetic studies are reliant on accurate phenotyping. A number of approaches to the measurement and assessment of facial form exist, for the interested reader, a brief summary of facial biometrics is found in the online Supporting Information (Supp. Tables S1 and S2). Emerging techniques utilizing 3D facial scanning and geometric morphometric analysis of high-resolution scan data are providing objective and automated means to identify subtle facial variation, and studies employing 3D facial analysis have offered novel approaches to examine cell biology [Tobin et al., 2008]. Current approaches using tens of thousands of data points from individual faces, which are compared with reference normal population data sets, are the foundation of these studies [Hammond et al., 2004]. Innovative approaches have been developed to account for “normal” facial variations that can impact on their discriminatory power. These approaches can be tailored to the assessment of facial phenotypes including facial asymmetry [Claes et al., 2014]. For example, to provide a measure of discrepancy in form, including asymmetry, the degree, distribution, and locality of discordance can be calculated and visualized by color histogram mapping of an individual’s scans (Figs. 2 and 2b).

Asymmetry, gender, (epi)genetics, and complex phenotypes

Although multiple facial phenotypes might correlate with immunological outcomes, asymmetry is a promising candidate. Facial asymmetry is described in a large number of genetic syndromes; a search of the Possum dysmorphology database © using the term facial asymmetry yields 239 conditions. A combined search with immunological dysfunction yields 39 results (database). Intriguingly, a number of these conditions, for instance, CHARGE syndrome and Deletion 22q11 disorder, are known to be associated with immunological compromise, including impaired vaccination responses, which generally improves with age. In addition, some of these conditions are known to involve epigenetic mechanisms. Notably, the CHD7 gene, in which mutations are identified in the majority of individuals with CHARGE syndrome, has a role in chromatin remodeling [Lalani et al., 2009] which is a fundamental epigenetic process [Feinberg, 2010]. Other disorders associated with facial asymmetry involve genes, such as those of the RAS-MAPK network, that are in developmental pathways important to cell lineage determination, growth, and differentiation (Genecards). Furthermore, components of the RAS-MAPK pathway may influence immunologically mediated phenotypes [Jand et al., 2007].
**Figure 2.** A: Dysmorphometric analysis of an individual with oculoauriculovertebral spectrum (OAVS). (A) Individual’s facial presentation. (B) “Normal Equivalent” face: the harmonious counterpart to the individual’s facial presentation. (C) Vector field of discordant regions (scale bar in millimeters) generated from robust superimposition of the mapped facial scan and its harmonious counterpart “normal equivalent.” The discordance covered 13% of the facial surface (relative significant discordance (RSD) score of 13%) and there was a summary severity score of 1.89 mm (root-mean-squared error (RMSE)). B: Facial asymmetry analysis of an individual with OAVS. (A) oblique (B) frontal, (C) worm’s eye vector fields (scale bar in millimeters). Images are generated from the robust superimposition of a mapped facial scan and its mapped reflected manifold to establish spatially dense correspondence. The asymmetry discordance covered 19% of the facial surface (relative significant asymmetry (RSA) score of 19%) with summary severity score of 4.8 mm (RMSE).

Facial and somatic asymmetries have been implicated in evolutionary processes and evolutionary processes are suggested to influence the pathogenesis of complex diseases [Le Souef et al., 2000]. Therefore, accurate determination of facial asymmetry may provide novel data for investigating complex phenotypes such as vaccine responses. Facial asymmetries have been implicated in mate selection preferences [Lie et al., 2008], which are known to influence evolution. In this context, symmetry in women, which correlated with non-MHC loci variation, has been established as an attractive trait as rated by men [Lie et al., 2008]. Also, in subjective comparisons of monozygotic twin pairs, the twin with perceived facial symmetry was rated the more attractive; this perceived attractiveness was directly related to the magnitude of the asymmetry [Mealey et al., 1999]; a biological underpinning for the relationship between symmetry and attractiveness may be the association of somatic symmetry and female fertility [Jasienska, 2006]. Studies also suggested an interactive effect between MHC variation and facial attractiveness on mate choice [Lie et al., 2008], and MHC variation is associated with many immunologically mediated diseases, in addition to resistance to infections. However, relationships between genetic factors and facial attractiveness are not limited to the MHC and there are gender-dependent differences [Lie et al., 2008]. Considering non-MHC loci, studies of cord blood T cells identified that the IL-4 cytokine gene induces components of the RAS-MAPK pathway [Lund et al., 2007]. Mutations in genes in this pathway occur in Noonan syndrome [Allanson, 2008], and facial asymmetry is one of the principal components of facial variation of this condition [Hammond et al., 2004].

