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Yazar, S; Forward, H; McKnight, CM; Tan, A; Soloshenko, A; Oates, SK; Ang, W; Sherwin, JC; Wood, D; Mountain, JA; Pennell, CE; Hewitt, AW; Mackey, DA (2013) Raine Eye Health Study: Design, Methodology and Baseline Prevalence of Ophthalmic Disease in a Birth-cohort Study of Young Adults. *Ophthalmic Genetics*, 34(4), 199-208.

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Title: Raine Eye Health Study: Design, methodology and baseline prevalence of ophthalmic disease in a birth-cohort study of young adults.

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Financial Disclosure: Startup funds were provided by the University of Western Australia and the Australian Foundation for the Prevention of Blindness.

Conflict of Interest: The authors have no proprietary or commercial interest in any materials discussed in this article.

Running head: Raine Eye Health Study

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ABSTRACT

Purpose: The Raine Eye Health Study (REHS) was conceived to determine the prevalence of and risk factors for eye disease in young adults, and characterize ocular biometric parameters in a young adult cohort. This article summarizes the rationale and study design of REHS and outlines the baseline prevalence of ophthalmic disease in this population.

Methods: The Western Australian Pregnancy Cohort (Raine) Study originated as a randomized-controlled trial of 2900 women recruited from the state’s largest maternity hospital. Their offspring (n=2868) have been followed at birth, ages one, two, three, five, eight, 10, 14, 17 and 21 years of age in a prospective cohort study. DNA has been collected from participants for genome-wide association studies. At the 21 year follow-up participants completed a comprehensive eye assessment that included visual acuity, orthoptic assessment and cycloplegic autorefraction, as well as several ocular biometric variables and multiple ophthalmic photographs of the anterior and posterior segments.

Results: A total of 1,344 participants (51.3% male) were assessed in a 24-month period. For the majority of examined participants (85.5 %) both parents were Caucasian. 63.3% had completed school year 12 or equivalent, 5.5% had myopia (spherical equivalent ≤ -3 diopters) and fifteen participants (1.2%) had unilateral or bilateral pterygia. Keratoconus, cataract, keratitis and uveitis were rare.

Conclusion: The REHS design and methodology allows comparison with other population-based studies of eye disease. The study established the prevalence of eye disorders in a large sample of predominantly Caucasian young Australian adults.

Keywords: epidemiology, participation, birth cohort study, recruitment, young adults, Raine Study

INTRODUCTION

Visual impairment is one of the leading causes of morbidity and poor quality of life. Many population studies in ophthalmology have been conducted targeting older adults (aged over 40 years) or paediatric groups (typically 15 years and younger). To date population-based data on prevalence of ocular disease and distribution of ocular biometry in young adults (ie aged between 20 and 40 years) has not been a major research or public health priority and therefore remains generally undefined.¹ There is a need for precise, up-to-date data on this age-group to help guide evidence-based practice and public health resource allocation. Although 82% of the world's blind are aged 50 and older, younger age groups affected by blindness and visual impairment should be a high priority due to the potential burden of many years ahead living with reduced vision.²

Identification of the prevalence and population attributable risk of diseases is critical to support policy decisions which will provide better health services to those in greatest need. However, it is also of importance to understand the underlying risk factors and their associations to prevent disease manifestation and progression thereby reducing their societal impact. Birth cohort studies are valuable for understanding such exposure-disease relationships in a defined population. For example, using a life course epidemiological approach, the 1958 British Birth Cohort Study has found that myopia in later life is influenced by prenatal and early life biological, social and lifestyle influences.³ Additional advantages of cohort studies include: 1) the estimation of distribution, prevalence and incidence of disease in the reference population; 2) the identification of risk factor trends over time; and 3) the evaluation of relationships among the available variables through both hypothesis generating and testing approaches.⁴ When a combination of genetic and phenotypic information is available in a birth cohort study, it may then be possible to determine whether genetic susceptibility to disease can be offset or exacerbated by particular life-course and socioeconomic trajectories.

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Delivery and Development of the Western Australian Pregnancy Cohort (Raine) Study

