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Forensically Relevant Applications of Genome-Wide Association Studies

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Abstract

The past century has seen great improvements in the science underpinning forensic investigations. The field has advanced from Lombardo's classification of the "criminal" physique, to the automated fingerprint matching systems. At the biological level, the field has moved from identification based on blood groups to automated genotyping, which is used to compare DNA extracted from saliva or blood left at a crime scene to reference DNA collected from individuals.

In a legal context, however, such improvements are of limited use if relevant parties cannot fully and accurately understand the evidence. This includes both sides of legal representation and, where a jury is involved, eligible jury members. Previous studies have examined the understanding of statistical evidence amongst jury-eligible individuals, finding that they commonly result in logical fallacies. These types of studies have typically focussed on the understanding of DNA matching methods. However, genomic analyses currently focus on the use of genome-wide association analyses (GWAS) which aim to identify single-base variants associated with the trait being studied. Many forensically relevant traits have been studied using GWAS approaches and there is substantial interest in the use of these findings to predict phenotypes of interest, and the implications of using these findings as supporting evidence during trials.

While there have been a number of GWAS studies of eye and hair colour and hair morphology, there are some notable gaps in current literature on genetic variants influencing forensically relevant complex traits. Although fingerprint patterns are commonly used for identification, there have been no studies seeking to identify genetic variants influencing these patterns. Similarly, although there are a number of papers focussing on gross hair morphology, there have been no studies on the micro-level morphology. In order to address these limitations, the current thesis aims to identify genetic variants that contribute to differences in fingerprint patterns, quantify the genetic contribution to and identify genetic variants influencing micro-level hair diameter and curvature.

In addition to the identification of genetic variants influencing forensically relevant traits, human factors are also of interest. In order to introduce GWAS evidence in a comprehensible manner to a legal and general audience, it is apparent that clear communication of scientific

and statistical evidence is essential. By studying attitudes and perception of GWAS evidence by a jury-eligible population, this thesis provides the first step towards an evidence-based approach to transmit knowledge reliably in a forensic context.

This thesis is comprised of both published and unpublished chapters. In Chapter 1, I present an introduction to the area and the methodology used in this thesis. This is followed by a preface to Chapter 2, which provides a literature review focussing on dermatoglyphics. Chapter 2 presents the first published study of the genetic influences on fingerprint patterns. The preface to Chapters 3 and 4 describes the structure and development of hair follicles. Chapter 3 provides the first study examining the heritability of micro-level hair diameter and curvature. Chapter 4 presents the first GWAS of micro-level hair curvature, comparing these findings to those for macro-level curvature in the same sample. I then provide a brief review of the literature on jury perception of scientific and statistical evidence in preface to Chapter 5. In Chapter 5, I present the findings of a study in which I examined attitudes towards DNA and GWAS derived evidence in a sample of jury eligible individuals. The final Chapter provides a discussion of the research findings, the limitations and potential directions for future work.

Declaration by Author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

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Variants within ADAMTS9-AS2 influence fingerprint patterns

Contributor	**Statement of contribution**
YW Ho (Candidate)	Preliminary descriptive and principal components analyses, GWAS, and meta-analyses (80%) Compiled relevant tables and graphs (90%) Wrote and edited paper (80%)
SE Medland	Conceived and designed study (100%) Fingerprint ridge count (100%)
SE Medland & NG Martin	Editing of manuscript (10%)
All other authors	Editing of manuscript (10%)

Contributions by others to the thesis

Some data analysed were collected as part of other research projects conducted at QIMR Berghofer, in particular for Chapter 2, ongoing studies of melanoma risk factors and memory and attention, conducted as part of the Brisbane Adolescent Twin Study conceived by NG Martin, and the Avon Longitudinal Study of Parents and Children (ALSPAC), based in the University of Bristol and led by G Davey-Smith. My contribution to this paper was to run the fingerprint GWAS analyses used as a negative control.

Hair curvature in Chapters 3 and 4 was coded by research nurses as part of the Brisbane Longitudinal Twin Study (BLTS) led by NG Martin. Light optic diameter and curvature measurements were provided by M Brims from BSC Electronics Pty Ltd.

Part of the bioinformatics database extraction in Chapter 4 was conducted by JN Painter, who also made the supplementary tables 4.2 to 4.4.

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quantitative genetics, forensics, psychology, dermatology, trichology, gwas, snp

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List of Abbreviations used in the thesis

ADAMTS9-AS2	ADAM Metallopeptidase With Thrombospondin Type 1 Motif, 9 - antisense RNA 2
AIC	Akaike information criterion
ALSPAC	Avon Longitudinal Study of Parents and Children
BLTS	Brisbane Longitudinal Twin Study
CSI	Crime Scene Investigation
DNA	deoxyribonucleic acids
DZ	dizygotic (twin)
E	environmental influences
ECPHR	European Convention for the Protection of Human Rights and Fundamental Freedoms
EDAR V370A	Ectodysplasin A receptor V370A
EGA	estimated gestational age
ENCODE	The Encyclopedia of DNA Elements
FoxD3	Forkhead Box D3
G	genetic influences
GWA(S)	genome-wide association (studies)
GxE	gene-environment interaction
H2/h2	broad sense/narrow sense heritability
HDAC2	Histone Deacetylase 2
HWE	Hardy-Weinberg equilibrium
IAFIS	Integrated Automated Fingerprint Identification System
IBD	Identical by descent
LD	linkage disequilibrium
MAF	Minor allele frequency
MC1R	Melanocortin 1 Receptor
MED13L	Mediator Complex Subunit 13-Like
mtDNA	mitochondrial DNA
MZ	monozygotic (twin)
NCIS	Naval Criminal Investigative Service
OFDA	optical fibre diameter analyser
OLA1	Obg-Like ATPase 1
p300	E1A binding protein p300
PCA	Principal components analyses
PCR	Polymerase chain reaction
POU1F1	POU Class 1 Homeobox 1
QC	quality control
QIMR	Queensland Institute of Medial Research
Q-Q	Quantile-quantile
RDC	reaction-diffusion-convection
RNA	Ribonucleic acid

SEM	structural equation modelling
▪ **A**	additive genetic influences
▪ **C**	common/shared environmental influences
▪ **D**	dominance genetic influences
▪ **E**	non-shared environmental influences
SIDS	Sudden Infant Death Syndrome
SMARCAD1	SWI/SNF-Related, Matrix-Associated Actin-Dependent Regulator Of Chromatin, Subfamily A, Containing DEAD/H Box 1
SNP	single nucleotide polymorphisms
SOX	SRY-related HMG-box
STR	short tandem repeat
TBX	T-box
TCHH	trichohaylin
varA	variance due to additive genetic effetcs
varG	variance due to genetic effects
varP	total variance
WNT	Wingless-Type MMTV Integration Site

Chapter 1: Introduction and General Methodology

1.1 General Introduction

Since before the advent of genetic technology, observable physical traits have been used in the identification of individuals and the study of genetic inheritance. These techniques were adopted by law enforcement agencies in profiling and documentation of suspects and incarcerated individuals, with a particular focus on phenotypes such as hair colour, eye colour, height, and fingerprints. Although best known for his methodological studies, Galton also conducted extensive studies on twin and family resemblance of fingerprint patterns (Galton, 1892; Holt, 1968), and developed a classification system that is used in legal systems to this day. While the importance of fingerprint evidence has decreased since the development of DNA genotyping technology, dermatoglyphic evidence remains important in the identification of suspects. These genotyping technologies have included methods such as short tandem repeat (STR) and mitochondrial (Mt) DNA matching, which have provided valuable information when considered collectively with other physical evidence and witness testimony (Gans et al., 2001; Justice, 2002). While the use and influence of DNA matching evidence have been well studied, genome-wide association (GWA) results have yet to be used in forensic contexts despite their broad application to disease and psychiatric phenotypes.

The aims of this thesis are to quantify the importance of genetics and identify genetic variants influencing three forensically relevant traits, fingerprint patterns and hair diameter and morphology. This is addressed in Chapters 2, 3 and 4, which investigates genetic contributions to dermatoglyphics and trichology, phenotypes that can be used in forensic investigations to confirm suspect, or victim identities due to their relative permanence compared to other types of evidence, as well as the individual uniqueness of these phenotypes. The main methodologies used in these chapters are described in the sections 1.2 and 1.3 below.

To investigate the potential translation of these findings, Chapter 5 describes a study measuring attitudes toward genetic evidence as well as factors that may influence perceptions of genetic and statistical evidence in potential jury members. In accordance with previous

literature examining the perception of DNA evidence, we also collected measures on education level, knowledge of statistics and genetics, frequency of media consumption, and perceived realism of media to examine their roles as potential confounds. Lastly, the major findings and conclusions of this thesis are discussed in Chapter 6.

1.2 Structural Equation Modelling and Twin Studies

Genetic epidemiological models that aim to predict phenotype expression utilize the variation in genetic effects by modelling data collected from individuals differing in the extent of their genetic (G) and environmental (E) relatedness. These models typically estimate the sources of variance due to G and E effects using Structural Equation Modelling (SEM). Genetic factors due to "Additive genetic" (A) and "Dominance" (D) can be estimated separately. Environmental factors shared by family members termed "Common environmental factors" (C) can be estimated separately to non-shared environmental effects known as unique or unshared Environmental influences (E) which include measurement error. While broad sense heritability (H^2) estimates the total portion of variation attributable to genetic effects (varG/varP), narrow sense heritability h^2 measures only additive effects within total variation (varA/varP). This quantification and partitioning of variance has allowed the development of linear models that allow the estimation of relative importance of the sources of variance.

Twin and family studies provide one of the most powerful methods to estimate heritability. Monozygotic (MZ) twins are assumed to share the same DNA inherited from their parents, whereas dizygotic (DZ) twins and siblings share approximately 50% of their segregating DNA. This difference in the degree of genetic relatedness provides the power to partition the between- and within-family variances, allowing the estimation of heritability. Specifically, the degrees of correlation for A and D are different for MZ versus DZ twins, but correlations for C and E are the same. Cross-twin MZ correlations for A and D are both 1, compared to .5 and .25 for DZs. Both MZ and DZ twins have an assumed correlation of 1 for C and uncorrelated E. Assuming both zygosities are drawn from the same population, C and D cannot be estimated simultaneously with twin data. An ACE or ADE model is usually selected based on the pattern of MZ versus DZ correlations. When rMZ is greater than 2(rDZ), an ADE model is fit to the data whereas an ACE model is used when 2(rDZ) > rMZ.

Further information such as sex differences can be gauged through the correlations between DZ opposite-sex twins, and influences of different environments and the mode of gene action can be seen by using biological information from other family members, such as half-siblings and cousins. Based on these principles, more complex models can be created, such as models taking into account covariates and gene-environment correlations and interactions, and multivariate models that estimate common genetic sources of variation between phenotypes.

One way of visualising and interpreting results from SEM is using path analysis. As illustrated in the univariate twin model below (Figure 1), the phenotypes of interest are represented within rectangles and latent G and E influences represented within circles. Causal paths are drawn using single-headed arrows linking from latent to observable traits, with path coefficients a, d, and e representing effects of latent variables on phenotypes. Double-headed arrows show correlations between latent factors. Using path tracing rules, genetic covariances may be calculated using paths linking between traits, i.e. A for DZ twins = a*.5*a.

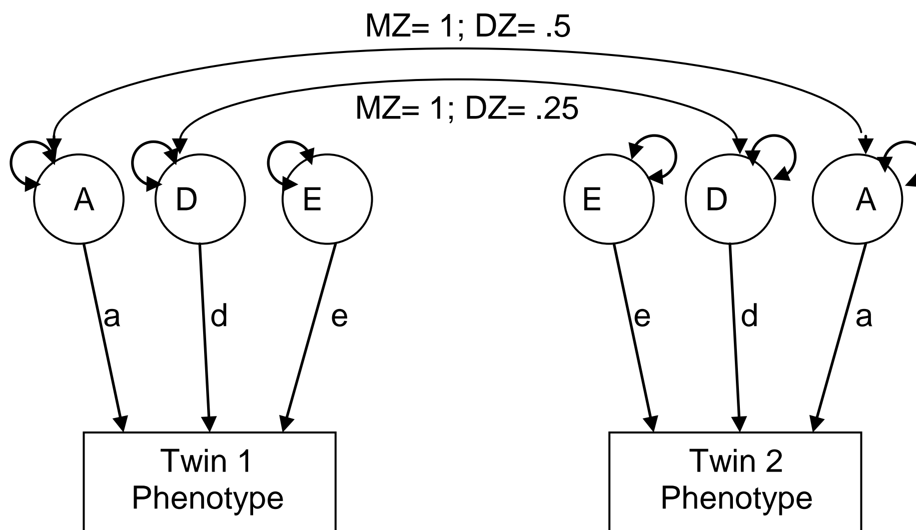

Figure 1.1 A univariate ADE twin model

A number of assumptions are involved in twin methodology. The equal environment assumption, for example, assumes that twins are affected to the same extent by their environment and upbringing. While there is evidence suggesting that MZ twins are treated more similarly than DZ twin pairs (Kendler et al., 1986) which could potentially inflate

heritability, it was also found that zygosity, environmental similarity and parental treatments did not predict twin similarities in personality, attitudes or cognitive ability (Kendler et al., 1993). More importantly, it is unlikely that the equal environment assumption is violated for traits analysed in this thesis.

Another limitation is generalisablity of twin study results to non-twin populations. Previous literature has shown that twins are on average more likely to be lighter and born pre-term, and more susceptible to *in utero* complications, than non-twins (Rijsdijk & Sham, 2002). However, it is possible to circumnavigate the problem of generalisability to non-twins by using extended family designs, such as including parents, children, and spouses of twins (Eaves et al., 1978; Heath & Eaves, 1985; Nance & Corey, 1976). The analyses in this thesis incorporates data from non-twin siblings to confirm the generalisability of the results.

Gene-environment effects such as assortative mating and Gene x Environment (GxE) interactions may also influence heritability estimates. Non-assortative mating refers to random patterns of genetic transmission, compared to assortative mating where people select partners who are phenotypically similar to themselves. This may lead to a higher correlation between DZ twins and an overestimation of C. To account for this, phenotypic correlation between parents could be measured (Heath et al., 1987). GxE interactions describe genetic sensitivity to environmental influences, such as genetic risk of type II diabetes triggered by dietary intake, or depression risk interacting with stressful life events.

The main software used for SEM in this thesis was OpenMx, which uses the R command language and was designed specifically to handle genetic and correlated data with flexibility (Neale et al., 2008). In order to model several zygosities simultaneously, covariance structure models were simultaneously fit to multiple groups, and optimised using maximum likelihood techniques. Covariates were also included to account for variance caused by extraneous variables.

These methods were used in Chapters 2 and 3 to estimate the heritability of fingerprint patterns across digits and hair diameter and curvature respectively. In Chapter 2, a series of univariate models were used to estimate the heritability of pattern type, while in Chapter 3 sex-limited univariate models were used for diameter, curvature, and consistency of diameter.

Bivariate models were also fit to the data to find correlations between the diameter and curvature, and diameter and consistency of diameter.

1.3 Genome-Wide Association Studies

While twin studies provide information on the proportion of genetic versus environmental sources of phenotypic variation, they do not provide any information about variants affecting the trait. In order to identify genetic variants affecting a phenotype, gene mapping methods are typically used. One early method of gene mapping was linkage studies, which followed the line of transmission of phenotypes and genotypes along family pedigrees (Ott, 1999). While this method was successful for genes of high penetrance and monogenetic conditions such as sickle cell anemia (Ashley-Koch et al., 2000), it was less capable in the detection of polygenetic disorders due to a lack of power (Risch & Merikangas, 1996). Instead, association mapping was suggested as an alternative, which offered higher power to detect common variants of smaller effect size and the ability to assess the importance of individual variants.

Early association studies were mostly conducted on candidate genes selected using *a priori* knowledge of the trait or genomic region of interest. This was a hypothesis-driven method that led to the discovery of genes affecting several diseases, such as late-onset Alzheimer's disease (Strittmatter et al., 1993). Contemporary association analyses have adopted a hypothesis-free approach, in which the same hypothesis of association is tested across the genome to identify novel variants that affect diseases or phenotypes of interest. This advance in methodology was largely facilitated by rapid technological developments from 2007 onward, such as high-density genotyping. The development of these genotyping chips were due to a large extent to the Human Genome Project, which provided the first complete map of the human genome, and the HapMap Project, which provided crucial information on haplotype blocks and linkage disequilibrium (LD), characterising the co-inheritance of two SNPs within a population. A major finding from the HapMap project was that in European populations, about 80% of common SNPs could be summarized with a subset of 500K SNPs (Li et al., 2008). More recently, the 1000 Genomes project provided population reference data from different ethnic populations, allowing the imputation of dense genetic data from the relatively sparse coverage provided by genotyping chips.

GWAS is conducted using variants across the genome to systematically assess evidence of association with the phenotype of interest. In the case of discrete traits, allele frequencies in cases and controls are compared for each marker and evidence for association is tested using a chi-square test or regression. For continuous traits, regression approaches are most common, and evidence of association is derived from the extent to which the SNP predicts the trait.

While this method appears to be straightforward, interpretation of GWAS results requires caution. For instance, the presence of LD within the genome means that a significant GWAS association for a SNP cannot be causally linked with the phenotype without further analyses. Moreover, the extremely high number of SNPs involved in GWAS leads to inflated Type I error rates, unless multiple testing is taken into account. For GWAS, the threshold for significance is typically corrected to $p < 5x10^{-8}$ (Pe'er et al., 2008). In addition, replication studies are usually conducted to minimise false positives from the discovery stage. Due to typically small effect sizes of genes, large cohorts are needed to achieve necessary power to detect signals, and this can be achieved using meta-analyses of data from discovery and replication stages, pooling cohorts across studies to increase power for detecting variants.

Some extraneous factors that may skew results of GWAS results include population stratification, where spurious associations arise due to differences in trait and genotype distributions within ethnic populations (Pritchard & Rosenberg, 1999). Population stratification in GWAS is typically assessed using the genomic control parameter λ, which can be found as the median of χ^2 statistics for all markers, divided by the median under the null (Devlin & Roeder, 1999). In contemporary analyses, more fine-tuned methods are typically used, including principal components based on genomic data (Price et al., 2006; Pritchard et al., 2000).

GWAS methods are employed in Chapters 2 and 4. In Chapter 2, the genetic variants influencing whorl pattern type are assessed across all ten digits of the hand, whereas in Chapter 4 GWAS was used to find variants influencing hair curvature.

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Preface to Chapter 2: Topic Overview - Dermatoglyphics

2.1 Fingerprint morphology and classifications

Dermatoglyphics refers to the scientific study of fingerprints and friction ridges that form on the palms and soles of primates (Kamboj, 2008). This chapter focuses on ridge patterns on the distal phalanges, as fingerprints are more commonly used in forensic contexts than sole prints.

As noted by Galton, dermal ridges on human fingers may be inconspicuous to the naked eye, but they raise significant questions about "heredity, symmetry, correlation and the nature of genera and species" (Galton, 1892). The evolutionary function of dermal ridges is unclear. While it has been generally accepted in past literature that these elevated folds on the skin serve the function of gripping of branches and objects (Warman & Ennos, 2009), there have also been suggestions that dermal ridges may play a role in enhancing touch sensitivity (Dandekar et al., 2003) and complex tactile perception of fine textures (Loesch & Martin, 1984; Scheibert et al., 2009). More recently, research has shown that individuals with adermatoglyphia - where a mutation in the *SMARCAD1* gene causes congenital absence of dermal ridges (Nousbeck et al., 2011) - show reduced number of sweat glands and increased propensity to acquire burn wounds. This suggests dermal ridges may play a role in heat diffusion or touch insensitivity.

The formation and structure of fingerprints have been analyzed since the early 1800s and have been documented with varying degrees of attention to details of the prints, termed minutiae (Galton, 1892). Common to most categorizing systems are the core and the triradii due to their utility in categorizing and recording patterns. It is possible to determine fingerprint type by counting number of triradii present (i.e. pattern intensity), and the ridge count by counting the number of ridges that lie on an imaginary straight line extending from a core to a triradius. A core is the centre of a fingerprint pattern, at the point where the innermost ridge folds in towards itself; it is possible to have more than one core (such as in the case of whorls and double loops). A triradius at the meeting point of three ridges, bracketing a triangular area. The number of triradii are informative in determining the major

type of fingerprint, for example arches do not have triradii whilst one triradius indicates a loop (Mendenhall, Mertens, & Jon, 1989).

Early studies of dermatoglyphics have also yielded some observations about morphological properties of human fingerprints, which influence the way dermal ridges are documented and used in research and daily life. The major characteristics being permanence over time, resistance to postnatal environmental influence, high degree of minutiae variance between individuals, and gross diversification into few major types (Holt, 1968). This implies that the fingerprints of an individual rarely change as a consequence of age or other external events. They are also relatively unique between individuals (the probability of two identical prints is estimated to be 1 in 64 billion (Galton, 1892)), which makes dermatoglyphics a unique and reliable biometric marker. As Figure 2.0.1 shows, patterns fall under a few major categories, namely arches (A) loops (B) and whorls (C) (Rife, 1947), which facilitates classification and research.

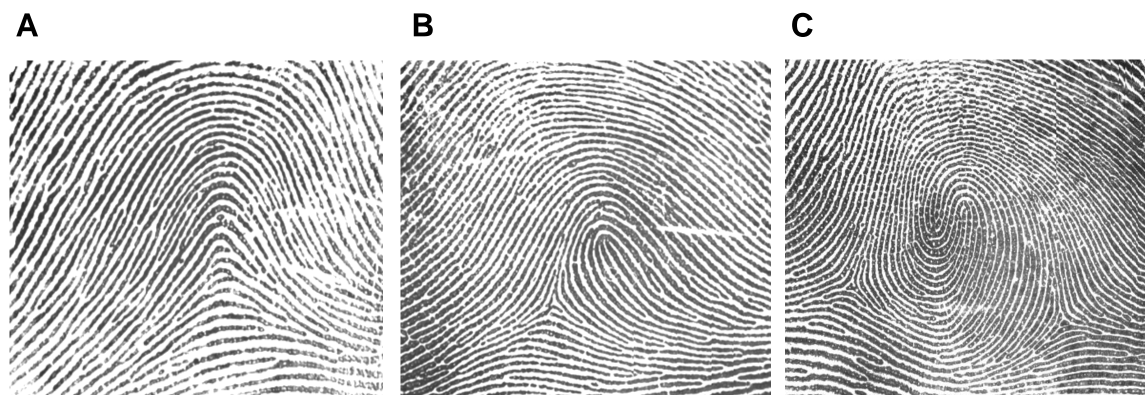

Figure 2.0.1 Three major categories of fingerprint patterns

As a consequence of these characteristics, dermatoglyphics can be utilized with modern technology as an effective way of identifying and differentiating individuals. Some specific academic and practical applications of fingerprinting include anthropological studies (Arrieta, & Lostao, C., 1988; Cheng, 2008), genetic and medical research (Bukelo et al., 2011; Oladipo et al., 2009), forensic investigations, customs documentation, and more recently, in personal and national security matters such as laptop computer and airport fingerprint scanners.

2.2 Biological development of dermal ridges

Early embryological and histological studies have led to detailed documentation of biological development of dermal ridges, which are crucial in our understanding of the factors that influence fingerprint formation.

Immediately after cell cleavage in gestation, cell differentiation occurs in gastrulation and results in discrete dermal layers, the ectoderm, endoderm, and mesoderm. The epidermis and friction ridge skin develops on the ectodermal layer, while the endoderm gradually forms muscle and vascular systems, and the mesoderm the internal organs. During late embryological development (3-8 weeks estimated gestational age) limbs develop rapidly in the process of morphogenesis. By the second month, arms, legs, knees and fingers begin to resemble adult form. A swelling of the mesenchyme, i.e. volar pads on the fingers, palms and soles, also takes place within this period, which plays a key role in the determination of ridge patterns (Raven & Johnson, 1992). Dermal ridges begin formation around 9-10 weeks estimated gestational age (EGA) (Babler, 1978; Cummins, 1929). In this stage of development, the growth of the volar pads decrease in rate relative to other areas, which gives the appearance of shrinkage or "regression" (Lacroix et al., 1984). By 16 weeks EGA the raised volar pads no longer differ in height to other areas of the fingers, palms, and soles (H. Cummins, 1929). Full dermatological patterns form around 21 weeks (Babler, 1978, 1991; Mulvihill & Smith, 1969). As a consequence of overlapping developmental timelines between dermal ridges and other parts of the body, developmental disturbance in utero may also result in observable changes in the ridge patterns (Loesch, 1983), such as Trisomy 21 which is reported to result in significantly higher instances of ulnar loops (Rajangam et al., 1995), and lower ridge counts in little fingers (Mglinets, 1991).

2.3 Theory of ridge formation

The development of ridges is complex process influenced by many genetic and environmental factors, such as growth stress *in utero* (Cummins, 1926), the physical and topographical forces on the volar pads during fetal development (Mulvihill & Smith, 1969), the widths of the volar pads in relation to their heights (Babler, 1991), and the general prenatal environment (Pechenkina et al., 2000). Various theories have been posited about the type and amount of influence from genetic versus environmental factors. A number of genetic influences have been identified including chromosomal abnormalities (Loesch, 1983). Previous studies have

found dermatogphyic traits to be highly heritable (Chakraborty, 1991; Li et al., 2003; Medland et al., 2007). An argument has also been made for epistatic influences (Heath et al., 1984); however, no study on pattern intensity has been able to assemble the sample size necessary to detect epistatic effects.

Different theories have arisen concerning the specific processes of pattern formation within the context of genetic influences. Some of the main biological hypotheses on the formation of dermal ridges are the “folding hypothesis”, the “fibroblast hypothesis”, and the “nerve hypothesis”, which will be briefly summarized below.

The folding hypothesis (Bonnevie, 1927) mainly highlights the rapid proliferation of basal cells. This is hypothesized to lead to a mechanical “buckling process” in which compression between cells lead to a shift in the location of cells and the shape of dermis, thus forming the primary ridges on the skin. Kücken (2004) further added to this hypothesis by showing forces around the fingertip and at the nail furrow influences ridge direction during the buckling process, which in turn plays a role in determining ridge pattern.

In contrast to mechanics-based theories and studies (Kücken, 2004), *the nerve hypothesis* mainly addresses the way nerve and blood vessels influence the positioning of primary ridges. Dell and Munger (1986) studied the volar pads of primate foetuses using electron microscopy, and found growth cones, actively developing neurites, in the epidermis are located equidistant between primary ridges. However, later experiments examining the impact of removing nerve fibres in opossums (Morohunfola et al., 1992) altered the ridge depth and developmental timing, and not in separation of primary ridges.

The fibroblast hypothesis was also proposed as a model of ridge pattern formation. The hypothesis is derived from the finding that there is a tendency for cells, particularly fibroblast cells, to form patterns in culture and align with a ridge structure (Green & Thomas, 1978). However, this theory has limited supporting evidence, as the pattern flow is different from fingerprint patterns.

Twin studies of dermatoglyphics have consistently shown significant genetic effects; the heritability of arches on any finger was found to be .91 in a sample of MZ and DZ twins. Similarly, total ridge count, which is related to pattern type via the intensity of ridges between

the core and triradius, shows high heritability and genetic influences (Holt, 1968; Machado et al., 2010).

2.4 Pattern differentiation

In contrast to studies of the development of ridges, pattern differentiation addresses the way different pattern types arise. The differential growth hypothesis (Mulvihill & Smith, 1969) proposed that pattern formation is determined by the ratio of volar pad height to width during developmental regression *in utero*, as a result of an interaction between genes underlying developmental timing and the inter-uterine prenatal environment, such as topographic growth forces (Cummins, 1923; Penrose, 1968). These theories have been supported to some extent by studies showing the role of volar pad size in dermal pattern development in rats (Okajima, 1991), the prevalence of arches in preterm abortuses (Babler, 1978), and aberrations of dermal patterns in populations with congenital malformation of the hands (e.g. polydactyly and syndactyly) (Bellelli, 1939; Cummins, 1926). Results from these studies suggest a volar pad larger in surface area would result in a whorl, while a smaller one would result in a loop, and the smallest an arch.

Mathematically, differentiation of pattern type has been modelled in terms of genetic and environmental influences. An early simple model suggested a common genetic influence on all fingerprint types, with differentiation caused by individual environmental influences (Holt, 1968). A later model represented pattern type as a result of a network of interactions between genetic and environmental factors, with both common and individual genetic and environmental influences acting upon each phenotype (Martin et al., 1982). Despite past research showing evidence suggesting a high heritability of ridge count and pattern types, no studies seeking to identify genetic variants affecting pattern type have been published.

2.5 Computer simulations of ridge formation

Complementary to the development of theories explaining the formation of dermal ridges, there have been attempts to create computer simulations of the process of ridge formation. Kücken (2004) conducted a computer simulation of ridge pattern formation according to the assumptions of boundary rules and compressive stress during regression of volar pads. These rules were used as parameters in a mathematical model for buckling pattern in order to approximate the development of ridge patterns *in utero*. The results from the simulation showed patterns closely resembling the experimental observations of Bonnevie (1927) which

provides support for the folding hypothesis and differential growth hypothesis. In contrast, Garzón-Alvarado (2011) proposed a reaction-diffusion-convection (RDC) model, which emphasizes biochemical influences, specifically glycosis, which allow buckling to take place. The results also showed ridge patterns approximate to fingerprint ridges. It is suggested that these biochemical processes could work in interaction with mechanical processes.

2.6 Dermatoglyphics in a forensic context

Dermatoglyphics have been used in forensic contexts for the documentation and identification of suspects and detainees since the early 19th century. While tenprint record systems, which documented ink-rolled prints of individuals' ten fingerprints on paper, are still in usage, they are now incorporated in a computerised system, such as the Integrated Automated Fingerprint Identification System (IAFIS) in use with the FBI. These systems allow users to submit latent prints to search for matches within the repository on a world-wide scale (Moses et al., 2012).

Latent prints are classified into patent, latent, and plastic impressions (Lee & Gaensslen, 2001) and may be collected from a variety of surfaces, although all three types may be broadly termed "latent prints". Patent prints refer to fingerprint deposits that are visible to the naked eye under good lighting conditions and may be constituted of sebaceous grease, blood, ink, or dust. Latent prints are typically invisible to the eye and could be detected through a chemical or physical process, including the use of latent print powders. Plastic prints are impressions left on malleable surfaces such as clay or soft wax, and may be detected under oblique lighting (Yamashita et al., 2012).

Unlike deliberate tenprint records, the conditions of latent print impressions are not controlled. The cleanliness, texture, contour, or porous nature of surfaces, as well as variations in temperature, humidity, and evidence handling processes are all factors that could complicate the appearance of latent prints before matching (Yamashita et al., 2012). As a consequence, fingerprint matching is a complex series of decision-making and requires a high level of training and expertise to perform with reliability and accuracy (Vanderkolk, 2012). While human experts have been shown to be adept at the task compared to non-experts (Thompson & Tangen, 2014), human factors nevertheless present possibilities for error due to fatigue and cognitive biases, and are relatively time-consuming. In comparison, IAFIS allows a certain level of improvement in cost-effectiveness and efficiency, but is yet to accomplish

the level of performance in manual fingerprint matching (Moses et al., 2012). In addition, both human and computerized matching methods require an existing tenprint entry for the latent print of interest, meaning individuals with no previous crime record cannot be identified using the matching process.

One way in which fingerprints could be used to identify individuals without using matching methods is to make use of the genetic basis of dermatoglyphs to find variants that influence pattern type. After variants have been identified, it may be possible to use DNA information collected from crime scene to construct a predictive mathematical method to estimate a suspect's pattern type.

2.7 Previous genetic studies

Table 2.0.1 below summarizes previous twin and genetic studies conducted on fingerprint phenotypes, including ridge count and pattern type. Case studies and studies investigating dermatoglyphics is disease populations were excluded. In the sample column, MZ/DZ denotes zygosity of the twins (monozygotic/dizygotic) and lowercase m/f denotes the gender (male/female).

Figure2.0.1 Summary of previous twin and genetic studies conducted on fingerprint phenotypes, including ridge count and pattern type.

Study Type	Author	Year	Sample	Finding
Twin studies	(Karmakar et al.)	2011	102 MZm pairs, 140MZf, 120 DZf	Significant dominance genetic influence (60%) for pattern type on all digits, significant environmental influence
	(Machado et al.)	2010	20 MZf pairs, 13 DZf pairs	Significant genetic influences (h2=.65-.96) for 12 dermatoglyphic characteristics. Higher arch frequency in the left hand. Positive heritability index with right hand loops and negative for whorls.
	(Reed)	2006	2484 twin pairs	267 twin pairs concordant for the presence of arch on any finger. Prevalence of arches to differed between males and females, h2=91%.
	(Sharma & Bakshi)	2005	64 twin pairs and 128 parents	No statistical difference in magnitude of bilateral asymmetry between MZ and DZ pairs. Sources of variation was significantly different between zygosities with evidence for greater environmental covariation for DZ twins.
	(Arrieta et al.)	1991	16 MZm pairs and 13 DZm pairs	Fluctuating asymmetry differed significantly between MZ twins, DZ twins, and singletons.
	(Heath et al.)	1984	60 MZm, 50 MZf, 62 DZm, 49 DZf, 3924 siblings, parents, and spouses	Significant proportion of variance in pattern intensity is due to epistatic interactions between additive deviations.
	(Martin et al.)	1982	60 MZm, 50 MZf, 62 DZm, 49 DZf, 80 opposite-sex siblings	Specific and general genetic and environmental factors influence ridge count on fingers.
	(Martin et al.)	1982	60 MZm, 50 MZf, 62 DZm, 49 DZf, 80 opposite-sex siblings	Non-additive genes and unequal gene frequencies increase ridge counts. Large additive genetic effects were found. Dominance and epistatic influences were non-significant.
	(Loesch & Swiatkowska)	1978	10 MZ and 111 like-sexed DZ twin pairs	High concordance rate in bilateral symmetry for twin fingerprint patterns
Genomic studies	(Dressler & Voracek)	2011	75 male and 75 female normal healthy adults	No significant association found between candidate markers of prenatal sex hormones and ridge count
	(Medland et al.)	2007	2114 twins and siblings from 922 families	Significant linkage of ridge count on ring, index, and middle fingers to the locus 5q14.1

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Chapter 2: Variants within *ADAMTS9-AS2* influence whorls in fingerprint patterns

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Study carried out in Brisbane, Queensland, Australia and Bristol, United Kingdom.

The Accepted Author Manuscript of this study is included at the end of this chapter.

