



A primary investigation on SNPs associated with eyelid traits of Chinese Han Adults



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ABSTRACT

Single/double eyelids present one of the most typical oriental externally visible characteristics in Asian populations. To investigate SNPs related to eyelid phenotypes in Chinese, genotyping of 4 candidate SNPs was performed with DNA from 96 (single/double eyelids 48 respectively) healthy Chinese adults through TaqMan[®] probes. SNP rs2277404 on the ABCC9 gene had a significantly different distribution for C/T and T/T genotype frequencies in single/double eyelids populations ($p < 0.05$); no difference was found for rs7316271 of the ABCC9 gene. Inconsistent genotype frequencies between single/double eyelids groups were found for SNP rs12635264 and rs1717652 on the MASP1 gene ($p > 0.05$). More samples and genetic markers should be tested in follow-up research to predict eyelid traits with high accuracy.

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1. Introduction

Comparative theory is widely used in forensic individual identification. When facing an unknown suspect in a crime case or unrecognizable remains in a disaster, it is difficult to confirm the identity. Forensic DNA phenotyping provides a way to predict human appearance from DNA intelligence [1] to lead the police investigation. Iris and hair color can be predicted based on SNPs with high accuracy by now, other SNPs associated with physical traits have been studied these years [1], but few reports involve Chinese specific appearance. In this study, 4 SNPs screened out from craniofacial development related genes were typed through TaqMan[®] probes to identify any possible relationship with the single/double eyelid traits in Chinese Han population.

2. Material and methods

Based on a previously reported paper [2,3], we classified people into two groups of varied eyelid phenotypes: typical single eyelid (no double eyelid line above the superior palpebral margin) and

double eyelid (clear and complete double eyelid lines were identified above and paralleled to the superior palpebral margin). 96 random unrelated individuals (48 for single/double eyelid groups respectively) were taken as volunteers with Informed Consent based on these inclusion criteria: healthy Chinese Han adults (age varied 18–50 years), no trauma, disease or surgical history of eyes and eyelids, no genetic diseases which could affect shape of eyes and eyelids.

A frontal high resolution electronic photograph of each volunteer's full face was taken with the eyes being at the same height of the camera lens. The phenotype of each volunteer's eyelids was evaluated and recorded as well as their basic information. A peripheral blood sample (1.5–2 ml) was collected from each volunteer, followed with genomic DNA extraction through phenol-chloroform protocol. The original extracted DNA (A260/280 1.70–1.94) was diluted to 1 ng/μl and was stored at 4 °C for subsequent genotyping.

Four candidates SNPs, i.e. rs2277404, rs7316271 on ABCC9 (Gene ID: 10060) and rs12635264, rs1717652 on MASP1 (Gene ID: 5648) have been selected and were typed through TaqMan[®] probes on the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instruction with 10 μl reaction mix: TaqMan[®] SNP Genotyping Assays, Human (ABI, PN: 4351379) 40 × 0.25 μl, TaqMan[®] Genotyping Master Mix (ABI, PN: 4351379) 2 × 5 μl, DNA dilution (1 ng/μl) 4.75 μl, PCR parameters: 95 °C 10 min for enzyme activation, 92 °C 15 s for

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Table 1
Genotype frequencies of rs2277404 on ABCC9 in single/double eyelids population.

Genotype	Single eyelid (%)	Double eyelid (%)	<i>p</i>
C/C	13 (27.1)	14 (29.2)	0.82
C/T	31 (64.6)	20 (41.7)	0.024
T/T	4 (8.3)	14 (29.1)	0.009
Total	48 (100.0)	48 (100.00)	–

denaturation and 60 °C 1 min for annealing/extension, 59 cycles on 96 wells plate.

Results of SNPs typing were auto-analysed by SDS v1.4 built-in 7500 Real-Time PCR System. All data derived from SDS v1.4 were analysed using SPSS 19 (SPSS Incorporation, Chicago, Illinois, USA) for the genotype frequency and χ^2 test between two groups—a significant difference between two variables should be confirmed when *p* value <0.05.

3. Results

Significant differences were found for C/T, T/T genotype frequencies for rs2277404 between the single/double eyelids groups (Table 1); each genotype frequency of rs7316271 was similar between two groups. No statistical significant differences were identified for rs12635264 and rs1717652 of MASP1, but the data did indicate inconsistencies between the single/double eyelids groups: the C/T and T/T genotype frequency of rs12635264 were 37.5% and 29.2% in single eyelid group, while 52.1% and 16.7% in double eyelid group; the C/T and C/C genotype frequency of rs1717652 were 56.3% and 27.1% in single eyelid group, while 45.8% and 39.6% in double eyelid group.

4. Discussion

Eyelids, including superior and inferior eyelid, covering the eyeball, are located in front of the orbit. A recognizable skin fold line above the superior palpebral margin forms the double eyelid (or superior palpebral fold), the contrary would form the single eyelid. The anatomic structure of the double eyelid varies between Mongoloid and Caucasian, which in turn leads to different eyelid phenotypes [4]. The single eyelid trait is rare in Caucasians. The occurrence rate of double eyelid is around 53% and 41% for single eyelid in Han population. The phenotype of single/double eyelids varies with ethnicity, age, geography and craniofacial development [5–7]. With good heritability and race specificity, eyelids are a complex trait affected by multiple genes and environmental factors. In brief, single/double eyelid phenotypes present a recognizable marked trait of oriental peoples' faces and are consequently good for gene marker association study.

The factors which could affect the eyelid phenotype, e.g. age, ethnic, trauma, (genetic) disease or surgery, were excluded from this study when collecting volunteers to limit the influencing factors to the genetic level, to subsequently get the isolated role of genetic markers.

Based on massive paralleling sequencing data from Miseq[®] (Illumina, San Diego, Ca, USA), 4 candidates SNPs on ABCC9 and MASP1 genes, which were both considered to be correlated with craniofacial development [8,9], were screened out from several genes in a previous experiment (data not showed). After performing genotyping of the 4 SNPs through TaqMan[®] probes using 96 single/double eyelids individuals' DNA, significant C/T and T/T

genotype frequency differences for rs2277404 on ABCC9 were identified between the two groups, while no difference for rs7316271 was found. Although no statistical differences were confirmed for rs12635264 and rs1717652 of MASP1 (see results) according to the χ^2 test, which might be due to the limited sample size in this study, the data did show asymmetry between groups. A larger sample size needs to be tested to get more reliable dataset in follow-up research.

5. Conclusion

According to this primary research, the genotype frequency for one SNP did correlate with the eyelid phenotype (C/T and T/T genotype frequency on rs2277404 of ABCC9 varied between single/double eyelid population). No statistical significant differences for other SNPs were found probably due to limited sample size. It can be expected that based upon enough samples and more SNP analysis in the future highly accurate eyelid phenotype prediction using genetic markers will be established.

Conflict of interest

None.

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