The age 21-year review of the Western Australian Pregnancy Cohort (Raine) Study investigated ophthalmic health and established the Raine Eye Health Study (REHS). The Raine Study is one of the largest ongoing prospective cohort studies of pregnancy, childhood, adolescence and young adulthood. From 1989 to 1991, 2,900 pregnant women were recruited at 16-18 weeks' gestation into a study at King Edward Memorial Hospital, Perth, Western Australia (KEMH). The original study was a randomized clinical trial investigating whether the use of intensive ultrasound and Doppler studies alter pregnancy outcome in terms of days of neonatal stay and the rate of preterm birth.⁵ An obstetrician at KEMH led the trial as a hospital-based research project. Subsequently, a specific research group was founded to incorporate the newborns of the recruited families into a cohort study. The aim of this cohort study was to determine how events during pregnancy and childhood influence health in later life. The study was initially funded by the Raine Medical Research Foundation at the University of Western Australia. Over the past 24 years the study maintained its existence through successful grant applications to various funding bodies including The Australian National Health and Medical Research Council. The cohort was evaluated in detail during childhood (1, 2, 3, 5, 8 and 10 years) and adolescence (14 and 17 years). Trained nurses and research assistants collected information across a variety of specialties including cardiovascular, reproductive, gastroenterology and respiratory medicine under the guidance of investigators from a wide range of disciplines. Each follow-up included one or more questionnaires as well as physical and psychometric assessments. Figure 1 illustrates the broad categories of measurements completed in each follow-up phase. A core set of measurements have been repeated in each follow-up. These include: 1) Social and physical activity at 5,8,10,14 and 17 years; 2) detailed dietary assessment at 8,10,14 and 17 years; 3) health services utilization at 1,2,3,5,8,10,14 and 17 years; and 4) school assessments at 8 and 10 years. The physical activity measures included fitness and

cardiovascular endurance and the International Physical Activity Questionnaire. Similarly, detailed assessment of diet included food frequency questionnaires and the Australian Commonwealth Scientific Investigation and Research Organisation nutritional assessments.

In addition to these general measurements, Raine cohort data includes some specialised measurements. For example, fetal biometric data measured by obstetric ultrasound at 18-20 weeks gestation are available on the entire cohort. Serial ultrasound and Doppler assessment of fetal growth and the umbilico-placental circulation at 24, 28, 34 and 38 weeks gestation were performed in approximately half of the cohort.^{5,6} Accurate measures of gestation in the cohort allow separation of the influence of birth weight from gestational age. The Raine cohort has detailed assessment of basal and stress-induced HPA-axis activity as part of ongoing investigations into the role of the HPA-axis in developmental programming. At 17 years of age, awakening (fasting) salivary cortisol level was measured on three consecutive days and serum cortisol and adrenocorticotrophic hormone (ACTH) were measured on the third day. Additionally, at 18 years of age, 1137 participants of the Raine study completed the Trier Social Stress Test (TSST)⁷

The Raine Study has proved a valuable scientific resource with some of the major findings to-date including that: i) infants who are breastfed longer than six months tend to have better mental health at six and eight years of age⁸; ii) a high quality breakfast which include foods from three different healthy food groups is associated with better mental health in teenagers⁹; and iii) children whose mothers were stressed or socially disadvantaged during pregnancy generally have a higher risk of developing behavioural and emotional problems.¹⁰ Detailed information on the key findings have recently been outlined by McKnight and colleagues (C. McKnight, personal communication, April 12, 2012).

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Raine participants underwent a comprehensive ocular examination for the first time at the 21-year follow-up. Major objectives of this eye health study were: 1) to determine the prevalence of ocular conditions such as refractive error, strabismus, amblyopia, pterygium and keratoconus in young adults; 2) to document the population distribution of disease-related endophenotypes (ie central corneal thickness, axial length) in young adults; 3) to determine genetic and environmental factors that influence ocular biometry and predispose to ocular diseases; 4) to establish an ocular baseline data for a population cohort that can be followed through later adult life; 5) to understand the association between early life factors (including maternal factors, pre-conceptual/perinatal factors, social, biological and lifestyle factors) and eye disorders and traits. This paper presents the REHS study methodology including its recruitment process and examination procedures. It also describes the baseline prevalence of ophthalmic disease in a young adult population.

STUDY DESIGN AND METHODOLOGY

Ethics Approval

The 21-year review of the Raine Study cohort obtained ethics approval from the Human Research Ethics Committee at the University of Western Australia. The REHS was conducted in accordance with the Declaration of Helsinki and informed consent was obtained from all participants. Previous ethics approvals for other aspects of data collection were completed for each of the earlier examinations.

Recruitment of Participants

More than 3000 pregnant women attending the public antenatal clinic at King Edward Memorial Hospital (KEMH), or nearby private practices between May 1989 and November 1991 were invited to

participate in the ultrasound imaging study. The selection criteria for enrolment were gestational age between 16 and 20 weeks, adequate level of English proficiency to understand the study implications, expected delivery at KEMH and intention of residence in Western Australia in the future such that follow-ups were possible. From the 2900 enrolled pregnancies; 2834 singletons, 64 sets of twins and 2 sets of triplets were born. Of the 2834 singletons, 1415 were randomized to an intensive ultrasound group and 1419 were randomized to a regular ultrasound group. 2804 mothers remained in the study with 2868 newborns recruited for the cohort follow-up. 2135 participants were 'active' (i.e. previously gave permission to be contacted for review) at the 21-year follow-up.