Abstract

Dermatoglyphs (dermal ridges on the distal phalanges, palms, and soles of primates) are complex quantitative traits that have provided a model system for genetic investigation for over one hundred years. The current study sought to identify the genetic influences on fingerprint patterns across all 10 digits, utilizing genome-wide association and meta-analysis. Data were collected from 4129 participants from two QIMR twin cohorts and 5339 children from the Avon Longitudinal Study of Parents and Children (ALSPAC). Genome-wide meta-analyses identified genome-wide significant variants within genes *OLA1* and *ADAMTS9-AS2* for whorls on different digits, and within an intergenic region between *TBX3* and *MED13L* on chromosome 12. Amongst significant results, rs1523452 within *ADAMTS9-AS2* presented a gradient influence for whorls across digits on the left hand and expressed the strongest signal on the little fingers (left, $p = 9.74 \times 10^{-27}$, $r = -.128$; right, $p = 7.62 \times 10^{-15}$, $r = -.097$) and ring fingers (left, $p = 2.16 \times 10^{-21}$, $r = -.113$; right, $p = 1.33 \times 10^{-17}$, $r = -.102$) of both hands. *ADAMTS9-AS2* is expressed in ganglion tissues, suggesting an influence on tactile perception, and is shown to be central in early morphogenetic development of organs such as the lungs, colon, testis and ovaries.

Introduction

The study of dermatoglyphics as a model genetic and quantitative trait has a long history. Notably, much of the early work (Galton, 1892) focused on the morphology of dermal ridges, and identified three major categories of fingerprint patterns: arches, whorls, and loops (Figure 2.1). Prior to the development of genotyping, dermatoglyphics were used (with dubious accuracy) to confirm paternity (Cotterman, 1951) and study ancestry (Rife, 1954), and while many papers have focused on the classification and association of dermatoglyphs with major chromosomal abnormalities (Loesch, 1983; Penrose & Loesch, 1970) such as trisomy 21 (Lu, 1968; Soltan, 1965), trisomy 18 (Uchida, Patau, & Smith, 1962), and Klinefelter's Syndrome (Alter, 1966; Penrose & Loesch, 1970), few have attempted to further understanding of the etiology of dermatoglyphics or to identify genetic variants contributing to the heritability of these traits.

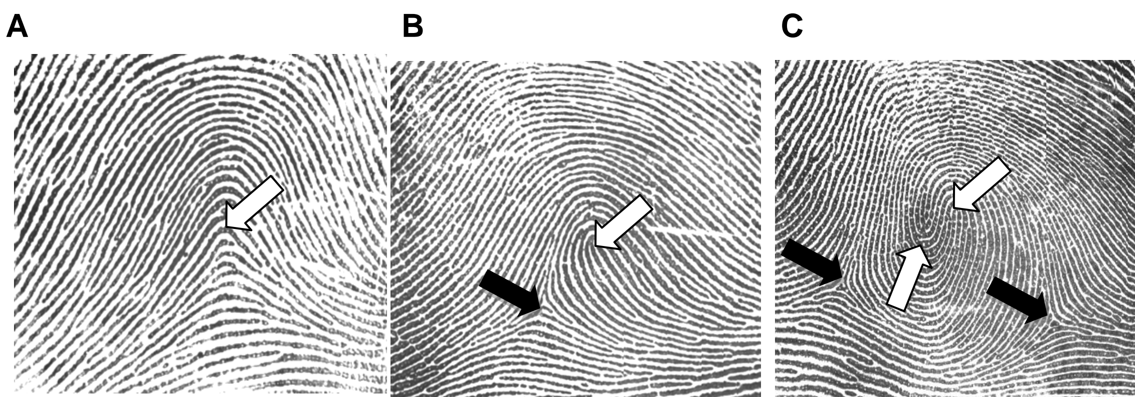


Figure 2.1. The three main categories of fingerprints with core and triradii indicated

A: arches; **B**: loops; **C**: whorls. The white arrows indicate the cores (i.e. where the ridges fold over themselves) and the black arrows indicate the triradii (i.e. where the ridges form a three-sided boundary). As evident from the figures, number of cores and triradii may determine pattern type; arches have no triradii, loops have one triradius, and whorls have two tridradii.

Early embryological studies show the formation of dermal ridges begins around 11 weeks gestation and these develop into full patterns around 21 weeks (Babler, 1978; Mulvihill & Smith, 1969). As shown in Figure 2.1, pattern types can be differentiated according to landmark structures formed by the dermal ridges, namely the triradii and the core (Holt, 1968). Evidence suggests that pattern formation is determined by the ratio of volar pad height to width during developmental regression *in utero* (Mulvihill & Smith, 1969), where the proliferation of epidermal cells on the digital pads initially overtake the areas surrounding it, and then as development equalizes, results in an apparent "regression" effect of the volar

pads. This process may be influenced by interaction between genes underlying developmental timing and the intra-uterine prenatal environment, such as topographic growth forces (Cummins, 1923; Penrose, 1968). These theories have been supported to some extent by studies showing the role of volar pad size in dermal pattern development in rats (Okajima, 1991), the prevalence of arches in preterm abortuses (Babler, 1978), and aberrations of dermal patterns in populations with congenital malformation of the hands (e.g. polydactyly (Cummins, 1926); syndactyly, (Bellelli, 1939)). A very high heritability has been reported for arches on any finger (Reed, 2006) and total ridge count, which is a measure that correlates with pattern type (Martin, Eaves, & Loesch, 1982; Medland, Loesch, et al., 2007).

Amongst the theories of genetic influences on limb development, the morphogenetic field theory (Gurwitsch, 1910) initially gained prominence (Roberts, & Coope, 1975; Roberts, & Greally, 1991). This theory proposes that cells in the embryonic stage respond to specific, discrete biochemical signals which determine the structure or organ that develops within that field (Jacobson, 1988), so that cells within a field for limb development would eventually differentiate into fore- and hind-limbs. Evidence supporting the field theory for dermatoglyphics includes correlations between digit patterns (Holt, 1951; Loesch, 1971; Loesch, 1986; Plato, 1975). Following criticisms of the genetic field theory, such as the limitations of the field theory as an obsolete, metaphysical, and speculative approach to anatomical development(De Robertis, 1991; Morgan, 1932), subsequent theories generally focused on specific genes, such as the *HOX* gene, which creates the anterior-posterior axis determining the placement of segment structure of vertebrates (Carraco, 1984; Carroll, 1994). The genetic approach can also be represented mathematically (Gilbert, 1996), giving rise to various models of finger ridge development. The most parsimonious of these models assumes a single genetic factor responsible for the correlation between pattern types across fingers and a set of random environmental influences that are responsible for differences in ridge count between digits (Holt, 1968). More complex models, however, suggest both shared and heterogeneous genetic factors influencing development and between-digit differences respectively, with a pattern of covariation between digits suggestive of a morphogenetic field effect (Martin et al., 1982). Multivariate linkage analyses also revealed a pattern of factor loadings for finger ridge count which supported this argument, and also found linkage to 5q14.1 driven by the middle three fingers (index, middle and ring) (Medland, Loesch, et al., 2007).

Building on previous findings, the present study sought to identify genetic variants that were associated with fingerprint patterns on each digit. Participants were recruited through two samples of twin and families from the Queensland Institute of Medical Research (QIMR), and from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study.

Materials and Methods

Study populations

In the QIMR first sample, 2301 adolescent twins, siblings, and their parents were recruited from the general population to take part in ongoing studies of melanoma risk factors and memory and attention, conducted as part of the Brisbane Adolescent Twin Study (Wright, 2004). Genotype and phenotype data were available for 2093 participants from 1096 families (402 MZ twin pairs, 591 DZ twin pairs, and 107 singletons). For the QIMR second sample, 1859 adults provided fingerprints for the thumb, index, and middle fingers, as part of a health and lifestyle study; genotype and phenotype data were available for 1287 participants from 744 families (391 MZ pairs, 151 DZ pairs, 2 sibling pairs, and 199 singletons). Given that finger patterns do not change with age, to increase power these datasets were combined and analyzed as one sample of 3301 participants from 1764 families.

ALSPAC is a population-based birth cohort study consisting of 14 541 women and their children recruited in the county of Avon, UK, in the early 1990s (Boyd, 2013). The children have been extensively followed from the eighth gestational week onwards using a combination of self-reported questionnaires, medical records and physical examinations. Biological samples including DNA have been collected from the participants. Ethical approval was obtained from the ALSPAC Law and Ethics Committee and relevant local ethics committees, and written informed consent provided by all parents. The study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary).

Fingerprint collection

For QIMR participants, fingerprints were collected for the adult sample using rolled ink prints on archival quality paper and an electronic ridge counting software (Medland, Park, Loesch, & Martin, 2007) was developed for the adolescent sample. Pattern intensity (the number of triradii) was then manually coded (by SEM and DZL). Within the ALSPAC

sample, pattern type for each digit was scored from photocopies of the palmar surface of the hands, which were collected for the purpose of measuring digit ratio (Medland et al., 2010). SEM manually coded pattern type into loops, whorls, and arches. Any digit where the fingerprint pattern was not clearly visible was coded as missing. As the full patterns of the thumbs were not clearly visible we excluded this digit from analyses.

Intensity and ridge count data were re-coded in terms of presence or absence of whorls and arches on each finger of each hand. Loops were coded as the reference category as they are the most common pattern type. To refer to the patterns on each finger, the thumb on each hand is coded 1 and the little finger 5, and the right or left hand is designated using the prefix L or R. Arches were not analyzed due to low pattern frequency and were excluded in the dataset. After initial quality control analyses, 10 variables were included in the study: the presence of whorls across all digits (L1-5, R1-5), except L1 and R1 for the ALSPAC cohort and L4, L5, R4, and R5 in the QIMR adult sample as it was difficult to obtain good prints of these digits.

Genotyping

As described previously, QIMR participants were genotyped on the Illumina 317, 370, and 610 SNP chips in the context of a larger GWAS (Medland et al., 2010). These data were then submitted to quality control to check for GenCall (< .7), HWE failure ($p < 10^{-6}$), SNP and individual call rates (< .95), and MAF (< .01). They were then screened for non-European ancestry, sex, pedigree, and Mendelian errors. In order to avoid bias due to use of different chip sets, a set of 274, 604 SNPs common across the genotyping platforms was used for imputation.

Within the ALSPAC cohort, 9912 participants were genotyped using the Illumina HumanHap550 quad genome-wide SNP chip (Illumina Inc., San Diego, CA, USA) by 23andMe subcontracting the Wellcome Trust Sanger Institute, Cambridge, UK and the Laboratory Corporation of America (LabCorp Holdings., Burlington, NC, USA). Individuals were excluded from analyses based on excessive or minimal heterozygosity, gender mismatch, individual missingness (>3%), cryptic relatedness as measured by identity by descent (genome-wide IBD >10%) and sample duplication. Individuals were assessed for population stratification using multi-dimensional scaling modelling seeded with HapMap Phase II release 22 reference populations. Individuals of non-European ancestry were

removed from further analysis. SNPs with a final call rate of <95%, minor allele frequency (MAF) <1% and evidence of departure from Hardy–Weinberg equilibrium (HWE) ($P < 5 \times 10^{-7}$) were also excluded from analyses (Fatemifar, 2013). Data from the remaining 5339 individuals who had both GWAS and finger pattern information were used in this paper. Both QIMR and ALSPAC samples were imputed using the Hapmap2 r22.36 CEU reference. SNPs that had a minor allele frequency (MAF) of >.01 and could be imputed with confidence (R^2>0.3) were used in these analyses. Only genotyped SNPs were used for chromosome X.

Statistical analysis

Heritability estimates were conducted in OpenMX (Boker et al., 2012; Boker et al., 2011), using binary coded data from the QIMR dataset and with sex as a covariate. Subsequently, a principal components analysis (PCA) was performed to investigate the latent factors within phenotypes that emerged after orthogonal transformation of correlations. GWAS analyses used an additive model which incorporated sex and population cohort as covariates. GWAS results from each sample were combined using Stouffer's Z score method in METAL to calculate meta-analytic p-values (Willer, Li, & Abecasis, 2010).

Results and Discussion

Heritability analysis

Table 2.1 shows the frequency of each pattern on each digit across the QIMR and ALSPAC samples. Whorls are most common on the thumb, index, and ring fingers of both hands. These results are consistent with previous studies of pattern frequency within a British population (Cummins, & Midlo, 1943) which reported a 22.1% overall frequency of whorls on the left hand and 28.7% on the right hand respectively.

Heritability analyses of these measures using the twin structure of the QIMR sample (Table 2.1) shows that pattern on each of the fingers is highly heritable. Further investigation showed a high correlation in pattern type between corresponding digits across hands (Table S2.1). Principal components analyses (PCA) with Varimax orthogonal rotation showed three underlying components of pattern type, namely one factor contributing to whorls on the middle three fingers (digits 2,3, and 4) on both hands, a second factor affecting whorls on the thumbs (digit 1), and a third factor influencing whorls on the little fingers (digit 5) (Table S2.2).

Table 2.1. Frequency and heritability of presence of whorls on all 10 digits.

	Mean frequency of traits in each sample		Heritability* (95% CI)
Trait/ Digit	**QIMR (N = 4129)**	**ALSPAC (N = 5339)**	**h^2 (QIMR)**
Whorls			
L1	.270	NA	.80 (.74-.86)
L2	.260	.297	.82 (.77-.87)
L3	.178	.167	.87 (.80-.91)
L4	.254^	.307	.82 (.75-.89)
L5	.177^	.107	.70 (.53-.83)
R1	.319	NA	.87 (.82-.91)
R2	.270	.325	.80 (.74-.85)
R3	.181	.173	.87 (.80-.91)
R4	.315^	.384	.83 (.75-.90)
R5	.123^	.133	.88 (.78-.94)

*Heritability derived from AE model from twins in the QIMR adolescent sample. Whorls were coded as 0 when absent and 1 when present, with loops used as the reference category.
^In the QIMR adult sample the ring and little fingers were not collected, resulting in an N of 2296; in ALSPAC the thumb was omitted, as it could not be reliably scored from the prints taken.
NA: Not available.

Genome-wide association and meta-analysis

GWAS of pattern type was conducted for each digit within the two cohorts (Table S2.3); as shown in the quantile-quantile plots (Q-Q plots) in Figure S2.4-S2.6, there was no evidence of systematic inflation in the QIMR or ALSPAC results. This is supported by the genomic inflation factors ranging from 1.004 – 1.027 (QIMR) and 1.007 – 1.034 (ALSPAC) across digits. Within the QIMR and ALSPAC samples, several genome-wide significant SNPs ($p < 5 \times 10^{-8}$) were found. Manhattan plots of all univariate analyses are provided in Figure S2.7 and S2.8. To summarize the evidence for association across the cohorts, we conducted meta-analyses. Q-Q and Manhattan plots for the METAL analyses are provided in Figures S2.6 and Figure 2.2 respectively. Across digits 2-5, we observed a region of strong signal on chromosome 3 influencing the presence of whorls. In order to further explore variants that

may be significant at a gene level, gene-based tests were conducted using the GATES procedure on KGG2.5 (Li, Gui, Kwan, & Sham, 2011) (Table S2.3).

Meta-analyses (Table S2.9) revealed the effects of two nominally significant (i.e. $p < 5x10^{-8}$) within-gene variants for the whorls phenotype, specifically *ADAMTS9-AS2* for the second digit of the right hand (WR2) and the 4th and 5th of both hands (WL4, WR4; WL5, WR5), as well as a variant within *OLA1* for whorls on the 2nd digit of the left hand (WL2; rs10201863, $p = 3.46x10^{-8}$).

For *ADAMTS9-AS2*, the rs1523452 SNP presented the strongest signal, WL5 ($p = 9.74x10^{-27}$) and WR5 ($p = 7.62x10^{-15}$; Figure 2.2) and accounted for 1.61% and 0.93% of the variance respectively (Table S2.9). The same SNP was also found to influence WL4 ($p = 2.16x10^{-21}$), WR4 ($p = 1.33x10^{-17}$), and WR2 ($p = 3.08x10^{-10}$). The signal was attenuated at WL2 ($p = 9.24x10^{-6}$) and was further reduced for WL1 ($p = 2.66x10^{-5}$) and WR1 ($p = 0.0001$). As these results indicate, there is a stronger association between significant SNPs on the left hand compared to the right. This bilateral asymmetry in pattern type mirrors that of prior literature regarding ridge count of individual fingers on the right versus left hands, showing that ridge count asymmetry has a low to medium genetic effect mediated by sex (Loesch, & Martin, 1982). Allele frequencies at this variant show that the G allele was associated with higher incidence of whorls in digit 5 (Figure 2.4a). Across fingers, the gene-based p-values ranged between $1.38x10^{-24}$ to $4.54x10^{-7}$. Interestingly, SNPs within a 2 kbp intergenic region within chromosome 12 were also significant for WL4 and WR4, peaking at rs1863718 ($p = 8.04x10^{-9}$ and $1.36x10^{-8}$ respectively). This region lies downstream of *TBX3* and upstream of *MED13L*. Other genes approaching significance included *EVI1* (rs1981745; $p = 7.52x10^{-7}$; gene-based $p = 5.89x10^{-5}$) for WR2.

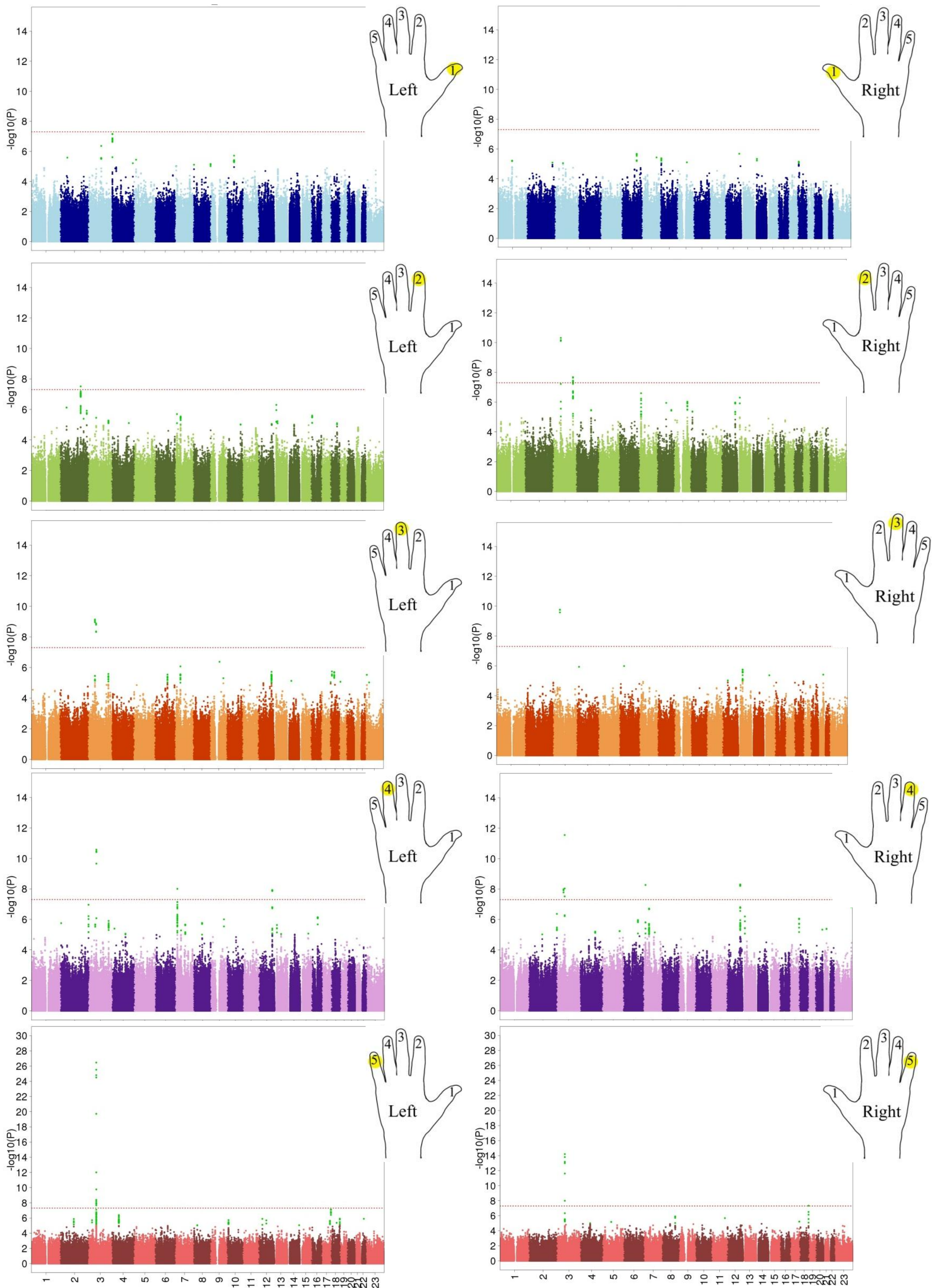


Figure 2.2. Manhattan plots for meta-analysis results

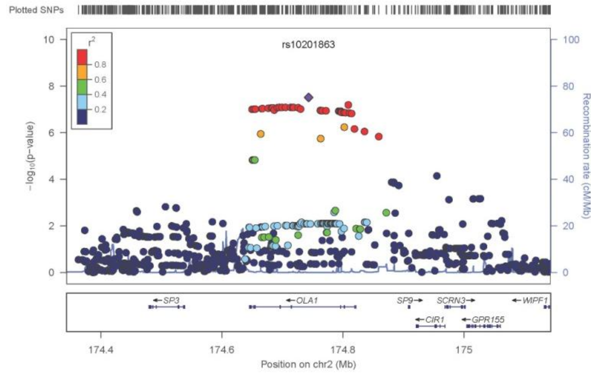
Association with oncogenes

As Figures 2.3a –d below illustrate, the signals found in the meta-analyses are mostly encompassed by single genes. Amongst these significant hits, *ADAMTS9-AS2* and *OLA1* are documented oncogenes. Specifically, real time PCR and loss/gain-of-function studies shows *ADAMTS9-AS2* is significantly underexpressed in glioma tumors with high WHO grade (III/IV) versus low grade tumors (I/II), and is reversely correlated with tumor growth (Yao et al., 2014).

Meanwhile knockdown gene studies of *OLA1* shows that in vitro cell migration was significantly inhibited for breast cancer cell lines (Zhang, Rubio, Zheng, & Shi, 2009); and real-time PCR has shown that *EVI1* overexpression leads to extremely poor prognosis of leukemia (Barjesteh van Waalwijk van Doorn-Khosrovani et al., 2003) and an increase in survival of treatment-resistant colon cancer cells (Liu, 2006). Fluorescence *in situ* hybridization also shows an overexpression of *EVI1* in ovarian cancer cells compared to normal ovarian cells (Brooks, 1996). These results suggest that genetic factors that regulate dermatoglyphic morphogenesis may also be influential in development and prognosis of several subtypes of cancer.

The chromosome 12 hits between 113904923 – 113903069bp are located with an intergenic region close to *TBX3*, which is known to cause ulnar-mammary syndrome (Bamshad et al., 1999; Coll, Seidman, & Muller, 2002; He, Wen, Campbell, Wu, & Rao, 1999) through a nonsense (Linden, Williams, King, Blair, & Kini, 2009; Sasaki et al., 2002) or frameshift mutation (Bamshad et al., 1997; Wollnik, Kayserili, Uyguner, Tukel, & Yuksel-Apak, 2002). Ulnar-mammary syndrome due to mutations in the *TBX* gene may be expressed in terms of a range of phenotypic abnormalities including hypoplasia of the terminal phalanx of the fifth digit (Schinzel, 1973), complete absence of the fourth and fifth digits (Schinzel, 1973), and posterior limb deficiencies in humans (Bamshad, Root, & Carey, 1996). *TBX3* also influences mammalian forelimb development in tandem with *TBX5* (Bamshad et al., 1997). This concurs with previous literature that limb development *in utero* is influential on subsequent fingerprint patterns that emerge (Mulvihill & Smith, 1969).

A



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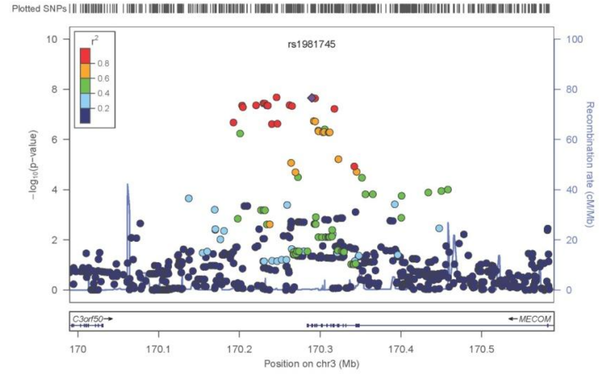

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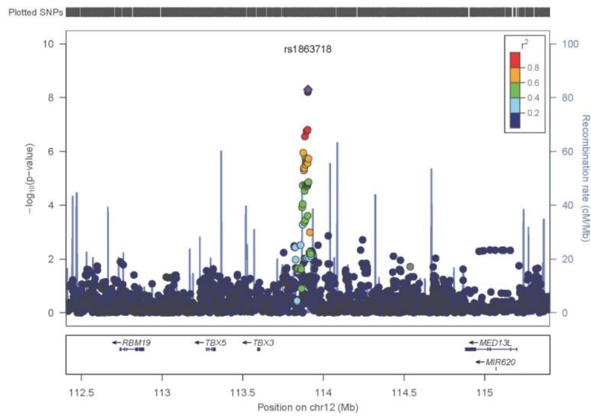

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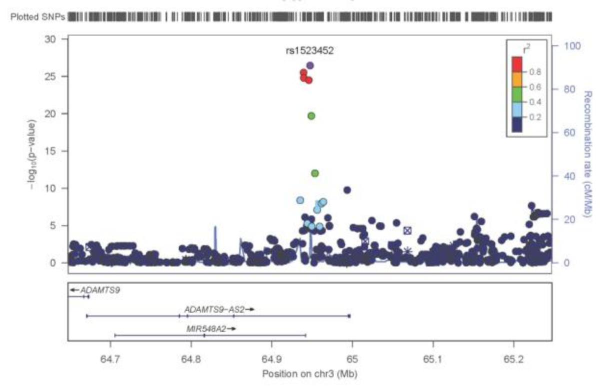

Figure 2.3. Locuszoom plots of meta-analysis results

A: WL2; **B**: WR2; **C**: WL4; **D:** WL5

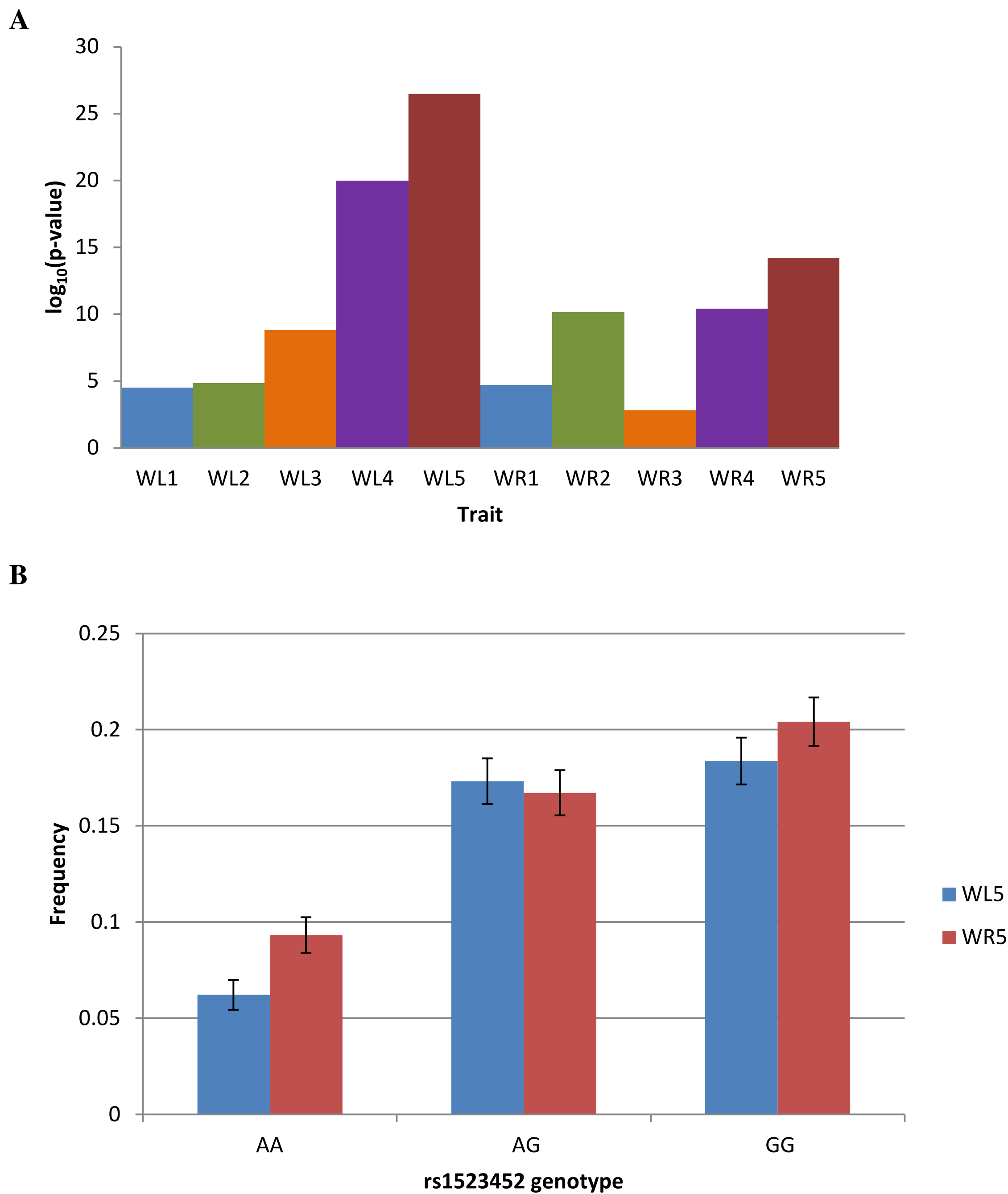

Figure 2.4. Histograms of **A**: meta-analyses -log10 (p-values) and **B**: Trait frequency as a function of allelic variation - Frequency of whorls on the left little finger (WL5; blue bars) and right little finger (WR5; red bars) as a function of the genotype rs1523452 in a sample of unrelated individuals from the $QIMR_1$ cohort (n[AA] = 708, n[AG] = 335, n[GG] = 49). With more G alleles, the proportion of whorls increases. Vertical bars correspond to the 95% confidence intervals on prevalence.

Gene tissue expression

ADAMTS9-AS2 is an antisense RNA adjacent to the gene *ADAMTS9*, a disintegrin and metalloproteinase with thrombospondin motifs 9, located at 3p14.1. Conventionally the role of antisense RNAs is to act as inhibitors to transcribed mRNAs through the process of base pairing (Weiss, 1999). The anti-sense RNA adjacent to *ADAMTS9* may play a role in inhibiting the expression of mRNA transcripts from *ADAMTS9*. Although there is no direct explanation of the role of *ADAMTS9-AS2* in development of whorls on the little fingers, RNAseq analyses show high expression of this gene in reproductive organs – ovaries, prostate, and testis – as well as in the colon and lungs, suggesting the role of this gene in early organ and sexual development. According to Gene Network databases, ADAMTS9-AS2 is also significantly expressed in ganglion, peripheral nerve cell that may play a part in texture differentiation. This concurs with literature suggesting a role of fingerprints in tactile perception via skin vibrations (Scheibert, Leurent, Prevost, & Debrégeas, 2009), although this tactile perception is optimized more in loop patterns than whorls (Loesch & Martin, 1984). Interestingly, variants in *ADAMTS9-AS2* also appear to influence whorls on all digits to differing levels of significance (Figure 2.4b) and variance explained (Figure S2.10).

In contrast to previous studies based on linkage analyses (Medland, Loesch, et al., 2007) and systems biology approaches such as the Turing model (Raspopovic, 2014; Sheth et al., 2012), our study is the first report investigating genetic factors influencing specific pattern type at the genotypic level. Our study used large sample sizes and a genome-wide association approach to discover novel SNPs that may be used to predict the presence of some dermal patterns. In conclusion, the results of this study did not yield direct evidence for the effects of single genetic variants on specific fingerprint pattern phenotypes; however, variants within *ADAMTS9-AS2* show a gradient influence on whorls across all digits.

Overlap between variants affecting medical and forensic phenotypes

Although the exact function of ADAMTS9-AS2 in the development of dermal ridge patterns is unclear, the current study presents an interesting overlap between oncogenes and a forensic phenotype. Due to current developments in applying genomic variant data to forensic processes, this has implications within the field of bioethics as forensic genotyping

technology may reveal medical information about the individual, which broadens the scope of discussion on the topic of real-world applications of genomic data.

Conflict of interest

The authors state no conflict of interest

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In memory of Bodgen Wdzewski, who contributed significantly in counting QIMR fingerprint intensity scores.

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Supplementary Tables and Figures

Table S2.1. Within-individual cross-digit correlations in the $QIMR_1$ sample (N=2492)

Trait	WL1	WL2	WL3	WL4	WL5	WR1	WR2	WR3	WR4	WR5
WL1	1									
WL2	.505	1								
WL3	.434	.667	1							
WL4	.437	.579	.717	1						
WL5	.447	.495	.517	.739	1					
WR1	.810	.542	.456	.466	.418	1				
WR2	.443	.815	.648	.626	.521	.526	1			
WR3	.351	.642	.786	.669	.456	.482	.688	1		
WR4	.336	.563	.657	.813	.570	.485	.634	.731	1	
WR5	.380	.502	.498	.704	.821	.461	.537	.519	.710	1

*Cross-hand correlations indicated in grey cells

Table S2.2. Principal Components Analysis calculated using data from the QIMR adolescent sample (N= 2296).

Rotated Component Matrix[a]

	1	2	3
WL1	.113	.135	.869
WL2	.665	.072	.354
WL3	.734	.149	.069
WL4	.556	.507	.137
WL5	.111	.829	.132
WR1	.225	.131	.828
WR2	.681	.129	.302
WR3	.752	.118	.024
WR4	.592	.402	.102
WR5	.192	.813	.105

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.[a]
a. Rotation converged in 5 iterations.

