



Trade Science Inc.

# BioTechnology

*An Indian Journal*

---

**FULL PAPER**

BTAIJ, 1(3), 2007 [112-116]

## Screening Of Human Eye Shape Gene Polymorphism By Polymerase Chain Reaction

Y.Farida Zuraina<sup>1\*</sup>, H.Khairedza Rahmi<sup>2</sup>, O.Khairul<sup>2</sup>, S.Rita Rohaizah<sup>3</sup>

<sup>1</sup>Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor (MALAYSIA)

<sup>2</sup>Biomedical Department, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia,  
Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, (MALAYSIA)

<sup>3</sup>INTEC Section 17, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, (MALAYSIA)

Tell : 6-03-55443889 ; Fax: 6-03-55444562

E-mail : fzuraina@salam.uitm.edu.my

Received: 7<sup>th</sup> August, 2007 ; Accepted: 12<sup>th</sup> August, 2007

### ABSTRACT

Rape victims have usually poor recollection of their rapist. In certain cases where victim is lucid, facial reconstructions through sketching are done by the help of trained forensic sketchers. Despite assistance, minor facial characteristics which would improve overall facial structure have always been overlooked due to the poor lighting at the site of incidence or the poor recollection of the victim. Since rapist DNA can usually be obtained from the rape site, a study was conducted to look at relationship between the eye shape and polymorphism of the eye lid gene. A total of 51 Malays, 23 Chinese and 18 Indians DNA were extracted using QIAamp DNA blood mini kit (QIAGEN, Hilden Germany). Amplification of the eye gene loci was done using GDB11509038For and GDB11509038 Rev primers. Later polymorphism was determined using restriction enzyme, BsmAI. Results indicated that 86 samples shown positive amplification with GDB11509038 primer pair. The polymorphism patterns are found to be inconsistent with eye shapes.

© 2007 Trade Science Inc. - INDIA

### KEYWORDS

Human's eye shape;  
DNA amplification;  
Polymorphism.

### INTRODUCTION

Human eye is part of human uniqueness. The Asian with almond-shaped eye differs from a typical Caucasian eye in having more fat around them. Both Asian and Caucasian eyes have fat-the latter simply have less<sup>[2,8,10]</sup>

Studies on multi-racial populations enable more accurate evaluation of inter-racial differences in preva-

lence rates by reducing confounding factors related to study design and methods<sup>[14]</sup>.

At present, three gene pairs controlling human eye color are known. Two of the gene pairs occur on chromosome pair 15 and one occurs on chromosome pair 19<sup>[5,6]</sup>. The *bey* 2(brown eye colour) gene, on chromosome 15, has a brown and a blue allele. A second gene, located on chromosome 19; the *gey*(green eye colour) gene, has a blue and a green allele<sup>[5]</sup>. A third gene, *bey*

1, located on chromosome 15, is a central brown eye colour gene<sup>[6]</sup>.

From previous studies, scientists found that chromosome 13 has the eye shape gene 'V'. Dominant genes code (VV, Vv), for almond shape and homozygous recessive (vv) is round<sup>[6]</sup>.

In 2006, the residents of Malaysia stood at 27.9 million, consisting of 58% Malays, 26% Chinese, 8% Asian Indians, 4% of other ethnic origins. It provides the opportunity to study the eye shape differences among different ethnic groups living in a similar geographic settings. Races in Malaysia have wide mixture of gene pool from all over the world because Malaysia have strong trade link between East and West since 14 B.C., and all these had a major impact on the culture, language and social customs of the country<sup>[7]</sup>, and perhaps also on the highly polymorphic eye shape.

Most of the eye shape studies among populations were performed on Caucasian<sup>[4,2,12,1]</sup> and Orientals from East Asian populations (i.e Chinese, Japanese or Koreans)<sup>[3,15]</sup> especially in the field of myopic research. Also, there have been no studies conducted on Malaysian major races. Thus, from this study, we hope to obtain and provide an estimated database not only for Malaysian population but also information for future studies.

## MATERIAL AND METHODS

### Sample collection

Three groups of subjects were recruited for this study, consisted of 51 Malays, 23 Chinese, and 18 Indians. The first group comprised of 19 students from UiTM Shah Alam, second group consisted of 46 students from Fakulti Sains Kesihatan Bersekutu, UKM, and the last group from 27 students of International education center (INTEC) UITM Shah Alam. The blood samples were collected from the three major ethnic groups in Malaysia namely Malay, Chinese and Indian. EDTA (1ml) blood was collected from each subject using *venipuncture* technique. The blood samples were stored at -20°C for further DNA extraction. Three generations of ethnic origin was sought back for each subject to ensure genetic purity.