Enrolment for Ophthalmic Examination

All active members of the original birth cohort were invited to attend at 21 years of age. There were no inclusion or exclusion criteria. Booking and assessment procedures are summarized in Figure 1. For each study day, the Raine Study administrative staff contacted participants by phone and invited participants to attend the follow up assessment. Prior to the appointment, each participant was mailed a detailed information sheet and follow-up questionnaires to complete, which could also be completed with assistance on the appointment day. In addition, directions, a map, contact telephone numbers, information on public transport and parking were provided. All participants received a reminder call two days prior to their appointment and a reminder text message on the day. All information on attending participants was verified for accuracy against the restricted access central Raine Study Database by the study co-ordinators. While eye research was the focus of the 21-year follow-up, data was also collected from the cohort on core longitudinal variables including anthropomorphic measurements, socio-economic and demographic data. There was ongoing follow-up of nutritional intake^{11,12}, exercise habits^{13,14} and cardiovascular disease^{15,16}. The cohort also underwent a Dual Energy X-ray

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Absorptiometry (DEXA) scan¹⁷ and a fibroscan¹⁸ . Heart rate, diastolic and systolic blood pressures were measured. Male participants were also invited to participate in a fertility substudy. The data collected for continuing cohort assessment and substudies will be utilized to investigate potential novel risk factors for abnormal ocular biometric parameters.

The ophthalmic examinations and physical assessments were conducted at the Lions Eye Institute and Sir Charles Gairdner Hospital, Perth, WA. A team consisting of an ophthalmologist, ophthalmology trainees, medical students, orthoptists, ophthalmic assistants and Raine Study research assistants (RAs) performed the eye examinations and physical assessments. A study manual, which included detailed information on the ocular examinations, was provided to all examiners to aid data collection and standardize the examination protocol and recordings. Certified orthoptists conducted the extraocular motility assessment.

Ocular examination

All the examination equipment was available at the research site. The eye examination protocol (Table 1) was arranged into 12 stations. A detailed explanation of each station can be found in the appendix. The participants rotated through the examination stations accompanied by a research assistant. Participants followed the order from station 1 to 12. If there was a delay at any station, the participant completed a different station with consideration of the requirement for cycloplegia.

Definitions utilised in prevalence of ophthalmic diseases

Colour photographs of the nasal and temporal conjunctiva in both eyes were assessed for the presence or absence of pterygium. Pterygium was defined as a fibrovascular conjunctival lesion with characteristic appearance extending to or across the limbus. Patients with myopia were defined as having a mean

spherical equivalent (sum of spherical error and half of cylindrical error) of both eyes $\leq$ to -3 diopters. Keratoconus was defined by the presence of unilateral high irregular corneal astigmatism, vort striae, Fleischer ring, or retinoscopic scissoring on slit-lamp biomicroscopy. The data on other ophthalmic diseases were obtained from participants' previous medical questionnaires. Prevalence rate is based on the findings per person and not per eye.

Questionnaires

Each participant completed a follow-up and a medical questionnaire, which included detailed information on sociodemographic data, ocular history, family history of ocular disease, general medical history and environmental risk factors with a focus on UV exposure. In addition, participants completed a extensive food frequency questionnaire that had been validated previously.¹²

DNA Sampling

DNA samples from previous assessments and consents for GWAS studies were available for most participants. If a DNA sample was not previously available, participant consent was sought to obtain DNA from blood or saliva sample. As part of the 21-year cohort review, the participants were asked to provide a fasting blood sample, which was collected on a separate day by the Raine Study phlebotomist at the participants' house. Appointments for blood sampling were made at the eye examination appointment to introduce participants to the phlebotomist and reduce the psychological stress of blood sampling. A 43 millilitre sample was drawn from the cubital fossa vein and delivered to the Royal Perth Hospital (RPH), Perth where blood analysis were performed. If required, DNA was extracted and sent for storage at KEMH. Surplus blood was stored at -80°C in freezers located at the RPH. Blood test results were sent to participants, with advice to consult their general practitioner regarding any results

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outside the normal range. Genome-wide genotyping has been performed using 250ng of DNA on Illumina 660 Quad Arrays. Genotype quality control and calling were undertaken on the Illumina BeadArray Reader at the Centre for Applied Genomics (Toronto, Ontario, Canada).

Data Entry and Statistical Analysis

The eye examination and physical assessment data were entered into a password-protected database created in Microsoft File Maker Pro (Version7). All data entry was checked by a second person and validation checks performed by the Raine Study Data Manager. Raine Study research assistants coded, scanned and verified questionnaires. Where available, crude eye examination data from the instruments were exported into the database.

