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Original Research

Forensic DNA phenotyping; Current updates and future prospects

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ABSTRACT
Forensic DNA Phenotyping (FDP) represents a transformative paradigm in forensic science, enabling the prediction of externally visible characteristics (EVCs)—such as hair, eye, and skin colour—from biological material recovered from a crime scene. In many instances, where short tandem repeats (STRs) approaches are not helpful to find the matches with the prospective unknown suspects at the crime scene, the technique of "biological witness" is of particular interest.
However, proceedings of a forensic case may be delayed if there is a mismatch (anonymous offender) or lack of database hits, and it becomes arduous for a forensic lab to make the suspect pool narrower. For phenotype to genotype prediction, the FDP efficiently narrows down the pool of suspects and help investigators to find the culprit. In this review we discuss in detail the valid genetic markers for skin tone, eye and hair colour, as well as more complex and emerging traits like facial morphology, age measurement, and biogeographic ancestry through epigenetic clocks.
These predictions could further be strengthened through highly sophisticated next generation sequencing analysis and bioinformatic approaches. This review further discusses the ethical and legal aspects surrounding the genetic privacy and potential discrimination. Given the advancements in modern molecular biology techniques, the scope and potential of FDP can be standardized and become an indispensable tool in forensic investigation, human identification and ultimately strengthening of criminal justice system.
Key Words: Forensic DNA phenotyping, Externally visible characteristics, Genetic markers, Short tandem repeats, Single nucleotide polymorphisms, criminal identification.

1. Background

The evolution of forensic genetics came with successive technological innovations in this field. The first wave of technological innovations marked the invention of forensic genetics, while the second wave resulted in the establishment, diversification, and application of forensic DNA databases. The third wave strove to go far off the match/no match out tums rendered by DNA databases (1). In the recent past, forensic DNA analysis concentrated majorly on DNA profiling and matching DNA material collected from crime scenes with the reference DNA from the investigated perpetrators. However, comparative DNA matching can only be successful if the suspect's DNA profile is already present in the forensic database. If the suspect has effectively run away from the investigation and the DNA profile information is missing from the database, then the STR, SNP, or YSTR profiles fail unless mass screening is performed. DNA mass screening also has some limitations in the context that suspects may avoid giving samples voluntarily with associated identification fear. In this scenario, some other powerful technique was required to resolve the crime cases (2). With the advent of FDP, it will become possible for the forensic scientists to investigate the complex criminal cases where DNA matching fails (3). In criminal investigations, FDP has been thought to play an emerging role in finding new clues in this context (1). FDP is a blanket term for all the practices and techniques, and it involves the prediction of phenotypic characteristics of a person based on the DNA material left on the crime scene (4). FDP predicts the external traits of a person when conventional DNA profiling flops. It is extremely advantageous as a supplementary investigative tool in forensics to present an idea of what the culprit may look like (5, 6). It can serve as a more efficient biological witness in solving forensic cases compared to human eyewitnesses, which tend to be less reliable (2). FDP principally started in the early 2000s, but it took much time to progress and to be implemented in forensic genetics because of limited knowledge about the genetic basis of human EVCs. It takes the same time and effort to conduct research on the inherited disorders and EVC genetics. The

same techniques and statistical procedures are involved in both fields, but the research funding strategies are more focused on enhancing our knowledge about disease-related genes than on identifying genes involved in EVCs (7). Exploring the unique normal characteristics of a person is equally important as identifying the disease-related SNPs and genes. Genetics and environmental factors significantly influence a person's phenotypic characters. To date, variations in the genes linked with pigmentation are extensively studied. Variations in the human iris colouration, skin and head hair are the best and the practical paradigm of FDP, as shown in **Figure 1** (8).