### DNA extraction and purification

DNA was extracted using QIAamp DNA Blood

Mini Kit (QIAGEN, Hilden Germany). The purity and concentration of DNA were measured spectrophotometrically using UV-Vis Spectrophotometer. Purified DNA was stored at -20°C until further used for DNA amplification.

### DNA amplification

The human eye shape gene was amplified by the polymerase chain reaction using Perkin Elmer GeneAmp PCR System 2400. The oligonucleotide primers used for amplification were; GDB11509038For- 5'-TCT GTC CTT GGC TGG TGA G-3' and GDB11509038 Rev-5'-GGA AGA AGA AAG GGG AGC AT-3' at a final concentration of 50pmol each in a PCR tube size 0.5mL. Five nanogram (ng) of DNA was used as template DNA and added to the PCR mixture consisting of 25µl ready mix™ Taq PCR reaction mix with MgCl<sub>2</sub>, pairs of oligonucleotide primers, and 18µl sterile distilled water. A sample containing distilled water instead of DNA template was placed to control for cross contamination during PCR runs<sup>[9]</sup>. The PCR conditions were initial denaturation at 95°C for 3min followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 57.3°C for 1 minute, and elongation at 72°C for another 1 minute. A final extension at 72°C for 5min was included. Two micro liters (µl) of PCR products were then electrophoresed on a 1.5% agarose gel to confirm the presence of the amplified human eye shape DNA fragment which is a 260-bp DNA product.

### Restriction enzyme digestion

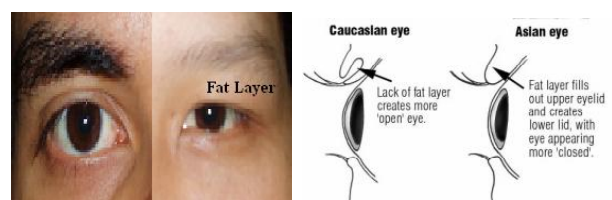


Figure 1 : Caucasian and Asian eyes differ in the amount of fat around the eye



Figure 2 : Comparison between almond-shaped eye and round eye

## FULL PAPER

PCR products were used for restriction enzyme digestion with BsmAI(New England Biolabs). BsmAI, a type II restriction-modification system from the gram negative anaerobic bacterium *Bacillus stearothermophilus* recognizes the sequence GTCTC was used for this study. The digestion mixture consisted of 1µl restriction enzyme buffer with bovine serum albumin(BSA), 1µl of PCR product, restriction enzyme at a final concentration of 1U/reaction and appropriate amount of RNase-free. The digestion mixtures were incubated for 1 hour at 37°C to ensure complete digestion and then transferred to 65°C to inhibit further digestion of the amplified DNA.

### Gel electrophoresis

The fragmented DNA was then separated using 1.5% agarose gel electrophoresis. Buffer for the process were Tris-borate-EDTA while voltage strength were set at 5V/cm. After two hours of electrophoresis the gels were then stained with ethidium bromide for subsequent visualization of PCR products under UV transilluminator<sup>[11]</sup>.

## RESULTS

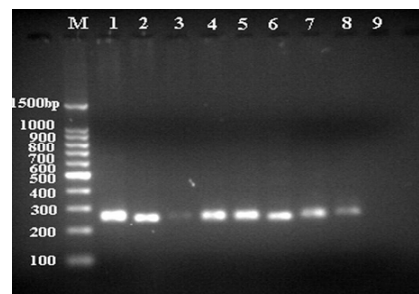
### PCR products

Figure 3 shows the PCR results at annealing time 57.3°C. From our studies, we recognized not all samples gave positive amplification. Negative control for each PCR run showed no amplification. It stated that the DNA samples and reagents were not contaminated.