All phenotype data were stored on secure servers at the Telethon Institute for Child Health Research (TICHR). All genotype data were stored on the iVEC supercomputer supported by the Western Australian Government and the Australian Federal Government. We will conduct univariate and multivariate genetic analysis utilizing our own software package SimHap and the PLINK package.¹⁹

National and international collaborations are encouraged in the Raine Study. The Raine Study Executive Committee are the custodians of the Raine Study data and biological samples. Interested investigators are required to seek approval from the Executive Committee for proposed projects. Access policies, data dictionaries, copies of questionnaires and assessment protocols for each follow-up are available to registered researchers on the Raine Study website (www.rainestudy.org.au).

Study Power for Genetic Discovery:

Power calculations for genetic analysis were performed using QUANTO.²⁰ For all calculations an additive model with 2 degrees of freedom was used and the marker allele was assumed to be in high linkage disequilibrium with the cause variant ($r^2 = 1$). Given our population-based design, we will have greater power to detect quantitative trait loci of modest effect size (Fig. 3).

RESULTS

From the original cohort of 2868 live births, 37 (1.3%) participants were deceased, 182 (6.3%) were lost to follow-up and 514 (17.9%) participants had withdrawn from the study by the time of the 21 year follow up. Of the remaining 2135 active Raine Study members, 1743 individuals (81.6%) verbally agreed to participate in the 21-year follow up. Of these 1743 participants, 1344 (77.1%) were examined in the 24-month period from March 2010 to February 2012. Participation in the 21-year follow up is shown in Figure 4. Among the 1344 examined participants, 119 attended only one of the last three follow-ups and 32 of participants had attended none (Figure 5).

Of the 1344 participants, 690 (51.3%) were males and 654 (48.7%) were females. Ethnic composition of participants is shown in Table 2. 1214 (90.3%) had a Caucasian mother and 1210 (90.0%) a Caucasian father. For 1149 (85.5%) participants, both parents were Caucasian.

In terms of occupation 37.1% of participants were full-time students and 5.7% were part-time students. 21.9% were in full-time employment and 26.3% were working part-time. 21.9% studied and worked at the same time. 63.5% of participants had completed the final year of high school or its equivalent.

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Baseline Prevalence of Ophthalmic Disease:

Prevalence of ophthalmic disease in the cohort is shown in Table 3. The most common ophthalmic condition was myopia. A total of 5.5 % participants had spherical equivalent of less than or equal to -3 diopters. No participant had been diagnosed with retinopathy of prematurity, retinal dystrophy or glaucoma.

DISCUSSION

The Raine Study cohort is unique in allowing prospectively collected antenatal, childhood and adolescent data to be correlated with outcomes of an ophthalmic examination. Detailed phenotypic data have been collected prospectively over the last 20 years, resulting in a database of over 16000 variables being available on each member of the cohort. This provides the possibility to study multiple exposures and multiple outcomes at many life stages, allowing identification of potential associations and risk factors in ocular disease process. Identification of these associations and risk factors may help to improve clinical practice and possible prevention of ocular disease.

We acknowledge that the limits of conventional epidemiology include being unable to confirm or refute causality, reverse causality, residual confounding and ensuring appropriateness of adjustment for confounders / mediators. However, it is possible to construct a ‘causal’ model that incorporates the interaction of many different risk factors at multiple life stages.³ Moreover, we will investigate genetic risk factors, which may show a causal association (eg Mendelian Randomization). Over 23 years of existence, the Raine Study has maintained high participation rates with minimal attrition, allowing establishment of more accurate prevalence rates. Further possible disadvantages of this prospective

cohort study include relatively low numbers of incident cases of some endpoints, and the problems of multiple significance tests conducted at different time points. We also acknowledge that there is a possible selection bias relating to the initial recruitment (ie. mothers giving birth in a public hospital might be of lower social class or different sociodemographic characteristics than those in a private hospital, or exclusion of non-English speaking mothers).

The REHS provides extensive data on ocular biometry and disease from a young adult, predominantly Caucasian population. Strengths of this follow-up include its large sample size, prospective information on neonatal and early life exposure and a standardized clinical assessment.

Characteristics of the original cohort published earlier²¹ were maintained in the REHS. The REHS had a slightly greater male than female participation rate. This is reflective of state population characteristics, where according to 2010 population estimates, 50.7% of the Western Australian residents were males.²²

The study sample is primarily Caucasian. This genetic homogeneity will be advantageous in analysis of quantitative trait loci through GWAS. Some may argue that finding variants in such a population will have limited application to the ethnically diverse Australian population. While this may be one limitation of the study, it should be remembered that ethnic differences in the prevalence and severity of disease and the responses to treatment are still part of the unsolved equation of genetic and environmental factors predisposing to ocular disease. Focusing on a particular population will allow us to obtain more specific results, which could be compared with outcomes from other populations.

With the development of new technologies, clinicians are more reliant on highly specialized equipment to make diagnostic decisions. Many clinicians now use these technologies as a part of their routine

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patient assessment to understand the development and progression of ocular diseases such as AMD and glaucoma. We used equipment such as the Spectralis HRA/OCT and HRT, which to date have not been used in large population studies. The unique set of results obtained from using this technology will construct baseline data for identification and tracking of disease.