Human facial asymmetry differs between genders [Claes et al., 2012c; Ercan et al., 2008], its magnitude is age dependent (unpublished observation) and it may be greatest earlier in life. Given that (1) there are age and gender-dependent variations in asymmetry; (2) facial asymmetry may have evolutionary foundations; and (3) these factors parallel aspects of immune system ontogeny, it is possible that factors mediating facial asymmetry may converge with those modulating vaccine responses.

The above observations may have relevance for other complex phenotypes. Studies on neurocognitive phenotypes with patho-genetic overlap, including schizophrenia and autism, demonstrated that (1) asymmetry varies with cognition in a gender-dependent manner [Hennessy et al., 2006]; (2) gender-specific facial asymmetry occurs in schizophrenia [Hennessy et al., 2004]; and (3) gender-dependent face–brain asymmetry has been identified in schizophrenia [Hammond et al., 2008]. In addition, tobacco, a known modifier of vaccine responses, both in isolation and as part of genetic interactions [Baynam et al., 2007a], influences the relationship between schizotypy, a mild nonclinical thinking style reminiscent of the one reported by individuals with a clinical diagnosis of schizophrenia, and hemispheric asymmetry [Herzig et al., 2010].

The interplay of factors mediating vaccine responses and facial biology support the proposition that high-resolution 3D facial phenotyping could be used to explore mechanisms mediating vaccine responses [Baynam et al., 2012] and other complex phenotypes. The relatively low cost of 3D facial analysis, including the absence of consumables, make it a suitable addition to existing cohorts and...
Moving Forward: The evolution of Facial Analysis
Understanding Normal Variation

The acquisition and understanding of normative data are the foundations for investigating the relationships between facial variation, health, and disease. A number of extant and evolving geographically dispersed datasets with variable characteristics [Hammond and Suttie, 2012] have been ascertained and include the Perth Face-Space which, when combined with related disorder sets, consists of an expanding set of approximately 1,500 imaged individuals. Optimally, this information would be coordinated in a global nodal network with common data elements; and to initiate this process, existing datasets and infrastructure should be consolidated and coordinated.

To understand normative variation, population groups underrepresented in current datasets would be included. This mirrors the situation with genetic studies that often exclude these populations, such as Indigenous Australians, who thus do not derive the full benefit of biomedical innovation. Addressing these issues may help to avoid the perpetuation of health disparities in these populations [Baynam, 2012; Kowal et al., 2011]. Furthermore, considering developmental considerations, longitudinal studies initiated in early childhood will be important and, by corollary, studies in elderly populations may also provide unique insights.

A Focus on Rare Diseases

Each rare disease (RD) is, by definition, of low prevalence. However, because there are 5,000–8,000 RDs, when taken cumulatively, the number of affected has been estimated at 1 per 10–18 people, or about 6–10% of the population [Donaldson, 2009; Knight and Senior, 2006; Minister of Health and Social Protection et al., 2004]. This cumulative prevalence equates to an estimated 1 to 2 million Australians, including more than 400,000 children and 27 million Europeans. Approximately, 80% of RDs are monogenetic or have a strong genetic basis, and some rare genetic syndromes have characteristic facies that have diagnostic utility. Accordingly, a number of studies have objectively documented facial phenotypes of individual RDs [Cox-Brinkman et al., 2007; Hammond et al., 2004, 2012; Tobin et al., 2008]. This work may form the foundation for developing tools that facilitate disease diagnostics, screening, differential diagnosis, and monitoring of therapy.

Normative studies of young individuals and of those affected with RD will be of paramount importance as an estimated 65% of RD appear in early life [Minister of Health and Social Protection et al., 2004] and timely diagnosis is central to optimal outcomes. Factors supporting facial analysis as particularly suitable for very young individuals include (1) it poses no health risk; (2) capture time is fast in comparison to other medical imaging; and (3) if required, image capture can be easily repeated until a suitable image is obtained [Kung et al., 2012].