Table S2.3. Results of univariate and meta-analyses GWAS for cohorts QIMR and ALSPAC on each digit ($p < 5x10^{-8}$)

Trait	Chr	BP	rs	P_{QIMR}	P_{ALSPAC}	P_{META}	β_{QIMR}	β_{ALSPAC}	Z_{META}
WL2	2	174743371	rs10201863	5.51E-04	1.31E-05	3.46E-08	0.066	0.999	5.516
WL3	3	55117470	rs709330	2.11E-03	5.64E-08	2.09E-10	-0.056	-0.490	-6.355
	3	55105358	rs358018	2.52E-03	6.94E-08	3.19E-10	-0.055	-0.489	-6.289
	3	55121051	rs697929	1.68E-03	5.40E-08	1.52E-10	0.057	0.491	6.404
WL4	3	55117470	rs709330	5.57E-04	9.81E-05	6.30E-09	-0.082	-0.383	-5.809
	3	55105358	rs358018	7.22E-04	1.70E-16	8.79E-09	-0.081	-0.435	-5.753
	3	64968467	rs4688533	1.61E-06	9.82E-16	8.25E-09	-0.222	-0.421	5.763
	3	64920911	rs2244503	7.47E-07	2.15E-09	1.21E-20	-0.100	-0.325	-9.316
	3	64923998	rs11924641	6.30E-05	3.20E-06	1.21E-12	-0.091	-0.311	7.105
	3	64922380	rs1523452	7.42E-07	3.55E-06	2.16E-21	-0.100	-0.311	-9.497
	3	55121051	rs697929	4.64E-04	3.15E-06	5.30E-09	0.084	0.311	5.838
	3	64914393	rs796973	1.98E-06	2.61E-05	1.95E-21	0.094	0.213	-9.508
	3	64928518	rs1851672	4.44E-05	5.60E-17	1.43E-08	0.085	0.435	-5.670
	3	64914646	rs17071864	9.83E-07	6.97E-17	1.20E-21	0.099	0.441	-9.558
	7	16005642	rs800704	9.66E-03	7.51E-08	4.21E-09	0.046	0.248	-5.876
	12	113903182	rs1317715	9.01E-04	1.94E-06	9.86E-09	-0.055	-0.207	5.733
	12	113904201	rs2098989	9.04E-04	1.98E-06	8.81E-09	-0.055	-0.207	-5.752
	12	113904623	rs1863716	9.05E-04	2.03E-06	8.43E-09	-0.055	-0.206	-5.760
	12	113904096	rs7957733	9.04E-04	2.07E-06	8.97E-09	-0.055	-0.206	-5.749
	12	113904923	rs1863718	9.04E-04	2.20E-06	8.04E-09	0.055	-0.206	5.768
	12	113904891	rs1863717	9.04E-04	2.28E-06	8.18E-09	0.055	-0.205	-5.765
	12	113903742	rs7957333	9.01E-04	2.28E-06	9.49E-09	-0.055	0.205	-5.740
	12	113904238	rs2098990	9.04E-04	1.89E-06	8.56E-09	-0.055	0.207	-5.757
	12	113903069	rs1317714	9.01E-04	1.86E-06	9.88E-09	0.055	0.207	-5.733
WL5	3	64938887	rs17071972	1.29E-02	9.20E-08	1.34E-08	-0.038	-0.436	-5.681
	3	64910252	rs7637774	9.39E-03	6.88E-08	3.32E-09	0.027	0.349	-5.915
	3	64933281	rs2222482	1.30E-02	1.91E-07	2.59E-08	0.038	0.432	5.567
	3	64936693	rs5010170	1.30E-02	1.47E-07	2.06E-08	0.038	0.436	5.607
	3	64933648	rs1916629	1.31E-02	1.91E-07	2.60E-08	-0.038	-0.432	5.566
	3	64968467	rs4688533	1.62E-06	1.43E-06	1.51E-10	-0.141	-0.632	6.404
	3	65197101	rs1384409	5.63E-04	3.55E-06	1.41E-08	-0.040	-0.325	5.672
	3	64920911	rs2244503	1.52E-08	3.76E-19	4.39E-25	-0.072	-0.650	-10.346
	3	64923998	rs11924641	5.24E-07	2.21E-15	5.04E-20	-0.073	-0.597	9.163
	3	64922380	rs1523452	7.12E-09	1.60E-20	9.74E-27	-0.074	-0.679	-10.704
	3	64933199	rs1713041	1.27E-02	1.91E-07	2.53E-08	0.038	0.432	5.571
	3	64914393	rs796973	5.47E-08	2.15E-20	8.39E-26	0.069	0.668	-10.503
	3	64928518	rs1851672	3.69E-05	4.01E-09	1.80E-12	0.054	0.423	-7.049
	3	64914646	rs17071864	5.42E-08	5.68E-20	2.21E-25	0.070	0.670	-10.411

Trait	Chr	BP	rs	P_{QIMR}	P_{ALSPAC}	P_{META}	β_{QIMR}	β_{ALSPAC}	Z_{META}
WR2	3	64920911	rs2244503	4.88E-04	8.17E-08	3.57E-10	-0.069	-0.280	-6.272
	3	64922380	rs1523452	5.93E-04	5.46E-08	3.08E-10	-0.068	-0.286	-6.295
	3	64914393	rs796973	5.73E-04	3.21E-08	1.85E-10	0.068	0.286	-6.373
	3	64914646	rs17071864	4.90E-04	2.01E-08	1.04E-10	0.070	0.295	-6.462
	9	110492772	rs1339878	1.25E-04	5.42E-05	3.98E-08	0.065	0.177	5.492
	9	110488429	rs1873759	1.17E-04	6.83E-05	4.75E-08	-0.065	-0.175	5.460
	9	110495122	rs12683387	1.41E-04	5.93E-05	4.83E-08	0.065	0.176	5.457
	9	110494725	rs1339879	1.40E-04	5.92E-05	4.81E-08	0.065	0.176	-5.458
	9	110495329	rs12685634	1.42E-04	5.93E-05	4.87E-08	0.065	0.176	-5.456
WR3	3	55117470	rs709330	8.51E-04	5.64E-08	1.76E-11	-0.058	-0.490	-6.725
	3	55105358	rs358018	9.04E-04	6.94E-08	2.63E-11	-0.058	-0.489	-6.666
	3	55121051	rs697929	8.65E-04	5.40E-08	1.73E-11	0.058	0.491	6.727
WR4	2	240943810	rs7602036	3.77E-03	2.03E-06	3.12E-08	0.068	0.210	5.535
	2	240941737	rs1320123	1.00E-03	2.06E-06	9.37E-09	-0.076	-0.218	5.742
	2	240940495	rs10933595	3.71E-03	6.39E-07	1.00E-08	0.063	0.220	5.731
	3	64920911	rs2244503	2.37E-03	9.82E-16	1.01E-16	-0.064	-0.421	-8.304
	3	64923998	rs11924641	3.45E-02	2.15E-09	1.03E-09	-0.050	-0.325	6.105
	3	64922380	rs1523452	1.65E-03	1.70E-16	1.33E-17	-0.067	-0.435	-8.541
	3	64914393	rs796973	1.82E-03	5.60E-17	5.63E-18	0.065	0.435	-8.640
	3	64914646	rs17071864	2.04E-03	6.97E-17	8.04E-18	0.065	0.441	-8.599
	7	16005642	rs800704	2.66E-03	7.51E-08	1.10E-09	0.056	0.248	-6.094
	12	113899167	rs1896333	3.29E-04	1.92E-05	4.04E-08	0.061	0.184	5.489
	12	113903182	rs1317715	1.05E-04	2.28E-06	1.68E-09	-0.067	-0.205	6.026
	12	113904201	rs2098989	1.06E-04	2.03E-06	1.49E-09	-0.067	-0.206	-6.045
	12	113897126	rs10744843	3.29E-04	1.87E-05	3.94E-08	-0.061	-0.185	5.494
	12	113895339	rs1896328	3.29E-04	1.91E-05	4.02E-08	0.061	0.184	5.490
	12	113904623	rs1863716	1.06E-04	1.94E-06	1.43E-09	-0.067	-0.207	-6.052
	12	113904096	rs7957733	1.05E-04	2.07E-06	1.52E-09	-0.067	-0.206	-6.042
	12	113904923	rs1863718	1.06E-04	1.86E-06	1.36E-09	0.067	0.207	6.060
	12	113902393	rs1896341	3.31E-04	1.74E-05	3.66E-08	-0.061	-0.185	5.507
	12	113904891	rs1863717	1.06E-04	1.89E-06	1.38E-09	0.067	0.207	-6.057
	12	113903742	rs7957333	1.05E-04	2.20E-06	1.62E-09	-0.067	-0.206	-6.032
	12	113904238	rs2098990	1.05E-04	1.98E-06	1.45E-09	-0.067	-0.207	-6.050
	12	113903069	rs1317714	1.05E-04	2.28E-06	1.68E-09	0.067	0.205	-6.026
	13	21482808	rs3117984	7.79E-05	5.61E-05	4.00E-08	-0.079	-0.210	5.491
WR5	3	64968467	rs4688533	3.27E-08	1.43E-06	6.66E-09	-0.183	-0.632	5.799
	3	64920911	rs2244503	1.03E-07	3.76E-19	1.18E-14	-0.077	-0.650	-7.718
	3	64923998	rs11924641	2.65E-07	2.21E-15	2.39E-12	-0.084	-0.597	7.010
	3	64922380	rs1523452	8.80E-08	1.60E-20	7.62E-15	-0.077	-0.679	-7.774
	3	64914393	rs796973	1.97E-07	2.15E-20	7.75E-14	0.074	0.668	-7.475
	3	64914646	rs17071864	1.35E-07	5.68E-20	7.37E-14	0.076	0.670	-7.481
	18	74480382	rs2004773	4.96E-03	2.40E-05	3.54E-08	0.041	0.325	5.512

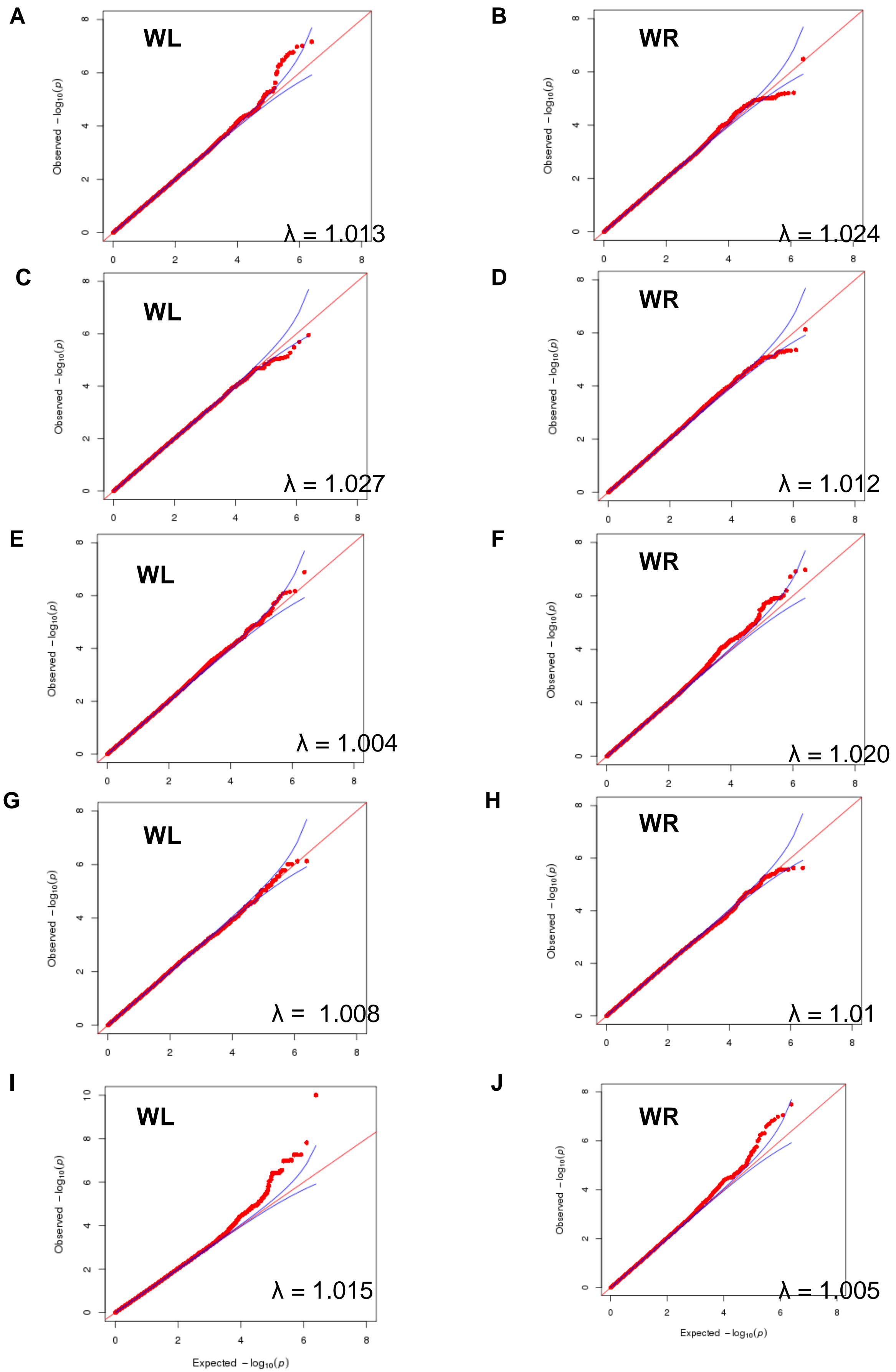

Figure S2.4. QIMR univariate quantile-quantile (Q-Q) plots

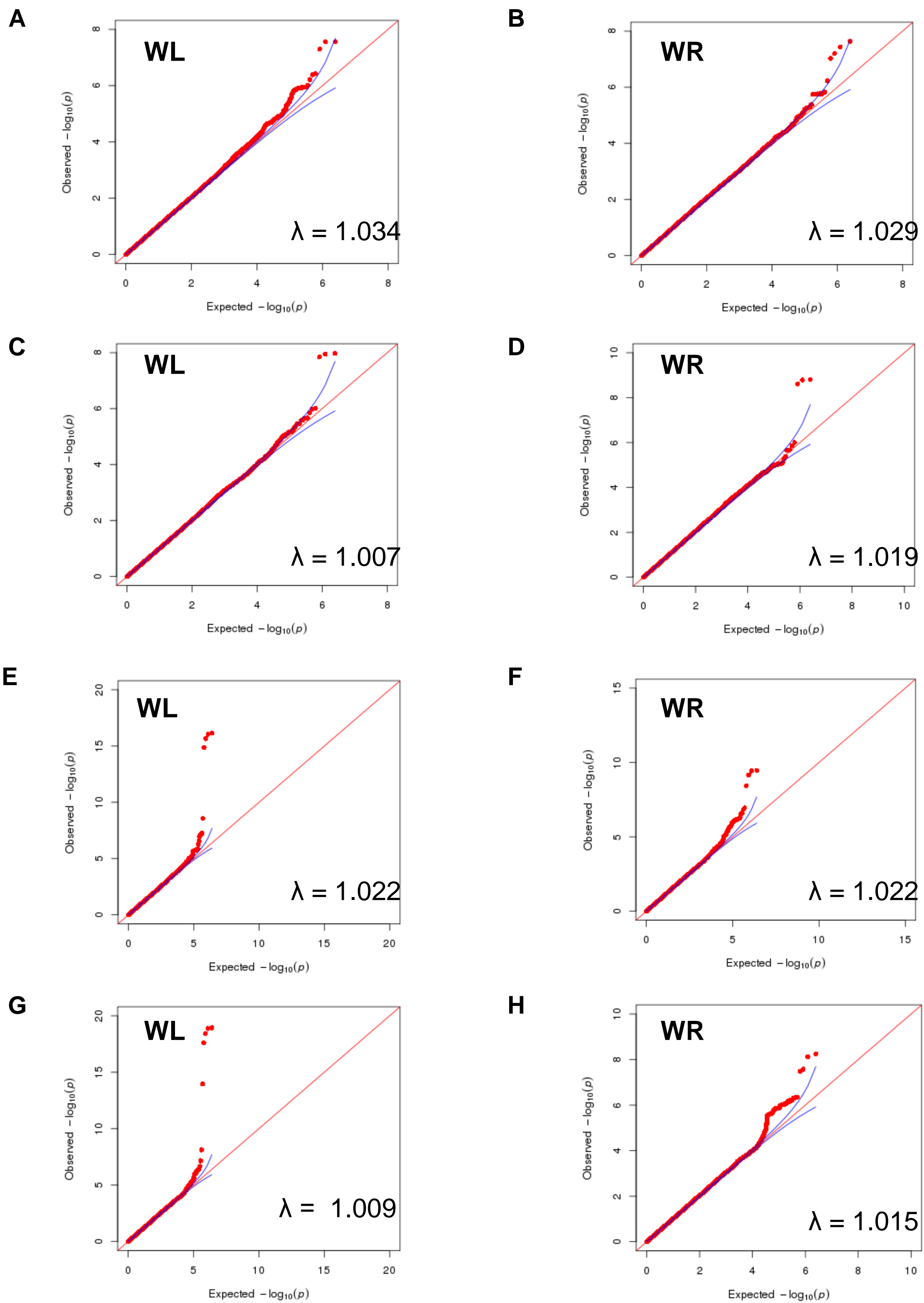

Figure S2.5. ALSPAC univariate quantile-quantile (Q-Q) plots
***The phenotypes WL1 and WR1 were not collected and was omitted from this table**

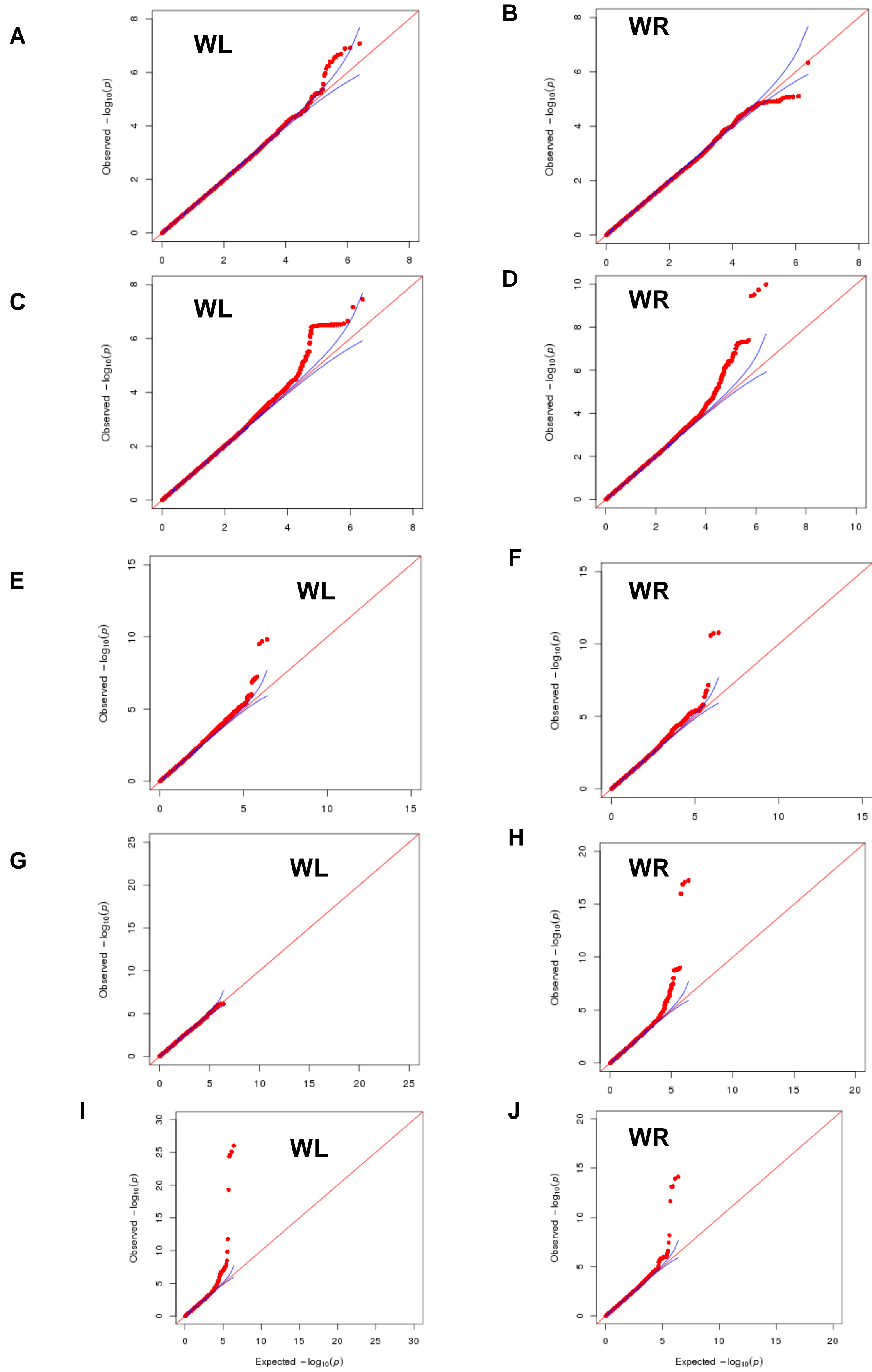

Figure S2.6. Meta-analytic quantile-quantile (Q-Q) plots

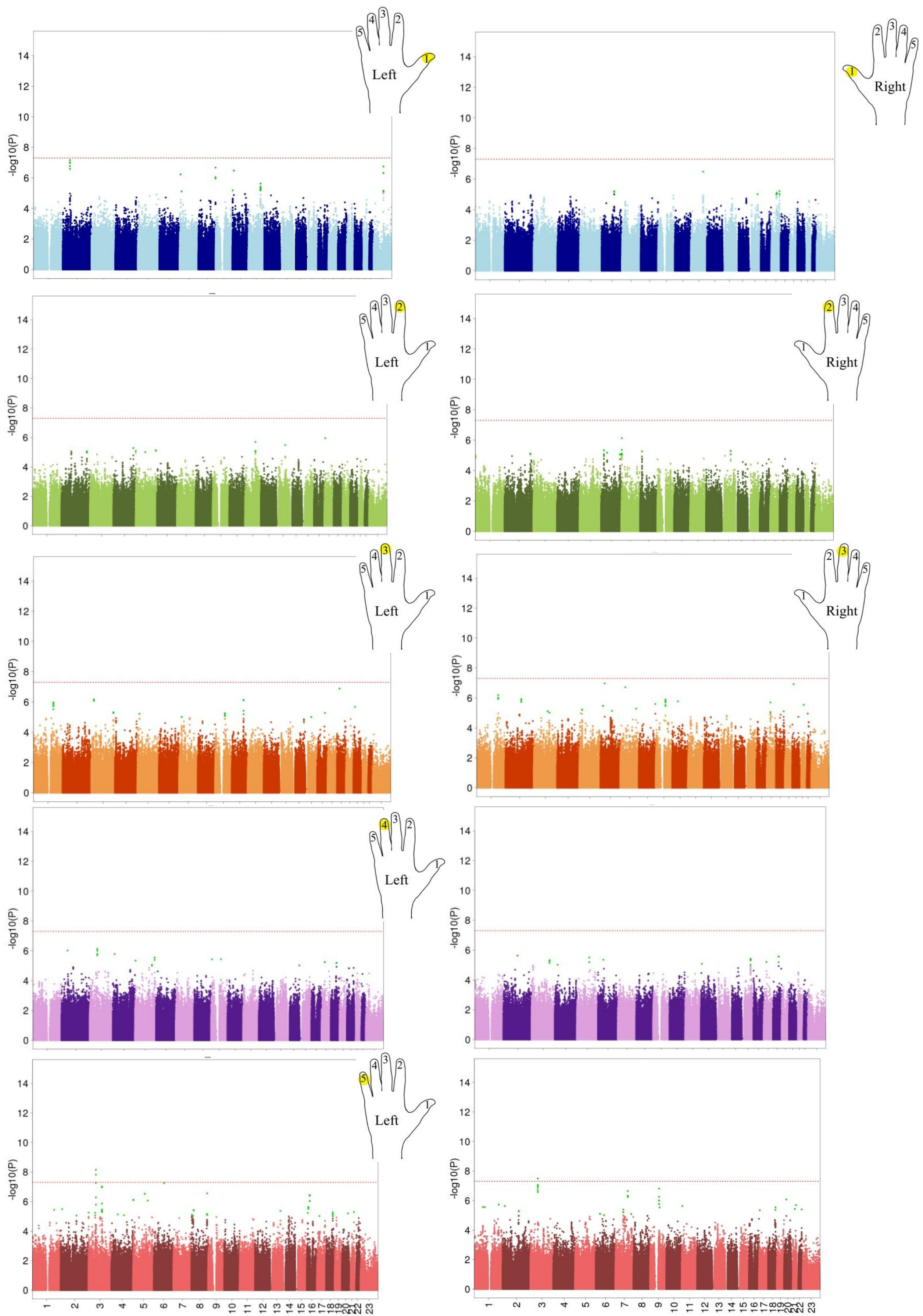

Figure S2.7. Univariate Manhattan plots for the QIMR cohort

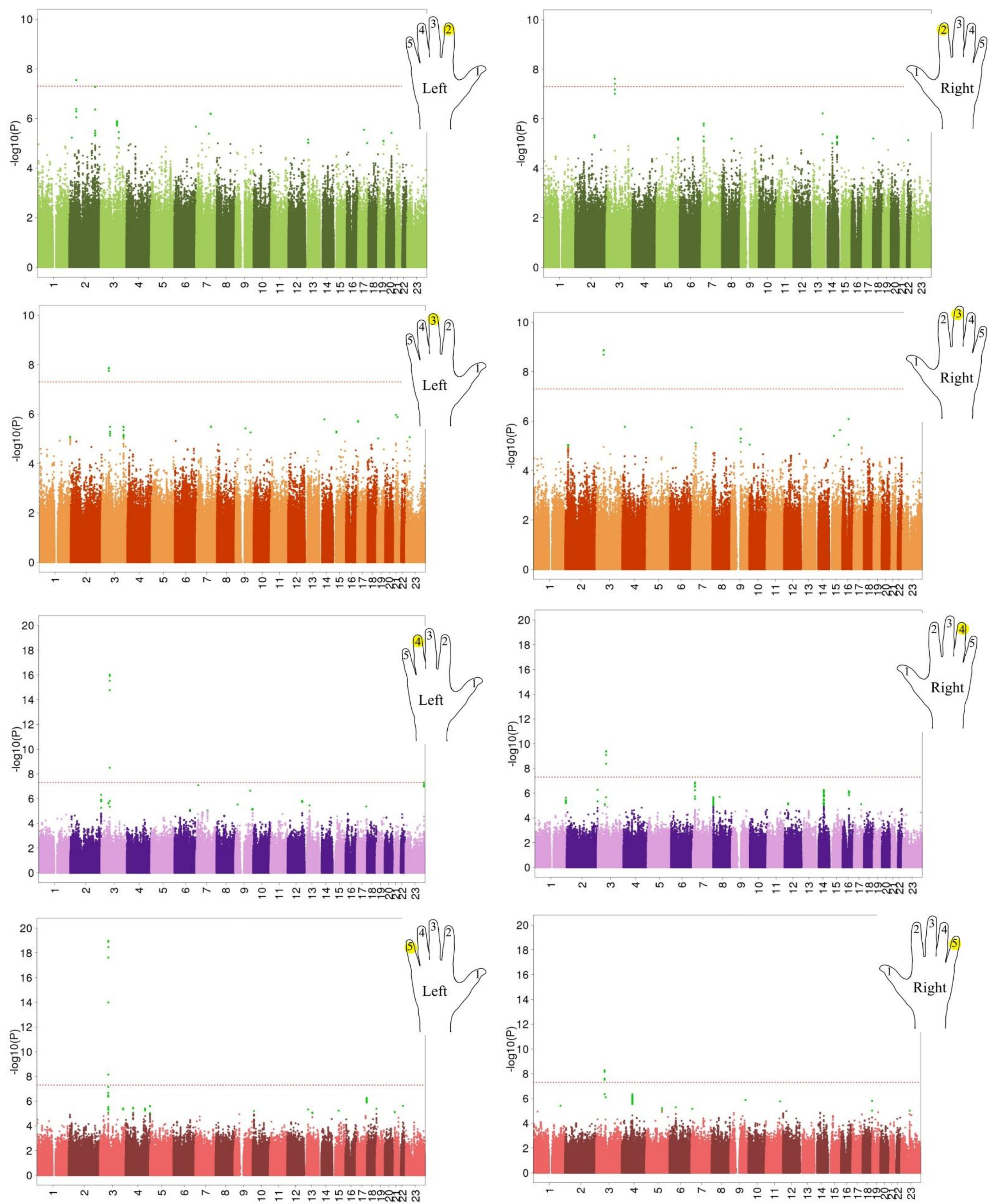

Figure S2.8. Univariate Manhattan plots for the ALSPAC cohort
***The phenotypes WL1 and WR1 were not collected and was omitted from this table**

Table S2.9. Results of meta-analysis for arches and whorls on each digit ($p < 5 \times 10^{-8}$)

Trait	CHR	rs	BP	P-val	GenePval	Gene	Var (Vg%)	Trait	CHR	rs	BP	P-val	GenePval	Gene	Var (Vg%)
WL2	2	rs10201863	174743371	3.46E-08	4.54E-07	OLA1	36.87	**WR2**	3	rs17071864	64914646	1.04E-10	1.52E-08	MIR548A2/ ADAMTS9-AS2	50.22
WL3	3	rs697929	55121051	1.52E-10			49.13		3	rs796973	64914393	1.85E-10		MIR548A2/ ADAMTS9-AS2	48.85
	3	rs709330	55117470	2.09E-10			48.38		3	rs1523452	64922380	3.08E-10		ADAMTS9-AS2	47.67
	3	rs358018	55105358	3.19E-10			47.39		3	rs2244503	64920911	3.57E-10		ADAMTS9-AS2	47.32
WL4	3	rs17071864	64914646	1.20E-21	1.72E-19	MIR548A2/ ADAMTS9-AS2	128.36		9	rs1339878	110492772	3.98E-08			36.33
	3	rs796973	64914393	1.95E-21		MIR548A2/ ADAMTS9-AS2	127.03		9	rs1873759	110488429	4.75E-08			35.91
	3	rs1523452	64922380	2.16E-21		ADAMTS9-AS2	126.74		9	rs1339879	110494725	4.81E-08			35.88
	3	rs2244503	64920911	1.21E-20		ADAMTS9-AS2	122.02		9	rs12683387	110495122	4.83E-08			35.87
	3	rs11924641	64923998	1.21E-12		ADAMTS9-AS2	71.34		9	rs12685634	110495329	4.87E-08			35.85
	7	rs800704	16005642	4.21E-09			48.90	**WR3**	3	rs697929	55121051	1.73E-11			53.99
	3	rs697929	55121051	5.30E-09			48.27		3	rs709330	55117470	1.76E-11			53.95
	3	rs709330	55117470	6.30E-09			47.80		3	rs358018	55105358	2.63E-11			53.02
	12	rs1863718	113904923	8.04E-09			47.13	**WR4**	3	rs796973	64914393	5.63E-18	7.94E-16	MIR548A2/ ADAMTS9-AS2	105.13
	12	rs1863717	113904891	8.18E-09			47.08		3	rs17071864	64914646	8.04E-18		MIR548A2/ ADAMTS9-AS2	104.15
	3	rs4688533	64968467	8.25E-09			47.05		3	rs1523452	64922380	1.33E-17		ADAMTS9-AS2	102.76
	12	rs1863716	113904623	8.43E-09			47.00		3	rs2244503	64920911	1.01E-16		ADAMTS9-AS2	97.19
	12	rs2098990	113904238	8.56E-09			46.95		3	rs11924641	64923998	1.03E-09		ADAMTS9-AS2	52.77
	3	rs358018	55105358	8.79E-09			46.89		7	rs800704	16005642	1.10E-09			52.58
	12	rs2098989	113904201	8.81E-09			46.87		12	rs1863718	113904923	1.36E-09			52.00
	12	rs7957733	113904096	8.97E-09			46.82		12	rs1863717	113904891	1.38E-09			51.95
	12	rs7957333	113903742	9.49E-09			46.67		12	rs1863716	113904623	1.43E-09			51.86
	12	rs1317715	113903182	9.86E-09			46.56		12	rs2098990	113904238	1.45E-09			51.83
	12	rs1317714	113903069	9.88E-09			46.56		12	rs2098989	113904201	1.49E-09			51.74
	3	rs1851672	64928518	1.43E-08		ADAMTS9-AS2	45.55		12	rs7957733	113904096	1.52E-09			51.69
WL5	3	rs1523452	64922380	9.74E-27	1.38E-24	ADAMTS9-AS2	161.09		12	rs7957333	113903742	1.62E-09			51.52
	3	rs796973	64914393	8.39E-26		MIR548A2/ ADAMTS9-AS2	155.19		12	rs1317715	113903182	1.68E-09			51.42
	3	rs17071864	64914646	2.21E-25		MIR548A2/ ADAMTS9-AS2	152.52		12	rs1317714	113903069	1.68E-09			51.42
	3	rs2244503	64920911	4.39E-25		ADAMTS9-AS2	150.65		2	rs1320123	240941737	9.37E-09			46.71
	3	rs11924641	64923998	5.04E-20		ADAMTS9-AS2	118.56		2	rs10933595	240940495	1.00E-08			46.53
	3	rs1851672	64928518	1.80E-12		ADAMTS9-AS2	70.50		2	rs7602036	240943810	3.12E-08			43.41
	3	rs4688533	64968467	1.51E-10		ADAMTS9-AS2	58.26		12	rs1896341	113902393	3.66E-08			42.98
	3	rs7637774	64910252	3.32E-09		ADAMTS9-AS2	49.75		12	rs10744843	113897126	3.94E-08			42.78
	3	rs17071972	64938887	1.34E-08		ADAMTS9-AS2	45.91		13	rs3117984	21482808	4.00E-08			42.73
	3	rs1384409	65197101	1.41E-08			45.76		12	rs1896328	113895339	4.02E-08			42.71
	3	rs5010170	64936693	2.06E-08		ADAMTS9-AS2	44.72		12	rs1896333	113899167	4.04E-08			42.70
	3	rs1713041	64933199	2.53E-08		ADAMTS9-AS2	44.15	**WR5**	3	rs1523452	64922380	7.62E-15	1.16E-12	ADAMTS9-AS2	93.40
	3	rs2222482	64933281	2.59E-08		ADAMTS9-AS2	44.09		3	rs2244503	64920911	1.18E-14		ADAMTS9-AS2	92.07
	3	rs1916629	64933648	2.60E-08		ADAMTS9-AS2	44.08		3	rs17071864	64914646	7.37E-14		MIR548A2/ ADAMTS9-AS2	86.55
									3	rs796973	64914393	7.75E-14		MIR548A2/ ADAMTS9-AS2	86.42
									3	rs11924641	64923998	2.39E-12		ADAMTS9-AS2	76.08
									3	rs4688533	64968467	6.66E-09		ADAMTS9-AS2	52.19
									18	rs2004773	74480382	3.54E-08			47.17 *

The phenotypes WR4 and WR5 for the $QIMR_2$ cohort were not collected and are omitted from this table

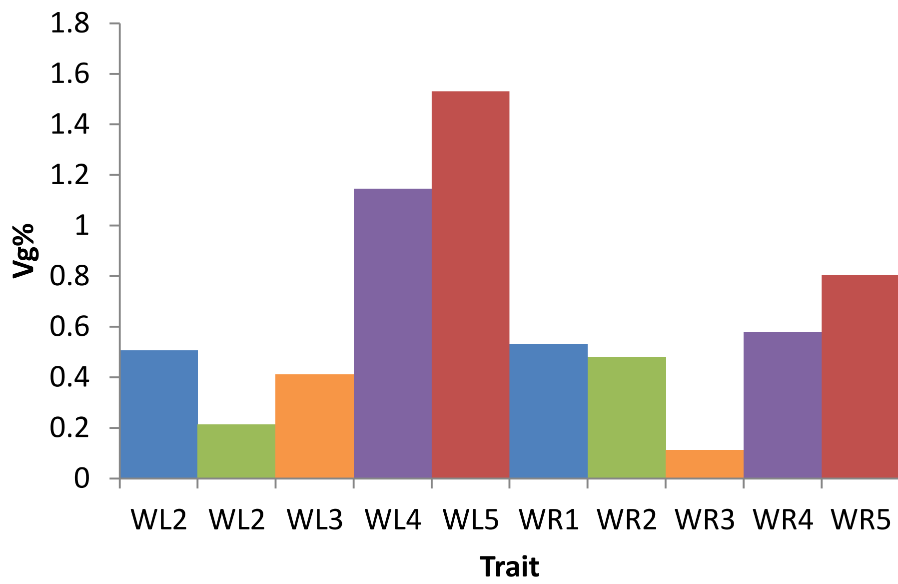

Figure S2.10. % variation explained by rs1523452 across digits, obtained by Z^2 /(N-2 + Z^2)

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Genetic variant influence on whorls in fingerprint patterns

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Study carried out in Brisbane, Queensland, Australia and Bristol, United Kingdom.