### Sequencing result

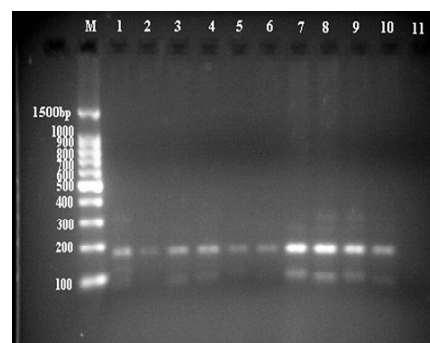
#### Confirmation of types by sequencing

PCR products were sequenced to verify the restriction enzyme digestion typing results. An aliquot of each PCR product was purified by electrophoresis on agarose and the PCR band was isolated and filtered with GFX PCR DNA and gel band purification kit (Amersham). Then, forward and reverse sequencing reactions were carried out using GDB11509038Rev and GDB11509038For. Dye terminator cycle sequencing was performed using big dye terminator Cycle Sequencing Kit(Applied biosystems) and analyzed with an ABI Prism 310 Genetic Analyzer. When more than one type was present in the sample, PCR products were



**Figure 3.** Agarose gel electrophoresis of PCR product. Product shown were obtained with PCR condition of denaturation temperature at 94°C, annealing at 57.3°C, using 1.5% agarose gel concentration at 50V for 2hours.

Lane from extremely left : M : DNA Marker 100bp, 1 : Malay 40(M40), 2 : Chinese 19(C19), 3 : Malay 41(M41), 4 : Malay 42(M42), 5 : Indian 11(I11), 6 : Indian 12(I12), 7 : Indian 13(I13), 8 : Malay 43 (M43), 9 : Negative Control



**Figure 4 :** The electrophoresis gel shows band pattern of each genotype of the eye shape gene polymorphism using BsmAI.

Lane from extremely left: M : DNA Marker 100bp, 1 : Malay 34(M34), 2 : Malay 35(M35), 3 : Chinese 15(C15), 4 : Malay 36(M36), 5 : Chinese 16(C16), 6 : Chinese 17(C17), 7 : Malay 37(M37), 8 : Chinese 18 C18), 9 : Malay 38(M38), 10 : Malay 39(M39). 11 : Negative Control

cloned using the PCR-Script Amp Cloning Kit as recommended by the manufacturer(Stratagene, Austin, TX) and then, up to five clones were sequenced. Identifications of genotypes were carried out by searches of sequences using the BLASTn tool.

## DISCUSSION

Observation of DNA extraction results shows that all DNA have successfully extracted by QIAamp DNA Blood Mini Kit(Qiagen, Hilden Germany). All DNA show a 23-kb with high intensity of the DNA.

From this study we found that 4 Malays(M5, M6, M17 and M39), 1 Chinese(C14) and 1 Indians(I8) gave

negative amplification with GDB11509038 primer pair. This might be due to the least amount of expression gene for protein development to encode eye shapes structure.

PCR products data varied widely among samples. For Malay the PCR products were 220bp, 228bp, 229bp, 231bp, 237bp, 238bp, 243bp, 244bp, 245bp, 247bp, 248bp, 249bp, 250bp, 251bp, 255bp, 258bp, 262bp, 265bp; whilst for Chinese 220bp, 229bp, 231bp, 236bp, 237bp, 238bp, 243bp, 245bp, 247bp, 251bp, 275bp; and Indians 231bp, 233bp, 236bp, 238bp, 243bp, 244bp, 247bp, 248bp, 250bp, and 251bp, respectively.

From the data obtained, there were 5 specific bands for Malays; 249bp(M10, M13), 255bp(M7), 258bp (M36), 262bp(M15), 265bp(M43). The specific band for Chinese was 246bp(C1) whilst for Indians 233bp (I14, I15).

Sequencing results followed by BLAST searches confirmed the genotyping by restriction enzyme digestion, even though not all the detected genotypes were isolated for sequencing. PCR products plotted from the graph are in line with the sequencing result which ranged from 220bp to 251bp. From the sequences, the percentage of GC contents (%GC) for each samples was calculated manually and indicated that Malays have 42% GC contents, Chinese 40% and Indians 39%. It shows that the PCR products need higher temperature needed to break the GC bonds during PCR.

DNA digested with restriction enzyme, BsmAI was significantly different for the races. All PCR products were cut at one restriction sites, generating two fragments. For Malay we found 110bp, 170bp, 171bp, 176bp, 178bp, 182bp, 183bp, 186bp, 188bp, 191bp, 195bp, 196bp, 200bp, 204bp, 209bp, and 224bp; and for Chinese were 178bp, 182bp, 183bp, 186bp, 188bp, 191bp, 195bp, 209bp, and 224bp; and for Indians were 176bp, 182bp, 183bp, 186bp, 188bp, 191bp, 195bp, 204bp, 209bp, and 224bp.

