RESEARCH NOTE

Polymorphisms of four pigmentation genes (*SLC45A2*, *SLC24A5*, *MC1R* and *TYRP1*) among eleven endogamous populations of India

MEETA MUKHERJEE[1], SANJUKTA MUKERJEE[1], NEETA SARKAR-ROY[2], TANIA GHOSH[1], D KALPANA[1] and ANIL KUMAR SHARMA[1]*

[1]*Central Forensic Science Laboratory, Directorate of Forensic Science Services, Ministry of Home Affairs, Govt. of India, 30 Gorachand Road, Park Circus, Kolkata 700 014, India*
[2]*National Institute of Biomedical Genomics, Netaji Subhas Sanatorium, 2nd Floor, Kalyani 741 251, India*

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Introduction

In humans, there is a wide range of skin pigmentation, within populations and among different populations. In the recent past, studies on skin colour genetics have reported more than a hundred genes actively involved in pigmentation pathway (Bennett and Lamoreux 2003). The genetic variants of pigmentation-related genes have been studied largely among intercontinental populations. Despite a landscape of biological and cultural diversity, Indian populations have been relatively poorly investigated for pigmentation gene polymorphisms. As a group, Indian population shows a wide range of skin pigmentation variation and, to a smaller extent, variation in hair and eye pigmentation. This preliminary study generates new allelic data for five single-nucleotide polymorphisms (SNPs) located in four pigmentation-related genes *SLC45A2*, *SLC24A5*, *MC1R* and *TYRP1* among 749 individuals from different populations (five caste and six tribal) of India, belonging to four major linguistic groups: Indo-European (IE), Dravidian (DR), Austro-Asiatic (AA) and Tibeto-Burman (TB), dispersed among six geographical locations.

Materials and methods

Sample collection

Blood samples from 749 unrelated healthy male individuals were collected with informed consent following protocols approved by the Institutional Ethics Committee of Central Forensic Science Laboratory, Kolkata, India. The individuals were from Balmiki (62), Sakaldwipi Brahmin (65), Kanyakubja Brahmin (78), Konkanastha Brahmin (71), and Mahadev Koli (65) populations belonging to IE group; Iyengar (66), Kurumans (67), and Gond (75) populations belonging to DR group; Tripuri (65) and Riang (67) populations belonging to TB group; and Munda (68) population belonging to AA group. Population details have been described in table 1.

DNA isolation and quantitation

Genomic DNA was isolated from the blood samples by the phenol–chloroform organic extraction method and was quantified using Quantifiler Human DNA Quantification Kit according to the manufacturer's protocol employing 7500 Real-Time PCR System (Applied Biosystems, Foster City, USA).

SNP selection and genotyping

Five SNPs having large differences in allele frequencies among Europeans, East Asians and African populations were selected from available literature (Parra *et al.* 2004; Graf *et al.* 2005). Detailed information on studied SNPs is given in table 2. Genotyping was performed using predesigned/validated TaqMan genotyping assays (Applied Biosystems, Foster City, USA). DNA amplification was carried out on a 7500 Real-Time PCR System using the following cycling conditions: 95°C for 10 min followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. To determine the genotypes, end-point fluorescence was read using SDS v. 2.0 software (Applied Biosystems, Foster City, USA).

Statistical analysis

Significant deviations from Hardy–Weinberg equilibrium (HWE) were computed using Arlequin program v. 3.1.15. The same package was used for analysis of molecular

*For correspondence. E-mail: aks4n6@gmail.com.

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Table 1. Details of the studied populations.

Population	Region	State	Linguistic group	Sociocultural group	Number of samples
Balmiki	North	Punjab	Indo-European	Caste	62
Sakaldwipi Brahmin	East	Jharkhand	Indo-European	Caste	65
Munda	East	Jharkhand	Austro-Asiatic	Tribe	68
Kanyakubja Brahmin	Central	Madhya Pradesh	Indo-European	Caste	78
Gond	Central	Madhya Pradesh	Dravidian	Tribe	75
Konkanastha Brahmin	West	Maharashtra	Indo-European	Caste	71
Mahadev Koli	West	Maharashtra	Indo-European	Tribe	65
Iyengar	South	Tamil Nadu	Dravidian	Caste	66
Kurumans	South	Tamil Nadu	Dravidian	Tribe	67
Tripuri	Northeast	Tripura	Tibeto-Burman	Tribe	65
Riang	Northeast	Tripura	Tibeto-Burman	Tribe	67

variance (AMOVA) to investigate the proportion of genetic variation within and among the studied populations (Excoffier *et al.* 2005). The phylogenetic relationships were estimated with multidimensional scaling (MDS) analysis. MDS analysis was performed by means of SPSS software v. 17.0 (SPSS, Chicago, USA) based on Nei's (Nei *et al.* 1983) standard genetic distance calculated with DISPAN software (Ota 1993).