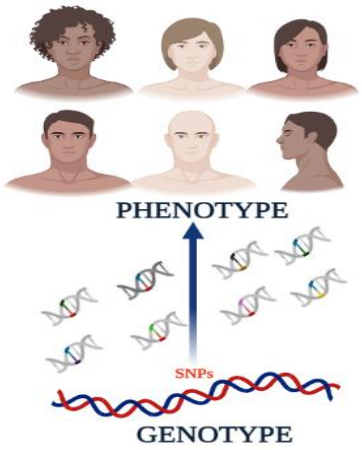

Figure 1. Genotype to phenotype

2. SNPs versus STRs profiling

Recently, STR testing has been more widely used in FDP techniques; however, SNP testing is now surpassing the existing STR methods. SNP testing successfully analyses DNA with low amounts and high degradation activity, an achievement not possible with the STR technique. Repetitive sequences, due to their high polymorphism in STRs, hinder the effective analysis of short sequences, making SNPs a more viable option for FDP (3). Moreover, the remarkably lower rate of mutation in SNPs and comparatively shorter length than STRs reduces the inter-sample contamination in polymerase chain reaction (PCR) processing (9). Another key feature of SNP testing lies in their greater abundance in the human genome than STRs, increasing the likelihood of building complete profiles compared to what is attainable with the STR approach (3); however, the downside of the SNP-based system is that it gives lower discriminating power and does not give good results with the mixed samples. Although there are numerous benefits of an SNP-based system, it is improbable that SNPs will supplant the STR-based system in the near future. Incompatibility of SNP-based systems with STRs and creation of new databases for SNPs and updating existing STR records with SNP profiles limit the replacement of existing systems with SNPs in FDP (3).

3. Phenotype prediction using Genetic Biomarkers

3.2 Eye Colour

Eye colour, which is the most prominent human trait, varies from lighter shades of blue to darker shades of brown, including intermediate colours like grey, yellow, hazel, and green, for example. This colour difference of the eye can be attributed to the number of melanosomes and amount of melanin present in the iris outer layer, just like the pigmentation pattern of skin and hair. Brown eyes have more melanosomes than the blue eyes (10). Irisplex System8 was one of the first phenotyping tools established and substantiated for 6 SNPs in different pigmentation genes (OCA2 (rs1800407), HERC2 (rs12913832), SLC45A2 (rs16891982), SLC24A4 (rs12896399), TYR (rs1393350), and IRF4 (rs12203592)) as shown in **Figure 2** (6). This remarkable system provides >90% accuracy in differentiation of brown and blue colour both in admixed and homogenous populations. It was recently tested in United States samples and corresponded to the accuracy in prediction as observed in Europeans. However, the accuracy of this tool was limited when tested in an Asian population (11). In another study from Iraq, the IrisPlex System was validated in the context of accuracy in the prediction of eye colour. Six SNP sets were tested using Sequenom MassARRAY Genotyping. It was tested for 3 categories, and specificity was 100% for intermediate, 100% for blue, and 78.13% for brown colour. This study included only 58 subjects, so a better idea about prediction accuracy could be achieved if a maximum population of different geographical origins is screened (12).

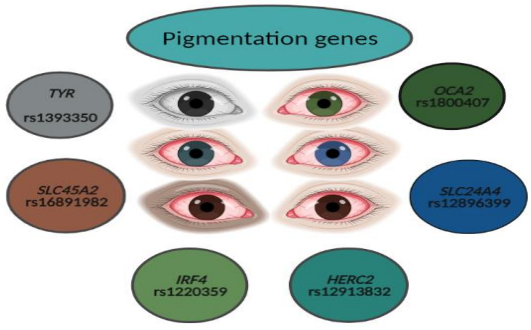

Figure 2. Major predictive genes for pigmentation in IrisPlex system

Besides this, intermediate eye colour is a major problem that requires further research in terms of the genetic variants associated with it. Accuracies achieved in the prediction of intermediate colour are much lower than those of blue and brown eye colour (13). Eye pigmentation is largely influenced by the gender. It has been extensively observed that in European countries, men tend to have lighter eye colour (mostly grey and blue) than women (mainly brown and green). However, no genetic factor has been discovered yet to explain this correlation. Substantial efforts are needed in the forensic field to explain these differences (4, 14). The SNP rs12913832 was considered the highly conserved SNP of the HERC2 gene predicting the eye colour. It is present on chromosome 15, upstream from the OCA2 promoter region (15). This SNP (rs12913832) in alliance with OCA2 SNPs has been observed to be highly associated with iris colour, primarily blue colour. In another independent study from Iran comprising 200 samples, the strong association of rs12913832 with iris colour was assured (16). Three SNPs, rs1800407 (OCA2 gene), rs1393350 (TYR gene), and rs12913832 (in the HERC2), were found to be significantly associated with eye colour in the Pakistani population of the Swat region. In this population brown colour was observed more than that of blue or intermediate colour. Like previous studies, accuracy of prediction remained the same (100%) for blue and brown colour; however, the IrisPlex tool incorrectly predicted the intermediate colour as blue or brown in this population. In this situation inclusion of some more genetic markers in the IrisPlex system is strongly needed to predict the intermediate colour with precision (17). No gene can predict the iris color solely, therefore, intergenic complexity and the population differences must be taken into account when predicting any phenotype (18).

