EMBO reports

CSI on steroids

DNA-based phenotyping is helping police derive visual information from crime scene samples to aid in the hunt for suspects

Howard Wolinsky

In January this year, the Police Department of Columbia, SC, USA, released a wanted poster for an African American man sought as a person of interest (POI) in connection with the murders of a 25-year-old woman and her 3-year-old daughter 3 years ago. It was the first wanted poster of its kind, delivered by CSI on steroids. The police used forensic DNA phenotyping—determining eye and hair colouring, freckling and genomic ancestry from a DNA sample—to produce a computer-generated image of the suspect, along with additional data. The poster and the tools used to create it provide a glimpse at a future in which genomic and genetic research are used in new ways to "identify" suspects, ironically at a time when forensic science has been facing a crisis in public confidence after a series of scandals.

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The murders of Candra "Candy" Alston and her daughter Malyasia Boykin in 2011 were considered cold cases. Mark Vinson, cold case investigator for the Columbia Police Department, said, "We do know that Candra was working as an escort using Internet sites to find clients. We've also released that [Alston and her daughter] were killed by different means, but we don't know exactly how they were killed. We have DNA from the scene we say would at least be a person of interest and would place a person being present around the time of the crime." The Police had interviewed nearly 200 people, 150 of whom submitted their DNA to authorities. But no DNA profile matched the sample from the crime scene.

To reboot the case, Vinson took a bold approach after he heard about a new forensic technique called Snapshot DNA Phenotyping, offered by Parabon Nanolabs, a Reston, VA-based company funded by the US Department of Defense. Columbia police spent about US$4,000 to have the sample processed. Parabon determined that the POI was a male whose ancestry is 92 percent West African and 8 percent European, and who has brown/black eyes and hair and no freckles. Using the DNA information, the Snapshot system produced a computer-generated image for the wanted poster.

Not everyone is impressed. Peter Claes, an expert in craniofacial morphometrics at the University of Leuven in Flanders, Belgium, thinks that the image had virtually no value. "It just looked like an average black man. It didn't have any characteristic features. That reconstruction didn't give any more information than the genetic background that they listed. This prediction is hardly specific so it doesn't really focus on an individual," he said. "[Parabon is] not going too far. It's going too fast." According to population geneticist Ellen Greytak, Director of bioinformatics at Parabon, her company has used some of the academic research from Claes and Mark Shiver, a population geneticist at the anthropology department at Pennsylvania State University in State College, PA, USA, who studies the genetic basis of facial morphology, to build their commercial product.

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Manfred Kayser, chair of forensic molecular biology at the Erasmus University Medical Center Rotterdam, the Netherlands, thinks that the Columbia police should have invested in a sex and ancestry DNA test instead. "Ancestry DNA testing provides very reliable results. You know with a large probability if a person is a male or a female or if a person comes from Europe or Africa or Asia. [Face Snapshot] is based on DNA markers that tell about genetic ancestry and at the same time may be involved in the face, but on the latter there's a big question mark, because our current knowledge on the genes that determine facial shape is very limited. I don't think you can predict a face from DNA with any practically useful level of detail, accuracy, and reliability, as of yet," he said.

Greytak maintains that, when a case has grown cold, forensic DNA phenotyping can help police at least exclude large portions of the population based on ancestry, eye colour and skin colour. Though, she concedes that Snapshot does not determine age. "That's a big drawback. Our prediction will come out with a person as a young adult just because our face database is young adults. But we can then apply aging to that if [investigators] want to see it at different ages, or if

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DOI 10.15252/embr.201540714

the case is 20 years old, then we know we need to age it 20 years," she said. Vinson said the poster has not yet yielded a person of interest. Still, he said, "I thought it was worth doing. We're still using it and are still hopeful."