To the Editor

Early work on dermatoglyphics identified three major categories of fingerprint patterns: arches, whorls, and loops, differentiated according to landmark structures formed by dermal ridges: the triradii and core (Holt, 1968). These pattern formations are determined by the ratio of volar pad height to width *in utero* (Mulvihill and Smith, 1969) influenced by gene interaction with intra-uterine environment (Penrose, 1968). Mathematical models suggested for dermatoglyphic development include heterogeneous genetic factors influencing development versus between-digit differences, with a pattern of covariation between digits suggestive of a morphogenetic field effect(Martin *et al.*, 1982). Multivariate linkage analyses revealed a pattern of factor loadings for ridge count which supported this argument, and also found linkage to 5q14.1 driven by index, middle and ring fingers (Medland *et al.*, 2007a). A very high heritability ($h^2 = .65$-$.96$) has been reported for up to 12 dermatoglyphic characteristics (Machado *et al.*, 2010), suggesting a genetic basis for pattern type. Building on previous findings, the present study sought to identify genetic variants associated with fingerprint patterns on each digit. Two samples of twin and families were recruited from the Queensland Institute of Medical Research (QIMR), and from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study(Boyd *et al.*, 2013). Participants and participant's parents provided written informed consent and ethical approval was obtained from the ALSPAC Ethics and Law committee and the Local Research Ethics Committees.

Adult and adolescent samples from QIMR were analyzed as one sample of 3301 participants from 1764 families. Fingerprints were collected for the adult sample using rolled ink prints on paper and an electronic archiving system (Medland *et al.*, 2007b) was developed for the adolescent sample. Pattern intensity (the number of triradii) and ridge count (for whorls, the greater of two counts was used) were then manually coded (by SEM and DZL). Within the ALSPAC cohort, 5339 individuals who had GWAS and finger pattern information were used in this paper (please note the study website contains details of data available through a fully searchable data dictionary, http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). Pattern type for each digit was scored and coded (by SEM) from photocopies of the palmar surface of the hands (Medland *et al.*, 2010). Any digit where the fingerprint pattern was not clearly visible was coded as missing information. As the full patterns of the thumbs were not clearly visible we excluded this digit from analyses. Intensity and ridge count data were then re-coded in terms of presence or absence of whorls and arches on each

digit, with loops as the reference category as they are the most common pattern type, and arches were not analyzed due to low pattern frequency. For reference, the thumb on each hand is coded 1 and the little fingers 5, and right/left hand designated using the prefix L or R. After quality control, 10 variables were included in the study: presence of whorls across all digits (L1-5, R1-5), except L1 and R1 for the ALSPAC cohort and L4, L5, R4, and R5 in the QIMR adult sample.

Both QIMR and ALSPAC samples were imputed using the Hapmap2 r22.36 CEU reference. SNPs that had a minor allele frequency (MAF) of >.01 and could be imputed with confidence (R^2>0.3) were used in these analyses. Only genotyped SNPs were used for chromosome X.

Heritability estimates were conducted in OpenMX (Boker *et al.*, 2012), using binary coded data from the QIMR dataset and with sex as a covariate (Table S1). Principal components analysis (PCA) with Varimax rotation was performed to investigate latent factors within phenotypes after orthogonal transformation of correlations. Results showed 3 underlying components of pattern type: whorls on the middle three fingers (digits 2, 3, and 4) on both hands, whorls on the thumbs (digit 1), and whorls on the little fingers (digit 5) (Table S2).

GWAS were conducted using merlin-offline for each digit and each cohort, and combined using Stouffer's Z score method in METAL to calculate meta-analytic p-values(Willer *et al.*, 2010). There was no evidence of systematic inflation in the QIMR (λ= 1.004–1.027) or ALSPAC (λ =1.007–1.034) results (Figures S3-S5) and several genome-wide significant SNPs ($p < 5x10^{-8}$) were found. As Table S6 shows, univariate GWAS for the QIMR sample yielded genome-wide significant p-values for the SNP rs1523452 ($p = 7.12x10^{-9}$) and adjacent SNPs rs 2244503 ($p = 1.52x10^{-8}$), rs796973 ($p = 5.47x10^{-8}$), and rs17071864 ($p = 5.42x10^{-8}$) for the WL5 phenotype. These were independently replicated in the ALSPAC sample (rs1523452, $p = 1.60x10^{-20}$; rs2244503, $p = 3.76x10^{-19}$; rs796973, $p = 2.15x10^{-20}$; rs17071864, $p = 5.68x10^{-20}$) and are encompassed by a single gene *ADAMTS9-AS2* (Figure S7). As Figure 1 shows, these signals were strengthened in meta-analysis. SNPs within a 2 kbp intergenic region downstream of *TBX3* and upstream of *MED13L* within chr12 were also genome-wide significant for WL4 and WR4, peaking at rs1863718 ($p = 8.04x10^{-9}$ and $1.36x10^{-8}$ respectively), and a variant within the *OLA1* gene region was significant for WL2 (rs10201863, $p = 3.46x10^{-8}$). To further explore variants at a gene level, gene-based tests were conducted using GATES procedure on KGG2.5 (Li *et al.*, 2011) (Table S8).

Amongst these hits, *ADAMTS9-AS2* and *OLA1* are documented oncogenes, affecting underexpression of glioma with high WHO grade (III/IV) tumors (Yao *et al.*, 2014) and inhibition of *in vitro* cell migration for breast cancer cell lines (Zhang *et al.*, 2009) respectively, suggesting genetic factors regulating dermatoglyphic morphogenesis may also be present in subtypes of cancer. Furthermore chr12 hits between 113904923 – 113903069bp are located in an intergenic region close to *TBX3,* which is known to cause ulnar-mammary syndrome. This concurs with previous literature that limb development *in utero* is influential on subsequent fingerprint patterns that emerge (Mulvihill and Smith, 1969).

ADAMTS9-AS2 is an antisense RNA located at 3p14.1, which may be an mRNA inhibitor for the adjacent gene *ADAMTS9.* Although there is no direct explanation of the role of *ADAMTS9-AS2* in development of whorls on the little fingers, RNAseq analyses show high expression in reproductive organs as well as in the colon and lungs, suggesting it may be influential in early organ development. *ADAMTS9* and *OLA1* are also expressed in low to medium levels in the skin. Interestingly, variants in *ADAMTS9-AS2* also appear to influence whorls on all digits to differing levels of significance and variances (Figure 2A, 2B). Allele frequencies at this variant show that the G allele was associated with higher incidence of whorls in digit 5 (Figure 2C).

In conclusion, although this study did not find direct evidence for the effects of single genetic variants on specific fingerprint pattern phenotypes, variants within *ADAMTS9-AS2* show a gradient influence on whorls across all digits.

Conflict of interest

The authors state no conflict of interest

Acknowledgements

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ALSPAC: We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. GWAS data was generated by Sample Logistics and Genotyping Facilities at the Wellcome Trust Sanger Institute and LabCorp (Laboratory Corportation of America) using support from 23andMe. The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC.. This publication is the work of the authors and DME will serve as guarantor for the contents of this paper. This work was supported by the Medical Research Council MC_UU_12013/4 to DME and MC_UU_12013/3 to N.J.T. DME is funded by an Australian Research Council Future Fellowship (FT130101709).

In memory of Bodgan Mdzewski, who contributed significantly in counting QIMR fingerprint intensity scores.

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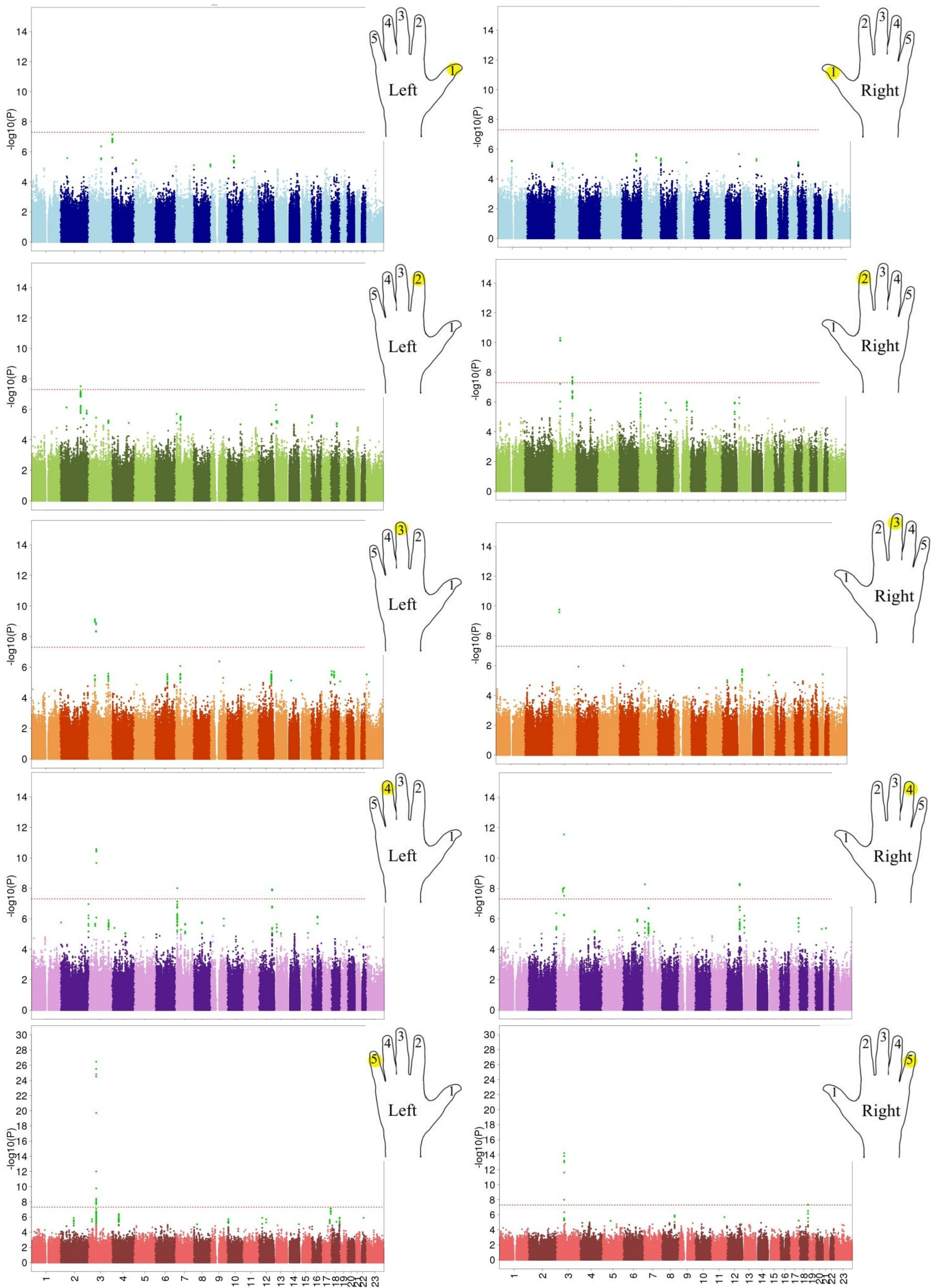

Figure 1. Manhattan plots for meta-analysis results. rs1523452 within the ADAMTS9-AS2 gene region presented the strongest signal for phenotypesWL5 ($p = 9.74x10^{-27}$) and WR5 ($p = 7.62x10^{-15}$), accounting for 1.61% and 0.93% of the variance. rs1523452 also influences WL4 ($p = 2.16x10^{-21}$), WR4 ($p = 1.33x10^{-17}$), and WR2 ($p = 3.08x10^{-10}$), attenuating at WL2 ($p = 9.24x10^{-6}$) and further reduced for WL1 ($p = 2.66x10^{-5}$) and WR1 ($p = 0.0001$).

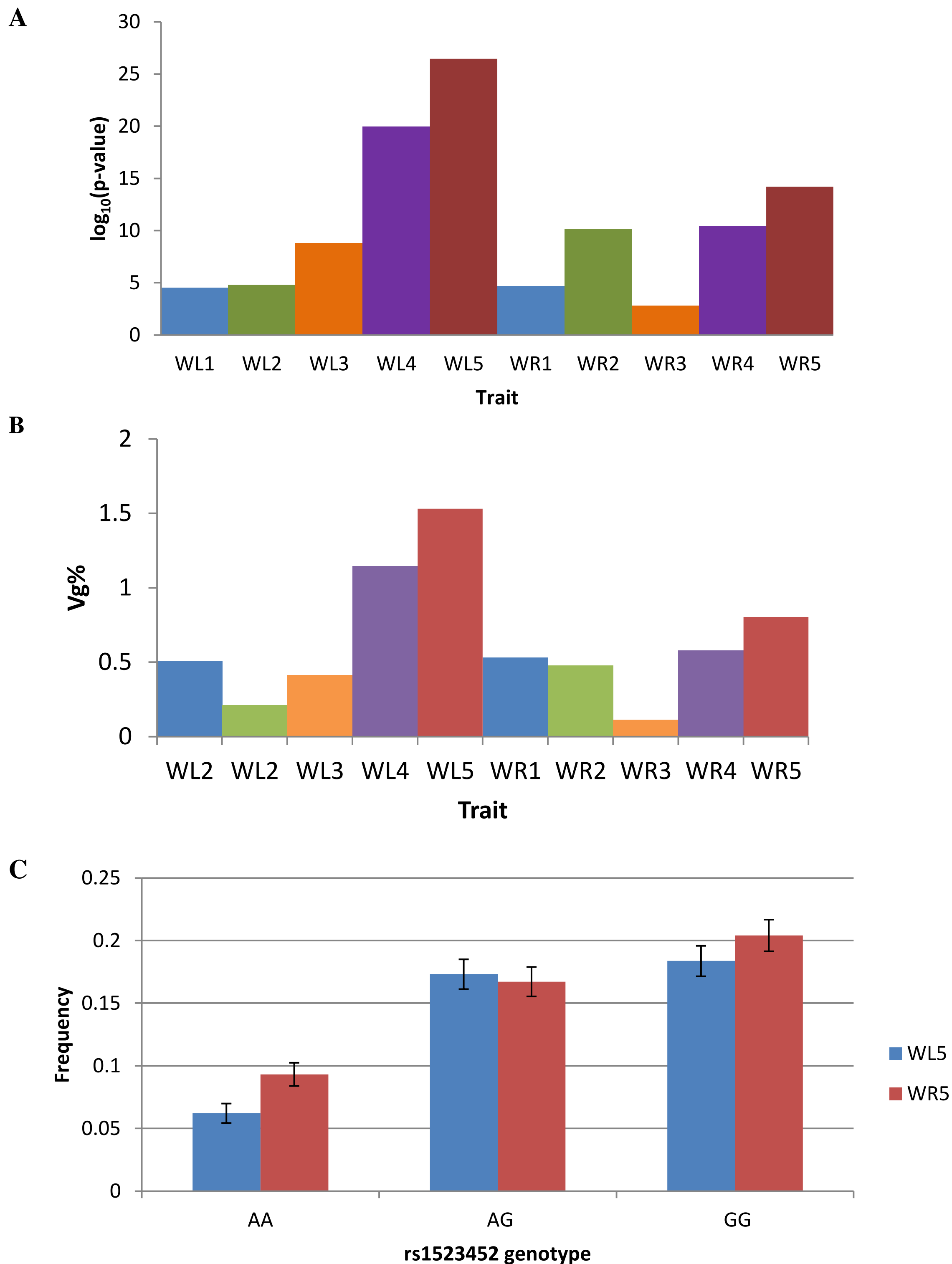

Figure 2. Histograms of **a**: meta-analyses -log10(p-values) and **b**: % variation explained by rs1523452 (within the ADAMTS9-AS2 gene region) across digits, obtained by $Z^2 /(N\text{-}2 + Z^2)$; **c**: Trait frequency as a function of allelic variation - Frequency of whorls on the left little finger (WL5; blue bars) and right little finger (WR5; red bars) as a function of the genotype rs1523452 in a sample of unrelated individuals from the $QIMR_1$ cohort (n[AA] = 708, n[AG] = 335, n[GG] = 49). With more G alleles, the proportion of whorls increases. Vertical bars correspond to the 95% confidence intervals on prevalence.

Preface to Chapters 3 & 4: Topic Overview - Trichology

3.1 Trichology in a forensic context

Although the evolutionary function of many types of hair have become obsolete with the speciation of homo sapiens (e.g. hair standing up when exposed to sudden and fear-inducing stimulus designed to put off predators), hair remains a salient physical characteristic of humans. Scalp hair in particular has been a topic of interest for social reasons such as status and fertility indicators, and scientific reasons, as a complex and heritable phenotype.

Within a forensic context, human scalp hair has been used in forensic investigations and submitted to court as trace evidence as a source of microscopical identification and toxicological testing since the 20th century. Due to the durability of hair fibres compared to other body parts such as skin and flesh, which decompose faster, human hairs are useful for identification purposes in forensic archeology. Characteristics of the hair such as skin tags or follicles attached to the follicle end and broken or damaged hair shaft may indicate force used in the removal of hair, and the color, thickness and curvature of the hair may provide suggestions to an individual's ancestry and age (SWGoMA, 2005). Since 1990, using human scalp hair in mitochondrial DNA matching has also become a common method for establishing identity (Melton, 2009).

3.2 Hair growth and morphology

The hair follicle is a complex mini-organ that produces hair via proliferation of keratinized cells at the hair bulb, which is located above the dermal papilla (Vogt et al., 2008) (Figure 1). The capillary loop provides nutrition for hair growth. These structures, together with the outer and inner root sheath that surrounds the hair shaft below the level of the sebaceous duct, are involved in follicular cycles. The permanent structures of the follicle do not change with the cyclic growth of hair and includes the sebaceous gland and the arrector pili muscle. While the outer root sheath contains mainly growth mediators and hormones, the inner root sheath is composed of three layers (cuticle, Huxley, and Henley) and is suggested to control longitudinal shape of the hair shaft.

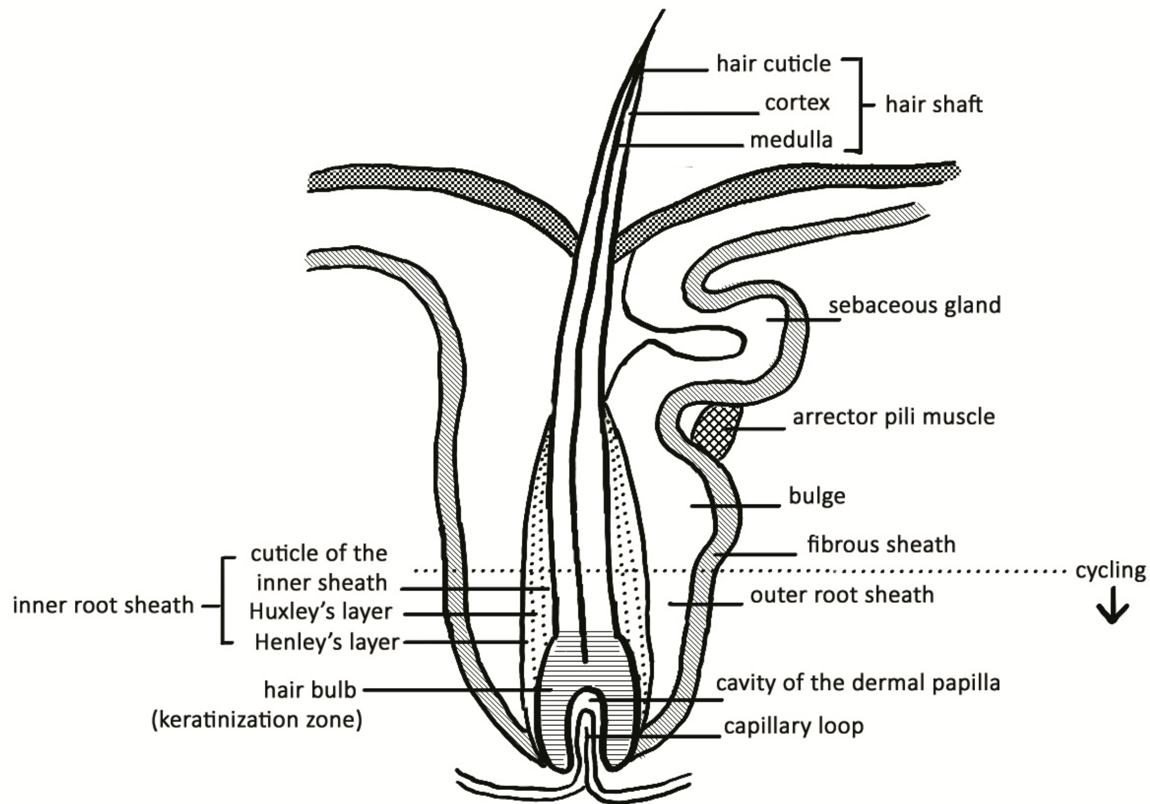

Figure 3.0.1. The hair follicle, *after Vogt et al. (2008).*

As the embryological development of hair follicles have been well-researched and documented in various biological and medical texts such as *Hair Growth and Disorders*, this section aims to provide a condensed summary of follicle formation and the genetics involved. Human follicle formation involves a series of stages (Schmidt-Ullrich & Paus, 2005) beginning in the 8th to 12th weeks of gestation, which can be categorized into the development of the follicle, hair fibre cell differentiation, and growth of the hair fibre (Paus & Cotsarelis, 1999). Previous research has shown that hair follicle morphogenesis is affected by a complex amalgam of autocrine, paracrine, and endocrine signaling pathways, as well as some regulatory pathways; however the nature of their interactions and relative significance is not well understood (Vogt et al., 2008).

Anthropological studies characterised the morphology of human scalp hair. Previous studies comparing European hair has found that lighter hair is thinner (e.g. blonde, 40-80 µm) while darker hair (e.g. dark brown, 50-90 µm) is thicker (Vogt et al., 2008). Across ethnicities, the shape of hair follicles determine the shape of the hair shaft and hence it curliness. While round follicles produce straight hair, a flat or oblong shape produces wavy or curly hair.

Previous studies have demonstrated that hair diameter is influenced by the size and secretory power of dermal papilla, which influences the size of anagen bulb, which in turn determines diameter and rate of hair growth. The overall appearance of hair fibres (i.e. diameter, cross-sectional shape, number of twists and windings per unit length of an individual hair) may vary with the position of hair bulb in relation to hair follicle, the size and shape of dermal papilla, and the curvature of the follicle along its length. Scalp hair follicle density also varies between ethnicities, with lighter haired Caucasians having the densest distribution on average (~130000) and East Asians having the least dense (~ 90000).

In addition to diameter and curvature, scalp hairs may be categorized into several types based on their shape, size, structure, and developmental timing, in order of structural complexity and pigmentation: languo, vellus, and terminal hairs. While languo hairs and vellus hairs are both unmedullated, fine and poorly pigmented, languo hairs are present at birth, while vellus hairs are present throughout lifetime. Approximately 7-25% of adult scalp hairs are vellus hairs.

Terminal hair, the main type of human scalp hair, has the largest cross-section diameter and is the most highly pigmented. The extraepidermal section of the terminal hair shaft consists of three layers, the cortex, cuticle, and medulla. The medulla is the innermost core of the hair shaft, surrounded by fibrous cells in the cortex. The outermost layer is the cuticle, which consists of translucent corneocyte layers, making the cortex pigmentation visible.

Postnatal hair growth occurs in growth-regression-resting cycles, through the anagen, catagen, and telogen phases (Figure 3.0.2). For 2-6 years during the anagen phase, the hair shaft is actively growing and cells undergoing mitosis grow upward toward the epidermis. The duration of the anagen phase is genetically determined and may last several years in terminal hair follicles. The catagen phase follows, a short resting phase divided into eight stages from late anagen to early telogen. In the next 2-3 weeks during the catagen stage, cell proliferation in the follicle halts and a period of programmed cell death occurs, leading to the regression of the hair follicle. The telogen stage takes place over the next 3 months. This involves the regression of the follicle to below the upper epidermis. Protein and DNA synthesis ceases and the telogen hair is retained in an epithelial sac. In the next exogen stage, the hair shaft sheds via a breakdown of proteins known as proteolysis. Lastly, in the kenogen stage, the hair follicle remains empty for an interval after the telogen hair is shedded. Scalp hairs do not

develop in parallel and one individual may carry hairs at all stages of development at any time. Typically, 85% of scalp hair is in anagen and 15% is in telogen.

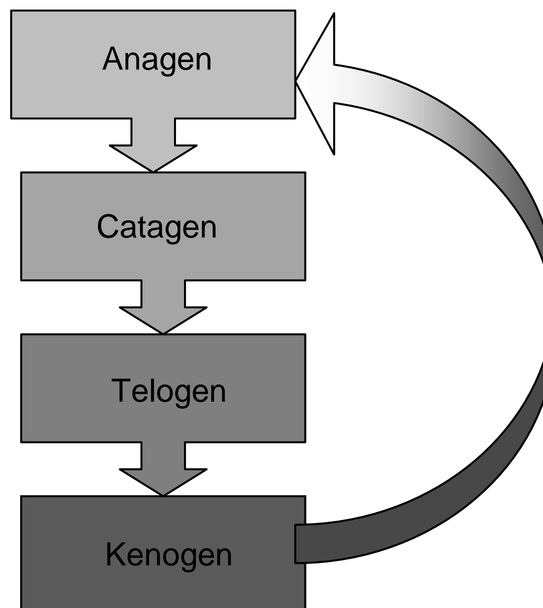

Figure 3.0.2. Hair growth phases.

3.3 Hair growth and genetics

A wide variety of genes has been found to influence aspects of hair development and characteristics. The *WNT* gene family has been implicated in the regulation of follicle morphogenesis, specifically the production of glycoproteins regulating cell proliferation, cell migration, and cell fate in embryos and adults. The hair fiber itself is formed of a large number of different keratin proteins, with recent genomic studies identifying 54 human keratins. The matrix proteins, also known as keratin-associated proteins, play a crucial role in forming a strong hair shaft. In terms of hair colour and curliness, previous genetic association studies have found red hair in Europeans is associated with variants in *MC1R* (Valverde et al., 1995); straight hair in Europeans is influenced by trichohyalin (*TCHH*) (Medland, Zhu, et al., 2009), and in East Asians by *EDAR V370A* (Tan et al., 2013). Additional 22 SNPs were also found by Han et al. (2008) in association with natural hair colour and tanning ability in women of European ancestry, such as the *SLC24A4* for light hair colour.

Previous studies have also established the heritability of self reported hair characteristics, such as hair colour in Dutch twins (Lin et al., 2015), hair curliness in European twins

(Medland, Nyholt, et al., 2009). A study of different hair types in an admixed Latin American sample also found 64% of the variance in hair shape and 74% of the variance in beard thickness to be heritable (Adhikari et al., In Press).

3.4 The present study

While previous genetic studies on trichological phenotypes focused on the overall morphology of the hair using data collected by self-report or observation, the current thesis focuses on the micro-morphology of hair curvature and diameter. The samples were collected using hair from twins and siblings, cut from the occipital bun by nurses as part of the Brisbane Longitudinal Twin Study (BLTS). These samples were then cut into smaller 2mm snippets using a guillotine and spread onto glass slides and analysed using a Optical Fibre Diameter Analyzer (OFDA 2000, BSC Electronics, Myaree, W.A., Australia), which measures hair diameter and curliness using light microscopy. Diameter was measured in terms of frequency within micron bins and curliness was measured by angle of curvature. Micro-level measurements of hair samples from the process were then computerized and collected for analyses.

Measurements of hair diameter and curvature were analysed using quantitative genetic methods. The following chapters present the first estimates of heritability of micro-measurements of hair diameter and curvature, as well as the variability of diameter. A genome-wide scan is then conducted to attempt to locate genes influencing these traits.

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Chapter 3: Variation and Heritability in Hair Diameter and Curvature in an Australian Twin Sample

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Abstract

Hair diameter and curvature are two salient characteristics of human scalp hair used in forensic contexts. While previous data show subjective categorization of hair curvature is highly heritable, the heritability of objectively measured curvature and diameter and variability of hair characteristics within each individual have not yet been established. The present study measured hair diameter and curvature using an optical fibre diameter analyser in a sample of 2332 twins and siblings. Heritability was estimated using maximum likelihood structural equation modelling. Results show sex differences in the magnitude of genetic influence for diameter and curvature, with the vast majority of the variance accounted for by genetic effects in males (Diameter= 86%, Curvature=53%) and females (Diameter =77%, Curvature =61%). The consistency of diameter was also highly heritable, but did not show sex limitation, with 67% of the variance accounted for by genetic factors. Moderate phenotypic correlations were seen between Diameter and Consistency (r= .3) but there was little correlation between Diameter and Curvature (r=-0.13). A bivariate Cholesky was used to estimate the genetic and environmental correlations between hair diameter and consistency, yielding genetic correlations of r_{gF}=.26 for females and r_{gM}=.18 for males.

Introduction

Human hair has been used in forensic investigations as a source for identification and toxicological testing since the 20th century. Characteristics of the hair such as skin tags or follicles attached to the follicle end and broken or damaged hair shaft may indicate force used in the removal of hair, and the colour, thickness and curvature of the hair may provide suggestions to an individual's ancestry and age (SWGoMA, 2005).

In general lighter hair is thinner while darker hair is thicker; in Europeans, fair hair measures about 40-80 µm, compared to dark brown hair, which has an average diameter of 50-90 µm (Vogt et al., 2008). Across ethnicities, the shape of hair follicle determines the shape of the hair shaft determining its curliness – round follicles produce straight hair while flat or oblong follicles produce wavy or curly hair. Previous studies have demonstrated that hair diameter is influenced by the size and secretory power of the dermal papilla, which influences the size of the anagen bulb and in turn determines diameter and rate of hair growth. The overall appearance of hair fibres (i.e. diameter, cross-sectional shape, number of twists and windings per unit length of an individual hair) may vary with the position of the hair bulb in relation to the hair follicle, the size and shape of dermal papilla, and the curvature of the follicle along its length.

The biological basis of hair diameter and curvature suggests these traits may be complex and heritable. Human follicle formation involves a series of stages (Schmidt-Ullrich & Paus, 2005) beginning in the 8th to 12th weeks of gestation, with the development of the follicle and hair fibre cell differentiation followed by growth of the hair fibre (Paus & Cotsarelis, 1999). Previous research has shown that hair follicle morphogenesis is affected by a complex network of autocrine, paracrine, endocrine signaling pathways, and regulatory pathways; however the nature of their interactions and relative significance is not well understood (Vogt et al., 2008).

Genes that have been hypothesized to regulate follicle morphogenesis include the WNT gene family, which produces glycoproteins regulating cell proliferation, cell migration, and cell fate in embryos and adults. Previous genetic association studies have found straight hair in Europeans is influenced by trichohyalin (*TCHH*) (Medland, Nyholt, et al., 2009) and in East Asians by *EDAR V370A* (Tan et al., 2013).

An earlier study (to which a subset of the present sample contributed) showed that 85 – 95% of the variation in nurse and self-rated hair curliness can be explained by genetic factors in a sample of twins of European ancestry (Medland, Zhu, et al., 2009). Similarly, a study of different hair types in an admixed Latin American sample also found a 64% of the variance in nurse-rated hair shape and 74% for beard thickness was due to genetic factors (Adhikari et al., In Publication). However, to date there have been no studies investigating the heritability of objectively measured hair curvature and diameter measurement. This study aims to address this by describing individual variation and estimating the heritability of physically measured hair diameter and curvature in a sample of 3057 twins.

Methods

Sample

Hair diameter and curvature data were collected from Australian twins and siblings as part of the Brisbane Longitudinal Twin Study (BLTS) (Wright, 2004). The BLTS has been an ongoing longitudinal study in Australia since 1992 focusing on adolescent population-based studies of melanoma risk factors and cognition, comprising twins and non-twin siblings. Participants were recruited through school and media appeals as well as word of mouth, written informed consent was obtained from participants and their parents or guardians, and data were collected at three time points at age 12, 14 and 16. Ethical approval for the study was gained from the QIMR Berghofer Medical Research Institute Human Research Ethics Committee. The present paper focuses on data from age 12 (visit 1) and 14 (visit 2). Hair samples were collected by research nurses who also provided subjective ratings on the hair texture in the same twin and sibling sample.

Measures

Diameter and curvature measurements were measured using an Optical Fibre Diameter Analyzer (OFDA 2000, BSC Electronics, Myaree, WA., Australia), based on light microscopy. This technique has been well-established for measuring wool fibre diameter and quality, providing accurate measurements of approximately 2,000 fibre samples in the space of a few minutes (Wortmann & Schwan-Jonczyk, 2006). Hair samples were cut into 2mm snippets using a guillotine, spread into 70mm square glass slides, and inserted into the machine. Diameter was measured in microns (µm) and sorted in to bins between170 – 590 µm. The OFDA provides the raw diameter data as frequency (number of snippets) per micron bin. For curvature, non-intersecting 200 µm long segments were used to find the angle of the curve and

multiplied by 5 to represent the angle of a 1mm segment of the hair strand. The OFDA provides the raw curvature data as frequency (number of snippets) per bin (with bins ranging from 0 to 360 degrees). In addition, the consistency of diameter and curvature within each sample were also provided as the standard deviation of each individual's distribution.