Novel approaches to 3D facial analysis, including the normal equivalent, have been developed that approach the issue of the rarity of individual RD [Claes et al., 2010b,c, 2012a,b; Hammond and Suttie, 2012]. These techniques allow for a more individualized assessment of facial dysmorphism. In an exploratory study, one of these techniques, dysmorphometrics was used to establish a facial signature for a rare and treatable disorder that had variable facial severity proportional to the clinical disease state. This finding will potentially provide adjunctive evidence for noninvasively monitoring treatment response in this condition [Kung et al., 2012]. In 2013, 33% of marketed innovative medicinal products in the United States were intended for an RD [FDA, 2011]. With the expansion of RD therapeutics, developments in treatment monitoring will be required. Equally, for those disorders for which treatments are not currently available these techniques will be important for objective natural history studies. The fine-scale, objective, scalable, cost-efficient, noninvasive, nonirradiating, and relatively portable nature of 3D facial analyses make these technologies unique candidates for these applications. It is likely that similar methodologies can be extended to nonfacial imaging, for example, for assessment of disproportion, and other aspects of habitus, by whole body scanning in those with skeletal dysplasias or assessment of digital changes in the mucopolysaccharidoses. Additional opportunities are provided by the ability to perform investigations with traditional imaging technologies. For example, paired with MRI for face-brain studies [Hammond and Suttie, 2012] or with cone-beam CT for skull-face assessments [Cheung et al., 2011]. Lastly, the relative inexpensive nature of this technology makes it suitable to provide phenotypic data to existing disease registries.

Understanding individual RDs has provided fundamental insights into basic biological processes and into the causes of common diseases. A recent example is the perspective provided from relationships between a rare lysosomal storage disorder, Gaucher disease, and the more common Parkinson’s disease, which may yield a specific therapeutic approach to Parkinson’s disease and related conditions [Mazzulli et al., 2011]; 3D facial analysis of RD may ultimately benefit common diseases. Along related lines, by endophenotyping and/or by elucidating biological pathways, a more speculative use of 3D facial analysis may be to assist with drug rescue, that is, research involving these techniques will be important for disorders that are not currently in development, and drug repurposing, that is, research on approved drugs for new indications. Pharmaceutical companies and biotechs might be able to employ 3D facial analysis research to scan their libraries for promising drug candidates or to initiate new drug entities. These screening applications may be a cost-effective mechanism compared to current new drug entity discovery which equates to over $1 billion dollars per new drug [Dinani and Grabowski, 2007]. This approach may be particularly relevant given the incentives and regulatory environment surrounding orphan drug development.

Human 3D facial analysis, when combined with other technologies, including facial analysis of mice, has been used to investigate disease biology [Tobin et al., 2008]. Between species (e.g., mouse-human) comparative phenotypic assessments will yield further insights into disease biology and such comparisons will be facilitated by mouse phenotypic, and genetic, resources that are being coordinated by the International Mouse phenotyping consortium (IMPC) and related projects [Chen et al., 2012]. Notably, the IMPC draft phenotyping pipeline includes dysmorphology [Schofield et al., 2012]. By the way of a further example of the potential of cross-species comparisons, recently a study demonstrated large-scale objective association of mouse phenotypes with human symptoms through structural variation indentified in patients with developmental disorders [Boulding and Webber, 2012]. Furthermore, explorations of disease biology, and other potential applications, may be aided by emerging methods that facilitate between-study integration, interrogation, visualization, and exchange of phenotypic and genotypic information [Adamusiak et al., 2012; de Bono et al., 2012; Pan et al., 2012].
It has been suggested that the most important responsibility of the physician is to observe the phenotype of their patients, and this has become particularly relevant in an era where genotype-phenotype relationships are rapidly being clarified by the use of high throughput molecular technologies. Regarding these genotype-phenotype investigations: methods for capturing and analyzing phenotypic data are one of the major bottlenecks in the understanding of human genome biology [Robinson, 2012]. Next generation sequencing (NGS) will require next generation phenotyping (NGP) [Baynam, 2012; Hennekam and Biesecker, 2012]; the full potential of phenome databases, such as OMIM, requires the aggregation of phenotypic information from multiple databases which depends on the availability of fine-grained phenotype ontology and feature frequency data [Oti et al., 2009]; and delineating the phenotypic components of disease allows (1) relationships between gene function and phenotype to be established, where one aspect of the phenotype is common to several genes in the same pathway, and (2) the discovery of interrelationships between disease pathways and genetic networks [Schofield and Hancock, 2012]. NGS has been described as the most powerful diagnostic tool developed since the roentgenogram [Hennekam and Biesecker, 2012]. Coupling NGP methods such as 3D facial analysis with NGS, and other -omics technologies, will enhance the use of these technologies in delineating disease causation. These factors support 3D facial analysis contributing toward reductions in the burden of RDs. In addition, the objective nature of 3D facial analysis may complement efforts to develop standardized descriptions of human phenotypic variation, such as the Elements of Morphology Project, that, in partner with standardized molecular nomenclatures, aim to facilitate robust genotype-phenotype correlations [Carey et al., 2012]; incorporation of phenotypic information into genomic variation databases may enhance clinical care and research [Riggs et al., 2012].