DNA profile matching against a database of people who have been arrested for or convicted of a crime is old news. Forensic DNA phenotyping is seen as the next step—establishing a suspect's visible characteristics and ancestry based on DNA samples from the crime scene against human genetic information about their geographic origin or ancestry. The Netherlands Forensic Institute kick started DNA phenotyping more than a decade ago with a grant to Erasmus University Rotterdam. "One idea was to be able to get human appearance information from a crime scene sample to get a picture from the sample donor and then use this image or information about the appearance from that person in the investigation," Kayser, who has headed the Department of Forensic Molecular Biology since its launch in 2004, explained. "We became successful quickly in the area of eye colour, then hair colour, and now we're adding skin colour, and we're working on a number of other appearance traits, the face, body height, hair structure, hair loss, and age. It's a classical issue of anthropology and anthropology genetics, but somehow, perhaps because of lack of funding, people were not really interested in looking at the genes that determine our appearance."

"Apart from predicting hair and eye colour, determining facial characteristics is another major goal for forensic phenotyping"

Susan Walsh, a forensic geneticist in the School of Science at Indiana University-Purdue University Indianapolis (IN, USA), who was part of Kayser's team, said Erasmus' researchers broke new ground when they tested every SNP related to eye colour in 2009: "Everyone was doing research into association with eye colour and finding all these genes, but no one was really thinking about how they could put it all together and make something that any scientist could run and get predictive results." The test, called IrisPlex, is now 95 percent accurate in predicting blue or brown eyes, but less accurate in predicting intermediate colours. "We're hoping to change all of that through research," Walsh said. "Predicting quantitative colour—not just blue or brown but the precise shade or pigment—in terms of eye, hair or skin colour of an unknown individual provides law enforcement, archaeologists and other investigators with information that can help identify a specific person or determine a potential pool of suspects that may or may not be of interest."

Walsh and Kayser, together with colleagues, developed another test, called HIrisPlex, which analyses 24 DNA markers from 11 genes and, together with a prediction model, allows parallel prediction of eye and hair colour. HIrisPlex achieves high accuracy for red hair (93%) and black hair (85%), and lower accuracies for brown and blonde hair (about 80%). "The major reason why at this moment DNA cannot predict blonde more accurately are people like myself who were blonde as children but their hair colour darkened considerably while growing out of childhood age," Kayser explained.

Apart from predicting hair and eye colour, determining facial characteristics is another major goal for forensic phenotyping. Penn State's Shriver said his research initially focused on ancestry as a phenotype and then looked for the link between genes and facial characteristics. Forensics was naturally the next step. "One of the tragedies of DNA application and forensic science is the number of rape cases that go unanalyzed because there is no suspect," he said. "If the perpetrator had not been previously convicted and in the national database, there is no way to make a match with traditional methods. But with phenotype, you can start to say something about the person and their ancestry, their background, their appearance, you can have new clues to stimulate leads from the public."

Shriver and Claes have developed a new model based on 1,700 faces, while 23andMe, the US genomics testing company, does the DNA testing (Fig 1) [1]. The researchers work with a set of 200 SNPs that are relevant for facial morphology. Shriver said he thinks it will be possible to predict faces based on DNA testing, but the technique is not ready for routine detective work. "Are these predictions useful? Do they make sense? Are they something we have to pay attention to? That has to be the first question. We need to address that. But a substantial amount of work remains," he said. "We have demonstrated that it's possible to make predictions and that the face is not too complex to study scientifically."

In cold cases, there is a nothing-to-lose attitude toward trying anything that could yield new information, and police investigators and scientists thus regard forensic DNA phenotype testing as an investigative tool that can lead to suspects or identify missing persons. Stacy Gallant, an investigator with the Toronto (Canada) Police Service, has been using phenotype information to thaw cold cases. Ontario's Center for Forensic Science is testing the IDENTIFY system developed by Identitas Corp., a New York City-based forensic genomics start-up. IDENTIFY is partly based on the work by the Erasmus group. The profile includes biogeographic ancestry, extended familial relationships, and physical features, including eye colour and hair colour. Gallant said the center has already supplied information on the sex of a potential subject from a sample from a crime scene. "Now we can get their ethnicity to the point whether they're black, white, Asian, or South Asian. [...] We now can narrow down [the suspect pool] to a male, white, with blonde hair and blue eyes, for example," he said.