Preliminary analyses shows that hair diameter means and consistency as well as curvature means vary greatly amongst individuals (Figure 3.1). As Figure 3.2 shows, the distribution of male and female hair diameter and curvature were largely normal and illustrates sex differences, while curvature was approximately log-normal. Table 3.1 shows the average number of guillotined hair snippets and length of hair fibers of each hair sample from males and females across visits 1 (aged 12) and 2 (aged 14).

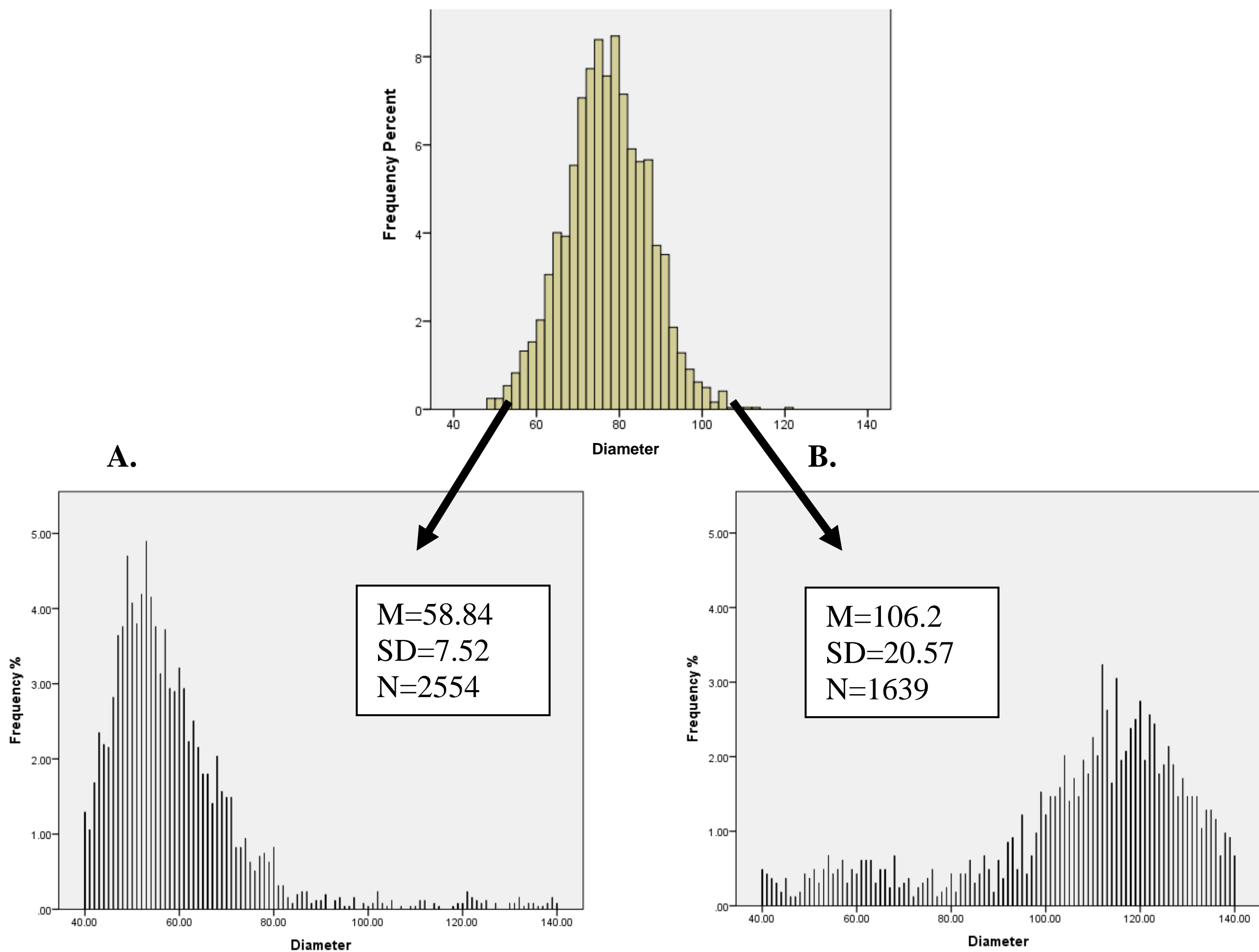
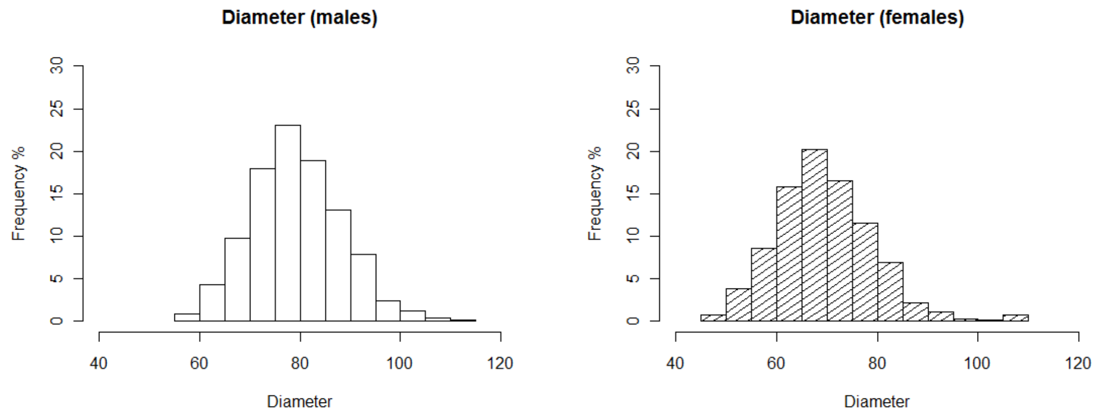
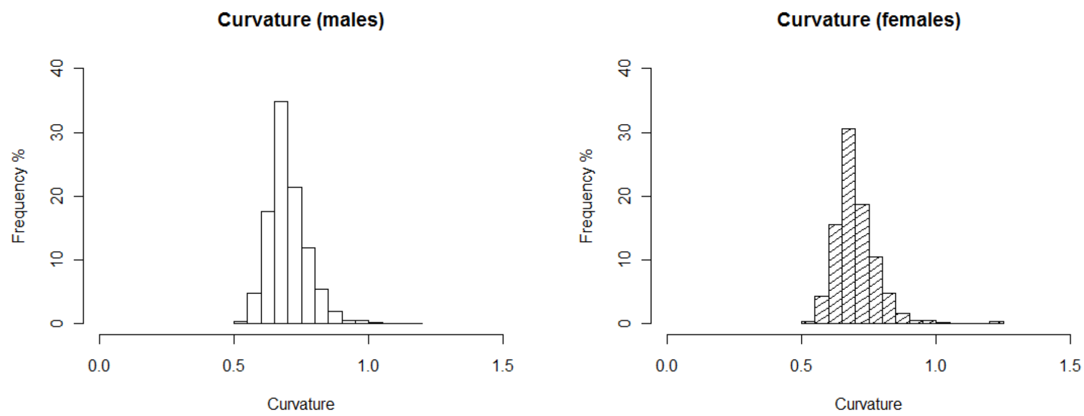


Figure 3.1. Histograms showing the distribution of hair diameter in example individuals. As shown in the histogram, across individuals the distributions were approximately normal. However, individual samples of hair show widely divergent distributions, as the graphs below show, individual A has thinner hair compared to individual B.

a.



b.



c.

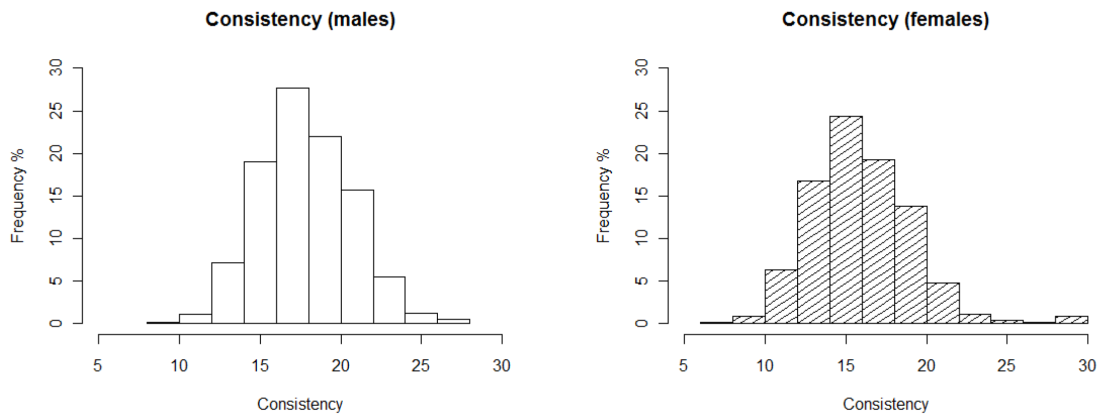


Figure 3.2. Histograms by sex (n_{female}=1458, n_{male}=1377) for frequency of a. mean diameter, b. mean curvature, and c. diameter consistency. These graphs show that mean diameter and curvature are normally distributed and that there are quantitative sex differences in diameter and curvature, with males having on average thicker and curlier hair. Moreover, males had higher diameter variance compared to females.

Table 3.1. Mean, standard deviations and range of number of hair fibres within each sample and hair length per sample

Variable	Visit	Sex	N	Mean	SD	Range
Number of 2mm hair fibres after guillotine cutting process	1	Male	1484	2258.56	883.49	220-6869
		Female	1566	2150.81	1051.58	121-8188
	2	Male	765	2348.57	836.2	167-5155
		Female	822	2315.28	1099.77	149-7619
Length of hair fibres (mm)	1	Male	1484	25.55	8.46	2-62
		Female	1566	28.14	10.61	4-78
	2	Male	765	28	10.1	2-70
		Female	822	27.78	11.82	2-74

Due to the nature of data we checked for outliers within individuals across measurements and within sample across a) individuals and b) families. Observations beyond 3 standard deviations above and below the mean were removed. To check for outliers at the family level we used the Mx %p function (Neale et al., 2003). This resulted in the removal of 44 and 47 families from the hair diameter visit 1 and 2 data respectively, and 5 and 4 families from the curvature data for visit 1 and 2.

Statistical methods

Prior to estimating of heritability, maximum likelihood analyses were conducted to test assumptions regarding the homogeneity of means, variances, and covariances using OpenMx (Boker et al., 2011). Sex, age, hair colour, and ancestry were included as covariates in all analyses.

Subsequent simplified models for equating means and variances tested for birth order effects (H_1), zygosity effects across same-sex twins (H_2), differences between same-sex and opposite-sex twin pairs (H_3), and differences between siblings and twins (H_4). H_5 equated all means and tested for effects of biological sex (Supp. Table 3.1).

To estimate heritability, an ADE model was used in this study as the MZ correlations were more than twice that of DZ correlations. Simplified AE and E models were tested for goodness of fit to determine whether parameters could be dropped from the full model. The change in model fit was tested using likelihood ratio chi-square tests. The distribution of twice the

difference in log likelihood (-2LL) approximates $\Delta\chi^2$, with the degrees of freedom (df) for this test equal to the difference in df between the models.

Results

Data were available for two time points for a large number of participants (Table 3.2) and test-retest correlations indicate substantial change over time (r^2_{diam}=.56; r^2_{curve}=.44, $r^2_{consistency}$=.60; Table 3.2). To maximize sample size and reduce the amount of environmental variance due to the use of hair dyes, products and treatments we used the data collected at age 12 to estimate heritability.

Table 3.2. Test-retest correlations (upper matrix) and number of individuals (lower matrix, in grey) for mean diameter, curvature, and consistency at visit1 (aged 12) and 2 (aged 14). Cross-trait correlations are given in intersecting cells for different traits. 95% confidence intervals are given in square parentheses.

	Diam1	Diam2	Curve1	Curve2	Consist1	Consist2
Diam1	2626	**0.58 [.54, .61]**	-0.13 [-.17,-.09]	0.02 [-.02, .07]	.30 [.26,.34]	.29 [.24,.34]
Diam2	1431	2001	0.02 [-03, .08]	-0.19 [-.23, -.15]	.20 [-.25,-.14]	.36 [-.40,-.32]
Curve1	2401	1430	2623	**0.44 [.40, .48]**	.13 [.09,.17]	.04 [-.01,.09]
Curve2	1432	1843	1431	2003	.04 [-.01,.09]	.13 [.08,.17]
Consist1	2420	1434	2419	1434	2420	**.59 [.55,.62]**
Consist2	1434	1847	1433	1847	1418	1847

After quality control, hair diameter and curvature data were available for 2332 twins and siblings from 1263 families. Data were available for 226 MZ female pairs, 217 MZ male pairs, 237 DZ females, 215 DZ males, 358 opposite sex DZ twins (181 female first-born and 178 male first-born), 260 female siblings, and 76 male siblings. The means and standard deviations of hair diameter and curvature means and variation are provided in Table 3.3.

Hypotheses regarding the means of variables (mean hair diameter, consistency, and curvature at age 12) were tested and no significant differences were found in comparisons except curvature between males and females at age 12 (Table S3.2). The same series of comparisons were applied to test differences between variances. Comparisons for male and female were

significantly different for all three variables. To account for this we included sex limitation in our analyses.

As shown in Table 3.3, the MZ correlation (r_{MZdiam}=.84, $r_{MZcurve}$=.58) is higher than the DZ correlation (r_{DZdiam}=.34, $r_{DZcurve}$=.30), suggesting a genetic influence in the mean diameter and curvature observed, as well as the variance in hair diameter. To examine the homogeneity of these correlations we tested a series of hypotheses as summarized in Table S3.1. As shown in Table S3.2, there were significant differences between male and female correlations for diameter, suggesting the presence of non-scalar sex differences.

Table 3.3. Means and standard deviations (in parentheses) of mean ($Mean_{diam}$, $Mean_{curve}$) of hair diameter and curvature visit 1 (aged 12), consistency ($Var_{consist}$) of hair diameter, twin correlations (r) for zygosity groups, and overall correlations for MZ and DZ groups

	MZF	MZM	DZF	DZM	DZFM	DZMF	MZ	DZ
Diameter								
N_{ind}	429	387	482	426	333	345	816	1586
N_{pair}	168	138	197	159	127	134	306	617
Mean	74.8	78.5	73.7	78.8	76.4	75.9	76.5	76.1
(SD)	(10.8)	(8.4)	(9.7)	(8.2)	(8.9)	(8.8)	(9.9)	(9.1)
r	0.81	0.88	0.42	0.34	0.31	0.2	0.84	0.34
Curvature								
N_{ind}	429	387	482	426	333	345	816	1586
N_{pair}	168	138	197	159	127	134	306	617
Mean	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
(SD)	(.2)	(.1)	(.2)	(.1)	(.2)	(.1)	(.2)	(.2)
r	0.63	0.48	0.34	0.37	0.25	0.22	0.58	0.3
Consistency								
N_{ind}	336	283	391	330	268	282	619	1271
N_{pair}	162	135	191	157	122	132	297	602
Mean	15.3	17.2	15.4	17.4	16.7	16.6	16.2	16.5
(SD)	(2.9)	(3.0)	(3.0)	(2.8)	(3.1)	(3.0)	(3.1)	(3.0)
r	0.63	0.71	0.37	0.3	0.17	0.2	0.72	0.27

Sex-limitation models were used to investigate difference in sources of variance between males and females. Curvature data was best described by a scalar model (AIC_{scalar}= 11923.31 vs $AIC_{nonscalar}$=11928.44) while a non-scalar sex limitation model provided the best fit to the diameter data (AIC_{scalar}=9593.77 vs. $AIC_{nonscalar}$=9587.03). Estimates of A, D and E variances are presented in Table 4. Although a dominant genetic effect (D) was found for male hair diameter ($NSSL_{diam}$ vs $NSSL_AE_{diam}$, $p=1.30x10^{-6}$), D was not significant for female hair diameter ($NSSL_{diam}$ vs $NSSL_{MaleADE,FemaleAE}$, p=.18) or curvature (ADE_{curve} vs. AE_{curve} , p=.588; Table 3.3).

The heritability of diameter consistency within each individual was also tested using sex limitation modeling. As Table 3.4 shows, there were no significant sex differences and a univariate ADE model provided the best fit for the data. Additive genetic effects accounted for 29% of the variance and dominant genetic effects accounted for 38%.

Based on the phenotypic correlations between variables (Table 3.2), a bivariate Cholesky was used to examine mean hair diameter and consistency of diameter. In order to test whether the source of genetic variation was the same in males and females, additional parameters m_{11}, m_{21}, and m_{22} were used to model additive genetic variability specific to males (Figure 3.3). However model fit was not significantly worse when these estimates were dropped (p=.24, Table 3.5). Sex limitation was found for diameter ($p=6.38x10^{-17}$) but not consistency (p=.22). Further models dropping covariance due to D (d_{21}) for both sexes, D for diameter for females (df_{22}) as well as the covariance due to E (ef_{21})for females also did not result in a significant drop in goodness-of-fit (p=.55). As shown in Figure 3.3, consistent with the univariate modeling, genetic factors accounted for 67% variance in consistency for both sexes, 87% of variance in diameter for males, and 78% for diameter for females. Cross-trait genetic correlations were for females (r_{gF}=.26) and males (r_{gM}=.18), and a small but significant cross-trait environmental correlation was present for males (r_{eM}=.09).

Table 3.4. Results of sex limitation modelling for diameter and curvature and consistency. Best fitting model is indicated in bold, $AIC_{NSSLdiam}$=9587.03; $AIC_{SSLcurve}$=11921.31; $AIC_{ADEdiamvar}$=6303.1. 95% Confidence Intervals (CIs) are provided for the full and best fitting models.

Model	Sex	Proportion of total variance A	D	E	rg/k	-2LL	df	$\Delta\chi^2$	AIC	p-value
Diameter										
GSL	Male	0.35	0.52	0.13	0.67	14353.88	2383		9587.88	
	(95%CI)	.01-.73	.14-.86	.10-.17						
	Female	0.47	0.31	0.22						
	(95%CI)	.09-.80	0-.70	.18-.27						
GSL_AE	Male	0.87	0.00	0.13	0.63	14364.07	2385	10.18	9594.07	0.006
	Female	0.77	0.00	0.23						
NSSL	Male	0.15	0.72	0.13	1.00	14355.03	2384	1.14	9587.03	0.285
	Female	0.51	0.27	0.22						
NSSL_Male ADE, Female AE	**Male**	**0.31**	**0.56**	**0.13**	**1.00**	**14356.81**	**2386**	**1.78**	**9574.81**	**0.182**
	(95%CI)	**.15-.51**	**.36-.73**	**.10-.17**						
	Female	**0.77**	**0**	**0.23**						
	(95%CI)	**.71-.81**		**.18-.29**						
NSSL_AE	Male	0.87	0.00	0.13	1.00	14382.14	2386	27.11	9610.14	1.30x10-6
	Female	0.76	0.00	0.24						
SSL		0.40	0.42	0.18	0.61	14365.77	2386	11.89	9593.77	0.008
ADE	Male	0.30	0.51	0.19	1.00	14440.14	2385	86.26	9666.14	0.000
	Female	0.30	0.51	0.19						
Curvature										
GSL	Male	0.59	0.00	0.41	0.72	16702.34	2387		11928.34	
	(95%CI)	.50-.63	0.00	.34-.5						
	Female	0.62	0.00	0.38						
	(95%CI)	.43-.66	0-.18	.34-.46						
NSSL	Male	0.52	0.07	0.41	1.00	16704.44	2388	2.09	11928.44	0.148
	Female	0.51	0.11	0.38						
SSL		0.60	0.00	0.40	0.67	16703.31	2390	0.97	11923.31	0.809
Scalar_AE		**0.60**	**0.00**	**0.40**	**0.67**	**16703.31**	**2391**	**0.97**	**11921.31**	**0.809**
	95%CI	**.52-.64**	**0.00**	**.36-.48**						
ADE	Male	0.53	0.07	0.41	1.00	16754.49	2389	52.15	11972.49	0.000
	Female	0.53	0.07	0.41						
AE	Male	0.58	0.00	0.42	1.00	16754.78	2392	52.44	11970.78	0.588
	Female	0.58	0.00	0.42						
Consistency										
GSL	Male	0.41	0.29	0.29	0.59	10739.88	2217		6305.88	
	(95%CI)	.004-.75	0-.72	.23-.38						
	Female	0.48	0.16	0.35						
	(95%CI)	.11-.70	0-.55	.28-.44						
NSSL	Male	0.15	0.56	0.29	1.00	10741.14	2218	1.265	6305.14	0.261
	Female	0.53	0.11	0.36						
NSSL_AE	Male	0.68	0.00	0.32	1.00	10752.2	2220	11.06	6312.20	0.011
	Female	0.61	0.00	0.39						
SSL		0.30	0.37	0.33	0.94	10744	2220	4.12	6304.00	0.249
ADE	Male	**0.29**	**0.38**	**0.33**	**1.00**	**10745.1**	**2221**	**5.22**	**6303.10**	**0.265**
	(95%CI)	**.05-.53**	**.13-.63**	**.28-.39**						
	Female	**0.29**	**0.38**	**0.33**						
	(95%CI)	**.05-.53**	**.13-.63**	**.28-.39**						
AE	Male	0.63		0.36	1	10757.1	2222	5	6313.1	0.002
	Female	0.64		0.36						

Abbreviations: GSL: General Sex Limitation; NSSL: Non-Scalar Sex Limitation; SSL: Scalar Sex Limitation; GSL_AE: General Sex Limitation AE Model; NSSL_AE: Non-Scalar Sex Limitation AE Model

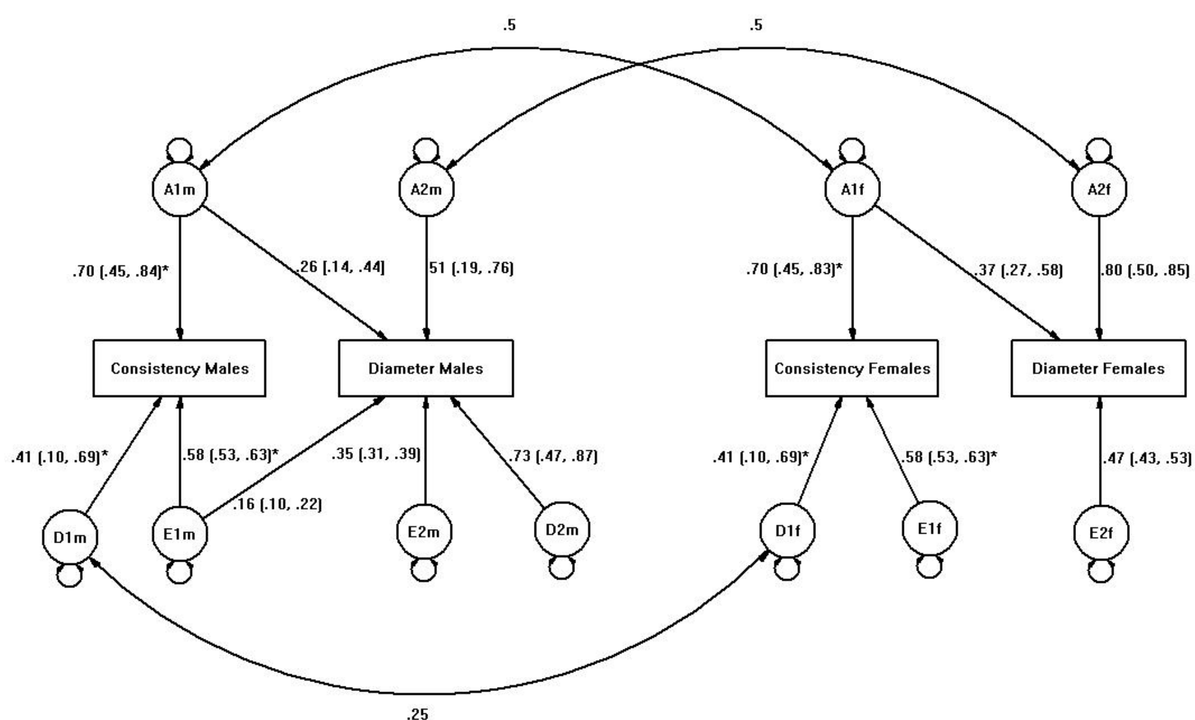


Figure 3.3. Bivariate analysis of mean diameter and consistency Cholesky model (ADE model). Path coefficients denoted with an asterisk (*) were equated across sexes.

Table 3.5. Bivariate analysis of mean diameter and consistency Cholesky (ADE model). Table shows fit for models equating standardised paths for consistency and covariation in D and E for males and females. Dropping D for males and females and E for females resulted in no significant change in fit. Best fitting model denoted with an asterisk (*).

	Model Fit					
	-2LL	**df**	**Δdf**	**Δχ2**	**AIC**	**pval**
Full Sex Limitation	20247.77	3781			12686.21	
Dropped male specific genetic variances (m11, m21, m22)	20255.72	3787	6	7.95	12681.72	0.24
No sex limitation	20352.56	3793	9	104.79	12766.56	6.38^{-17}
No sex limitation on consistency	20255.98	3787	3	8.21	12681.98	0.22
No sex limitation on consistency, dropping df21, ef21, dm21	20261.24	3790	3	13.47	12681.24	0.14
No sex limitation on consistency, dropping df21, df22, ef21, dm21*	20261.6	3791	1	13.8	12679.6	0.55
No sex limitation on consistency, dropping df21, df22, ef21, dm21, em21	20285.96	3792	1	24.68	12702.29	6.76^{-7}

Discussion

Hair curvature and diameter are highly polymorphic traits. Using data from twins and siblings in a quantitative genetic analysis, this study has shown that in a large sample of 12-year-old twins and siblings of European ancestry, physical measurements of these traits are significantly heritable, with genetic factors accounting for up to 87% of total variance for diameter, 60% for curvature, and 67% for consistency of diameter.

Compared to previous studies of heritability of hair curvature in Europeans (Medland, Zhu, et al., 2009), which reported heritabilities of 85-95%, this study found a 60% of the variance could be explained by genetic factors. However, the previous study assessed macro level curvature (categorical self-report) while the present study assessed micro level curvature. Using the *polycor* package (Fox, 2010) in R, polyserial correlations were calculated between nurse report and OFDA measurement data, yielding a correlation of .44, p=.02, n=1181, suggesting that while microsopical measurement of hair curvature may have different properties to macroscopic evaluation, there is substantial common variation. There was also evidence of sex limitation for female diameter and curvature, suggesting specific differences in genetic and environmental variances in hair diameter and curvature as well as an overall difference in variance for male and females in hair diameter. However, there was no significant sex limitation for consistency. There was evidence of dominant genetic influences on diameter for males and on curvature for both sexes.

Possible environmental factors that influence hair diameter, consistency, and curvature include the use of shampoo and hair fixatives, deliberate straightening or curling of hair, and exposure to chemicals such as chlorine and calcium in water. Some other issues to consider when interpreting these results include the effect of differences in hair length and hair treatments between males and females. In addition, these data were collected from young adolescents and the generalization of these results to children or adults needs to be assessed.

In conclusion, this study shows that hair diameter, consistency, and hair curvature are significantly heritable. For mean diameter and curvature, this effect is moderated by sex. In the next stage of this investigation, genome-wide analyses will be conducted to try to identify loci that influence hair diameter and curvature.

Acknowledgements

Funding was provided by the Australian Research Council (ARC), the Australian National Health and Medical Research Council (NHMRC), Visigen, Identitas Inc., the Australian Federal Police Forensics, and the Victorian Police Forensic Services Centre, applications submitted by Dennis McNevin and Runa Daniels. We would like to thank all twins and their families who participated in the BLTS and provided their valuable genotype and phenotype measurements, as well as nurses who participated in the categorical coding of hair curvature. Additional thanks are due to Mark Brims for making available hair measurements from the OFDA.

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Supplementary Tables

Table S3.1. Contrasts used to test hypotheses about means and variances in twin and sibling pairs.

	Zygosity group	H_0		H_1		H_2		H_3		H_4		H_5	
		T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
1	MZF	1	1a	1	1	1	1	1	1	1	1	1	1
2	MZM	2	2a	2	2	2	2	2	2	2	2	1	1
3	DZF	3	3a	3	3	1	1	1	1	1	1	1	1
4	DZM	4	4a	4	4	2	2	2	2	2	2	1	1
5	DZFM	5	6	5	6	5	6	1	2	1	1	1	1
6	DZMF	6	5	6	5	6	5	2	1	2	1	1	1
7	Female Sibs	7	7	7	7	7	7	7	7	1	1	1	1
8	Male Sibs	8	8	8	8	8	8	8	8	2	2	1	1

Contrasts used to test hypotheses about covariances in twin and sibling pairs (Visit 1, age 12).

	Zygosity group	H_0	H_1	H_2	H_3	H_4	H_5	H_6
1	MZF	1	1	1	1	1	1	0
2	MZM	2	1	1	1	1	1	0
3	DZF	3	2	2	2	2	1	0
4	DZM	4	2	2	2	2	1	0
5	DZFM	5	5	5	2	2	1	0
6	DZMF	6	6	5	2	2	1	0
7	Female Sibs	7	7	7	3	2	1	0
8	Male Sibs	8	8	8	4	2	1	0
9	F/M Sibs	9	9	9	5	2	1	0

Contrasts used to test hypotheses about covariances in twin and sibling pairs (Visit 2, age 14).

	Zygosity group	H_0	H_1	H_2	H_3	H_4	H_5
1	MZF	1	1	1	1	1	0
2	MZM	2	1	1	1	1	0
3	DZF	3	2	2	2	1	0
4	DZM	4	2	2	2	1	0
5	DZFM	5	5	5	2	1	0
6	DZMF	6	6	5	2	1	0

Table S3.2. Difference in log likelihood for tests concerning means, variances, and covariances of hair diameter and curvature in 12-year-old twins.

Model	Δdf	Diameter	Curvature	Consistency
$H1_M$	4	3.218	2.953	0.954
$H2_M$	2	0.793	1.1	1.17
$H3_M$	2	1.293	0.022	2.24
$H4_M$	2	0.667	1.986	2.195
$H5_M$	1	2.9	7.948*	1.46
$H1_V$	4	5.753	5.373	3.012
$H2_V$	2	2.646	0.162	1.898
$H3_V$	2	6.963	1.186	2.956
$H4_V$	2	2.844	2.082	1.680
$H5_V$	1	51.657*	76.887*	0.466
$H1_C$	2	11.056*	0.501	1.820
$H2_C$	1	0.01	0.016	1.831
$H3_C$	1	2.441	0.536	0.012
$H4_C$	2	5.026	4.52	0.479
$H5_C$	1	187.467*	87.145*	179.530*

*$p \leq .01$

Table S3.3. Parameter estimates for full bivariate Cholesky of hair diameter and consistency and best fitting model marked with an asterisk (*).

	Parameter	Male		Female	
		Estimate	95%CI	Estimate	95%CI
Full Sex Limitation	**a11**	0.45	[0.03, 0.81]	0.79	[-0.84, 0.84]
	a21	0.26	[-0.23, 0.6]	0.23	[-0.2, 0.48]
	a22	0.38	[-0.03, 0.82]	0.78	[-0.86, 0.86]
	d11	-0.48	[-0.83, 0.82]	-0.14	[-0.64, 0.59]
	d21	-0.51	[-0.91, 0.91]	-0.35	[-0.76, 0.76]
	d22	-0.43	[-0.86, 0.86]	0.00	[-0.62, 0.62]
	e11	0.54	[0.48, 0.62]	0.60	[0.54, 0.66]
	e21	0.14	[0.08, 0.21]	0.07	[0, 0.14]
	e22	0.35	[0.31, 0.4]	0.47	[0.42, 0.52]
	m11	-0.52			
	m21	0.34			
	m22	0.29			
No sex limitation on consistency, dropping df21, df22, ef21, dm21 *	**a11**	0.70	[0.45, 0.83]	0.70	[0.45, 0.83]
	a21	0.26	[0.14, 0.44]	0.37	[0.27, 0.58]
	a22	0.51	[0.19, 0.76]	0.80	[0.5, 0.85]
	d11	-0.41	[-0.69, -0.1]	-0.41	[-0.69, -0.1]
	d21				
	d22	-0.73	[-0.87, -0.47]		
	e11	0.58	[0.53, 0.63]	0.58	[0.53, 0.63]
	e21	0.16	[0.1, 0.22]		
	e22	0.35	[0.31, 0.39]	0.47	[0.43, 0.53]

Chapter 4: Novel genetic variants influencing light optic measurement of hair curvature

Yvonne YW Ho, Jodie N Painter, Grant W Montgomery, Nicholas G Martin, Sarah E Medland

Abstract

Hair curliness has previously been shown to be highly heritable. Here we present the first genome-wide association (GWAS) of objectively measured hair curvature and compare the results to those obtained by observable classification. The observational data were collected using nurse report measures while objective data were acquired using an Optical Fibre Diameter Analyser (OFDA). Results show significant association with a previous associated variant rs11803731within the *TCHH* gene region for both observational and objective measures ($p_{OFDA} = 1.14x10^{-10}$, β=.19, R^2=.97; p_{NURSE}=$1.10x10^{-15}$, β =.14). GWAS of the objectively measured data also resulted in the detection of an additional novel variant, rs142826872 (p_{OFDA} =$7.04x10^{-10}$, β =-.46, R^2=.77; p_{NURSE} =$1.20x10^{-5}$, β =-.19) on chromosome 15 within the *PWRN2* gene region. Bioinformatics analyses shows transcriptionally active regions for the signal in *TCHH*. These results support the hypothesis that hair morphology is a polygenic phenotype and that objective measures of physical traits may yield novel information about the genetic variants that influence them in comparison to observational measures.

Introduction

Variability of scalp hair characteristics such as colour, curliness (curvature) and thickness are topics of investigation within the field of forensics, due to their salient role in identification and in anthropological studies as predictors of ethnicity. Such studies suggest the biological basis of hair diameter and curvature are both heritable and affected by multiple genetic variants.

Beginning in the 8th to 12th weeks of gestation, human hair follicles develop in a series of stages (Schmidt-Ullrich & Paus, 2005) leading to the formation of the follicle and hair fibre cells, as well as growth of the hair fibre (Paus & Cotsarelis, 1999). Previous research has shown that hair follicle morphogenesis is affected by a complex interaction between growth and homeostasis hormone pathways, as well as regulatory pathways, although the nature of their interactions is not well understood (Vogt et al., 2008).

The heritability of self-reported hair characteristics has typically been found to be high; hair colour was reported to show a 73 – 99% broad sense heritability in a Dutch sample (Lin et al., 2015), while hair curliness was found to be 85 – 95% heritable in an Australian cohort (Medland, Zhu, et al., 2009). A study of different hair types in an admixed Latin American sample found a 64% heritability for hair shape and 74% for beard thickness (Adhikari et al., In Press). Moreover, a previous analysis using the same sample utilized in this present study shows that hair diameter and curvature are heritable traits with strong genetic influence, and evidence of sex limitation with slightly higher broad sense heritability for males ($H^2_{diameter}$= 86%, $H^2_{curvature}$=53%) than females ($H^2_{diameter}$=76%, $H^2_{curvature}$=61%).

