

DNA Identikit: Use of DNA Polymorphisms to Predict Offender Appearance

Angela van Daal, Salini Manoharan, Ming Ni

Faculty of Health Sciences & Medicine, Bond University, Gold Coast, QLD 4229
Australia

Abstract

Forensic DNA analysis currently requires a suspect to be identified for comparison to an evidence DNA profile. The evidence DNA profile does not provide significantly useful investigative information, with the notable exception of gender and perhaps ancestry, on its own. The ability to determine the physical characteristics of the source of a crime scene sample has the potential to provide important investigative information. The most obvious descriptors of an individual's appearance are height, colouring and facial features, which are all highly heritable, as exemplified by the striking similarity of identical twins. It should therefore be possible to determine the genetic polymorphisms responsible for the different phenotypic traits. To date most work in this area has concentrated on pigmentation, since the genetic basis of hair, skin and eye colour is quite well understood. Greater than 120 genes have been identified in the mouse that affect coat and skin colour and the human homologues of these genes are known. Single nucleotide polymorphisms (SNPs) in a number of these genes have been associated with various human hair, skin and eye colour phenotypes and a number of these SNPs have been shown to have functional effects. The height and facial morphology traits have been less well studied in humans.

Introduction

DNA profiles are now routinely obtained from biological stains found at crime scenes. However current methods of DNA analysis require identification of a suspect for comparison purposes such that potential suspects must either be known to investigators or be identified by searching the questioned profile against a database of DNA profiles. In those instances where there no suspect is identified or no database match is made, the DNA profile *per se* provides limited information to investigators, with gender and possibly ancestry information being the exceptions.

The determination of the physical characteristics of the person who left the crime scene sample would provide important probative information. The striking physical similarity of identical twins suggests that the physical appearance of an individual is encoded in his/her genome. While some characteristics are significantly affected by the environment, others are largely determined as a result of genetic make-up and are highly heritable. These include the major physical descriptors of a person, namely their colouring, height and facial features [1]. Human phenotypic traits such as pigmentation are caused a number of polymorphisms in a number of genes [2]. The polygenic nature of complex traits means they are likely to be influenced by a number of single nucleotide polymorphisms (SNPs) found in a variety of genes and intergenic regions that act together in specific combinations. Additional genetic variation such as DNA methylation and copy number variants may also contribute to these phenotypes. Knowledge of the genetics of complex polygenic traits should permit DNA analysis of a crime scene sample to provide investigators with information akin to eyewitness identification.

Pigmentation

The wealth of knowledge on the genetics of pigmentation and the considerable understanding of the genetics of mouse coat colour determination has assisted the understanding of normal human pigment variation [3]. Greater than 120 pigmentation genes have been identified in the mouse and the human homologues of these are known. SNPs in a handful of these genes have been associated with various human hair, skin and eye colour phenotypes [4-6].

Human pigmentation is determined by the type and amount of melanin, which functions as a photoprotective agent. A failure to synthesize melanin results in oculocutaneous albinism (OCA) of which there are four forms [7]. Melanin is synthesized from tyrosine as either black/brown eumelanin or yellow/red pheomelanin within specialized post-Golgi lysosomal organelles known as melanosomes within neural crest-derived cells known as melanocytes [8]. The mature melanosomes containing melanin are transported into keratinocytes via the dendrite tips of the melanocytes. The membranes of both keratinocytes and melanocytes fuse, resulting in the 'pinching off' of the melanocytic dendrites. Once in the keratinocyte, the dendritic membrane is degraded and the melanosomes are released into the keratinocyte cytoplasm. Pigment is eventually deposited over the nucleus of the keratinocytes, giving colour and photoprotection from ultraviolet light to human skin. Melanosomes differ in their shape, size, number, and distribution,

depending on the type of melanin they contain, and this determines the pigmentation phenotype of hair and skin. melanosomes.

Two key proteins, melanocortin 1 receptor (MC1R) and agouti signalling protein (ASIP), are involved in the switch from eumelanin to pheomelanin synthesis. MC1R, a melanosome transmembrane G protein coupled receptor, is the key regulator of this process [9]. Stimulation of MC1R by α -melanocyte stimulating hormone (α -MSH) results in an increase in cAMP and the production of eumelanin. Antagonism of this interaction by ASIP results in a cAMP decrease and production of pheomelanin. The first human gene identified that showed an association with normal pigment variation was MC1R. Several MC1R polymorphisms have been identified that show a strong association with red hair and fair skin [5, 10-12]. Most redheads are compound heterozygotes or homozygous for one MC1R polymorphism. There are three common MC1R polymorphisms, R151C, R160W and D294H, which account for about 90% of red hair and fair skin phenotypes in some populations [13]