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Gallant added that the technique has not yet successfully identified an offender, as some of the cases he has tried it on are as old as 20 or 30 years. "It takes time to go through these cases and look at all the individuals who were persons of interest back then and eliminate them," he said. Moreover, even though forensic DNA phenotyping gives the odds of a suspect having blue or brown eyes, the results are

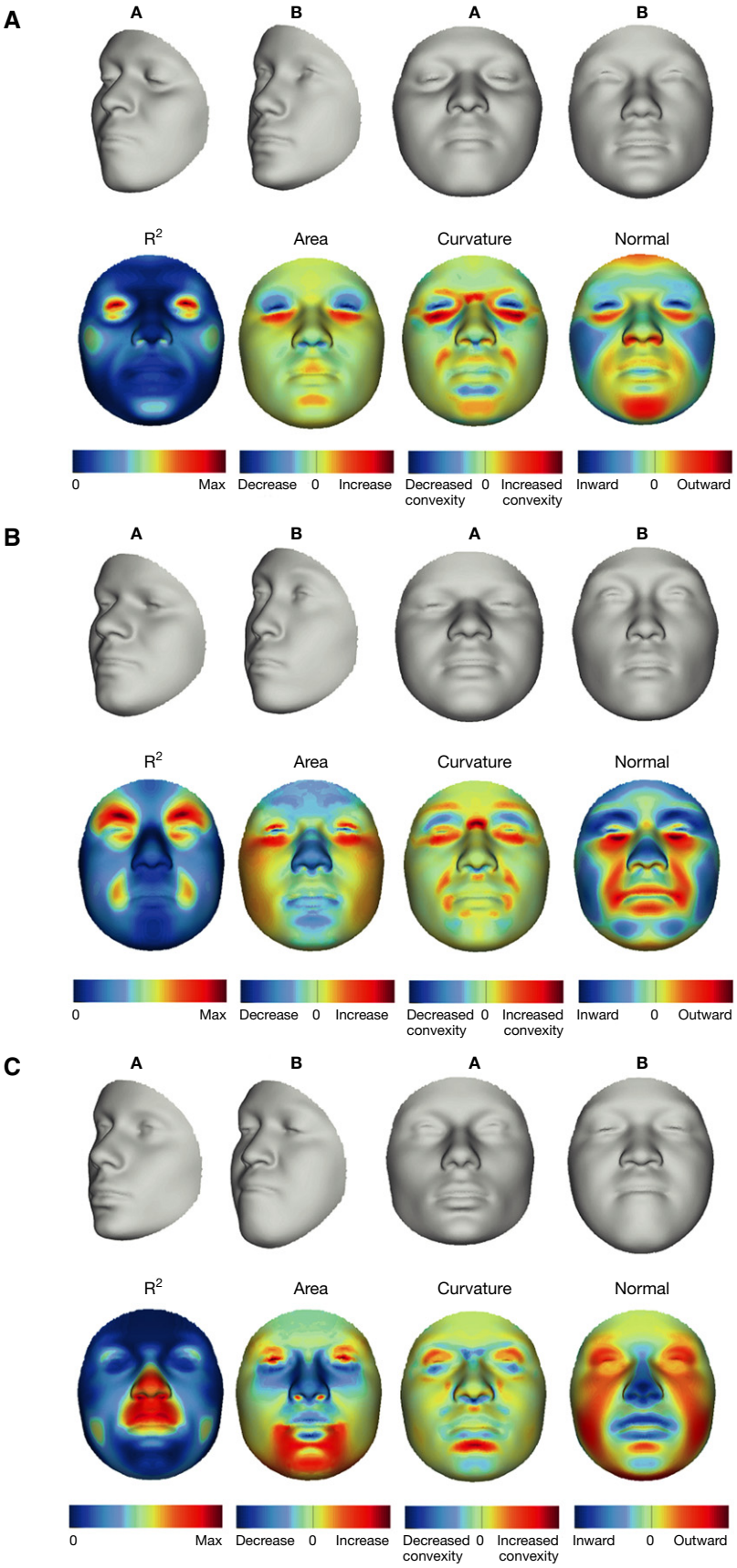

Figure 1. Transformations and heat maps showing how variants in 3 genes affect face shape.
The initial predictor variables are SNPs in the genes (A) *SLC35D1*, (B) *FGFR1*, and (C) *LRP6*. The top row of each panel shows the shape transformations near the extreme values of the genetic markers. The second row shows the proportion of the total variation; the three primary facial shape change parameters: area ratio, curvature difference, and normal displacement.
Source: Claes *et al* [1].

not definitive. Hair dye, Botox treatments, plastic surgery, facial deformities, scars, and colour lenses could mislead investigators. Kayser maintains that criminals have known "for more than 100 years that wearing gloves avoids leaving physical fingerprints; nevertheless, physical fingerprints remain a successful tool because so many criminals do not use gloves. Now you are asking for these individuals to artificially change their appearance, which involves much more planning than simply wearing gloves. I therefore expect that faking appearance traits will not become a practical hurdle for forensic DNA phenotyping in many cases."

Age is another major area of focus for forensic phenotype research. Gallant thinks it would be very useful if such profiling could determine the age of a person at the time they left the sample. Researchers have considered telomere length and the accumulation of mitochondrial DNA deletions as measures of age, but these have not panned out, according to Shriver. He added that Kayser's research on signal joint TCR excision circles (sjTRECs), which are small DNA rearrangements in t(hymus) cells, is proving more reliable: as the thymus ages, the number of sjTRECs increases. The technique works on blood samples, Kayser explained; he is currently improving age estimation by combining it with age-related gene expression and DNA methylation markers. Shriver expects that this forensic tool could be a mainstay within 5 to 10 years.

Kayser is also testing the predictive value of SNPs associated with male baldness. "For this and any other age-dependent appearance traits, it will be vital to combine DNA-based appearance trait prediction with DNA-based age prediction," he said. Another area with some potential, according

