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Analysis of genetic polymorphisms associated with the presence of freckles for phenotypic prediction

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ABSTRACT

The prediction of externally visible characteristics (EVCs) is a commonly used practice by the forensic sciences as an important resource in the investigation of criminal cases in which the identity of perpetrators or victims is unknown or even to recognize decomposed cadavers. With this purpose, genetic markers associated with pigmentation traits have been widely studied by forensic scientists and, nowadays, it is possible to predict phenotypic characteristics such as hair, eyes and skin colour, as well as the presence of skin freckles by analysing single nucleotide polymorphisms (SNPs). In this study, we analysed the association of six SNPs located in pigmentation genes to the presence of freckles in individuals from the Brazilian population for forensic DNA phenotyping. The study was based within the context of a larger project on a population sample of 534 adult Brazilians of both sexes and different skin colours. DNA was extracted from peripheral blood and genotyped using the *TaqMan® OpenArray® Real-Time PCR System* (ThermoFischer Scientific) technique. Statistical analyses were carried out with the R software (version 4.0.2). As for the results obtained, three SNPs were shown to be statistically associated to the freckling, *rs12203592*, *rs1800404* and *rs222847*, with CT, AG and AA genotypes being the main contributors, respectively. Variables such as sex of the individuals and skin colour were found to also contribute to the manifestation of this pigmentation trait. Further statistical analyses will be carried out to evaluate the possibility of using the SNPs in this study for phenotyping prediction of the Brazilian population, improving existing DNA phenotyping models in forensic sciences.

1. Introduction

Genetic markers present in pigmentation genes have been used to perform phenotypic prediction tests in order to assist forensic investigations in cases where the identity of suspect or victim is unknown. These tests involve the genotyping of selected SNPs to determine one or more phenotypes, such as hair, eye and skin colour [1], the presence of freckles, among other traits. Freckles, or ephelides, are externally visible characteristics (EVCs) that consist of hyperpigmentation of certain regions of the skin, and it is currently known that allelic variants of the MC1R gene are the main contributors to the formation of freckles, already described in the Caucasian and Japanese populations [2–6]. Studies on human pigmentation report that the presence of polymorphisms in the IRF4, ASIP, TYR, BNC2, HERC2, OCA2 genes and SLC45A2 haplotypes are associated with freckling [3–12] and influence the amount, size, colour and pattern of manifestation of this EVC. The phenotypic prediction of ephelides has great importance for forensic sciences and together with other pigmentation traits such as eye, hair and skin colour can provide more complete and accurate information about an individual's appearance, complementing existing models and prediction systems. In this study, we analysed the association of six SNPs located in pigmentation genes to the presence of freckles (*rs1042602*, TYR; *rs1800404*, OCA2; *rs11636232* and *rs2238289*, HERC2; *rs12203592*, IRF4; *rs2228479*, MC1R), evaluating the association of polymorphisms with freckling in the total sample, considering the sex of the individuals in the study cohort and their skin colour (white, black, brown and yellow).

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2. Material and methods

The study cohort consisted of 534 adult Brazilians of both sexes and different skin colours (white, black, brown and yellow). The presence of freckles was classified by self-declaration of the participants (present or absent) and by the analysis of photographs taken of the area of the volunteers' eyes. As for the skin colour, the participants were classified by two researchers according to the four skin colour categories used by the *Instituto Brasileiro de Geografia e Estatística* (IBGE) to categorize the Brazilian population, which are white, brown, black and yellow.

The selection of the SNPs was based on a review of the literature about phenotypic prediction of freckles and pigmentation genes, being considered the polymorphisms which association with the presence of freckles has been established with a minimum statistical significance of less than 5 % ($p < 0{,}05$). Out of the 34 SNPs found associated with freckling, 6 were already present in the laboratory database, previously genotyped, composing a larger project, which objective was to analyse 41 polymorphisms for phenotypic prediction of hair, eyes and skin colour in the Brazilian population. The freckling phenotype was evaluated for the first time in this context.

DNA was extracted from peripheral blood through the salting-out method [13], and genotyped using the *TaqMan® OpenArray® Real-Time PCR System* (ThermoFischer Scientific) technique. Statistical analyses were carried out with the R software (version 4.0.2) and all selected SNPs as well as the variants sex and skin colour were submitted to the Pearson's Chi-Square test and Odds-Ratios with 95 % confidence intervals (CI) were determined. Only the DNA variants that indicated an association with the freckling phenotype were considered in the analyses that included sex and skin colour variants. Values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

From the statistical analyses performed, it was observed that out of the six selected SNPs, three showed association ($p < 0.05$) to the presence or absence of freckles, *rs12203592*, *rs1800404* and *rs222847*, with CT, AG and AA genotypes being the main contributors, respectively (Table 1). Although the *rs222847* did not present statistically significant OR (Odds-Ratio) values, it was included in the analysis of the sex of the individuals and skin colour, due to its $p < 0.05$.

In the analysis of the sex variable for the manifestation of freckles, a value of $p = 4.0872 \times 10^{-7}$ was obtained, which indicates that the variant is relevant to freckling in the study cohort. Evaluating the SNPs individually, it was observed that *rs12203592* presented statistically significant values for both male and female sexes, with $p = 2.3857 \times 10^{-7}$ for females and $p = 2.203 \times 10^{-4}$ for males. On the other hand, SNPs *rs1800404* and *rs2228479* only indicated an association between females and freckling, whose p values were $p = 9.414 \times 10^{-3}$ and $p = 0.02683$, respectively. It is known that there is a higher prevalence of freckles in females than males, as seen in the results obtained for the *rs1800404* and *rs2228479* in other studies [6,9]. Skin colour was found to be relevant for the occurrence of freckling ($p = 9.1262 \times 10^{-5}$) and within the four colours analysed, white and brown skin colours indicated a statistical association to the manifestation of the EVC, $p = 5.214 \times 10^{-8}$ and $p = 0.04585$, respectively. The white skin colour is frequently reported in literature to the presence of freckles due to allelic variants located in pigmentation genes [6] and as for the brown skin colour, its association with freckling has not yet been reported in literature and therefore further analyses should be carried involving this skin colour and the EVC.

4. Conclusion

Phenotype prediction using genetic markers associated to pigmentation traits has become an important resource in the investigation of criminal cases in which the identity of perpetrators or victims is unknown. Based on the results obtained in this study, three SNPs *rs12203592*, *rs1800404* and *rs2228479*, as well as the variables skin colour and sex of the individuals, indicate promising results for forensic prediction. Further analyses including the six SNPs and a DNA-based predictive model to freckling will be performed in order to evaluate the possibility of using those polymorphisms for forensic phenotyping prediction of the Brazilian population.

Table 1
SNPs that indicated association with freckling and their respective *p*-values estimated by Pearson's Chi-Square, Odds-Ratio (OR) values and 95% confidence interval (CI).

SNP ID	Genotype	*p*-value	OR and 95% CI
IRF4 *rs12203592*	CC	1.395×10^{-9}	0.24 (0.15 – 0.39)
	CT		4.12 (2.56 – 6.70)
	AG		1.77 (1.14 – 2.79)
OCA2 *rs1800404*	AA	0.0205	0.56 (0.36 – 0.88)
	GG		0.57 (0.13 – 1.04)
	GG		0.61 (0.33 – 1.19)
MC1R *rs2228479*	AG	0.0114	1.63 (0.84 – 3.06)
	AA		18.16 (0.86 – 381.14)

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Declaration of competing interest

None.

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