1.2 Hair colour

Among the most noticeable EVCs, hair colour stands in a prominent place. Two types of melanin determine the hair colour. Eumelanin (brown/black) and pheomelanin (red/yellow) are the major two types of melanin pigment responsible for variations in hair colour (19). Red-haired individuals possess more pheomelanin than eumelanin, while dark hair has elevated levels of eumelanin (20). MCIR has been thought to demonstrate a good discriminating power for whiter skin colours, freckles and red hair. Later, a predictive model comprising 22 SNPs (including HERC2, SLC45A2 and SLC24A5) was developed with 81%–93% accuracy for different hair colour categories (21). With advances in FDP, HIrisplex System was created in 2013 that contained 18 marker alongwith 6 preexisting Irisplex SNPs (5). Scientists exploited the genotypic and phenotypic correlation between hair and eye colour with the aim of expanding our understanding of the genetic influence of hair colour variations and prediction models. Secondly, the combined assay was thought to be useful, giving an SNP profile for hair and eye colour in a single run and saving resources and time. The expanded assay combined with preexisting Irisplex SNPs entitled as HIrisplex (5) Hirisplex entails markers from the *SLC45A2, KITLG, IRF4, ASIP, TYR, SLC24A4, HERC2, OCA2, EXOC2*, and *TYRP1* genes (21).

Hair color statistical probabilities were checked initially from 1243 polish, Greek, and Irish subjects. After testing many alleles at specific genotypic loci model for hair color was developed that included 11 variants from *MC1R* gene N29insA, rs885479, rs1805008, rs11547464, rs1805007, rs1805005 rs1805009, rs1805006, Y152OCH, rs1110400, rs2228479, and rs12821256 (*KITLG*), rs28777 (*SLC45A2*), rs16891982 (*SLC45A2*), rs1800407 (*OCA2*), rs683 (*TYRP1*), rs4959270 (*EXOC2*), rs2378249 (*ASIP/PIGU*), rs12913832 (*HERC2*), rs1042602 (*TYR*), rs2402130 (*SLC24A4*), rs12203592 (*IRF4*) (5) This system gives prediction of four categories of hair color black, brown, red and blond including probabilities of the lighter or darker shades as presented in **Figure 3**. A final hair colour prediction can be made based on the genotypic and phenotypic information extracted from them. This system has been widely used in the recent past, giving reliable and accurate results; however, more individuals should be added to the existing databases covering the individuals outside the European descent. Given its robustness and specificity, the HirisPlex system has been found suitable for DNA analysis in evolutionary and anthropological studies (5). Moreover, the parallel analysis of hair and eye colour using the HIrisPlex system makes it most suitable for forensic cases. In a recent study from the North Eurasian region, the accuracy of the established prediction system was checked on populations living in border regions of Asia and Europe. Researchers found that the precision of the well-established systems for predicting eye and hair colour is lower than estimated in Europeans. Population differences need to be considered for wide applications of these systems. More variants should be screened in different populations, and newly discovered SNPs can be added to the pre-existing panel to make it uniformly usable for other populations (22).

3.3 Skin colour

Skin colour prediction has become a hot topic in the forensic field with increasing advances and its applicability in the FDP. However, the deficit of the strong consensus on the approaches for documenting the skin colour phenotype and the other muddling factors, including epistasis and environmental influence, for instance, tanning from sun exposure, and various unmarked genetic variants, pose a greater difficulty in the development of the skin colour prediction test (23). It has been studied that variations in the skin pigmentation evolved chiefly in response to ultraviolet rays (UV) in different planetary regions. People living in the regions closer to the equator line encounter high UV radiation and selective pressure, leading to darker skin colour, while the distant regions present lighter skin colour (24).