Previous genetic association studies have found several loci affecting hair curvature within specific populations. Straight hair in Europeans is associated with variants located in trichohyalin (*TCHH*) (Eriksson et al., 2010; Medland, Nyholt, et al., 2009) and within *ectodysplasin* A receptor *(EDAR)* in East Asians (Tan et al., 2013). These studies utilized categorical measures of hair curvature assessed via self-report or researcher classification. While these previous studies have demonstrated that simple classification schemes can be used to identify genetic variants influencing hair curvature, the present study sought to assess whether the use of objective quantitative assessments of hair curvature would increase the power to identify additional loci influencing this trait.

Methods

Sample

As previously described, hair clippings were collected from 3057 twins and siblings in Australia and measured using an optical fibre diameter analyser (OFDA). The hair diameter and curvature data was collected as part of the Brisbane Longitudinal Twin Study (BLTS) (Wright, 2004), an ongoing longitudinal study in Australia focusing on melanoma risk factors and cognition. Participants were recruited through school and media appeals as well as word of mouth. Written informed consent was obtained from participants and their parents or guardians, and data was collected at three time points at age 12, 14 and 16. Ethics approval for the study was obtained from the QIMR Berghofer Medical Research Institute Human Research Ethics Committee.

Measures

Hair diameter and curvature were measured using an Optical Fibre Diameter Analyzer (OFDA 2000, BSC Electronics, Myaree, WA., Australia), an industry-standard technology used for measuring wool fibre diameter and quality (Wortmann & Schwan-Jonczyk, 2006). Hair clippings of 2-4 cm were sampled from the scalp on the occipital bun and spread onto 70mm square glass slides. Non-intersecting 200 µm segments were used to find the angle of the curve in degrees. This was then multiplied by 5 to represent the angle of a 1mm segment of the hair strand, and sorted into degree bins. Data was presented as frequency of hairs within each degree bin. Segment measurements beyond 3 standard deviations above and below the mean were removed from analyses and then averaged for each individual. Data were then log-normalised. These quantitative micro-level OFDA curvature measures were then compared to classifications made by trained research nurses who rated participants hair curvature as straight, wavy or curly.

Imputation and association analyses

The analyses in the present study uses a sample of 2623 individuals for whom nurse reports, OFDA measurements and genome wide genotypes were available. Participants were genotyped using Illumina 317K, 370K, or 610K platforms. Following quality control (QC), genotypes common across these platforms were imputed using the Haplotype Reference Consortium reference set using MACH (Li et al., 2009). Genome-wide association analyses were conducted using rare-metal worker (Feng et al., 2014) which assesses the evidence for association at each variant, using the imputed dosage format imputed genotypes,

parameterized as $x_{ij}= \beta_{dose}+\beta_{sex}+\beta_{age}+\mu$ where *j* denotes the trait value for an individual within family *i*, while accounting for familial relatedness and zygosity.

Univariate GWAS analyses were performed on the quantitative measures of hair curvature and on the three categories of nurses' classification in the same individuals. Sex and age were included as covariates in both GWAS. Following each GWAS analysis, we applied standard quality control (QC) procedures using thresholds of quality score R^2 >.5 and minor allele frequency (MAF) > .005, including only those SNPs reaching QC thresholds in subsequent analyses. The individual SNP results were also used to perform gene-based association tests using the Knowledge-based mining system for Genome-wide Genetic studies (KGG) software (M. Li et al., 2011). Log-likelihood tests were used to determine which SNPs could be reasonably considered to tag causal SNPs by comparing the log-likelihoods obtained for SNPs with $p < 1x10^{-6}$ with that of the most significantly associated SNP at each locus. SNPs with likelihood rates of <1:100 of being the top SNP were prioritized for bioinformatics analyses below.

Bioinformatics analyses

To investigate possible effects of SNPs on gene function, bioinformatics analyses were performed on the top candidate causal tagging SNPs per locus using the HaploReg program (Ward & Kellis, 2012), which utilises publically available data from the ENCODE (Birney et al., 2007), and Roadmap Epigenomics (http://www.roadmapepigenomics.org/) projects to determine whether SNPs lie in potentially functional genomic regions. This is indicated by the presence of, for example, promoter and enhancer histone marks, open chromatin, transcription factor binding and methylation. The location of super-enhancers relative to candidate causal SNPs was assessed using the dbSUPER database (http://bioinfo.au.tsinghua.edu.cn/dbsuper/index.php).

Results

Two genome-wide significant ($p < 5x10^{-8}$) association signals were found for OFDA curvature measure on chromosomes 1 and 15, with a number of suggestive ($p < 1x10^{-5}$) signals present on additional chromosomes (Supp. Table 1). Quantile-Quantile (Q-Q) plots (Supp. Fig. 1) and corresponding lambdas show no evidence of population stratification. The most significant signal was observed on chromosome 1 in the *TCHH* exonic gene region, top SNP rs11803731 (A/T, $p_{OFDA} = 1.14x10^{-10}$, β=.19, R^2=.97; p_{NURSE}=$1.10x10^{-15}$, β =.14; Fig. 1) increasing the likelihood of straight hair. This result replicates previous findings (Medland, Nyholt, et al., 2009) of the influence of rs11803731 on nurse reports of hair curvature. Association analyses conditioning on rs11803731 revealed no other SNP was significant at $p< 8x10^{-4}$.

SNP rs11803731 on chromosome 1 is a non-synonymous SNP located within exon 3 of *TCHH*, the presence of which would result in the substitution of a methionine for leucine at amino acid position 790 (L790M). The effect of this change has been explored previously (Medland et al). In the current study, log-likelihood tests revealed a further 11 SNPs in LD with rs11803731 had odds of <100:1 of being the top SNP, and could reasonably be considered as potential functional candidates: numerous SNPs fall within regions indicated to be transcriptionally active by the presence of histone and transcription factor binding, DNase I hypersensitivity, and of super-enhancers in cell lines derived from skin (Supp. Table 2). The second locus reaching genome-wide significance for the OFDA measure was on chromosome 15, with the minor T allele of the top SNP rs142826872, located within the *PWRN2* gene region, increasing the likelihood of curly hair (p_{OFDA} =$7.04x10^{-10}$, β =-.46, , R^2=.77; p_{NURSE} =$1.20x10^{-5}$, β =-.19). No other SNPs were significant in analyses conditioning on rs14282872, indicating a single association signal also at this locus.

For the chromosome 15 top SNP rs142826872, the log-likelihood tests revealed a further 5 SNPs in LD with odds of <100:1 of being the top SNP, and could reasonably be considered as potential functional candidates at this locus. Bioinformatic analysis indicates this region has very little transcriptional activity, making the determination of the possible functional effects difficult. All six SNPs change at least one transcription factor binding motif, but there is no evidence that any of the same TFs bind to this region in any cell line, including those derived from skin, studied to date (Supp. Table 3).

Using data from annotation of noncoding variants on HaploReg (v4.1), rs142826872 was found to interact with 5 types of DNA motifs *FoxD3, HDAC2, Pou1f1, Sox*, and *p300*. Data from the Encyclopedia of DNA elements (ENCODE) shows it also falls within an H2K4Me1 Mark region for K562 cells of the leukemia line.

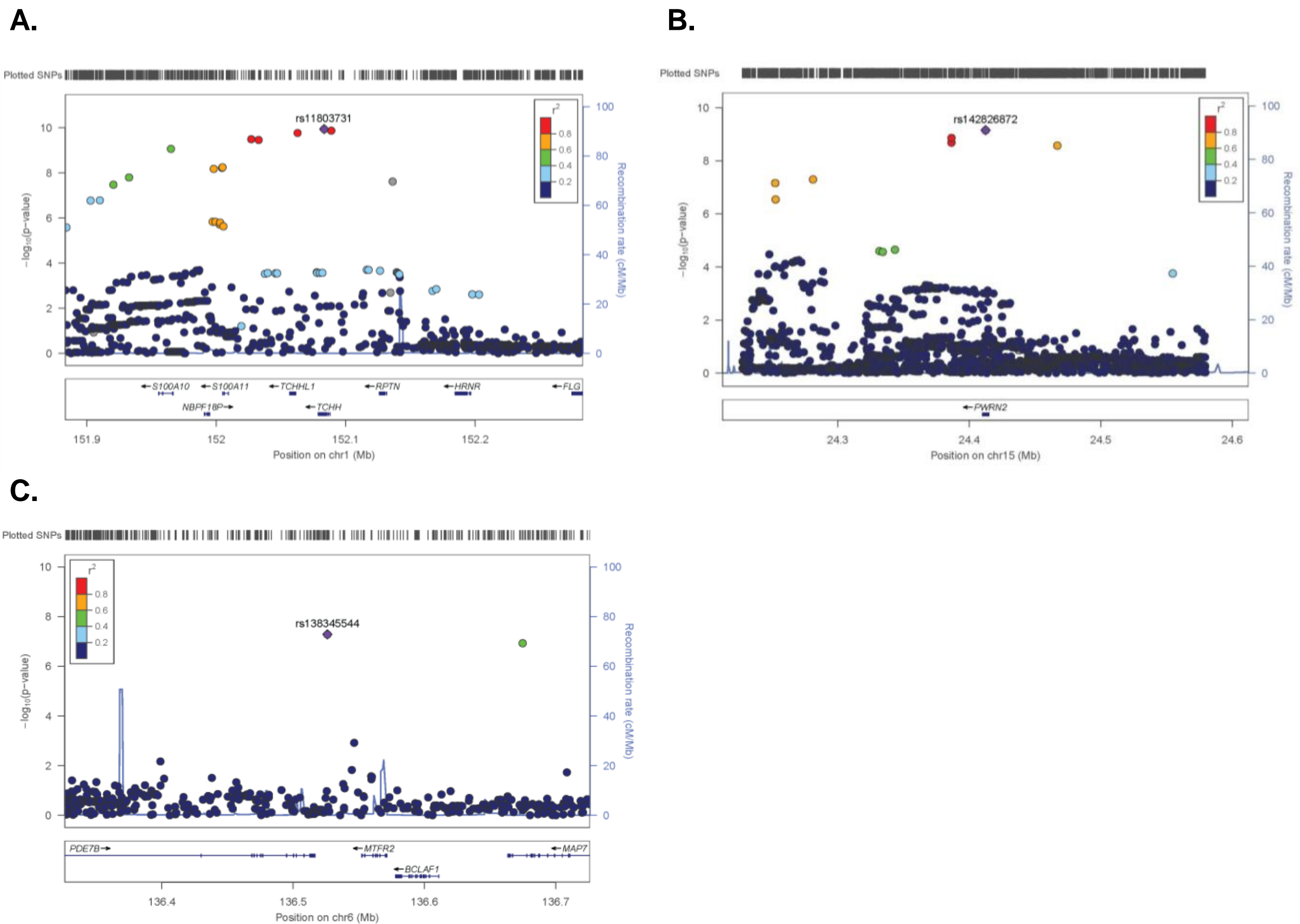

Figure 4.1. Locuszoom plots of genes including markers within 200kb from each direction of top SNPs for A. rs11803731 in *TCHH*, **B.** rs142826872 in *PWRN2*, **and C.** rs138345544 in *RP1-143G15.4.*

KGG gene-based analyses of the OFDA curvature measure supported the individual SNP findings, with a gene-based p-value of $p=1.27 \times 10^{-10}$ for *TCHH* (Supp. Table 1) and $p=7.04 \times 10^{-10}$ for *PWRN2*.

An additional signal was found on chromosome 6 located within a long non-coding RNA (lncRNA) *RP1-143G15.4* (top SNP rs138345544, $p_{OFDA} = 5.23 \times 10^{-8}$, $\beta = -.59$; $p_{NURSE} = .0009$, $\beta = -.21$). While the genotyping quality ($R^2 = .54$) was lower compared to the other two SNPs, subsequent analyses was conducted for exploratory purposes. Conditional analyses on this SNP resulted in no other variant significant at $p < 1 \times 10^{-5}$. KGG gene-based analyses of the

OFDA curvature measure supported the individual SNP findings, with a gene-based p-value of $p=1.27x10^{-10}$ for *TCHH* (Supp. Table 1) and $p=7.04x10^{-10}$ for *PWRN2*. Log-likelihood tests revealed only one SNP (rs145221413) in addition to rs138345544 had odds of <100:1 of being the top SNP, although these SNPs are in very low LD ($r^2=0.51$) with each other. This region is also very transcriptionally quiet, with few marks of transcriptional activity seen in any cell line (Supp. Table 4).

Discussion

The curvature of human hair has been shown to be a highly heritable trait. While previous association studies have identified some genetic variants influencing hair curvature using observational measures this is the first study to use quantitative measures of curvature. A comparison of the quantitative and categorical measure of hair curvature suggest that the micro level assessment of curvature provided by the OFDA analysis can detect additional variation in hair morphology that is not detected by the macro level classification.
Although no replication data were available, the genome-wide association analyses of the quantitative curvature measures identified novel variants influencing micro-level hair curvature. The strong signal observed in the *TCHH* locus replicates previous findings, serving as a positive control to assess the utility of quantitative hair curvature measures. The extent of support for the *TCHH* signal was stronger for the categorical measure than that observed on the quantitative measure. We hypothesise that this is due to either macro versus micro scale of measurement or to the sampling of only a relatively small number of hairs taken from a single area on the head. As Figure 4.2 shows, when markers are matched between OFDA and nurse report results, the p-values are predictive of each other, suggesting that OFDA methods are comparable to nurse report in the detection of variants predicting hair curvature.

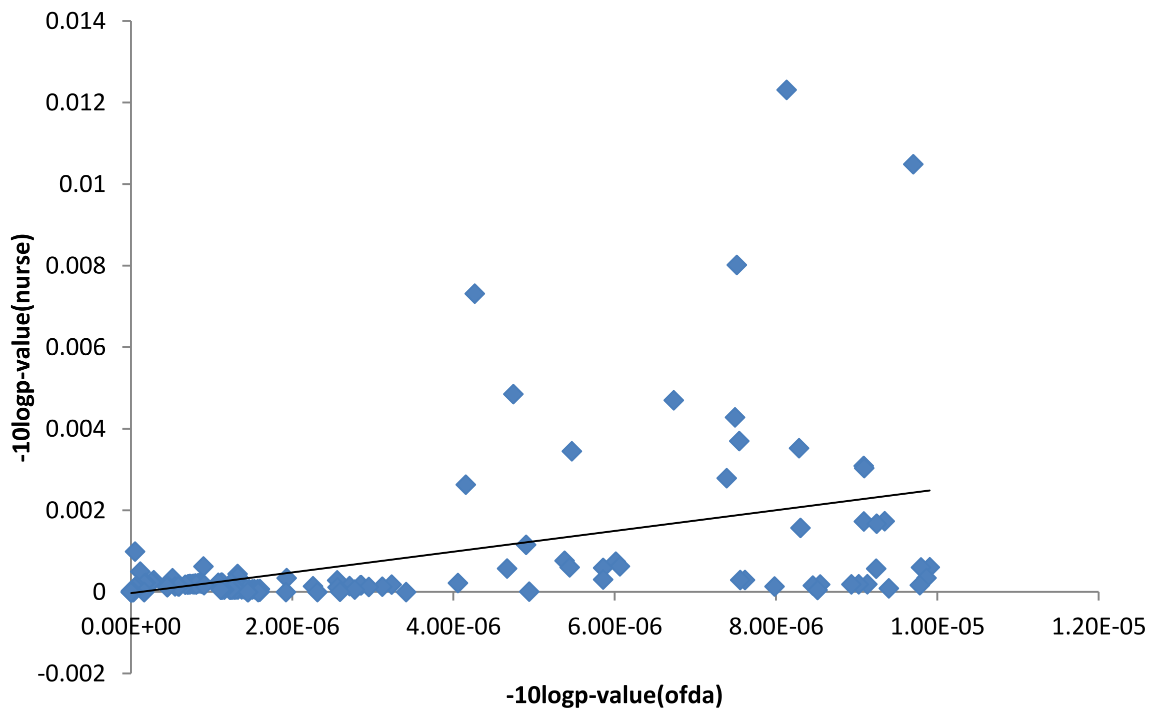

Figure 4.2. Scatterplot of SNPs at p<.00001 for OFDA measures and nurse report measures. As this figure shows, there is a positive correlation (r^2=.46) between p-values of these two measures, suggesting OFDA measures are a good predictor of nurse report curvature.

It is also of interest to note that *EDAR*, previously associated with straight hair in Asians (Tan et al., 2013), approaches the suggestive threshold with p=4.87x10^{-5}, although the variant (rs10174201) detected in this sample do not coincide with those seen in East Asians (rs3827760) and is not in LD with this variant.

While *TCHH* and *EDAR* are known to influence hair curvature, rs142826872 in *PWRN2* (Prader-Willi Region Non-coding RNA) is a novel variant non-coding RNA (ncRNA), expressed in the testis and kidney (Buiting et al., 2007) as a result of deleted or unexpressed genes transcribed only from the paternal chromosome. Previous literature regarding *PWRN2* focuses on its involvement in the development of Prader-Willi /Angleman Syndrome (PWS), characterized by cognitive and developmental abnormalities and obsessive eating, unusually fair skin and hair, and early adrenarche (growth of pubic or axillary hair) before age 8 despite delayed onset of puberty (Kalser & Chamberlain, 2015). PWS is also clinically characterized by some disorders in hair growth in neonatal and pubertal stages, which suggests *PWRN2* may also play a role in fetal development and hormonal growth (Kalser & Chamberlain,

2015). Transcriptional and regulatory factors also appear to be relevant with influences on *FoxD3*, related to the expression of vitiligo (Alkhateeb et al., 2005), suggesting this variant within *PWRN2* may be associated with dermatology related traits.

The *RP1-143G15.4* region on chromosome 6 is a long non-coding RNA (ncRNA), a non-protein coding transcript exceeding 200 bases that may play a role in gene-specific transcription, post-transcription regulation of mRNA processing, and epigenetic regulation. However, this SNP also appears to be of low imputation quality (R^2=.54) and would require further investigation via fine-mapping and future replication.

In summary, in the present study comparing observational and objective measures of hair curvature, the known *TCHH* variants influencing hair curvature were found by both methods, and regulatory functions of *TCHH* were explored in additional to replication of previous findings (Medland, Nyholt, et al., 2009). Transcriptionally active regions were also found within TCHH, which would benefit from replication and functional experiments. New variants within the *PWRN2* and *RP1-143G15.4* regions were also found using the objective measures. In terms of application, the use of continuous measures is an improvement on categorical due to the minimization of differences between individual conceptualization of categorical information (Kayser & de Knijff, 2011). This result is analogous to a previous association study of digital quantification of human eye colour, which yielded three new loci in comparison to categorical eye colour measures (Liu et al., 2010). This suggests that quantitative measures may be one way of finding novel pleiotropic variants across phenotypes.

In addition to new information on transcriptional and regulatory factors involved with the replicated variant in *TCHH*, it is interesting to note that genetic variants in *PWRN2* affecting this forensically relevant trait appears to overlap with variants implicated in a medical syndrome. In terms of future studies and potential applications, researchers may benefit from considering more medically pertinent genes within forensic genetics, and the bioethical implications of applying such knowledge in real life forensic processes.

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Supplementary Tables and Figures

Supplementary Table 4.1. Results of hair curvature GWAS, list of variants at p<1x10^{-5}. SNPs at 5x10^{-8} are shaded in grey. Subscript "OFDA" denotes results from objective measures and "nurse" denotes results from observed curvature. P-val$_{gene}$ shows gene-based p-values.

Chr	rs	BP	p-val$_{OFDA}$	OR$_{OFDA}$	p-val$_{nurse}$	OR$_{nurse}$	p-val$_{gene}$	Gene
1	rs74052807	4533536	9.24E-06	0.684544	5.74E-04	0.842497		
1	rs10047182	4534321	9.80E-06	0.68526	6.05E-04	0.84307		
1	rs11812066	4534661	9.91E-06	0.68537	6.09E-04	0.843132		
1	rs72694821	151883303	4.94E-06	1.45404	1.10E-05	1.23368		
1	rs72694822	151884287	2.59E-06	1.47165	8.03E-06	1.2385		
1	rs56147627	151902892	1.67E-07	1.54819	7.79E-07	1.27223		
1	rs111623044	151910049	1.63E-07	1.52286	2.02E-06	1.24997		
1	rs67081753	151920480	3.30E-08	1.51001	2.02E-07	1.25377		
1	rs56390241	151932589	1.58E-08	1.57781	3.99E-09	1.31991		
1	rs41265236	151964935	8.54E-10	1.56788	3.26E-09	1.28804		*S100A10*
1	rs2999559	151997199	1.45E-06	1.3388	1.66E-09	1.23773		
1	rs34974291	151998015	6.55E-09	1.45263	1.32E-12	1.30582		
1	rs3007671	151999347	1.46E-06	1.33643	2.18E-09	1.23414		
1	rs2999547	152002663	1.92E-06	1.3329	2.57E-09	1.23367		
1	rs3007674	152002984	1.58E-06	1.33534	2.24E-09	1.23411		*S100A11*
1	rs12134184	152004435	5.90E-09	1.45358	1.58E-12	1.30424		*S100A11*
1	rs12123975	152004804	5.61E-09	1.45344	1.26E-12	1.30515		*S100A11*
1	rs2999550	152005482	2.31E-06	1.32868	2.71E-09	1.23241		*S100A11*
1	rs12130862	152027015	3.18E-10	1.53346	2.52E-15	1.37064		
1	rs55668963	152032745	3.44E-10	1.53194	2.81E-15	1.36975		
1	rs17646946	152062767	1.68E-10	1.53835	7.17E-16	1.37568		
1	rs11803731	152083325	1.14E-10	1.55927	1.10E-15	1.38267	1.27E-10	*TCHH*
1	rs36010924	152088844	1.33E-10	1.55955	1.84E-15	1.38098		
1	rs4845418	152136230	2.40E-08	1.52501	7.61E-13	1.37533		
2	rs145240189	5016486	9.25E-06	2.67774	1.68E-03	1.49532		
3	rs6789549	41021263	9.87E-06	0.788046	3.47E-04	0.893687		
3	rs334550	119840731	7.54E-06	0.784832	3.70E-03	0.91239		
3	rs35450153	119844982	9.09E-06	1.27221	3.04E-03	1.09836		
3	rs73177923	119846955	9.09E-06	1.27208	3.09E-03	1.09812		
3	rs17811405	119863330	7.39E-06	1.27848	2.79E-03	1.10037		
3	rs4676832	119935080	1.51E-06	1.57241	6.54E-05	1.24747		*GPR156*
3	rs6777971	119936272	1.47E-06	1.57315	6.49E-05	1.24759		*GPR156*
3	rs4676834	119936850	1.44E-06	1.57369	6.45E-05	1.24768		*GPR156*
3	rs9811483	119936972	1.43E-06	1.5739	6.44E-05	1.24772		*GPR156*
3	rs6787458	119944395	1.24E-06	1.57754	6.19E-05	1.24817		*GPR156*
3	rs1388755	119947711	1.23E-06	1.5778	6.24E-05	1.24795		*GPR156*
3	rs6438569	119953272	1.27E-06	1.57699	6.39E-05	1.24761		*GPR156*
3	rs4321558	119953697	1.28E-06	1.57645	6.45E-05	1.24743		*GPR156*
3	rs4501147	119953764	1.29E-06	1.5764	6.46E-05	1.24741		*GPR156*

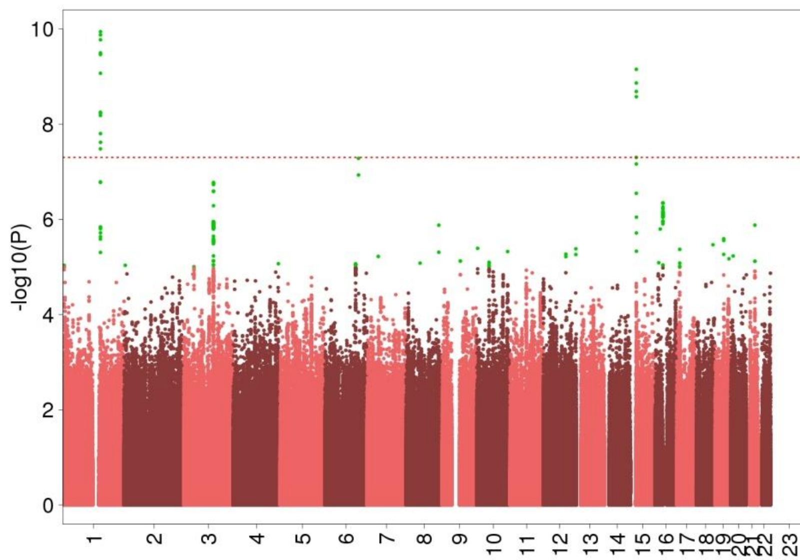
Chr	rs	BP	p-val$_{OFDA}$	OR$_{OFDA}$	p-val$_{nurse}$	OR$_{nurse}$	p-val$_{gene}$	Gene
3	rs1492271	119961596	1.37E-06	1.57451	6.70E-05	1.24682		*GPR156*
3	rs6803611	119963432	1.41E-06	1.57389	6.80E-05	1.24662		*GPR156*
3	rs6772226	119968485	1.46E-06	1.57257	6.94E-05	1.24622		
3	rs4583685	119973068	1.53E-06	1.57115	7.14E-05	1.24573		
3	rs2101656	119974065	1.57E-06	1.57036	7.21E-05	1.24555		
3	rs1907680	119976017	1.60E-06	1.56979	7.33E-05	1.24528		
3	rs1352932	119979146	1.11E-06	1.58134	5.90E-05	1.24916		
3	rs1352931	119979305	1.12E-06	1.58119	5.93E-05	1.24909		
3	rs980839	119980196	1.13E-06	1.58079	5.97E-05	1.24897		
3	rs9637364	119981494	1.16E-06	1.58009	6.01E-05	1.24882		
3	rs4263318	119985678	1.23E-06	1.57825	6.22E-05	1.24829		
3	rs4314205	119985833	1.23E-06	1.57842	6.24E-05	1.24826		
3	rs796824	119986776	1.25E-06	0.633799	6.25E-05	0.801154		
3	rs813697	119989611	1.30E-06	0.634245	6.34E-05	0.801297		
3	rs787185	119990987	1.33E-06	0.634448	6.39E-05	0.801368		
3	rs79019771	120002287	2.95E-06	0.6451	1.26E-04	0.809237		
3	rs2943755	120004384	3.12E-06	0.645764	1.33E-04	0.809824		
3	rs814182	120004402	2.56E-06	0.643129	1.17E-04	0.808326		
3	rs787211	120005723	2.71E-06	0.64441	1.47E-04	0.811197		
3	rs2971213	120007104	2.26E-06	0.642037	1.44E-04	0.810821		
3	rs1085624	120008549	2.85E-06	0.644964	1.68E-04	0.812629		
3	rs1085622	120008607	2.86E-06	0.645015	1.69E-04	0.812679		
3	rs1085620	120008762	3.23E-06	0.646653	1.84E-04	0.813689		
3	rs75888934	120014568	5.86E-06	0.652863	3.08E-04	0.819047		
3	rs2673704	120134431	5.17E-07	0.76304	3.40E-04	0.893471		*FSTL1*
3	rs1259295	120140731	2.59E-07	1.32051	2.51E-04	1.12219		*FSTL1*
3	rs1259297	120141209	2.55E-07	1.32074	2.49E-04	1.12229		*FSTL1*
3	rs2700260	120142164	1.85E-07	1.32561	2.27E-04	1.12333		*FSTL1*
3	rs916509	120143827	1.75E-07	1.32656	2.15E-04	1.12387		*FSTL1*
3	rs916512	120144064	1.68E-07	1.32702	2.08E-04	1.12415		*FSTL1*
4	rs55972184	183664946	8.51E-06	1.2876	5.18E-05	1.14401		*ODZ3*
6	rs77434204	125020121	9.13E-06	0.54932	1.93E-04	0.74696		
6	rs117010803	125020345	8.55E-06	0.54816	1.87E-04	0.746369		
6	rs117848331	125020880	8.93E-06	0.54896	1.90E-04	0.746695		
6	rs80296224	125020959	9.03E-06	0.549247	1.92E-04	0.746912		
6	rs138345544	136526012	5.23E-08	0.254179	9.94E-04	0.612961		
6	rs145221413	136674658	1.17E-07	0.248023	5.03E-04	0.580699		
7	rs34006338	45957318	6.01E-06	0.352324	7.46E-04	0.635522		*LOC100129619 IGFBP3*
8	rs7840417	56596831	8.30E-06	0.743409	1.57E-03	0.884246		
8	rs77435039	132503606	1.33E-06	0.456321	3.73E-04	0.712578		
8	rs17625395	132528118	4.90E-06	0.475626	1.16E-03	0.732919		
9	rs79624074	73486444	7.51E-06	1.86489	8.02E-03	1.23885		
9	rs72729789	73537881	7.49E-06	1.75222	4.29E-03	1.23063		

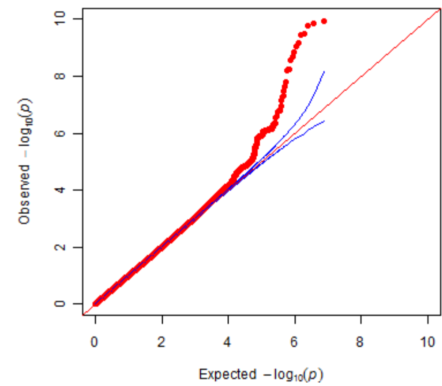
Chr	rs	BP	p-val$_{OFDA}$	OR$_{OFDA}$	p-val$_{nurse}$	OR$_{nurse}$	p-val$_{gene}$	Gene
10	rs78851323	3590343	4.06E-06	2.23852	2.22E-04	1.45605		
10	rs2663053	49984053	7.98E-06	1.28007	1.37E-04	1.13101		*WDFY4*
10	rs2928393	49989419	8.46E-06	1.27898	1.60E-04	1.12952		*WDFY4*
10	rs4838648	49992689	9.78E-06	0.783126	1.70E-04	0.885661		*WDFY4*
10	rs2127590	50013594	9.40E-06	0.780647	9.14E-05	0.880282		*WDFY4*
10	rs12253552	125265544	4.74E-06	0.343634	4.86E-03	0.674674		
12	rs117220582	91507625	6.06E-06	0.407822	6.33E-04	0.673541		
12	rs3138288	91540103	5.38E-06	0.403999	7.69E-04	0.67645		*DCN*
12	rs113975734	132179531	5.47E-06	1.31639	3.45E-03	1.10887		
12	rs73160760	132182015	4.15E-06	1.32297	2.63E-03	1.11277		
15	rs144486314	24252440	6.85E-08	0.427515	1.56E-04	0.706867		
15	rs117580763	24252731	2.84E-07	0.423805	2.91E-04	0.704077		
15	rs117329723	24281161	4.99E-08	0.431359	1.12E-04	0.706257		
15	rs141054249	24386421	2.06E-09	0.340351	1.74E-05	0.637634		
15	rs184287717	24386435	1.37E-09	0.339469	1.16E-05	0.634195		
15	rs142826872	24412369	7.04E-10	0.348112	1.30E-05	0.645772	7.04E-10	*PWRN2*
15	rs183342783	24466920	2.65E-09	0.301801	3.09E-05	0.615234		
15	rs140064998	24749760	4.66E-06	0.420333	5.78E-04	0.685463		
15	rs77727448	24773432	1.93E-06	0.392794	3.44E-04	0.665421		
15	rs141829480	24796348	9.00E-07	0.411614	6.28E-04	0.69838		
16	rs62039521	15194989	8.13E-06	0.506327	1.23E-02	0.799582		
16	rs111715700	21183307	1.60E-06	0.349309	8.21E-06	0.563021		
16	rs6565290	31864938	5.88E-07	0.935613	1.39E-04	1.01755		
16	rs8058598	31865194	6.00E-07	0.378974	1.42E-04	0.799443		
16	rs2358778	31868109	9.08E-07	0.387296	1.70E-04	0.671631		
16	rs12919368	31869688	8.11E-07	0.385324	1.85E-04	0.672792		
16	rs12924931	31873146	1.24E-06	0.392455	2.25E-04	0.676737		
16	rs1828131	31874104	1.13E-06	0.390132	2.30E-04	0.676366		
16	rs9929494	31877797	1.12E-06	0.390725	2.33E-04	0.677075		
16	rs6565291	31880101	1.08E-06	0.389888	2.30E-04	0.676375		
16	rs4889563	31880599	8.97E-07	0.387061	2.01E-04	0.674065		
16	rs7200162	31881705	8.83E-07	0.386851	2.01E-04	0.673988		
16	rs7203345	31892236	8.21E-07	2.6066	2.04E-04	1.48759		*ZNF267*
16	rs4026654	31893441	7.75E-07	0.386093	1.98E-04	0.673907		*ZNF267*
16	rs4889565	31897308	8.75E-07	0.388335	2.14E-04	0.675404		*ZNF267*
16	rs3913871	31898912	8.79E-07	0.388196	2.21E-04	0.675811		*ZNF267*
16	rs8059500	31901787	8.70E-07	0.387545	2.19E-04	0.67525		*ZNF267*
16	rs3913870	31902209	8.66E-07	0.387548	2.13E-04	0.674797		*ZNF267*
16	rs4889567	31903541	8.69E-07	0.387416	2.18E-04	0.675038		*ZNF267*
16	rs4026653	31904017	7.25E-07	0.384198	1.92E-04	0.672417		*ZNF267*
16	rs4026652	31904177	7.22E-07	0.383668	1.92E-04	0.671969		*ZNF267*
16	rs4889517	31905355	7.40E-07	0.384004	1.95E-04	0.67225		*ZNF267*
16	rs3913868	31905551	7.33E-07	0.383983	1.94E-04	0.672186		*ZNF267*
16	rs3843960	31905717	7.29E-07	0.383795	1.94E-04	0.672084		*ZNF267*
16	rs4889518	31907797	7.85E-07	0.384719	1.90E-04	0.671623		*ZNF267*

Chr	rs	BP	p-val$_{OFDA}$	OR$_{OFDA}$	p-val$_{nurse}$	OR$_{nurse}$	p-val$_{gene}$	Gene
16	rs3861268	31908168	7.32E-07	0.383189	1.94E-04	0.671558		*ZNF267*
16	rs9928704	31908963	7.24E-07	0.383123	1.91E-04	0.671351		*ZNF267*
16	rs3861270	31909782	8.47E-07	0.385332	2.16E-04	0.673413		*ZNF267*
16	rs7185691	31910324	7.22E-07	0.382765	1.91E-04	0.671102		*ZNF267*
16	rs8056158	31914232	8.29E-07	0.384485	2.12E-04	0.672592		*ZNF267*
16	rs77410910	31914422	8.34E-07	0.384761	2.12E-04	0.672859		
16	rs113454670	31915335	7.99E-07	0.383985	2.01E-04	0.671694		
16	rs138421978	31916693	9.35E-06	2.7326	1.74E-03	1.4819		
16	rs8060276	31918246	8.01E-07	0.383499	2.06E-04	0.671718		*ZNF267*
16	rs8048908	31918418	7.07E-07	0.381008	1.86E-04	1.03442		*ZNF267*
16	rs4889568	31920209	8.03E-07	0.382756	2.07E-04	0.67112		*ZNF267*
16	rs9788838	31920223	7.96E-07	0.3826	2.06E-04	0.671064		*ZNF267*
16	rs4889569	31920229	8.00E-07	0.382691	2.06E-04	0.671073		*ZNF267*
16	rs1827959	31920835	7.64E-07	0.381957	1.96E-04	0.670056		*ZNF267*
16	rs148590777	31923102	9.09E-06	2.74959	1.73E-03	1.48521		
16	rs7205034	31923949	7.05E-07	0.379808	1.85E-04	0.668296		*ZNF267*
16	rs3850114	31926619	7.82E-07	0.381694	2.03E-04	0.670187		*ZNF267*
16	rs7197804	31928620	4.59E-07	0.373001	1.58E-04	0.664841		*ZNF267*
16	rs3843961	31931289	6.75E-07	0.378687	1.81E-04	0.667431		
16	rs11150650	31935901	5.48E-07	0.374909	1.41E-04	0.662086		
16	rs11150651	31936095	4.55E-07	0.372735	1.25E-04	0.660354		
16	rs4889520	31936869	4.50E-07	0.372312	1.23E-04	0.659851		
16	rs12599066	31937829	4.52E-07	0.371678	1.25E-04	0.659512		
17	rs9911061	11534848	8.29E-06	0.7965	3.53E-03	0.916441		*DNAH9*
17	rs10521184	11536464	9.70E-06	0.798043	1.05E-02	0.926312		*DNAH9*
17	rs7217504	11538145	4.26E-06	0.789697	7.32E-03	0.922379		*DNAH9*
18	rs7506960	67304536	3.41E-06	0.770947	4.76E-07	0.848115		*DOK6*
19	rs117428370	34767593	5.44E-06	0.351095	6.07E-04	0.63158		
19	rs144511794	34852656	2.56E-06	0.338905	2.88E-04	0.615199		
19	rs8191365	34858598	2.78E-06	0.387956	6.16E-05	0.618088		*GPI*
19	rs79830083	55952899	6.73E-06	0.399458	4.70E-03	0.717588		
20	rs73093818	9182966	5.86E-06	0.466252	5.93E-04	0.710141		
21	rs2834251	35084921	1.32E-06	0.644421	4.44E-04	0.830318		*ITSN1*
21	rs8129602	35120983	7.56E-06	0.654814	2.96E-04	0.819295		*ITSN1*
21	rs2276227	35122725	7.61E-06	0.654931	2.97E-04	0.81933		*ITSN1*

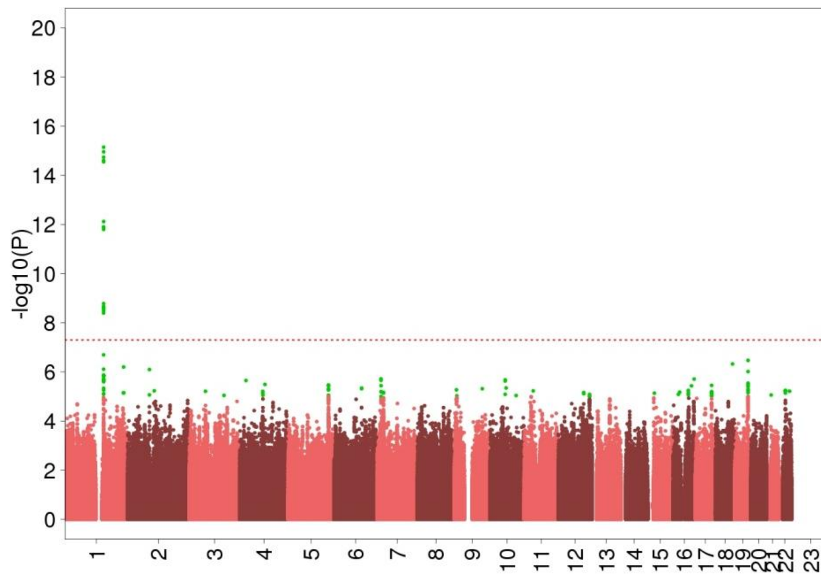
Supp. Figure 4.1. Manhattan and quantile-quantile (Q-Q) plots of univariate GWAS for a. OFDA curvature; b. nurses' report of curvature. Top hits on chromosome 1 for OFDA curvature and nurse report curvature are within the *TCHH* gene region, rs11803731, $p_{OFDA} = 1.14 \times 10^{-10}$; $p_{NURSE} = 1.10 \times 10^{-15}$. Genomic inflation for OFDA measures was $\lambda = 1.002$; nurse report $\lambda = 1.011$.