to Kayser, is height. In 2014, the Giant Consortium found that 700 SNPs were linked to height variation in a study of 250,000 people, but these 700 SNPs only explained about 15% of the height variation in the study population. Kayser and colleagues recently showed that extremely tall stature can be predicted from 180 of SNPs with about 75% accuracy, which he expects to increase by using more SNPs.

Even though the science of forensic phenotyping is advancing quickly, there are still hurdles to its use by law enforcement officials, not least of which are legal restrictions. According to molecular biologist Peter Schneider, of the Institute of Legal Medicine at the University Hospital of Cologne, Germany, the legal frameworks covering forensic DNA phenotyping vary widely between countries in Europe and between states in the USA. "We have countries that have introduced databases very early on and who are very permissive in what kind of cases are being analyzed and who is going to be entered into the database, because in some countries it's allowed to enter the DNA profiles of convicted offenders, whereas in some countries you can also enter the DNA profiles of suspects in ongoing investigations," he said.

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Schneider, who is also coordinator of the EUROFORGEN-NoE (European Forensic Genetics Network of Excellence), which involves police agencies, justice departments, and scientists in eight countries, to create the European Virtual Centre of Forensic Genetic Research, recalled that Dutch legislators approved the use of forensic DNA phenotyping to predict externally visible characteristics a decade ago. "They did that at a time when there was not a single marker available, but it was very clear that this would be coming up quite soon. What they did was they put it on the agenda of the parliament, creating public debate," he said. "They came to a conclusion that anything that you can see from the outside of a person is not private information." In contrast, Schneider's native Germany does not allow the use of any predictive markers for forensic analysis. "All genetic information is private, and once you cross the line into the coding part of the genome, where do you stop?" German parliamentarians will not touch the topic for historic reasons related to the persecution of Jews, he explained: "We did have one situation where we identified people by their genetic traits, which was done in a very negative way in the Third Reich period."