Previously, genome wide association analysis (GWAS) studies were conducted in European (25, 26) and Asian regions (27) for gene mapping of skin colour. However, GWAS studies are not suitable for genetically heterogeneous group of populations. Skin colour variations exist between

Figure 3. Illustration of the HIrisPlex system for hair color prediction (Figure adopted from Walsh et al. 2013)

the different continents, so limiting the complete mapping of genes responsible for pigmentation. This was best demonstrated by Valenzuela et al. where they performed a study on multi ethnic study group for 3 SNPs named *SLC24A5* rs1426654, *SLC45A2* rs16891982, and *ASIP* rs2424984. These SNPs achieved a R2 value of 76.3% for hair color, 76.4% for eye color whilst only 45.7% for skin color (27). Many studies have been done with 6-7 SNPs set to predict the skin color but inconclusive outcomes were obtained with low error rates (28-30).

In 2014, skin reflectance was measured by the Maronas et al. (2014), where they used 59 SNPs set formerly linked with hair and eye colour (4). This study included 280 non-European and European individuals, and skin color information was collected through the structured questionnaire. In this comprehensive study the authors identified a subset of 29 SNPs that was significantly correlated with the skin color variations as presented in **Table 1**. These 29 SNPs were marked prime SNPs providing basis for separation of most black/intermediate skin colored persons and most white skin colored individuals. Previously suggested 10 SNP sets, including *SLC24A5* rs1426654, *SLC45A2* rs13289, *SLC24A4* rs2402130, *SLC45A2* rs16891982, *ASIP* rs6058017, *KITLG* rs10777129, *OCA2* rs1448484, *TPCN2* rs3829241, *TYRP1* rs1408799, and *ASIP* rs6119471 have been reported to predict the skin colour variations with success in iterative naïve Bayes analysis. As the study encompassed relatively smaller sample size, so further data from larger groups is needed to validate and judge the accuracy and reliability of these results (29, 30).

Several evolutionary factors limit the genotype/phenotype associations in mapping studies. The resulting correlation applies to a specific group of population, ultimately admixed populations do not give the same discriminatory power (31). Reckon with this evolutionary bar, a global model was established expanding 36 markers of 16 pigmentation genes (4). In this model three (dark–black, dark, light) and five skin tones (dark–black, dark, intermediate, pale, very pale) were considered with prediction accuracy of 83-97% and 72%-97% respectively.

Figure 4. Illustration of skin color prediction model provided with the HIrisPlex-S DNA test system (Figure adopted from Chaitanya et al., 2018)

A new, forensically validated HIrisPlex-S DNA test system (S for skin) was also developed for prediction of skin, hair and eye color at a time from biological sample DNA. This advanced system comprised of two SNaPshot-based multiplex assays. HIrisPlex-S system targets total 41 SNP, 17 for skin color and 24 SNPs for hair and eye color prediction previously part of HIrisPlex assay as presented in **Figure 4**. HIrisPlex-S encompasses 3 statistical models for simultaneous prediction of eye (6 SNPs IrisPlex model), hair (22 SNPs HIrisPlex model), and skin color (newly incorporated 36 SNPs HIrisPlex-S model) (4).

Table 1: The 29 most associated SNPs with the skin color in SHEP (skin, hair, eye pigmentation assay) 1, 2 and 4.

Markers	Gene	Position	Chr.	Assoc[a]	References	SHEP
rs13289	*SLC45A2*	33986409	5	H/S	(21, 26, 32, 33)	
rs16891982	*SLC45A2*	33951693	5	E/H/S	(27, 34) (30, 35)	
rs26722	*SLC45A2*	33963870	5	E/H/S	(32, 36)	1
rs3782974	*DCT*	95092896	13	S/A	(36)	
rs1426654	*SLC24A5*	48426484	15	H/S	(27, 30, 34, 37, 38)	
rs1015362	*ASIP*	32738612	20	E/H/S	(21, 39)	
rs1805005	*MC1R*	89985844	16	H	(5, 21)	
rs1805009	*MC1R*	89986546	16	H/S	(5, 21, 35, 40)	
rs1540771	*IRF4*	466033	6	E/H/S	(25, 26, 39)	