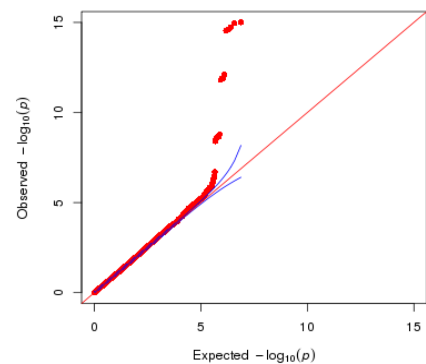
A





B





Supplementary Table 4.2. Haploreg results for the most likely causal candidate SNPs increasing the likelihood of straight hair in the TCHH gene region on chromosome 1.

SNP	Pos (hg38)	Ref	Alt	EUR freq	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	Motifs changed	NHGRI/ EBI GWAS hits	GRASP QTL hits	Selected eQTL hits	GEN-CODE genes	dbSNP func annot
rs11803731	152110849	A	T	0.22	ESC, ESDR, LNG, IPSC, FAT, STRM, BRST, BLD, VAS, LIV, BRN, GI, PANC, O	ESDR, IPSC, BLD, BRN, LNG, GI	ESC,ESDR,ESC,IPSC,BRST,**SKIN,SKIN,SKIN**,LNG,GI,BRN	CTCF,POL2		1 hit	2 hits	4 hits	TCHH	missense
rs36010924	152116368	A	G	0.22					RXRA			2 hits	2.3kb 5' of	
rs17646946	152090291	G	A	0.23		FAT, GI	GI,PLCNT,CRVX	STAT3	Foxo,Pax-4,STAT	1 hit	1 hit	4 hits	1.2kb 5' of	
rs12130862	152054539	A	T	0.22		PANC			TCF4			3 hits	6.6kb 5' of	
rs55668963	152060269	T	C	0.22					Mef2,PU.1,Pbx-1,Znf143			2 hits	12kb 5' of	
rs41265236	151992459	C	G	0.2	ESC, ESDR, LNG, IPSC, FAT, STRM, BRST, BLD, MUS, BRN, **SKIN**, VAS, LIV, GI, KID, PANC, PLCNT, HRT, OVRY, SPLN, CRVX, BONE	ESDR, BLD, BRN, HRT, THYM, GI	ESC,ESDR,ESDR,ESDR,ESC,LNG,IPSC,IPSC,BRST,BLD,BLD,BLD,**SKIN,SKIN,SKIN,SKIN**,HRT,GI,GI,KID,LNG,MUS,MUS,PLCNT,GI,THYM,GI,OVRY,PANC,MUS,GI,LNG,CRVX,BRST,MUS,MUS,VAS,BLD,BLD,BRN,SKIN,SKIN,LNG	GR,POL2,ERALPHA_A,POL24H8,TCF4,CFOS,CJUN,AP2ALPHA,BAF155,BAF170,BRG1,CMYC,E2F4,GTF2F1,INI1,JUND,MAX,MXI1,P300,POL2S2,RAD21,RFX5,SMC3,STAT1,STAT3,TAF1,HAE2F1,POL2B,GATA3,ZNF263,NRSF	En-1,Gbx1,Hoxa3,Hoxa7,Hoxb3,Hoxb7,Msx2,Nkx1-1			1 hit	S100A10	intronic
rs12123975	152032328	A	G	0.25		FAT, BRST, BLD, **SKIN**, ADRL, GI	ADRL,BRST,BLD,SKIN	POL2B	CTCF,Cart1,Foxj1,GATA,HNF1,Sox,TCF11::MafG,Zfp105			1 hit	AL591893.1	
rs12134184	152031959	C	T	0.25		FAT, BLD, BRN, ADRL,	**SKIN**	POL2	Myc,RFX5				AL591893.1	
rs34974291	152025539	A	G	0.25		**SKIN**, BRST			FAC1,Foxd3,Foxp1,Foxp3,Irx,Pax-4,TATA				AL591893.1	
rs56390241	151960113	T	G	0.18					Foxa,Foxi1,Foxj1,Foxj2,Foxl1,Foxp1,Foxq1,Pou2f2,Pou6f1,p300			1 hit	23kb 3' of S100A1	
rs4845418	152163754	G	C	0.2		ESDR, FAT, BLD, MUS, **SKIN**, GI,	BLD		AP-1,Irf,Maf,Nanog,PRDM1,STAT,TATA,TCF12			3 hits	4.5kb 5' of RPTN	
rs67081753	151948004	C	T	0.2		**SKIN**, ADRL, CRVX, BRST		CTCF	AP-1,BCL,CACD,CHD2,Egr-1,Ets,MOVO-B,Pax-4,RREB-1,SP1,SRF,STAT,UF1H3BETA,VDR,ZNF219,Zfp281,Zfp740,Znf143			6 hits	35kb 3' of S100A10	

Supplementary Table 4.3. Haploreg results for the most likely causal candidate SNPs increasing the likelihood of straight hair in the PWRN2 lincRNA region on chromosome 15.

SNP	Pos (hg38)	Ref	Alt	EUR freq	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	Motifs changed	NHGRI/EBI GWAS hits	GRASP QTL hits	Selected eQTL hits	GENCODE genes	dbSNP func annot
rs142826872	24167222	C	T	0.02					Foxd3,HDAC2,Pou1f1,Sox,p300				PWRN2	
rs184287717	24141288	T	C	0.01					BATF,BCL,Irf,PU.1,Pax-5,RXRA,STAT,p300,p53				21kb 3' of PWRN2	
rs141054249	24141274	A	G	0.01					DMRT4,TCF4				21kb 3' of PWRN2	
rs183342783	24221773	C	G	0.01					Gfi1,NF-Y,SP1				RP11-580I1.1	
rs117329723	24036014	C	T	0.02					AP-1,AhR::Arnt,Arnt,CHOP::CEBPalpha,Pax-8				33kb 3' of RP11-484P15.2	
rs144486314	24007293	C	T	0.02									3.9kb 3' of RP11-484P15.2	

Supplementary Table 4.4. Haploreg results for the most likely causal candidate SNPs increasing the likelihood of straight hair in the RP13-143G15.4 gene region on chromosome 6.

SNP	Pos (hg38)	Ref	Alt	EUR freq	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	Motifs changed	NHGRI/EBI GWAS hits	GRASP QTL hits	Selected eQTL hits	GENCODE genes	dbSNP func annot
rs138345544	136204874	G	A	0.02					SIX5				RP13-143G15.4	
rs145221413	136353520	T	C	0.01		PANC			SIX5				MAP7	intronic

Preface to Chapter 5: Topic Overview – Perceptions of DNA Evidence

The legal systems of most Western countries are evidence-based and the verdict largely depends on the direction and quality of evidence presented in trial. The previous chapters focused on two types of commonly presented evidence, fingerprints and hair. The scientific basis of these types of evidence has made it possible to objectively scrutinize the validity and accuracy of modern forensic practices. In accordance with major court decisions such as Daubert v. Merrell Dow Pharmaceuticals, 1993 and its antecedents (General Electric v. Joiner, 1996; Kumho Tire v. Carmichael, 1999), together referred to as the Daubert trilogy, this scrutiny of the reliability and scientific validity of evidence governs admissibility of evidence to court.

5.1 DNA Evidence

One type of evidence that has undergone particularly rigorous scientific and forensic scrutiny is DNA matching evidence. Since the discovery of short tandem repeats (STRs) - highly variable and heritable repetitive DNA patterns - and the development and inclusion of "DNA fingerprinting" methods within law enforcement investigation systems, several highly publicised cases have occurred where the scientific validity of DNA evidence has been put on trial.

One notable example is the recent case of Knox and Sollecito, 2007, which came under focused media attention when the DNA evidence sourced from a murder scene contributed to a widespread debate regarding the accuracy and standards of DNA matching evidence, ultimately leading to the exoneration of the lead suspects.It should be noted that the Knox and Sollicito case utilised low copy number (LCN) DNA analysis, which necessitates increased polymerase chain reaction (PCR) cycles to produce a full profile from a small DNA sample. This makes interpretation of results more difficult due to higher possibility of contamination. The controversy surrounding LCN DNA analyses led to an investigation into the validity of this method (Caddy et al., 2008), concluding that while LCN DNA was a robust scientific method, the strength and weight of evidence should still be a salient area of consideration by jury members.

"Classical" DNA profiling used in cases such as these typically involves the enzymatic breakdown of latent DNA sources, such as blood, saliva, and semen, which is tagged by DNA probes containing microsatellites or oligonucleotide sequences. In the Australian context, human identification kits such as ProfilerPlus are used which provides information on nine tetranucleotide STRs and the amelogenin locus via PCR, As technology improved, more sophisticated technique of DNA profiling developed, such as the multiplex PCR which allows the user to identify several types of target DNA sequences or SNPs simultaneously (Hayden et al., 2008). DNA profiling kits currently in use have become much more sensitive than the past, making it possible to analyse DNA traces not only from blood and saliva samples but also from "touch DNA" – biological material transferred from human to surface through direct contact (Cale, 2015). These DNA amplification kits, combined with the power of next generation sequencing, are capable of generating suspect profiles from as little as 1 trillionth of a gram of DNA material.

While corresponding technological and methodological advances have improved the quality and efficiency of "DNA fingerprinting" evidence in general, the nature of the evidence presents several limitations. The often degraded quality and quantity of DNA material retrievable from a latent scene, makes matching methods difficult. The dependence on an existing record of genotypes means that despite the thoroughness and impartiality of the laboratory responsible, a suspect's DNA profile cannot be identified unless they have had previous criminal records that justified the collection of their DNA samples.

5.2 Statistics and the Jury

In addition to these technical limitations, advances in DNA fingerprinting have also brought forward new challenges for standards of evidence. For example, with the advancement of genome-wide genotyping methods, new analytical approaches have arisen that can allow the identification of genetic variants influencing physical traits, the forensic implications of which are unexplored. There is a possibility that phenotypes could be predicted from genetic information gathered from latent blood and saliva samples at crime scenes, providing additional information about the perpetrator's physical appearance. Sufficiently accurate phenotypic prediction from genetic variants has been achieved in the past for eye and hair colour (Walsh et al., 2013).

These analytical approaches are not without shortcomings. The type I error rate in GWAS is high due to the large number of variants being tested. The GWAS results are not necessarily replicable or robust. Communicating this specialised, biostatistcial concept to a legal and jury-eligible audience would be extremely challenging. While no studies have been conducted on the layman's understanding of statistics of association studies, research and legal precedence, shows understanding of probabilities in a legal context is increasingly crucial with scientific expert witness testimony. For example in R v Clark (2003), a case relating to sudden infant death syndrome (SIDS) and the indictment of the child's mother for infanticide. In this case an expert paediatrician stated that the overall SIDS risk is 1 in 73 million, which led to the mother being found guilty. However, the sentence was overturned on appeal when it was shown that the environmental and genetic factors leading to higher SIDS risk (i.e. the prior probability) were not taken into account. In the context of the application of probabilistic DNA evidence in court, the "prosecutor's fallacy" is a common phenomenon, where the prior probability of a suspect's innocence given a DNA match i.e. P(Innocent| Match) is erroneously equated with the probability of a match given a suspect's innocence, i.e. a false positive: P(Match| Innocent). Despite the importance and usefulness of these mathematical principles, previous research has shown that probabilistic evidence is frequently underweighted, misinterpreted, or ignored in favour of evidence specific to a case or situation. This may be partially explained by the counter-intuitive nature of statistical probabilities, and outlines the need for a clear and unbiased presentation and explanation of statistical evidence to jury members.

In terms of association studies, relevant statistical concepts would include the idea polygenicity, replicability, generalisability and "Winners's Curse". Polygenicity refers to the idea that many genes may be influencing a trait, while replicability and generalisability refers to characteristics of scientific results, whether the results could be replicated in an identical experimental condition, and whether results obtained from a sample are applicable to the general population. "Winner's Curse" is a phenomenon observed in GWAS studies, where a significant result is found in one study, but subsequent replications fail to repeat the finding due to a "regression toward the mean", meaning the tendency of data to reach the average with more observations (Ioannidis, 2008).

5.3 The "CSI" Effect

While cognitive and common-sense fallacies within a jury audience affect legal proceedings, it is also necessary to investigate the influence of media on jury perceptions. The "CSI effect" is a well-known hypothesis that gained media attention and traction around the early 2000s, positing two main effects on jury verdicts. Firstly, increased exposure to popular police procedural, crime and legal dramas such as CSI, NCIS and Bones led to increased expectations of forensic science availability and accuracy, hence contributing to failures to convict when such expectations were not met in a real court of law (Podlas, 2006). While this hypothesis is pervasive in the media and attracted much attention with researchers in the behavioural and social sciences, there has been to date a lack of empirical evidence to support this phenomenon (Hui & Lo, 2015; Kim, Barak, & Shelton, 2009; Schweitzer & Saks, 2007).

The second way the "CSI effect" may influence jury decision is inflated confidence in DNA evidence and an increase in convictions when DNA evidence is provided in court (Schweitzer & Saks, 2007). One study of jury member perception in Australia found that convictions were 23-50 times more likely when scientific evidence was provided (Briody, 2006). In one recent study investigating the perception of DNA evidence compared to other typed of evidence in a mock jury, Liebermann (2008) found across three studies that DNA evidence was rated as significantly more accurate and persuasive compared to other types of evidence such as fingerprint and eyewitness statements, even when damaging witness cross-examination discredited the DNA evidence. Moreover, scientific evidence such as DNA and fingerprint evidence was found to be more accurate and persuasive compared to testimonial evidence.

Subsequent literature has extended research on the "CSI effect" by addressing other aspects of media consumption, such as perceived realism rather than frequency of exposure, and found that amongst Canadian psychology undergraduates in a mock jury setting, both exposure frequency and perceived realism had direct and indirect influences on attitudes toward DNA evidence (Maeder & Corbett, 2015).

In addition to the "CSI effect", other factors that may affect jury perception of evidence includes the demographic of eligible members, their previous experience as jurors, and mathematical, biological, and legal education.

Similar to DNA matching evidence, if genetic association results are to be applied to a forensic context, the implications on jury perception of evidence and their views on association must be studied, in order to formulate an efficient way to communicate the biostatistical aspect of genetic evidence. The following chapter describes a survey study carried out within a jury-eligible sample, using measures for media consumption, perceived media realism, previous education and genetic and statistical comprehension.

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Chapter 5: Attitudes and evaluation of Genome-Wide Association Studies (GWAS) in a jury-eligible sample

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Abstract

Previous studies have found a high level of perceived accuracy of DNA evidence, however these studies mainly focused on DNA profiling methods. With the improvement of genetic technology, newer ways of utilizing genetic information to identify individuals have emerged. Genome-wide association analyses (GWAS) have been conducted focusing on the discovery of variants associated with forensically relevant traits. The results of these analyses could potentially be used to predict these traits to aid in the identification of suspects. In order to survey attitudes and comprehension of GWAS in the context of forensic evidence, the present study recruited 412 psychology first-year participants and collected data on attitudes toward DNA and GWAS evidence in comparison to other evidence types, as well as the potential usefulness of GWAS evidence in different forensic procedures. Measures were included to assess potential "CSI" and realism of media effects, as well as participants' education levels and their comprehension of genetic and statistical information. Results show the predictors explained a significant amount of variance for "importance of GWAS in convicting a suspect when other evidence is inconclusive" (R^2=18%, $F(14, 166) = 2.618$, $p=.002$) and this was mainly driven by genetics comprehension ($\beta=-.168$, $p=.021$), and perceived realism of media ($\beta= .187$, $p =.016$). This study concludes that scientific literacy and an understanding of basic genetics may be helpful in the evaluation of DNA and GWAS evidence. Moreover, the "CSI effect" was found to be dependent on media type and perceived realism of media. These results show the importance of transmission of scientific and genetic knowledge to jurors before expert testimony is given.

Introduction

The use of DNA profiling in criminal cases is well established, and has acquired widespread recognition as a valid method of determining the presence of an individual at a crime scene. Although methods have improved over the years with technology and research, there are limitations to this kind of evidence. For instance, the amount of trace DNA left at the crime scene or on the victim may be insufficient for some types of analyses. In addition, matching depends on genetic databases of prior offenders, or the ability to collect samples from suspects, which can severely limit the usefulness of current DNA profiling methods.

In addition to the use of DNA evidence for identification and confirmation of suspects, there is interest in the use of genetic evidence in the information gathering stages of an investigation. Results of genome-wide association studies (GWAS) could potentially provide a way to predict aspects of a person's physical appearance from their DNA. While the amount of variance explained by a single variant is rarely higher than 1%, cumulatively it may be possible to accurately predict a complex trait by calculating a risk score. In corroboration with testimony and/or other physical evidence, GWAS may allow a higher degree of confidence in the evidence presented or help guide the search for suspects.

In the forensic context, research into the statistical nature of DNA evidence is of interest as these types of evidence can be used to strengthen or debate cases for a defendant. Common examples of statistical evidence include the probability of a blood type or bullet lead match between that found on a suspect or victim and that found on an established crime scene (Kasa, 2007; Thompson, 1989). Previous studies have looked at how juries respond toward DNA evidence. A large US survey conducted by Carlson (2005) showed 27% of participants believed that DNA evidence is completely reliable and 58% thought it was very reliable. In a other series of studies in the US, Liebermann (2008) compared DNA evidence with other types of physical evidence commonly found in crime scenes (fingerprints, hair fibers, eyewitness testimony, victim testimony), using a self-administered questionnaire and manipulating the type of evidence found, the nature of evidence (incriminatory or exculpatory) and the type of crime (murder or rape) in a scenario. In their second and third studies, they added damaging cross-examination testimony and relevant jury instructions. The results across studies showed an extremely high confidence in the accuracy of DNA evidence (95%) and its indication of the suspect's guilt (94%) compared to other physical evidence,

and only a qualitative cross-examination of DNA evidence led jurors to realize the limits of this type of evidence.

While existing literature provides some insight into the statistical misconceptions and fallacies that may affect jurors, prosecutors and defenders in the application of DNA profiling, there is yet to be any investigation on whether GWA evidence may affect juries' decision-making process, and whether this information is readily comprehensible to jury-eligible individuals. This study aims to examine the interface between human perception and GWA evidence in legal settings, specifically the perceived importance and confidence in GWA evidence. In future investigations, the results of this study may help determine whether evidence based on GWA results is a useful tool in informing verdicts, and how statistical evidence in the context of expert witness testimony may be presented to optimize understanding within juries and law enforcement.

Methods

Participants

In exchange for first year psychology course credit, 412 jury-eligible participants aged 17 and above were recruited for this study described as an "online questionnaire measuring attitudes toward DNA evidence". Seventy-one percent of this sample was female, with a mean age of 19.94 years, and the majority of participants reported their ethnicity as "European" (37.9%) or "Asian" (34.7%) and speaking English as their first language (72%). The vast majority of the sample (94.7%) did not have prior experience as a juror. Most participants reported their highest level of education as Year 12 (77.2%) and were currently in their 1st to 2nd year Bachelors degree (78.2%), and had some form of science or mathematical training (72.6%) in senior high school.

Materials

An online questionnaire was developed using the Qualtrics software, using concepts from Lieberman's (2008) study that compared different types of evidence with DNA matching. In the present study, participants were provided with a brief description of the concepts of GWAS which was based on public information available on the National Institute of Health website (http://www.genome.gov/20019523; Appendix A). To test participants' comprehension of this information, the presentation of the information was followed by a multiple-choice comprehension test (Appendix B).

Participants' media consumption was measured in order to control for what literature has termed the "CSI effect", where perception of forensic evidence accuracy is apparently inflated by increased exposure to popular crime and legal dramas. A Likert-like sliding scale with five levels was used where participants were asked about their general media consumption habits as well as their specific TV show preferences (Appendix C). Perceived realism of the TV shows was also measured using a Likert scale (Appendix C). Mathematical and statistical knowledge was also measured, as understanding of probability is relevant to comprehending and applying statistical information. A short timed statistical quiz composing of 9 questions was added to the end of the questionnaire (Appendix D). Questions were developed by referencing first-year statistical exam questions from the University of Queensland and paraphrasing the question content or changing numerical figures. In order to determine a suitable level of difficulty for this section, a pilot study was carried out prior to data collection and questions where the percentage of correct answers were under 5% were changed or removed.

In order to assess attitudes toward GWAS evidence, measures for usefulness of GWAS in different forensic practices were included in the questionnaire (Appendix E) (i.e. finding suspects, narrowing down a list of suspects, confirming suspect identity, convicting a suspect when other evidence in inconclusive, and convicting a suspect when other evidence is somewhat conclusive). We also asked participants about the relative trustworthiness and usefulness of GWAS evidence compared to other types of forensic evidence (i.e. eyewitness testimony, expert witness testimony, psychologist testimony, video and photographic evidence, fingerprint matching, DNA matching, postmortem examination reports; Appendix F).

Other data collected included past juror experience and gambling habits, as these may inform participants' attitudes toward statistical evidence. Self-report levels of understanding of maths and statistics, DNA evidence, other types of evidence, and legal proceedings were also measured.

Procedure

Prior to the study, participants were given information sheets containing background information and the aim of the study, and notified that participation was voluntary and that they would not be penalised for withdrawal from the study at any time (Appendix G). They were then instructed to follow a website link to the online questionnaire and complete it within 24 hours of receiving instructions. The first page of the questionnaire included a webpage requiring participants to give full informed consent before being forwarded onto the initial demographics survey. All participants completed the same questionnaire. After the study, participants were given a debriefing sheet (Appendix H) detailing the research literature behind the study, and asked to contact the researcher (YW Ho) by phone or email if questions or concerns about the study arise. Ethical approval was given by the University of Queensland School of Psychology (Ethics approval number: 14-PSYCH-PHD-39-JS).

Statistical Analyses

Total scores for maths and genetic comprehension were calculated by summing total of correct answers for each section. To ensure validity of measures, 155 cases where time elapsed for the maths test was less than 3 minutes set to value "missing". Multivariate outliers were examined which resulted in the exclusion of one case. Level of education for each type of knowledge area (i.e. mathematical, legal, and scientific) and total amount of crime and legal consumption were also summarized by using unit-weighted composite scores in SPSS.

Results

Results of maths and genetics knowledge tests shows that 86.1% of participants scored above 6.5/13 for genetics comprehension and 26% scored above 5/10 for the maths test. Independent t-tests showed that across different forensic procedures, the importance of GWAS was considered significantly higher than the midpoint of the scale (i.e. 2.5, Table 5.1) with "importance of GWAS in narrowing down a list of suspects" scoring the highest ($M= 3.55$, $p < .001$), and "importance of GWAS in convicting a suspect when other evidence is inconclusive" the lowest ($M=2.80$, $p<.001$). However, all other types of evidence were perceived as significantly more trustworthy ($p < .05$, Table 5.1) than GWAS evidence ($M=4.95$, $SD = 1.20$), and DNA matching was perceived as the most trustworthy ($M=5.85$, $SD = .87$, $p < .001$).

Table 5.1. Means, standard deviations, and range of participant responses for dependent variables

Variable	N	Mean	SD	Range
Importance of GWAS in finding suspects	294	2.9422	1.19729	0-5
Importance of GWAS in narrowing down list of suspects	295	3.5559	1.07353	0-5
Importance of GWAS in confirming suspect identity	298	3.2987	1.28238	0-5
Importance of GWAS in convicting a suspect when other evidence is inconclusive	294	2.7959	1.30339	0-5
Importance of GWAS in convicting a suspect when other evidence is somewhat conclusive	297	3.0909	1.14276	0-5
Trustworthiness of eyewitness testimony	301	3.9900	1.00162	1-7
Trustworthiness of expert testimony	303	5.0660	.98781	2-7
Trustworthiness of forensic scientist testimony	301	5.4518	.93551	1-7
Trustworthiness of psychologist testimony	302	4.7715	1.03307	2-7
Trustworthiness of video/photographic evidence	302	5.5033	1.15781	2-7
Trustworthiness of fingerprint matching evidence	301	5.5615	.93828	3-7
Trustworthiness of DNA matching evidence	302	5.8510	.86707	3-7
Trustworthiness of postmortem examination evidence	302	5.2748	1.05998	1-7
Trustworthiness of GWAS evidence	299	4.9532	1.20311	2-7
Confidence in GWAS evidence needed to consider "beyond reasonable doubt"	303	77.3234	20.20543	5-100

Multiple regressions were performed on ratings of GWAS evidence trustworthiness and usefulness on different forensic procedures compared to other types of evidence. Predictors included in the model were composite scores of education levels in five areas (maths, law, science, psychology, and other), composite scores for media consumption for two types of television shows (crime and legal), perceived realism of consumed media, total scores in the genetic comprehension test, and the total test score in the statistical quiz, controlling for ethnicity, sex, and age. In addition, as interaction between realism and frequency of media consumption was found in prior literature (Maeder & Corbett, 2015), an interaction term was included to test its significance within the model.

In order to address the problem of multiple testing, a Bonferroni correction was used on the p-value for each set of tests, resulting in an adjusted threshold of p=.006 for trustworthiness of evidence and p=.008 for importance of GWAS.

In terms of comparing GWAS evidence with other types of evidence, the overall model did not reach the corrected significant threshold for any of the dependent variables. Moreover, the model was nominally significant for psychologists testimony and fingerprint matching only.

Results for usefulness of GWAS evidence shows that the predictors explained a significant amount of variance for importance of GWAS in convicting a suspect when other evidence is inconclusive (R^2=18%, F (14, 166) =2.618, p=.002; Table 5.3). This was significantly predicted by genetics comprehension (β=-.168, p =.021), and perceived realism of media (β= .187, p=.016). No other models were significant at p<.008. However, it is interesting to note that genetics comprehension appeared to also have a significant negative effect on the perceived usefulness of GWAS on confirming a suspect identity (β =-.18, p=.017) and convicting a suspect when other evidence is somewhat conclusive (β =-.17, p=.017), and a positive effect on threshold needed to consider GWAS evidence "beyond reasonable doubt" (β =.18, p=.023).

Table 5.2. Standardised coefficients (β) for multiple regressions on trustworthiness of evidence types (eyewitness, expert, forensic scientist, psychologist testimony, video/photographic, fingerprint matching, post-mortem, DNA matching, and GWAS evidence) **using predictors** (Maths and genetics test total, maths, law, science, psychology education, frequency of media consumption and perceived realism of media)**(standard errors in parentheses, significant predictors shaded in grey), and variance explained by predictors for total model,** $p_{bonferroni}$=.006.

	Trustworthiness of evidence								
	Eyewitness testimony	Expert testimony	Forensic scientist testimony	Psychologist testimony	Video/ photo evidence	Fingerprint matching evidence	Post-mortem report	DNA matching evidence	GWAS evidence
Maths test total	0.09 (0.08)	0.1 (0.07)	0.09 (0.07)	0.09 (0.08)	-0.08 (0.09)	-0.05 (0.08)	0.13 (0.08)	0.06 (0.06)	0.02 (0.07)
Genetics test total	0.01 (0.08)	0.11 (0.07)	0.1 (0.07)	0.02 (0.08)	0.12 (0.09)	-0.08 (0.07)	0.09 (0.08)	-0.06 (0.06)	-0.11 (0.06)
Maths education	0.03 (0.11)	0.02 (0.11)	-0.02 (0.1)	-0.07 (0.11)	-0.06 (0.13)	0.16 (0.11)	0.03 (0.11)	0.05 (0.09)	0.01 (0.13)
Law education	0.04 (0.09)	0.07 (0.08)	-0.04 (0.08)	0.15 (0.09)	-0.02 (0.1)	-0.13 (0.09)	-0.06 (0.09)	-0.08 (0.07)	-0.02 (0.1)
Science education	0.02 (0.19)	0.1 (0.17)	0.16 (0.16)	-0.01 (0.18)	-0.1 (0.21)	-0.01 (0.18)	0.03 (0.18)	0.19 (0.15)	0.2 (0.21)
Psychology education	-0.04 (0.11)	0.02 (0.1)	0.02 (0.09)	0.11 (0.1)	-0.01 (0.12)	-0.06 (0.1)	0.12 (0.11)	-0.02 (0.09)	-0.01 (0.12)
Other education	0.08 (0.25)	0.03 (0.24)	0.06 (0.22)	0.09 (0.25)	-0.01 (0.28)	-0.09 (0.24)	0.09 (0.25)	-0.04 (0.21)	0.07 (0.28)
Freq of media consumption (Crime)	-0.21 (0.1)	-0.05 (0.1)	-0.04 (0.09)	0.1 (0.1)	-0.16 (0.12)	-0.14 (0.1)	0.12 (0.1)	-0.08 (0.09)	0.03 (0.12)
Freq of media consumption (Legal)	0.15 (0.09)	-0.08 (0.08)	-0.11 (0.08)	-0.16 (0.09)	-0.08 (0.1)	0.05 (0.08)	-0.15 (0.09)	0.17 (0.07)	-0.01 (0.1)
Perceived realism	0.2 (0.09)	0.11 (0.09)	0.12 (0.08)	0.24 (0.09)	0.09 (0.1)	0.22 (0.09)	0.07 (0.09)	0.15 (0.08)	-0.05 (0.1)
Freq of media x Perceived Realism	0.05 (0.11)	0.14 (0.1)	0.07 (0.09)	0.01 (0.1)	0.03 (0.12)	0.08 (0.1)	0.17 (0.11)	0.04 (0.09)	0.18 (0.12)
Rsq	9.5%	10%	7.6%	14.1%	1.8%	14.2%	11.9%	11.9%	9,7%
p-value	.24	.21	.48	.02	.21	.02	.08	.08	.22

Table 5.3. Standardised coefficients (β) for multiple regressions on importance of GWAS for finding suspects, narrowing down a list of suspects, confirming suspect identity, convicting suspect and threshold for "beyond reasonable doubt" in GWAS evidence **using predictors** (Maths and genetics test total, maths, law, science, psychology education, frequency of media consumption and perceived realism of media) **(standard errors in parentheses, significant predictors shaded in grey), and variance explained by predictors for total model**, models significant at $p_{bonferroni}$=.008 marked with asterisk (*).