Strategies for High Retention rates:

Maintenance of sample size in longitudinal prospective studies is critical to maximize statistical power and reduce bias. The Raine Study has sustained high retention rates although the number of active participants has reduced over the years. Participants move away from the metropolitan area, and go interstate or overseas. There is the loss of contact details through change of address, change of name and loss of contact details of relatives. Some participants withdraw from the study. The Raine Study tries through various methods to establish a feeling of ownership and belonging amongst participants, and reduce cohort attrition. Raine Study staff are highly trained, regular newsletters are sent out and participants are involved in study planning.

Participation by active members of the cohort (those not lost to follow up, deceased or withdrawn) in the REHS was high (81.6%). At age 20 participants had a variety of work, study and family commitments, and other issues such as examination periods and school holidays had to be accommodated. The cohort assessment was planned at times that were adaptable to the needs of the cohort, which included weekends, public holidays and weekdays. Childcare was available for participants with infants and young children. A motivating factor for participation could have been the access to a comprehensive eye-health examination and the immediate discussion of the individual results with an Ophthalmologist.

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3 In this article, we aimed to describe standardized methodology and practical guidelines to utilize in
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5 future eye research. We provided the baseline prevalence of ophthalmic disease in a young adult
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7 population. Western Australian data collection ceased in March 2012, although there is provision for
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9 follow up of participants in other states of Australia. Further analysis of data will define the prevalence
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11 of refractive error, strabismus, corneal dystrophy and amblyopia in a young adult population.
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13 Investigation of GWAS and early life data could identify genes and environmental factors that influence
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15 ocular biometry and predispose to ocular disease. The 23-year cohort follow-up of the Raine Study
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17 commenced in March 2012 with a focus of sleeping patterns and asthma in young adults. We anticipate
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19 repeating the eye follow-up when the cohort reaches 40 years of age when many chronic, age-related,
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21 ocular diseases begin to manifest.
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32 **ACKNOWLEDGEMENTS**

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35 We acknowledge the major contribution from the Raine Study participants and thank the Raine Study
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37 research staff for cohort co-ordination and data collection. Initial funds for the Raine Eye Health Study
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39 pilot were provided by the University of Western Australia and the Australian Foundation for the
40
41 Prevention of Blindness. We are grateful to the Australian National Health and Medical Research
42
43 Council for the long-term contribution to funding the study over the last 20 years and to The University
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45 of Western Australia (UWA), Raine Medical Research Foundation, UWA Faculty of Medicine,
46
47 Dentistry and Health Sciences, The Telethon Institute for Child Health Research, The Women and
48
49 Infants Research Foundation and Curtin University for providing funding for Core Management. We
50
51 thank Professors John Newnham, Fiona Stanley, Louis Landau, Lawrie Beilin and Ian Puddey. We
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53 would like to also thank Fred Reinholz, Naeem Fatehee, Chandra Balaratnasingam, Kate Hanman, Bob
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and Dawn Street and the 2010 and 2011 UWA Research and Discovery Unit medical students for assisting with eye assessments. We also acknowledge the support of the National Health and Medical Research Council of Australia (Application ID APP1021105).

For Peer Review Only

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Figure 1. Broad categories of assessments in the Raine cohort follow-ups.

URL: <http://mc.manuscriptcentral.com/nopg> E-mail: ophthalmicgenetics@yahoo.com

Figure 2. Summary of booking and examination process

Participation Rates in REHS

Figure 4. Participation of active cohort members in the Raine Eye Health Study assessment

Figure 3. Power of this study to detect a quantitative trait locus conferring 1.5–3.5% of the continuous trait’s variance at the genome-wide threshold ($\alpha = 5 \times 10^{-8}$).

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Table 1. Raine Eye Health Study ocular examination protocol

Station 1

- a. Pre-cycloplegia Autorefraction (Nidek ARK-510A, NIDEK Co.Ltd, Japan)
- b. Vertometry (CL-200 Computerized Lensmeter, Topcon Medical Systems, Inc, NJ)
- c. Colour Vision (Ishihara's Tests for Colour Deficiency (24 Plate Edition), Kanehara Trading Inc,Tokyo, Japan)

Station 2 Best corrected vision tests

- a. Visual Acuity (Test Chart 2000 XPert, Thomson Software Solutions, UK)
- b. Contrast Sensitivity (Test Chart 2000 XPert, Thomson Software Solutions, UK)
- c. Vernier Acuity (Test Chart 2000 XPert, Thomson Software Solutions, UK)

Station 3 Orthoptic Binocular vision function tests

- a. Cover Test
- b. Assessment of Extraocular Movements
- c. Other findings (4 Diopter Prism Test, Nsytagmus)
- d. Assessment of Stereoacuity (Langs II and Titmus)
- e. Assessment of Ocular Dominance (Miles test)