	EXOC2					
rs3829241	*TPCN2*	68855363	11	H	(21)	2
rs1408799	*TYRP1*	12672097	9	E/H/S	(21, 39, 41)	
rs35264875	*TPCN2*	68846399	11	E/H/S	(21, 39, 42)	
rs6058017	*ASIP*	32856998	20	E/H/S	(21, 43)	
rs4911414	*ASIP*	466033	20	H/S	(39)	
rs17305657	*C20orf71*	31806588	20	S	(44)	4
rs1448484	*OCA2*	28283441	15	H/S	(35, 36)	
rs2424984	*ASIP*	32850375	20	H/S	(34)	
rs1545397	*OCA2*	28187772	15	F	(30)	
rs1805006	*MC1R*	89985918	16	H/S	(5, 21)	
rs12931267	*FANCA*	89818732	16	H/F	(44)	
rs619865	*EIF6*	33867697	20	S	(45)	
rs1805007	*MC1R*	89986117	16	H	(34), (5, 35, 46)	
rs2402130	*SLC24A4*	92801203	14	H/S	(5, 35, 39)	
rs28777	*SLC45A2*	33958959	5	H	(26), (5)	
rs6119471	*ASIP*	32785212	20	H/S	(30, 35)	
rs642742	*KITLG*	89299746	12	S	(47)	

Assoc: association with H, hair; S, skin; A, AIM (ancestry-informative marker) ; E, eye; F, freckles.

3.4 Height

Height is considered the most complex trait in human genetics that follows non-Mendelian inheritance (22). To date, four genome-wide association analysis (GWAS) have been conducted with considerable sample size by international Genetic Investigation of Anthropometric Traits (GIANT) Consortium (48). These studies have clearly demonstrated the complexity of height genetics involving hundreds of independent SNPs with very small to very large effects. Scientists have predicted that there may be thousands of SNPs that independently contribute to this trait. In a recent study on the height of the GIANT Consortium by third GWAS meta-analysis, 512 new markers have been identified. This analysis was based on 693529 Europeans and predicted the tall stature with high precision (49). Given the forensic requirement to minimize the marker number and maximizing the precision accuracy, another effort was made by Liu et al., (2015) to introduce a subset of 412 predictor SNPs from 689 SNPs. Accuracy achieved by 689 SNPs corresponded to subset of 412 SNPs. However, markers for full height range remained to be evaluated. To date, many genes have been found associated with human height prediction, including *ZBTB38*, *ESR*, *GDF5-UQCC*, *VDR*, *HMGA2*, *CYP-19* and *HHIP*. Many of these predictor genes are not playing direct role in height determination but through growth-signaling pathways. Even with substantial increase in number of variants for height prediction still there are no significant values for accurate prediction of this phenotype across different populations (50). Most current studies have been shown to give accuracy of 75% and failed to achieve higher percentage. When talking about height, it should also be taken into account that this phenotype may have different etiology apart from genetic factors such as nutrition, environmental and hormonal factors, pathologies, and lifestyle (51). There are still many gaps in validating the previously reported SNPs for tall stature. Moreover, height cannot be exactly grouped into distinct categories like eye or hair colour due to the continuous variations. There is much more to do in this particular field of FDP to launch the assays for accurate prediction in every population.

3.5 Age

Age assessment is an exigent task for forensic experts in FDP. There are several methods by which age estimation can be done, such as morphological examination of bones and teeth and other changes of biomolecules associated with aging process (e.g. aspartic acid racemization protein glycosylation, mitochondrial deletions and telomere shortening) (52, 53). Typically, age estimation can be achieved by mRNA mutation, telomere length, or by sjTRECs but the prediction accuracy is not sufficient enough to proceed with a forensic case (54). Epigenetic markers have gained utmost importance as a substantial forensic tool.