	Importance of GWAS					
	Finding suspects	Narrowing down list of suspects	Confirming suspect identity	Convicting suspect (other evidence inconclusive)	Convicting suspect (other evidence somewhat conclusive)	Confidence in GWAS evidence needed to consider "beyond reasonable doubt"
Maths test total	-0.07 (0.1)	-0.04 (0.08)	0.01 (0.1)	-0.02 (0.1)	-0.01 (0.08)	0.02 (1.41)
Genetics test total	-0.04 (0.09)	-0.07 (0.08)	-0.18 (0.09)	-0.17 (0.09)	-0.18 (0.08)	0.18 (1.38)
Maths education	-0.04 (0.14)	0.02 (0.12)	-0.07 (0.14)	-0.09 (0.14)	-0.03 (0.12)	0.13 (2.02)
Law education	-0.08 (0.11)	0.02 (0.1)	0.02 (0.11)	0.01 (0.12)	-0.06 (0.1)	-0.01 (1.62)
Science education	0.14 (0.23)	0.12 (0.2)	0.13 (0.23)	0.1 (0.23)	0.17 (0.2)	-0.03 (3.32)
Psychology education	-0.17 (0.13)	-0.12 (0.11)	-0.1 (0.13)	-0.14 (0.13)	-0.15 (0.11)	-0.04 (1.9)
Other education	0 (0.31)	-0.09 (0.27)	0.03 (0.31)	-0.03 (0.3)	-0.05 (0.27)	0.02 (4.52)
Freq of media consumption (Crime)	-0.03 (0.13)	-0.09 (0.11)	-0.07 (0.13)	-0.06 (0.13)	0.01 (0.11)	0.02 (1.87)
Freq of media consumption (Legal)	0.02 (0.11)	-0.03 (0.1)	-0.08 (0.11)	0.09 (0.11)	0.05 (0.1)	-0.05 (1.59)
Perceived realism	0.01 (0.11)	0.04 (0.1)	0.16 (0.11)	0.18 (0.11)	0.11 (0.1)	-0.01 (1.65)
Freq of media x Perceived Realism	0.05 (0.13)	0.12 (0.11)	-0.03 (0.13)	0.05 (0.13)	-0.04 (0.11)	0 (1.89)
Rsq	9%	10%	12%	18%	13.7%	7.1%
p-value	.28	.18	.08	.002*	.03	.54

Discussion

Previous studies of perceptions of DNA evidence focused on STR matching methods, which present some limitations when the suspect does not have an existing DNA sample and the sample cannot be obtained. In these situations, genotyping variants that influence appearance found through GWAS may help identify suspects. The present study provided the first assessment of the perception of GWAS evidence in a jury-eligible sample. The study considered eleven predictors: total score of maths quiz, total score of genetics comprehension test, composite scores of frequency of media consumption for law and crime dramas respectively, perceived level of realism in media, education levels in maths, law, science, psychology, and other, and a realism x frequency of media consumption interaction term. The dependent variables were attitudes toward trustworthiness of eyewitness testimony, expert testimony, forensic scientist testimony, psychologist testimony, post-mortem reports, video/photographic evidence, fingerprint matching evidence, DNA matching evidence, and GWAS evidence. We also assessed the perceived usefulness of GWAS evidence for finding suspects, narrowing down a list of suspects, confirming suspect identity, convicting a suspect in the face of inconclusive or somewhat conclusive evidence.

In comparison to previous studies, this study provides some evidence in support of the CSI effect in that frequency of media consumption affects perception of scientific evidence. However, this may not be in the direction hypothesized, and may depend on the type of media. For perception of DNA matching, crime media was negatively correlated to perceived trustworthiness while legal dramas were positively correlated (Table 5.2). Moreover, perceived realism of the media was also positively correlated to perception of DNA evidence, and no significant effect interaction between realism and consumption frequency was found (Table 5.2). These effects, however, do not extend to GWAS evidence, possibly due to the absence of evidence derived from association methods in crime or legal dramas.

One variable that appeared consistently to affect perception of scientific evidence was science literacy, specifically that a higher level of science education is correlated to a higher regard for scientific evidence, shown by a higher rating of trustworthiness for DNA and GWAS evidence compared to other types of evidence (Table 5.1). On the other hand, a higher score in genetics comprehension led to a lower level of readiness to accept the importance of GWAS in confirming suspect identity or its use in convicting a suspect in the face of

inconclusive evidence, as well as a higher confidence threshold for plausible deniability. This suggests that scientific literacy may be an influential factor in determining whether scientific evidence is favoured over testimony evidence, and that an understanding of basic genetics may be helpful in the evaluation of GWAS evidence.

In conclusion, the present study investigated the relationship between predictors pertaining to media consumption, education level and genetic and statistical knowledge to perception of GWAS and DNA evidence, compared to testimony evidence. It was found that scientific and genetic knowledge significantly influenced attitudes toward GWAS and DNA evidence. Moreover, the "CSI effect" was found to be dependent on media type and perceived realism of media. These results have potential implications on juror education in cases where scientific evidence may be used, and the importance of transmission of relevant knowledge to jurors before expert testimony is given in order to ensure information is understood correctly.

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Appendices

Appendix A: Description of genetic variation based on public information available on the National Institute of Health website (http://www.genome.gov/20019523)

Almost all human traits and diseases have a genetic basis. In understanding the role of genetics, we may be able to predict human traits and develop better ways to prevent and cure disease. Cells are the fundamental structures of every living organism. Their nuclei contains genetic information in the form of deoxyribonucleic acid (*DNA*) sequences. These sequences are made up of basic chemical units (*bases*): guanine, cytosine, thymine, and adenine (G, C, T, and A), which match up to form *base pairs*. The human *genome* – any individual's complete set of DNA, containing 3 billion base pairs – are arranged into 23 separate *chromosomes*. Specific sequences within chromosomes (*genes*) direct the type and amount of proteins to create. However, only 29% of the human genome is comprised of genes. The rest *non-coding regions* exist to provide regulatory functions for genes.

The human species share 99.9% of their genetic information. Minute variations such as *mutations* and *polymorphisms* in genetic sequences between individuals explain the differences in our physical traits and disease risk. Mutations are generally rare and easily associated with specific diseases; polymorphisms, however, are more common, and their relationship to disease and physical trait is more ambiguous. For example, single nucleotide polymorphisms (*SNPs*) are changes in one single base pair within a DNA sequence, and could be found every 100 to 300 bases along the genome, within both genes and non-coding regions. A single individual may have millions of SNPs. The relationship between SNPs and disease expression is complex, as it may involve several other genes (*pleiotropy*), gene-gene interaction (*epistasis*), as well as genetic-behavioral-environmental interactions. It is the goal of many clinician researchers and scientists today to investigate the specific roles of these factors for different diseases.

Attitudes toward association data

In this section you will be asked about your opinions toward certain types of forensic evidence. There are no right or wrong answers. When you have finished, click the forward button (>>).

Building upon your knowledge from the previous section, suppose there is now a way of predicting a person's physical trait (*phenotype*) using their genetic profile (*genotype*). To do this, researchers use a statistical procedure called genome-wide association (*GWA*) to find the relationship between one or several SNPs and a physical trait, such as height, fingerprint patterns, and hair thickness. Interestingly, SNPs that are found to affect traits explain only a very small amount of variance in the physical trait (<2%).

Appendix B: Multiple choice comprehension test for genetics

Cell nuclei contain:

- ◯ the human genome
- ◯ DNA
- ◯ genes
- ◯ DNA and genes
- ◯ I don't know

True or false: The entire human genome is responsible for directly conducting protein transcription.

True	False
◯	◯

Non-coding regions:

- ◯ are insignificant in the functioning of the human body
- ◯ comprise 29% of the human genome
- ◯ provide regulatory functions for genes
- ◯ do not contain SNPs
- ◯ none of the above

True or false: in terms of DNA, all humans are almost exactly the same.

True	False
◯	◯

Which of the following is/are false? (you may select more than one option)

- ☐ polymorphisms are huge differences between individuals' DNA
- ☐ SNPs are a type of polymorphism
- ☐ mutations do not directly affect disease risk
- ☐ the relationship between polymorphisms and disease risk is clear
- ☐ mutations are as rare as polymorphisms

Which of the following is/are true about SNPs? (you may select more than one option)

- ☐ it stands for several nucleotide polymorphisms
- ☐ it occurs every 100-300 base pairs along the genome
- ☐ it is the variation of one base pair within individuals
- ☐ it occurs only in gene regions
- ☐ everyone has them

True or false: Several different genes could affect the same disease or trait.

- ◯ Yes - this is called "pleiotropy"
- ◯ Yes - this is called "epistasis"
- ◯ No - only one gene could affect one trait
- ◯ No - only gene-gene interaction could affect disease expression

Which of the following could be reasonably hypothesized to contribute to the cause of a complex disease or physical trait?

- diet
- the interaction between two or more genes
- a SNP within a non-coding region
- where a person lives
- all of the above

In general, scientists nowadays:

- know everything about genetics
- are not interested in learning about other influences on disease apart from genetics
- are still trying to find out about how and why disease and physical traits vary
- do not share a common interest with medical practitioners
- do not rely on research funding

Appendix C: Survey of general media consumption habits, specific TV show preferences and perceived realism of the TV shows.

How many hours do you watch television shows/movies per week? (Including Internet downloads and online streaming)

- Less than 5
- 5-10
- 11-20
- More than 20

How often do you watch the following television shows (including Internet downloads and online streaming)? Please drag the marker along the sliding scale to indicate frequency.

	Never 0	Not Often 1	2 Most Weeks	3 Every Week	4	More than once a week 5
CSI (CSI: Miami; CSI: New York, etc)						
Law & Order (L&O: Criminal Intent, L&O: SVU, etc.)						
NCIS						
Criminal Minds						
Bones						
Dexter						
Hawaii Five-O						
Hannibal (TV show)						
White Collar						
Other police procedural /detective shows						

How often do you watch the following television shows (including Internet downloads and online streaming)? Please drag the marker along the sliding scale to indicate frequency.

	0	10	20	30	40	50	60	70	80	90	100
Ally McBeal											
Boston Legal											
The Good Wife											
Suits											
Other legal drama											

In your opinion, how realistic are the following shows?

	Not at all 0	1	Moderately 2	3	4	Extremely 5
CSI (CSI: Miami; CSI: New York, etc)						
Law & Order (L&O: Criminal Intent, L&O: SVU, etc.)						
Other crime shows and police procedurals						

Appendix D: Short timed statistical quiz

If I tossed a coin 4 times and got 4 heads, the probability of getting a tail on the 5th toss is:

- 1/2
- less than 1/2
- greater than 1/2
- Not enough information
- I don't know

In a playoff series, the probability that Team A wins over Team B is 5/7, and the probability that Team C wins over Team D is 2/3. If the probabilities are independent, the probability that Team A loses and Team C wins is

- 10/21
- 5/21
- 4/21
- 1/21
- I don't know

A deck of 52 cards is shuffled and one is randomly picked from the pile. What is the probability that the card selected is an even number?

- 1/13
- 5/13
- 10/13
- Not enough information
- I don't know

Ten coins are tossed at the same time. In how many of the outcomes will the third coin turn up a head?

- 2^9
- 2^{10}
- 3×2^9
- 3×2^{10}
- I don't know

In a class of 25 people, what is the probability that two people share the same birthday? Assume 365 days in a year.

- 0.068
- less than 50%
- greater than 50%
- Not enough information
- I don't know

In a class of 25 people, what is the probability that two people share the same birthday? Assume 365 days in a year.

- 0.068
- less than 50%
- greater than 50%
- Not enough information
- I don't know

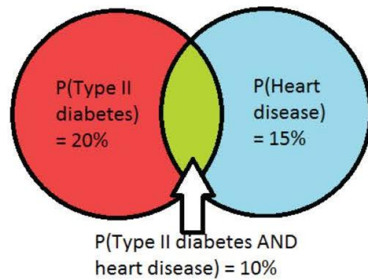
If a doctor has a 2/5 probability of answering any statistics question correctly, what is the probability he would pass a statistics test (i.e. achieve 50% or above) of 20 questions?

- 0.4^{10}
- $0.4^{10} \times 0.6^{10}$
- 2/5
- Not enough information
- I don't know

Type II diabetes and heart disease are prevalent within a certain population. It is estimated that 20% of the population will develop type II diabetes sometime during their lives, 15% will develop heart disease eventually, and 10% will develop both.

Given that a randomly selected person has developed at least one of these medical problems, what is the probability this person will present with both problems?

[Hint: P(A or B) = P(A) + P(B) - P(A and B)]



- 0.25
- 0.10
- 0.40
- Not enough information
- I don't know

In a court of law, probability statistics are often used to strengthen a case for and against a defendant's innocence.

If a prosecutor states a probability of 1 in 1 billion (i.e. 1 in 100,000,000) that trace DNA at a crime scene could match any random person's DNA, what is the probability that the defendant is indeed the source of the trace DNA?

- 99.9%
- 1%
- 0.00000001%
- Not enough information
- I don't know

An expert witness now takes the stand. The witness works in the DNA laboratory responsible for typing and matching DNA profiles. According to the testimony given, the laboratory makes mistakes in profiling on an average of 2 out of 100 times.

The probability of two events A and B both occurring could be calculated by the following equation:
P(A and B)= P(A) + P(B) – [P(A) x P(B)]

What is the probability that the trace DNA matches the defendant's by chance (1 in 1 billion according to the prosecutor), AND the laboratory made a profiling error?

- about 2 out of 100
- about 1 in 1 billion
- about 99.99%
- about 1%
- I don't know

Appendix E: Likert-like scale surveying attitudes toward usefulness of GWAS in different forensic practices

How important do you think GWA evidence would be, in terms of:

	Completely irrelevant 0	1	Somewhat important 2	3	4	Crucial 5
Finding a suspect						
Narrowing down a list of suspects						
Confirming the identity of a known suspect						
Convicting a defendant when other types of evidence are *inconclusive*						
Convicting a defendant when other types of evidence are *somewhat* conclusive						

Appendix F: Likert-like scale surveying relative trustworthiness and usefulness of GWAS evidence compared to other types of forensic evidence

How would you rate the trustworthiness of following types of evidence?

	1 Not At All	2 Barely	3 Quite Untrustworthy	4 Quite Trustworthy	5 Very Trustworthy	6 Absolutely Trustworthy	7
Eyewitness							
Expert (people with qualifications in areas relevant to the case)							
Forensic Scientist							
Psychologist							
Video/photographic							
Fingerprints							
DNA matching							
Postmortem report							
SNPs identified from GWA studies							

Appendix G: Information sheets containing background information and the aim of the study

School of Psychology
Participant Information Sheet
Jury Perception of DNA Evidence

The purpose of the study
The purpose of this study is to examine the factors that affect juror's perceptions of forensic evidence. This study is being conducted by Yvonne Ho as part of her PhD degree at the University of Queensland, under the supervision of Dr. Sarah Medland. *No previous experience or study of biology or law is required for participation.*

Participation and withdrawal
Participation in this study is completely voluntary and you are free to withdraw from this study at any time without prejudice or penalty. If you wish to withdraw, simply stop completing the questionnaire. If you do withdraw from the study, the answers that you have provided up to that point may be included in analyses.

What is involved?
In this study you will be asked to:

- provide some demographic information and answer some questions to about your understanding of probability
- read some information about DNA evidence and answer some questions about your opinions on the use of this kind of evidence in a court of law

Participation in this study will take approximately one hour.

Risks
Participation in this study should involve no physical or mental discomfort, and no risks beyond those of everyday living. If, however, you should find any question or procedure to be invasive or offensive please leave this questions blank.

Confidentiality and security of data
All data collected in this study will be stored confidentially. Only members of the research team will have access to identified data. All data will be coded in a de-identified manner and subsequently analysed and reported in such a way that responses will not be able to be linked to any individual.
The data you provide will only be used for the specific research purposes of this study.

Ethics Clearance and Contacts
This study has been cleared in accordance with the ethical review processes of the University of Queensland and within the guidelines of the National Statement on Ethical Conduct in Human Research. You are, of course, free to discuss your participation with project staff (email: yvonne.ho@qimrberghofer.edu.au or sarah.medland@qimrberghofer.edu.au). If you would like to speak to an officer of the University not involved in the study, you may contact one of the School of Psychology Ethics Review Officers**:** Jolanda Jetten (j.jetten@psy.uq.edu.au, tel 3365 4909), Jeanie Sheffield (jeanie@psy.uq.edu.au, tel 3365 6690), Thomas Suddendorf (tsuddend@psy.uq.edu.au, tel 3365 8341) or Alex Haslam (uqshasla@uq.edu.au, tel 3346 7345). Alternatively, you may leave a message with Danico Jones at tel 3365 6448 for an ethics officer to contact you, or contact the University of Queensland Ethics Officer, Michael Tse, on 3365 3924, e-mail: humanethics@research.uq.edu.au

If you would like to learn the outcome of the study in which you are participating, you can contact me at the email above around October 2015, and I will send you an Abstract of the study and findings.

Thank you for your participation in this study.

Appendix H: Informational debriefing sheet

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA
School of Psychology

Informational Debriefing Sheet – For All Participants

Jury Perception of DNA Evidence

The use of DNA profiling in murder and sexual assault cases is well-established, and has acquired widespread recognition as a valid method of determining the presence of an individual (usually the defendant) at a crime scene. Current methods of DNA profiling are based on fragment analysis (Panneerchelvam, 2003), in which DNA sequences of a certain length are extracted and compared against another sample. Although methods have improved over the years with technology and research, there are limitations to this kind of evidence. For instance, the amount of trace DNA left at the crime scene or on the victim may be insufficient for some types of analyses. If a suspect is not physically present for DNA extraction, matching would be dependent on genetic databases of prior offenders, which severely limits the usefulness of current DNA profiling methods.

Recently, research in human genetics has made possible a method of linking physical attributes to specific genes, and even to single base pairs (or single nucleotide polymorphisms [SNPs]) of DNA. The gene MC1R, for example, is directly related to the expression of red hair, and three SNPS in the OCA2 gene region is known to cause albinism. These genome-wide association studies (GWAS) provide a way to predict a person's physical outlook based on their DNA. Due to the size of the human genome (~ 3 billion base pairs), the amount of variance in complex traits explained by one SNP is rarely higher than 10%. This means the existence of one or several contributing SNPs may not be complete proof of a physical trait. However, when in corroboration with testimony and/or other physical evidence, GWAS may allow a higher degree of confidence in the verdict. In the absence of DNA admissible for fragmentary analyses, GWA data may also provide some clues as to the DNA and physical profile of a perpetrator.

In the forensic context, DNA and other statistical evidence have been topics of research interest as they can be used to strengthen or debate cases for a defendant, such as the probability of a blood type/bullet lead match between that found on a suspect or victim and that found on an established crime scene (Kasa, 2007; Thompson, 1989). In the past, some studies have looked at how juries respond toward DNA evidence. One poll conducted by (Carlson, 2005) showed that amongst 1012 Americans, 27% believed that DNA evidence is completely reliable and 58% thought it was very reliable. Another series of studies (Liberman, 2008) compared DNA evidence with other types of physical evidence commonly found in crime scenes (fingerprints, hair fibers, eyewitness testimony, victim testimony), using a self-administered questionnaire and manipulating the type of evidence found, the nature of evidence (incriminatory or exculpatory) and the type of crime (murder or rape) in a scenario. In their second and third studies, they added damaging cross-examination testimony and relevant jury instructions. The results across studies showed an extremely high confidence in the accuracy of DNA evidence (95%) and its indication of the suspect's guilt (94%) compared to other physical evidence, and only a qualitative cross-examination of DNA evidence lead jurors to realize its limited and flawed nature.

While existing literature provides some insight into the statistical misconceptions and fallacies that may affect jurors, prosecutors and defenders in the application of DNA

fragment matching methods, there is yet to be any investigation on whether GWA evidence may affect juries' decision-making process, and whether this information is readily comprehensible to jury-eligible individuals. This study aims to examine the interface between human perception and GWA evidence in legal settings, specifically the perceived importance and confidence in GWA evidence. In future investigations, the results of this study may help determine whether GWA evidence is a useful tool in informing verdicts, and how statistical evidence in the context of expert witness testimony may be presented to optimize understanding within juries and law enforcement. Your perceptions and attitudes toward different types of forensic evidence and your understanding of genetic information was tested in the form of a multiple-choice questionnaire. Mathematical and statistical knowledge was also measured to provide a control variable, as understanding of probability is crucial to comprehending and applying statistical information. All participants completed the same questionnaire, and the data collected may be analyzed using ANOVA.

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Chapter 6: General Discussion and Conclusions

6.1 Overview

Human biometrics such as eye and hair colour has long been used in the identification and confirmation of identity in a forensic context. The classification of traits at an observational level has allowed for extensive studies on frequencies and variations. Complementing this, the study of genetic influences has been quite successful in terms of predicting the inheritance and expression of these traits. As genetic technology and methodologies have advanced, newer methods of identifying genetic influences such as GWAS have been developed, which have resulted in the discovery of new variants influencing these complex traits. While these methods have been invaluable in the context of medical and psychiatric disorders, there has been less focus on applying GWAS to forensically relevant traits.

The aim of this thesis was to identify novel genetic variants influencing forensically relevant traits, providing a first examination of the roles these variants may play in investigations and legal proceedings. In order to do this, I followed two main lines of study, firstly to quantify the genetic contributions to, and identify new genetic variants influencing forensically relevant phenotypes, fingerprint patterns and hair diameter and morphology, using GWAS and twin study methodologies as illustrated in Chapters 2-4, and secondly to assess attitudes towards novel types of genetic evidence within a jury-eligible sample in Chapter 5. The following sections summarize the main findings from the above Chapters, and discuss their implications within the broader context of this thesis.

6.2 Synthesis of Results

Identifying new genetic variants

In Chapter 2, finger print pattern data from a large cohort of adolescent twins was used with univariate AE models. Results showed high heritability (h^2= .7 to .88) for whorls across digits (Chapter 2). Chapter 2 also describes a GWAS for dermatoglyphs. In the discovery analyses using the QIMR sample, one SNP rs1523452 was found to be highly significant ($p=7.12 \times 10^{-9}$) for the little finger of the left hand, and this result was replicated in the ALSPAC sample ($p=1.60 \times 10^{-20}$) with λ= 1.015 and 1.009 respectively, demonstrating this finding is robust and

replicable. Subsequently the results from these cohorts were meta-analysed to increase power, revealing that rs1523452 also influenced the presence of whorls other digits, culminating in a gradient effect more apparent on the left hand. Rs1523452 is located with *ADAMTS9-AS2,* an antisense RNA that is implicated in glioma. This study also found signals within *EVI1* and *OLA1*, which are involved with leukemia and breast cancer respectively. In addition, a variant was found in an intergenic region on chromosome 12 located close to the *TBX3* gene region, which is known to cause ulnar-mammary syndrome, which can result in the missingness of the fourth and fifth phalanges.

Overall, these results show that dermatoglyphs is a complex pleiotropic trait, influenced by genes that have been implicated in a number of cancers. While the functional role of *ADAMTS9-AS2* in pattern development is not yet clear, the gradient effect across digits was particularly interesting in the context of previous mathematical modeling of fingerprint pattern development, both general and specific genetic and environmental influences were shown to affect ridge count across digits (Martin, Eaves, & Loesch, 1982). Ridge count is closely linked to pattern type where a higher ridge count is indicative of a larger pattern, which would likely result in a whorl, and a lower count would be indicative of an arch. As the *ADAMTS9-AS2* variant was influential across all digits, the results of this study suggest it may play a role as a general genetic influence on the development of whorl patterns.

In Chapter 3, heritability was estimated for objective, micro-level hair morphology measures obtained using computerized light optic methods (Optical Fibre Diameter Analyser, OFDA), and analysed using univariate ADE models on twin and sibling data. Bivariate models were also fit to measure extent of correlation between diameter and consistency. Univariate heritability for diameter and curvature shows significant sex limitation effects, suggesting the sources of genetic variability were different for males compared to females for both variables, $H^2_{diam.M}$= 86%, $H^2_{curve.M}$=53%, $H^2_{diam.F}$=76%, $H^2_{curve.F}$=61%. Meanwhile heritability for consistency of hair diameter within individuals was not sex limited ($H^2_{consist}$= 70%). Bivariate methods yielded phenotypic correlations, showing the correlation between hair diameter and consistency (r ~ .20) is predominantly due to shared additive genetic influences.

A previous study reported 85-90% of the variance in categorical reports of hair curvature was due to genetic effects (Medland, Zhu, & Martin, 2009), while parent-offspring correlations for hair diameter were reported to be 53% (Das-Chaudhuri & Chopra, 1983). The present

study reports a heritability of 53-61% for hair curvature and 76-86% for diameter and a 70% for consistency of diameter. The discrepancy in curvature heritability between studies may be attributed to differences in measurement and the micro versus macro level data analysed. Our findings of sex differences in diameter concurs with existing knowledge, albeit on Japanese (Ikoma, 1973) and Indigenous Australian (Das-Chaudhuri & Chopra, 1983) samples.

Chapter 4 presents a GWAS analysis of hair curvature as measured by an OFDA, providing objective data on hair curvature. This was compared to previously collected nurse report hair curvature matched to the same sample. The results from GWAS of OFDA measures replicated a previously found variant for nurse-reported observations of hair curvature within *TCHH*, and the nurse reports on the same sample replicated this result at a genome-wide significant level ($p_{OFDA} = 1.14x10^{-10}$; $p_{NURSE}=1.10x10^{-15}$), supporting the validity of the novel variants found in this study. Another major signal found for this phenotype was chromosome 15 in the *PWRN2* gene region which was also replicated with matched nurse report data ($p_{OFDA} =7.04x10^{-10}$; $p_{NURSE} =1.20x10^{-5}$). The reported *PWRN2* variant is involved in the development of Prader-Willi Angleman Syndrome, which may present with abnormal skin and hair colour, and interacts with several DNA motifs, including *FoxD3,* which is involved with the development of vitiligo.

Compared to nurse-reported hair curvature, objective measures not only replicated previous signals but also yielded novel variants. This supports the idea that additional knowledge can be mined from known genetic phenotypes by changing the way such phenotypes are considered from categorical to continuous measures. A previous study shows the quantification of eye colour also resulted in the discovery of three new loci which led to the development of a model accounting for over 50% of eye colour variance (Liu et al., 2010). Other traits that may benefit from association analysis of quantified phenotypes includes hair colour.

Perceptions of DNA evidence

Chapter 5 measured attitudes toward DNA and scientific evidence were surveyed with a sample of university students of jury-eligible age. This was done using a survey based on Liebermann's (2008) study of DNA evidence versus testimony evidence in university students. Measurements of usefulness and trustworthiness of DNA and GWAS evidence were

collected, as well as variables that may affect perception of scientific evidence. These included education level, maths and genetics comprehension, frequency of media consumption, and perceived realism of media. Multiple regressions were conducted to analyze the relationship between the predictors and the dependent variables. Results showed that while the CSI effect was present in the sample, the direction of effect was dependent on type of media, and that perceived realism of the media also influenced perception of DNA evidence. Across types of scientific evidence, science literacy was most influential predictor, leading to a higher level of perceived trustworthiness for genetic evidence compared to other types of evidence. Genetic comprehension affected views toward GWAS evidence in terms of raising the threshold for plausible deniability.

One interesting discovery apart from the influence of science and genetic education on views toward GWAS evidence was the significance of the "CSI effect". Previous studies showed conflicting results for the existence of this phenomenon, where increased exposure to popular crime television shows led to an inflation of perceived accuracy and efficiency of DNA matching amongst jury members. The present study suggests that the CSI effect may exist but is influenced by type of media, as legal versus crime dramas led to effects in opposite directions. Previously found interactions between media consumption frequency and perceived realism of media were not found in the current study, although perceived realism had a significant effect in increasing the perceived trustworthiness of DNA evidence.

6.3 Limitations

Some limitations of classical twin studies include the use of twin data to generalize to potential non-twin populations. While some aspects of these studies utilized only twin data, such as that for diameter and consistency bivariate Cholesky, there were no significant difference in the distributions between the twins and their non-twin siblings. It is also unlikely that the measures studied in this thesis would be subject to violations of the equal environment assumption. These chapters applied classical twin modeling on nominal and continuous level data. Overall, the results of these heritability studies showed that fingerprint patterns and hair morphology are strongly heritable, which motivated genome-wide association analyses aiming to identify variants affecting these phenotypes.

Potential limitations of GWAS studies include complex influences of sex and age. For hair, age is an influential factor due to the participants' pubertal development around age 12. In order to address this, sex and age were both included as covariates in all analyses.

Following from the twin studies and GWAS analyses, chapter 5 describes a study on jury perception of GWAS evidence. Results showed that should GWAS results be allowed as court evidence, a jury consisting of individuals who have accomplished high school education and above would be capable of understanding this type of genetic information. However, to be able to critically evaluate GWAS evidence, a certain level of genetic knowledge is required. While this study identified some variables affecting perception of GWAS evidence, further studies are needed to determine whether these perceptions would influence verdict decision making in a jury.

6.4 Broader Ethical Implications

While the main aim and content of this thesis does not encompass a philosophical discussion on storage and utility of DNA data, ethics and privacy issues may incur complications on the application of the results found in this thesis. In this section, I would like to provide a brief, non-expert statement of my opinions on the topic for completeness.

One of the main concerns with the gathering and use of genetic data, when reduced to its fundamental issue, is the contradiction between the need to protect the majority's safety through authoritarian means (security) through the identification and apprehension of suspects, and an individual's right to bodily integrity and privacy (liberty). This is an especially salient question where sensitive medical information may be revealed via genetic analyses. Within democratic societies applying the rule of law, legislations on this issue reveals to some extent the priorities of that community (Patyn & Dierickx, 2010).

While there is no legislation specifically governing the use of GWAS results in forensic settings, the use of DNA databases is widespread and some underlying principles may be applied on GWAS. In terms of the use of DNA databases, the consensus within the European Union appears to be permissive but also emphasizes the stringent conditions under which DNA data is to be stored, therefore attempting to balance protecting social interests and individual rights. For example, the European Convention for the Protection of Human Rights and Fundamental Freedoms (ECPHR; Council of Europe, 1950) stipulates non-interference

with individual privacy except when authorized in the interest of public security and crime prevention (article 8). Similarly, the International Declaration of Human Genetic Data (UNESCO, 2003) allows for the genetic data of suspects involved in civil or penal investigations to be made available for a period of time, although a later revision, as does the ECPHR, states that such data could only be stored after a guilty conviction in court processing.

These legislations reflect my own opinion that DNA databases are no doubt of value to law enforcement investigations; however, in order to preserve societal trust in the legal system to protect their rights, it is important to observe the civil liberties this type of information affects, and put in place appropriate safeguards. In this regard, Guillen et al. (2003) suggested that for the creation of DNA databases, three criteria should be considered: the gravity of offence, the degree of recividism, and the possibility of latent biological evidence. Individual rights should be suspended only in the cases of specific types of offence, higher recividism, and if the type of crime leaves biological evidence. This may serve as a guideline for the use of GWAS results. In addition to this, it is also necessary to ensure that policies regarding the use and retaining of genetic data are shaped and decided upon through social involvement, that the data is treated appropriately and securely, and used in a valid and ethical manner.

6.5 Concluding Remarks

This thesis set out to identify genetic variants that might be used to predict forensically relevant traits. This has been addressed firstly through the series of studies presented in chapters two to four, which quantified the heritability of two salient forensic phenotypes, fingerprint pattern and hair curvature and identified novel variants. The variants contribute to the knowledge of quantitative genetics as applied to forensic investigations. Within this thesis, I used three main methodologies, structural equation modeling using twin and family data, genetic association tests, and multivariate modeling. The results of these analyses have led to a better understanding of the genetics underlying these complex traits and the potential for these types of findings to be used as evidence in a forensic context.

These methodologies may also be applied to other genetic traits of forensic relevance. However, caution should be exercised in the selection and definition of phenotypes for association analyses, such as attention to the heritability of the phenotypes and potential

environmental influences. In addition to heritability, practical selection of phenotypes should also be considered in the context of forensic usefulness. Some identifying physical traits such as body size and weight are difficult for witnesses to accurately report, are relatively changeable, and would be of limited use to the identification of unknown suspects. In consideration of this, dermatoglyphs and trichology were chosen, as they are both easily observed and relatively unique to each individual on a micro level. Other traits that may be practical in this context include extensions of the research in this thesis to loop and arch type fingerprint patterns, as well as other phenotypes such as gait, ear lobe attachment, philtrum width and depth, and facial symmetry.

In light of this new knowledge, there is work yet to be done to broaden and fine-tune our understanding of the heritability and underlying genetic influence of these traits before it could be realistically applied to current forensic investigations. For instance, our sample is composed of individuals of European ancestry, which necessitates further investigation to find whether the same variants influence other ethnicities. However, while the effect size of these variants are small, as is typical in association studies, these variants provide an initial step towards prediction and may add be of use to existing predictive algothrims and provide additional targets for customized forensic genotyping arrays.

The implications of these results can be extended to a courtroom context in several ways. In the process of forensic investigations, results of association analyses may be applied in predictive algorithms to estimate phenotypes, which after validation can be used for the identification of suspects where the phenotype of a suspect is unknown and in the narrowing down of a list of suspects. While the effect sizes of the variants identified here are too small to be used as predictors in isolation, in the future we may see trait prediction from GWAS results presented as evidence. In order to investigate potential effects of GWAS evidence in a forensic setting, the second line of investigation in this thesis surveyed attitudes toward such types of information. Results suggest that although media effects are unlikely to influence jury perception of GWAS, it is important to communicate the genetic and statistical background of this methodology. Informative studies for future directions in this line of study may consist of ecologically valid simulations of court proceedings that include GWAS evidence, in order to investigate the ways of transmitting genetic and statistical knowledge and how it affects the final verdict.

While complex trait genetics and forensic sciences have different considerations, at the intersection of the two fields there lies a possibility for the application of genetic methods to forensically relevant traits, which presents some unique challenges. One of these challenges is gaps in the literature on genetic variants affecting forensically relevant traits, a few of which were addressed by the research within this thesis. However, there lies a broader and more socio-behavioral question of whether these results could be, and should be used in forensics, and how their use would affect judicial structures and functions in real life. Given the cumulated amount of knowledge on complex trait variants, we are closer than ever to compiling a sufficiently accurate and cost- and time-effective way to predict traits. With continued research on how best to present results from association studies in a legal setting, it will be possible to establish ways to educate jury members to ensure understanding of the evidence presented.

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Appendices

I contributed to the GWAS aspect of this study, which was a published during my candidature. This study is not included as part of my thesis.

Link to article:
http://enigma.ini.usc.edu/wp-content/uploads/2016/02/ENIGMA-PGC_genetic_connectivity.pdf