Station 4 Eye Photography

- a. Eyelid Position Photography (Nikon Coolpix E995, Tokyo, Japan)
- b. Eye Colour Photography (Nikon D100 digital camera, Tokyo, Japan with 105 mm 147 f/2.8 Micro Nikkor lens (Melville, New York, USA)
- c. Conjunctival Autofluorescence Photography with filtered electronic flash (Nikon D100 digital camera, Tokyo, Japan with 105 mm 147 f/2.8 Micro Nikon lens, Melville, New York, USA)

Station 5

- a. IOP Measurement (Icare TAO1i Tonometer, Icare Finland Oy, Helsinki, Finland)
- b. Instillation of Tropicamide 1%, Phenylephrine 10%.
- c. Measurement of Eyelash Length

Station 6

Measurement of Corneal Higher Order Aberrations (Zywave II Wavefront Aberrometer, Bausch&Lomb, Inc., Rochester, NY)

Station 7

Ocular Biometry (IOLMaster (V.5), Carl Zeiss Meditec AG, Jena, Germany)

Station 8

- a. Anterior Segment Tomography (Oculus Pentacam, Oculus Optikgerate GmbH, Wetzlar, Germany)
- b. Endothelial Cell Count Analysis (EM-3000 Specular Microscopy, Tomey Corp., Nagoya, Japan)

Station 9

- a. Fundus Photography: 60° Optic Disc centred colour/ Fovea centred colour/ Red Free Disc centred monochrome (Canon CF-60DSi and CF-60UVi Digital Fundus Camera, USA)
- b. Stereo Disc Photography (Optic Disc Photography 3-Dx Fundus Camera, Nidek co, Japan)

Station 10

Optical Coherence Tomography (Spectralis HRA+OCT, Heidelberg Engineering, Heidelberg, Germany)

Station 11

Post- cycloplegia Autorefraction (Nidek ARK-510A, NIDEK Co.Ltd, Japan)

Station 12

Retinal Tomography (Heidelberg Retina Tomograph 3, Heidelberg Engineering, Heidelberg, Germany)

Ethnicity	Mother's ethnicity (%)	Father's ethnicity (%)
Caucasian	1214 (90.3)	1210 (90.0)
Chinese	54 (4.0)	36 (2.7)
Indian	36 (2.7)	39 (2.9)
Indigenous Australians and Torres Islanders	9 (0.7)	12 (0.9)
Polynesian	10 (0.7)	11 (0.8)
Vietnamese	5 (0.4)	6 (0.5)
Not stated/ Unknown	16 (1.2)	30 (2.2)

Table 2. Ethnicity demographics

Ophthalmic Disease	Number of affected participants (n=1344)	Percentage (%)
Myopia ($\leq -3D$)*	74	5.5
Pterygium*	15	1.22
Cataract		
Congenital cataract	3	0.22
Traumatic cataract	1	0.07
Keratoconus*	2	0.15
Uveitis		
Idiopathic acute anterior uveitis	1	0.07
Juvenile idiopathic arthritis - related anterior uveitis	1	0.07
Herpes simplex keratitis	1	0.07

* See text for clinical definitions.

Table 3. Baseline prevalence of ophthalmic disease in REHS.

Appendix:

The REHS 12-Station Protocol

All the examiners were instructed to print out a copy of the results where possible, complete the LEI examination recording form and insert their initials for each station.

Station 1

Pre-cycloplegic Autorefraction: Pre-cycloplegic autorefraction and keratometry were performed with Nidek ARK-510A (NIDEK Co.Ltd, Japan). Participants were instructed to relax their eyes and look at the internal image through the eyepiece and try not to bring it into focus. The measured prescription and keratometry results in diopters were recorded in the examination form.

Vertometry: If the participant had a pair of glasses at the time of examination, CL-200 Computerized Lensometer (Topcon Medical Systems Inc., Oakland, NJ) was used to measure the prescription of the glasses.

Colour vision: Participants with colour vision deficiency were identified with Ishihara's Test for Color Deficiency (24 Plate Edition, Kanehara Trading Inc., Tokyo, Japan)

Station 2: Best corrected vision

Distance Visual Acuity: Visual acuity was measured using a logarithm of the minimum angle of resolution chart (Test Chart 2000 Xpert, Thomson Software Solutions, UK), which was run on Window XP computer. Except for the larger size, each row of the chart contained five letters and letter size reduced in steps of 0.1

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logMAR between one row and the next. The monitor was placed at three meters and viewed via a mirror. Participants were tested monocularly and with glasses or contact lenses if worn. The letters were alternated between the eyes. All participants were pinholed, even in the event that one or both eyes achieved 6/6 vision or better. A matching HOVT card was available for disabled participants. If no letters were identified on the chart, counting fingers, hand movements and perception of light were assessed.