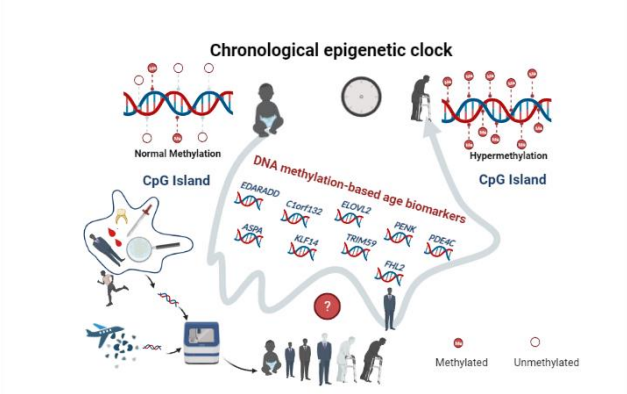

Figure 5: Age prediction by DNA methylation approach (Figure produced through Biorender)

In the recent past, growing knowledge of epigenetics has provided evidence for a direct correlation of age and DNA methylation alterations in genome (55). DNA methylation involves the modification of cytosine nucleotide at the 5' position with the presence of methyl group followed by guanine nucleotide called as CpG site (56).

With the progressing age, DNA methylation level changes, hypermethylated and hypomethylated located in CpG island or outside the CpG island respectively, as demonstrated in **Figure 5** (57). In this context, various studies have reported prediction models built on different tissues, genes, age predictive analysis and detection technologies. Presently, DNA methylation is contemplated as most suitable biomarker for age prediction. To date, several genes have been discovered for age prediction such as *FHL2*, *ASPA*, *PDE4C*, *ELOVL2*, *PENK*, *EDARADD*, *TRIM59*, *C1orf132*, and *KLF14* (58). Among all, *ELOVL2* gene present at chromosome 6 is considered as the best age predictor. with the aging process *ELOVL2* promoter undergoes hypermethylation (59). Despite promising results in several independent studies, further candidate genes are required to enhance these results for more accurate age estimates. *PDE4C* (phosphodiesterase 4C) and *ASPA* (aspartoacylase) were the most widely used genes along with *ELOVL2* in prediction models (60).

Blood and mineralized tissue analysis has revealed hypermethylation of CpG sites in *ASPA* and *PDE4C* (57). In a recent study, CpG sites were evaluated for DNA methylation in *ELOVL2*, *PDE4C* and *ASPA* genes. The results of this study demonstrated that *ELOVL2* and *PDE4C* can be a good option for chronological age estimation instead of telomere length. The quantile prediction model for age prediction developed in this study could improve the prediction accuracy in future studies of forensics (61). In another recent study, DNA methylation level was analyzed for *ELOVL2*, *EDARADD*, *FHL2* and *PDE4C* and the results of this study for age estimation were in accordance with previous studies (62). However, the DNA source should be taken into account in estimation studies, as DNA methylation pattern can be varied for blood samples, tooth or other tissues. The most important thing to be taken care before its widespread adoption in forensic field is to evaluate epigenetic variation in more ethnic populations so that environmental and genetic influences on DNA methylation level can be demonstrated.

3.6 Facial features

The uniqueness of every person's face lies in the spatial arrangement (horizontal, vertical and depth) and shape of different facial features (e.g. lips, eyes, nose etc.). Facial morphology prediction is of great importance when it comes to the suspect's DNA analysis**.** Amid all EVCs, facial features prediction is the foremost objective that can narrow the pool for suspect/targeted person identification (63). GWAS have explored millions of SNPs in the context of facial unique features. To date more than 50 loci have been found to be associated with the facial variations that is great landmark in FDP (64). There are genes associated with more than one features. Different genes associated with facial traits are documented in table 2. Most important point, considering different variants associated with facial features, congenital anomalies should not be ignored. Embryonic development patterns greatly influence the facial features so all these factors should be disentangled before making an association (65). Cranio-facial

candidate genes have been tested for facial phenotyping in recent past. This approach was employed for Brazilians, US Americans, and Cape Verdeans and large differences in frequency were observed. The authors scrutinized 24 SNPs from 20 genes with preliminary association with facial features (66). Keeping in view the very little work done on this particular aspect of FDP, it is very much clear that we are at initial stage of comprehending which gene (SNPs) dictate facial variations. To unveil all the factors involved in facial features, there is a long way to go in FDP (65).