Synergies and Future Directions

Unique aspects of 3D facial analysis provide the potential to develop traditional and novel collaborative networks. In addition to existing fruitful collaborations between Computer Engineers, Clinical Geneticists, Paediatricians, Dentists and Surgeons, networks could be expanded to include a diverse range of medical professionals and scientists. The engineered bases of these technologies will also benefit from progress within the greater bioinformatic community. Further input may be provided from commercial bodies, for example, noting the similarities between financial risk analysis and diagnostic algorithms, and between geological surface analysis and analysis of human surfaces.

There is a relative dearth of genetic and phenotypic information in some developing regions and amongst some populations, by virtue of geographic isolation or other factors. The low-cost and portability of 3D scanners can address these issues.

When assessing an individual’s facial morphology, Clinical Geneticists often “subtract” familial variation obtained from direct observation of a consultant’s relatives or familial photographs. The ability to develop objective facial imaging to assess this, that is, “familial morphometrics,” will improve the sensitivity and specificity of 3D facial analysis. In general, morphometric techniques, an object’s or organism’s form (a concept that encompasses size and shape independent from orientation or position) is the structural information and the conventional observation of interest. These observations, however, are made in isolation and a context-based observation of shape currently does not exist. The introduction of context information as in computational linguistics [Qiu et al., 2011], can lead to an improved understanding of observations made. The idea is to combine contextual information with structural information already coded in the shape of an individual’s face through the relationship of that face to others (e.g., parents). A context-based shape independent from orientation or position (i.e., the structural object’s or organism’s form) is the structural object’s or organism’s form that encompasses size and ability to develop objective familial imaging to assess this, that is, observation of a consultand’s relatives or familial photographs. The neticists often “subtract” familial variation obtained from direct portability of 3D scanners can address these issues.

A powerful diagnostic tool developed since the roentgenogram [Hennekam and Biesecker, 2012]. Coupling NGP methods such as 3D facial analysis with NGS, and other -omics technologies, will enhance the use of these technologies in delineating disease causation. These factors support 3D facial analysis contributing toward reductions in the burden of RDs. In addition, the objective nature of 3D facial analysis may complement efforts to develop standardized descriptions of human phenotypic variation, such as the Elements of Morphology Project, that, in partner with standardized molecular nomenclatures, aim to facilitate robust genotype-phenotype correlations [Carey et al., 2012]; incorporation of phenotypic information into genomic variation databases may enhance clinical care and research [Riggs et al., 2012].

Synergies and Future Directions

Unique aspects of 3D facial analysis provide the potential to develop traditional and novel collaborative networks. In addition to existing fruitful collaborations between Computer Engineers, Clinical Geneticists, Paediatricians, Dentists and Surgeons, networks could be expanded to include a diverse range of medical professionals and scientists. The engineered bases of these technologies will also benefit from progress within the greater bioinformatic community. Further input may be provided from commercial bodies, for example, noting the similarities between financial risk analysis and diagnostic algorithms, and between geological surface analysis and analysis of human surfaces.

There is a relative dearth of genetic and phenotypic information in some developing regions and amongst some populations, by virtue of geographic isolation or other factors. The low-cost and portability of 3D scanners can address these issues.

When assessing an individual’s facial morphology, Clinical Geneticists often “subtract” familial variation obtained from direct observation of a consultant’s relatives or familial photographs. The ability to develop objective facial imaging to assess this, that is, “familial morphometrics,” will improve the sensitivity and specificity of 3D facial analysis. In general, morphometric techniques, an object’s or organism’s form (a concept that encompasses size and shape independent from orientation or position) is the structural information and the conventional observation of interest. These observations, however, are made in isolation and a context-based observation of shape currently does not exist. The introduction of context information as in computational linguistics [Qiu et al., 2011], can lead to an improved understanding of observations made. The idea is to combine contextual information with structural information already coded in the shape of an individual’s face through the relationship of that face to others (e.g., parents). A context-based shape independent from orientation or position (i.e., the structural object’s or organism’s form) is the structural object’s or organism’s form.
Appendices

 benefit of individuals with both common and RDs [Rath et al., 2012]

Conclusion

The high-resolution, noninvasive, nonionizing, scalable and relatively inexpensive nature of 3D facial analysis offers opportunities for novel investigations of rare and common diseases. A knowledge of evolutionary factors may be helpful for instructing and interpreting these studies which will best proceed by innovative collaborative nodal networks.

References


References