Nevertheless, Schneider said that legislation is slowly adapting to the science. France, which has one of the largest DNA databases in Europe, had a very restrictive approach to DNA phenotyping, but the Cour de Cassation, the final court for civil and criminal questions, recently ruled that pigmentation markers for eyes, hair, and skin could be used in the trial of a serial rapist in Lyon. The court still ruled that ancestry data were private information. Forensic biologist and researcher Mark Benecke, based in Cologne, Germany, commented that the legal tangle will affect the acceptance of forensic DNA phenotyping. "Legislation will follow and take care of it all," he said. "Sometimes, it just needs a boost from people who believe in higher justice."

Ironically, forensic phenotyping is gaining attention as forensic science itself has come under increasing scrutiny as a result of a series of scandals dating back to the 1990s. One notorious example was the "Phantom of Heilbronn", an alleged female serial killer sought by the German police for 16 years until 2009, when it was discovered that the cotton swabs used to take the original samples from 40 crime scenes had been contaminated by a female laboratory worker during production. As a result, her DNA was erroneously thought to be present at every crime scene, making her the prime suspect. Another prominent example came to light in 2013, when a review of a single technician's work suggested that DNA evidence in more than 800 rape cases over a 10-year period had been potentially mishandled or overlooked in New York City, resulting in investigators receiving incorrect reports. As it performed the review, the medical examiner's found other, unrelated problems where the DNA evidence in 19 rape cases had been commingled with DNA from other cases (http://www.nytimes.com/2013/01/11/nyregion/new-york-reviewing-over-800-rape-cases-for-possible-mishandling-of-dna-evidence.html?_r=0).

More shockingly, in April 2015, the US Justice Department and the FBI acknowledged that nearly every examiner in the FBI's forensic unit had provided flawed testimony in virtually all trials in which they offered evidence on non-DNA-based morphological hair analysis for more than 20 years. "Of 28 examiners with the FBI Laboratory's microscopic hair comparison unit, 26 overstated forensic matches in ways that favored prosecutors in more than 95 percent of the 268 trials reviewed so far..." (http://www.washingtonpost.com/local/crime/fbi-overstated-forensic-hair-matches-in-nearly-all-criminal-trials-for-decades/2015/04/18/39c8d8c6-e515-11e4-b510-962fcfabc310_story.html). The National Association of Criminal Defense Lawyers and the Innocence Project, which aided the government in the investigation, said these cases include 32 defendants who had received a death sentence, 14 of whom have been executed or have died in prison.

Is there a risk that these high-profile botched cases could affect the acceptance of DNA phenotyping as a new tool in uncovering potential criminals? Benecke commented that these scandals represent "typical failures" of bureaucratic systems that should not hold back promising new technologies. "The lesson is the same as always: Don't believe yourself, do not believe the expert, do not believe anything until it is tested often and double-blinded," he said. Similarly, Roger Koppl, an economist at Syracuse University in the USA who studies forensic laboratory administration, commented that laboratories, independent of the techniques they use, always need checks and balances, in particular cross-laboratory redundancy.

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However, investigators and phenotype researchers contend that phenotype prediction will only serve as an investigative tool,

not a source of evidence such as DNA fingerprinting or hair analysis. Walsh stressed that forensic DNA phenotyping is not intended for use in court. "There will never be a time that DNA phenotypes will convict someone. At the end of the day, STR (short tandem repeat) profiling will always be the best. Forensic DNA phenotyping would be used for police to narrow down their list of suspects," she said. Whether DNA phenotyping will ever even catch anyone, however, remains to be seen.

Reference

1. Claes P, Liberton DK, Daniels K, Rosana KM, Quillen EE, Pearson LN, McEvoy B, Bauchet M, Zaidi AA, Yao W *et al* (2014) Modeling 3D facial shape from DNA. *PLoS Genet* 10: e1004224