Contrast Sensitivity: The traditional method of measuring contrast sensitivity is sine wave grating. It was shown that the low-contrast letter charts could provide valuable information in clinical settings. We test contrast sensitivity by using a low-contrast letter chart (Test Chart Xpert, Thomson Software Solutions, UK). The viewing distance was set to six meters. Participants were tested monocularly and with glasses if worn. Participants were asked to read letters displayed in triplets of decreasing contrast from top to the bottom until they could no longer read two of the three letters. The contrast of the last row read was recorded in units of logarithm of the minimum angle of resolution.

Vernier Acuity: We determined the minimum angular separation required to detect that two lines placed end to end were not co-linear using the Test Chart Xpert 2000. The participants tested monocularly with glasses, if worn. They were instructed to inform the examiner as soon as they see the straight line splitting into two on the screen. The test was repeated three times for each eye. Mean of the three measurements were recorded in arc seconds.

Station 3: Binocular Vision Function

Cover Test/Alternate Cover Test: To determine the presence of heterotropia and heterophoria, a cover test and an alternate cover test were performed at both distance (6m) and near (1/3m). The tests were repeated with and without glasses. Prism bar cover test was performed to measure any observed misalignment.

Ocular Motility: Eye movements were examined in nine-positions of gaze with a pen torch. The participants were instructed to hold their head stationary and follow the light with their eyes. Abnormal motility was scored on +4 (gross overaction) to -4 (gross underaction) with increments of 0.5. Bielshowsky head tilt test was performed when a palsy of superior oblique muscle was suspected.

Four Diopter Base Out Prism Test: This test is used to detect a micro-strabismus and associated central suppression scotoma if present. Participants were instructed to fix on a small fixation target at near (1/3m). A four-diopter base out prism was placed in front of one eye and a fixation movement to confirm fusion of images was observed. In the event of no movement was observed to overcome prism and restore binocular single vision, the test outcome recorded as a negative that was presence of a central suppression. The test repeated on the other eye. If no movement was observed when prism was placed in front of either eye, the response was recorded as equivocal.

Nystagmus: Any nystagmus observed was recorded with its direction and subtype.

Stereoacuity: Lang II test was completed to assess gross stereopsis or depth perception. The identified images were recorded with corresponding arc seconds. Then the Titmus circle test was completed to assess a finer grade of stereoacuity. Number of correctly identified circles were recorded.

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Ocular Dominance: Dominant eye was determined by using the Miles test. Participants were instructed to extend their arms straight in the air and create a triangle with their hands, then frame some letters from the letter chart through that triangle. The examiner covered one eye at a time and instructed participant to report when the letters were no longer in his/her view. The eye framing the letters upon covering of the contralateral eye was recorded as the dominant eye. Also, the participants were questioned on their chirality (handedness).

Station 4: Eye Photography

Eyelid Position: To determine the symmetry between the eyes and also the parts of around the eyes including eyelids, a photo covering the area from temple to temple was taken with a standard pocket camera (Nikon Coolpix E995, Tokyo, Japan).

Eye Colour: To determine presence of pinguecula or pterygia, a colour photo of iris in primary position, medial and lateral conjunctiva in dextroversion and laevoversion were taken using a digital camera (Nikon D100, Tokyo, Japan) fitted with 105 mm f/2.8 Micro Nikkor (Nikkor, Melville, New York, USA) lens.

Conjunctival UV Auto-fluorescence: A camera system developed by Coroneo and colleagues (Ooi 2006, 2007) was used to take the conjunctival UV auto-fluorescence photos for each participant. The camera system included a height adjustable table equipped with subject head-rest, camera positioning assembly, digital single-lens reflex camera (Nikon D100 (Nikon, Melville, New York, USA)), 105 mm f/2.8 Micro Nikkor (Nikkor, Melville, New York, USA) lens, and filtered electronic flash. Both nasal and temporal regions of the eyes were photographed at 0.94 magnification.

All images were saved in RGB format at the D100 settings of JPEG Fine (1:4

compression) and large resolution (3,000 2,000 pixels).

Station 5:

Intraocular Pressure (IOP) measurement: IOP of the eyes were measured with ICare TAO1i Tonometer (Icare Finland, Oy, Helsinki, Finland). Six consecutive measurements were taken for each eye and mean measurement was recorded.

Cycloplegia: If the IOP was less than 22mmHg in each eye, one drop of Tropicamide 1% and one drop Phenylephrine 10% were administered for dilation of pupils. In the event of high pressures, ophthalmologist checked for the closure of the angle of the anterior chamber of the eye.

Eyelash measurement: One upper eyelash from each eye was measured with a ruler and average of the two measurements was recorded in millimeters.