The *ASIP* gene was screened for SNPs in individuals of varying skin and hair colour. A single polymorphism, g.8818A→G, in the 3'-untranslated region (UTR) was identified and was found at a higher frequency in African-Americans compared with other population groups [14]. Significant differences were also seen in allele frequencies between European-Australians and indigenous Australians ($P < 0.0001$) with carriage of the G allele highest in Australian Aborigines. In the Caucasian population sample a strong association was observed between the G allele and dark hair colour ($P < 0.004$). The postulate that this SNP affected mRNA stability was tested by measuring ASIP mRNA levels in melanocytes carrying the variant and non-variant alleles. Using quantitative real-time polymerase chain reaction the mean ASIP mRNA ratio of the AA genotype to the AG genotype was 12 ($P < 0.05$), suggesting that the 3'-UTR polymorphism results in decreased levels of ASIP and therefore less pheomelanin production [15].

The membrane-associated transporter protein (MATP) gene, recently renamed solute carrier family 45 member 2 (SLC45A2) is another key gene involved in pigmentation variation. Two SNPs in the *MATP* gene, p.Phe374Leu and p.Glu272Lys, were genotyped in 608 individuals from four different population groups [16]. The 374Leu allele was seen more commonly in all non-Caucasian population groups ($P < 0.0001$). A significant increase in 272Lys allele frequency was observed in the Asian and African-American populations compared to Caucasians ($P < 0.0001$). The

polymorphisms showed highly statistically significant associations with dark hair, skin, and eye pigmentation in the Caucasian population. The promoter region of MATP was also screened for SNPs resulting in identification of one novel and two previously identified SNPs [17]. The three promoter polymorphisms were found to be in linkage disequilibrium with each other but not with the two previously reported coding region polymorphisms. The allele frequency of this haplotype was significantly different in diverse population groups and was significantly associated with olive skin colour in Caucasians. Luciferase reporter assays in a melanoma cell line were used to show that this haplotype significantly decreased MATP transcription.

We recently genotyped sixty SNPs in 26 candidate pigmentation genes. Greater than half of these genes showed significant associations with one or more of skin, hair and eye colour (unpublished results). All four genes that are defective in albinism show SNPs that are associated with normal pigment variation. The high success rate of SNP associations suggests that the genetic basis of normal human pigmentation variation is decipherable and future forensic DNA tools will include the ability to provide predictive investigative information to crime scene officers.

Height

The genes involved in the determination of height are less well understood. Height is a complex, highly heritable trait. Adult height is related to the length of the long bones in the body. This is determined by elongation at the epiphyseal growth plates. Many hormones, growth factors and transcription factors mediate bone elongation. The main hormonal regulator is growth hormone, which mediates bone growth through the action of insulin-like growth factor1. Estrogen, which is responsible for inducing the pubertal growth spurt and fusion of the epiphyseal growth plates, is one of the most important hormones effecting longitudinal bone growth [18]. Vitamin D and its receptor also play an important role in bone elongation. Polymorphisms that show associations to adult height have previously been identified in a number of genes including aromatase (CYP19), vitamin D receptor (VDR) and estrogen receptor α (ER α). Genome wide linkage studies have also pointed to chromosomal regions associated with stature, with two of the regions identified containing the ER α and VDR genes. Other genes are suspected to play a role in height inheritance and those associated with longitudinal bone growth appear to be the best candidates.

Facial Morphology

Facial morphology would seem to be the most complex human physical characteristic to decode. The striking resemblance of identical twins suggests a strong genetic component for facial structure and features. Just as the understanding of the genes involved in albinism has revealed information about the normal pigmentation process, so too might diseases such as craniosynostosis help us understand normal development of the human face. Craniosynostosis is the premature fusion of calvarial sutures, which results in mild to severe facial abnormalities including long or beaked noses, broad eyes, large brow ridges, extremely broad, narrow, or asymmetrically shaped skulls. Point mutations in six genes have been identified that cause craniosynostosis syndromes [19]. A SNP that shows association with a decrease in the cephalic index, the standard measure of head shape, was identified in *FGFR*, one of the genes causing craniosynostosis [20]. In order to elucidate the genetic basis of something as complex as the human face other techniques such as differential expression array analyses and bioinformatic approaches using the abundance of SNP databases and population frequency data, will be required. These and other methods should allow the unravelling of the genetic basis of these complex characteristics.

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