Table 2: Association of different genes with facial features in normal individuals (Data adapted from **(63)**

Phenotype	**Gene**
Eye nasion distance	*COL17A1, PAX3*
Nose height	*PRDM16*
Nasion,eye,zygoma,ear distance	*C5orf50, TRPC6*
Inter tragi	*FOXA1, MAFB, MIPOL1,PAX9, SLC25A2*
Nose tip	*BC039327, CASC17, KCTD15, PAX3, SOX9,* Intergenic
Gonion-eye angle	*OSR1-WDR35*
Alae to nose tip	*CHD8, CACNA2D3, PDRM16, ZNF219*
Alae breadth	*PAX1, PRDM16*
Forehead	*EYA4,GL13,RPS12,TBX15*
Bridge of nose	*EPHB3, DVL3, PAX3, RUNX2, SUPT3H*
Eye shape	*HOXD1-MTX2, WRDR27*
Nasal sidewalls	*PAX3, SUPT3H,* Chr1p32.1-intergenic
Mid-face height	*PARK2, MBTPS1 (profile)*
Alae	*DCHS2, DVL3, EPHB3, KCTD15, SOX9*
Nose prominence	*CACNA2D3, DCHS, ZNF219,CHD8,PRDM16*
Lips	*ACAD9, FREM1, HOXD* cluster, *RAB7A*
Mental fold	*PKDCC*
Chin	*ASPM, DLX6,DYNC1L1, EDAR*

4. Social, Ethical and Legal concerns in Forensic DNA phenotyping

Misleading and incorrectly undertaken analysis of phenotyping can have a negative impact on society. If phenotyping analysis reveals a phenotype that is

associated with a certain group in the population, it can lead to social stigmatization, which is serious concern for some scientist to proceed with phenotyping (67). Biogeographical markers greatly influence the traits that are associated with certain ethnic groups, such as skin colour. DNA phenotyping at the same time makes it possible to narrow down the suspected individuals pool to exclude the innocents from the criminal investigation, thus providing a great social advantage as well. The most widely discussed ethical concern in FDP was invasion of privacy (68). Some of the invisible phenotypes that will be drawn from DNA can violate the privacy of a person. There is lack of agreement between many countries on the practical use of FDP (69). Some countries have implemented the strict laws on use of FDP in context of phenotype. The only information that can be used is the sex inference drawn from the DNA source. Moreover, the legal basis of this technique has not been yet defined clearly. It can be used for investigative purposes but will not have any validation in the court (70).

5. Future prospects

The role of molecular genetics in forensic science is extremely important. Forensic DNA phenotyping is the advancing technique in forensics that have an impressive potential to make landmarks in the crime investigation. It can be widely employed in the cases of disaster, missing persons or other crime cases. To date, several biomarkers have been discovered and tested for different human phenotypes, but most of this research was focused on the European population. Genetic differences existing in different populations questions the prediction accuracy of these markers for individuals from Asian or Western descent. Prediction accuracies achieved so far needs further validation in other ethnic groups and some additional markers need to be added to enhance the predictability. There is much more work to be done to define and build this field by cross-border collaborations for the betterment of community. The knowledge we have so far is still limited about EVCs in terms of the expression profile of the biomarkers identified up till now, how they contribute to a certain phenotype and characteristic remained to be evaluated. The biomarkers identified for different phenotypes should be combined to multiplex assays for better implication of FDP. The persuasiveness of this emerging technique lies in its reliability in different age groups as well. Another important concern employing SNPs in FDP is its role in the disease susceptibility. There are many genetic conditions that appear in the later age but the genetic susceptibility is there. So it must be considered that the SNPs which are found to be associated with a phenotype may not have a role in some genetic disorder. Moreover, maximum biomarkers have been evaluated and reported for skin, hair and eye color and less markers exist for other phenotypes such as ear lobe shape, teeth shape, and facial features. Profound research is required to evaluate these aspects to predict the complete composite face in terms of congruency. The legal basis of FDP should be defined clearly in future to make it applicable in the field and the general population should be made aware of this technique and the possible marginal errors to avoid any ethical or social issues. Misconceptions and stigmatization should not limit its use in the future; instead, laws should be made to regulate its restricted use. A day will come when the suspect's photo will be away from a single click if the consistency and ambition to seek the truth and justice is maintained.

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