Whereas DNA digested by BsmAI for Lane 2 for Malays were 96bp, 100bp, 102bp, 104bp, 107bp, 112bp, 115bp, 118bp, 120bp, 121bp, 123bp, 125bp, 126bp, 129bp, 132bp, 141bp, 148bp, and 155bp. For Chinese the bands were 104bp, 105bp, 107bp, 110bp, 112bp, 115bp, 118bp, 120bp, 121bp, 123bp, 126bp, 141bp, and 148bp; and for Indians were 100bp, 110bp,

115bp, 118bp, 120bp, 121bp, 126bp, 141bp, 148bp, and 155bp, respectively.

We have also observed specific bands from those data which for Malay were 110bp(M10), 170bp(M11, M12, M13, M15, M16, M34), 171bp(M1), 196bp (M9), and 200bp(M44) for Lane 1 and 96bp(M16), 102bp(M34,M35), 129bp(M31), and 132bp(M37) for Lane 2. The specific band for Chinese was only in the Lane 2 which was 105bp(C8), while Indians did not have any specific band.

Human eye shape gene has been found to be amplified using GDB11509038 primer pairs. From this study, we discovered that there is no significance different between races regarding to the human eye shape gene. All other major ethnic groups differed significantly from each other with respect to their haplotype distributions<sup>[13]</sup>. Thus, human eye shape gene should be very useful as anthropological markers and forensic science associated cases.

#### ACKNOWLEDGMENT

This study was supported by IRDC Research Grant from Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia and Ministry of Science, Technology and Innovation (MOSTI).

#### REFERENCES

- [1] D.A.Atchison, N.Pritchard, K.L.Schmid, D.H.Scott, C.E.Jones, J.M.Pope; *Investigative Ophthalmology and Visual Science*, **46**, 2698-2707 (2005).
- [2] W.P.Chen; *Ophthalmic Plastic and Reconstructive Surgery*, **3(3)**, 135-140 (1987).
- [3] H.M.Cheng, O.S.Singh, K.K.Kwong, J.Xiong, B.T.Woods, T.J.Brady; *Ophthalmology Vision Sci.*, **69**, 698-701 (1992).
- [4] J.F.P.Deller, A.D.O.Connor, Sorsby; *Proc.R.Soc.B. Biol.Sci.*, London, **134**, 456-467 (1947).
- [5] H.Eiberg, J.Mohr; *Clinical Genetic*, **31(3)**, 186-91 (1987).
- [6] H.Eiberg, J.Mohr; *J.Hum.Genetic, Europe*, **4**, 237-241 (1996).
- [7] R.Ismail, L.K.Teh, J.Amir, Z.Alwi, C.G.Lopez; *Journals of Clinical Pharmacy and Therapeutics*, **28**, 279-284 (2003).
- [8] S.Jeung, B.N.Lemke, R.K.Dortzbach, Y.G.Park,

**FULL PAPER**

- H.K.Kang; Arch.Ophthalmology, **177(7)**, 907-912 (1999).
- [9] P.Kay, K.Meehan, A.Williamson; Journal of Virological Methods, **105**, 159-170 (2002).
- [10] W.K.Lim, K.Rajendran, C.T.Choo; Ophthalmic Plastic and Reconstructive Surgery, **20(3)**, 207-211 (2004).
- [11] K.Mullis, F.Ferre, R.A.Gibbs; Birkhauser Boston, (1994).
- [12] G.F.Schmid, B.L.Petrig, C.E.Riva, E.Logean, R.Walti; Int.Ophthalmol, **23(4-6)**, 317-20 (2001).
- [13] A.Sjalander, R.Birgander, N.Saha, L.Beckman, G.Beckman; Human Heredity, **46**, 41-48 (1996).
- [14] C.S.Tan, N.Venketasubramanian, V.Ramasamy, W.Goa, S.M.Saw; Parkinsonism and Related Disorder, **11**, 233-239 (2005).
- [15] X.D.Zhou, F.R.Wang, S.Z.Zhou, J.S.Shi; 'A computed tomographic study of the relation between ocular axial biometry and refraction', In T.Tokoro (Eds). 'Myopia updates', Hakone, Japan, Tokyo Springer, 112-6 (1996-1997).