Station 6:

Zywave II Wavefront Aberrometer: To detect the higher order aberrations in the optic media, we measured the refractive error by using Zywave II Wavefront Aberrometer (Bausch&Lomb, Inc., Rochester, NY). The measurements were completed prior to the cycloplegia. Measurements with no readings repeated post-cycloplegia. In the presence of keratoconus, a secondary measurement was completed with Orbscan II (Bausch&Lomb, Inc., Rochester, NY)

Station 7:

IOL Master: Ocular biometric parameters including axial length, corneal curvature, anterior chamber depth and horizontal corneal diameter were measured with

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IOLMaster V.5 (Carl Zeiss Meditec AG, Jena, Germany). For AL, five consecutive measurements were taken until the following criteria were satisfied: measurements within $\pm 0.02\text{mm}$ of each other, good wavefrom – no double peaks, acceptable signal-to-noise ratio >2.0 . Any measurement outside the mentioned criteria deleted and repeated. During keratometry, three measurements within 0.3D within each meridian with careful alignment and focus were recorded. Next, five consecutive ACD measurements were taken when the fixation point was sharply focused. Finally, three corneal diameter measurements within 0.2mm were recorded for each eye. Right and left eye measurements were within 0.02mm .

Station 8:

Oculus Pentacam: Anterior segment tomography of each dilated eye was taken with Oculus Pentacam (Optikgerate GmbH, Wetzlar, Germany). Quality of the images were checked and repeated if necessary.

Tomey Specular Microscopy: Endothelial cell count analysis helps to evaluate the endothelial cell density, variation in size (polymegathism), variation in shape (plomorphism) and other corneal factors such as injury, inherent disease, and inflammatory or foreign material. EM-3000 Tomey Specular Microscopy (Tomey Corp., Nagoya, Japan) was used to complete endothelial cell count analysis on each participant. Unclear screen shots were repeated.

Station 9:

Fundus Photography: Three sets of retinal photographs were taken for each eye using Canon CF-60DSI Digital Fundus Camera (USA). The lens angle was set to 60 degrees and the illumination dial was turned to level 2. First, the participants were

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3 instructed to stare directly ahead into the camera and a macula-centered photo was
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5 taken. Then the participants were instructed to look at the fixation light attached to the
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7 top of the headrest and the disc-centered photo was taken. Finally, the photo setting
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9 on the computer screen was changed from “colour” to “red-free”, red free filter of the
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11 camera was removed and the illumination was increased level 3 to take the disc-
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13 centered red-free photo. If the quality of the images were unclear or there was an
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15 artifact ie eyelashes, the photographs were retaken.
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20 **Stereo-disc Photography:** Optic Disc Photography 3-Dx Fundus Camera (Nidek Co,
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22 Japan) was used in this study to examine the anatomy of the optic disc in three-
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24 dimension. Participants were appropriately positioned in front of the camera and
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26 instructed to look at the internal or external fixation lights during photography shot.
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28 The quality of the image was assessed. When it was unclear, poorly illuminated or the
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30 discs were in different colours, the photograph was retaken.
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38 **Optical coherence tomography:** Images were captured using a Spectralis
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40 HRA+OCT (Heidelberg Engineering, Heidelberg, Germany). Volume scans were
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42 obtained for the macula (30° x25°, 31 slices at 240 microns) and optic disc (15°x10°,
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44 49 slices at 30 microns). Retinal nerve fiber layer (High speed, ART16) and choroidal
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46 thickness images (2 x horizontal & 2 vertical EDI, High speed, ART100) were also
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56 **Post-cycloplegic autorefraction:** Autorefraction and keratometry results were
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58 repeated after adequate pupillary dilation.
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Station 12

Heidelberg Retina Tomography: Glaucoma is more common in individuals aged over 40, however it could occurs at any age. Confocal scanning laser ophthalmoscope is used in analysis of glaucoma manifestation and progression. A baseline glaucoma analysis was done on each participant using the Heidelberg Retina Tomography (Heidelberg Engineering, Heidelberg, Germany). Participant was appropriately positioned in front of the camera and instructed to stare at the flashing light. The position of the camera was adjusted to illuminate and sharpen the image of the optic disc. Poor images were repeated. Each scan was reviewed at the end and the mean standard deviation of less than 20 μ m was maintained for quality check.

Participant Eye Report (Feedback)

At the end of the examination session, each participant was given a detailed debriefing on the results of the completed examinations, provided with a summary of his/her current ocular health status and given the opportunity to ask questions. Any newly diagnosed pathology was discussed with the participants and any individuals requiring an ophthalmological intervention were referred to the clinical experts in sub-specialties. A feedback report outlining the participant's ocular status as well as a copy of macular-centred retinal photos were provided to all participants. Later, an analysis of the preliminary signs of UV damage and a copy of the conjunctival UV auto-fluorescence photos were mailed to each participant.