Results and discussion

The distribution of allele frequencies of the five SNPs are summarized in table 3 and figure 1. Although the markers associated with skin pigmentation are subject to selection pressure, none of the studied SNPs showed any significant deviation from HWE in the present study. The 111T allele of rs1426654 and the derived allele of rs2733832 were observed with relatively higher frequencies in the caste populations in comparison to the tribal populations of IE and DR groups. The rs16891982 was least polymorphic in all the studied populations. The selective advantage of 374 L allele of rs16891982 against ultraviolet radiation (UVB) reported in previous studies (Soejima and Koda 2007) may explain the lower prevalence of the derived allele in the Indian subcontinent. The 163Q and 272 K alleles of rs885479 and rs26722 respectively have been reported previously with a high frequency of 70% in East Asian and Southeast Asian populations and with much lower frequencies in Indians and Europeans (Rana *et al.* 1999; Makova and Norton 2005). In the present study, rs26722 showed no significant variation among the studied populations. The 163Q allele of rs885479 was observed with a frequency of more than 50% in the tribal populations of TB family. The comparable frequency of 163Q allele of rs885479 in TB and East Asian

Table 2. Genetic information about the five selected SNPs.

Reference SNP ID	Character	Gene	Chromosomal location	Protein	Allele	Amino acid change	Mutation type
rs16891982	Skin colour	*SLC45A2* (solute carrier family 45, member 2)	5p13.3	MATP: membrane-associated transporter protein	C/G*	L374F	Coding, nonsynonymous
rs26722	Skin colour				C/T*	E272K	Coding, nonsynonymous
rs1426654	Skin colour	*SLC24A5* (solute carrier family 24, member 5)	15q21.1	NCKX5: potassium-dependent sodium calcium exchange protein	G/A*	A111T	Coding, nonsynonymous
rs885479	Skin and hair colours	*MC1R*	16q24.3	MC1R: melanocortin 1 receptor	G/A*	R163Q	Coding, nonsynonymous
rs2733832	Skin colour	*TYRP1*	9p23	TYRP1: tyrosinase-related protein 1	C/T*	–	Intron

*Derived allele of the particular SNP.

Table 3. Allele frequencies of five SNPs in Indian populations.

Reference SNP ID	Gene	Allele	Allele frequency[a]										
			KOLI	BALM	SKBR	KKBR	KONK	IYGR	KURM	GOND	MUND	TRPR	RING
rs16891982	*SLC45A2*	c/G	0.0846	0.0565	0.1692	0.1154	0.0563	0.0672	0.0746	0.0200	0.0294	0.0000	0.0149
rs26722	*SLC45A2*	c/T	0.1462	0.2419	0.2000	0.1923	0.2606	0.1493	0.2090	0.1200	0.0809	0.2385	0.3582
rs1426654[b]	*SLC24A5*	g/A	0.7231	0.6694	0.7692	0.8846	0.9789	0.7463	0.4104	0.2333	0.0956	0.0923	0.1119
rs885479[b]	*MC1R*	g/A	0.0154	0.0081	0.0231	0.0641	0.0282	0.0149	0.0299	0.1000	0.1397	0.5923	0.6940
rs2733832	*TYRP1*	c/T	0.2308	0.3226	0.2231	0.2949	0.3732	0.2388	0.2090	0.1267	0.1324	0.0308	0.0373

[a]Allele frequency of minor allele (capital letter);
[b]SNP with large differences in frequency of minor allele.
SKBR, Sakaldwipi Brahmin; KOLI, Mahadev Koli; KONK, Konkanstha Brahmin; KKBR, Kanyakubja Brahmin; BALM, Balmiki; GOND, Gond; IYGR, Iyengar; KURM, Kurumans; TRPR, Tripuri; RING, Riang; MUND, Munda.

populations indicates their common ancestral origin and the historical route of migration undertaken by the TB speakers from southern China through the northeastern corridor of India (Majumder 2008).

The AMOVA results in table 4 showed that irrespective of any grouping, about 70% variation is attributable to differences within the studied populations. A higher percentage of variation was observed among groups based on linguistic affinity (25.48%) followed by groups based on social equivalence (19.73%). Groups based on geographic equivalence showed lowest genetic divergence (9.88%). This indicates that latitudinal differences alone cannot explain the existing skin pigmentation diversity among the Indian populations.

To understand the phylogenetic relationships between studied and HapMap populations (YRI, Yoruba people of Ibadan, Nigeria; CEU, US residents with ancestry from northern and western Europe; CHB, Han Chinese from Beijing; JPT, Japanese from Tokyo; GIH, Guajarati Indians from Houston, USA) MDS analysis was performed. The overall pattern (figure 2) showed that the TB populations were completely differentiated from the rest of the linguistic groups on the first and second dimensions and were close to the East Asian populations of the HapMap dataset. The separate cluster of TB speakers indicates the limited genetic exchange with neighbouring populations primarily due to the geographical isolation leading to their distinct genetic structure. The separate cluster of TB speakers also supports the established fact of convergent evolution of light skin colour among East Asians and Europeans (Norton *et al.* 2007). This recommends search for other genetic determinants largely responsible for light skin phenotype in TB speakers than rest of the populations of Indian subcontinent. Based on the allele frequency data, it can be assumed that out of the five SNPs in the present study, only rs1426654 and rs2733832 of *SLC24A5* and *TYRP1* genes respectively, along with some other genes, may be involved in influencing pigmentation differences across India. Mutation in *SLC24A5* gene is responsible for golden phenotype in zebra fish (Lamason *et al.* 2005). Recently, a nonsynonymous mutation (rs1426654) of the human orthologue of this putative exchanger gene was reported to be responsible for large pigmentation differences between African and

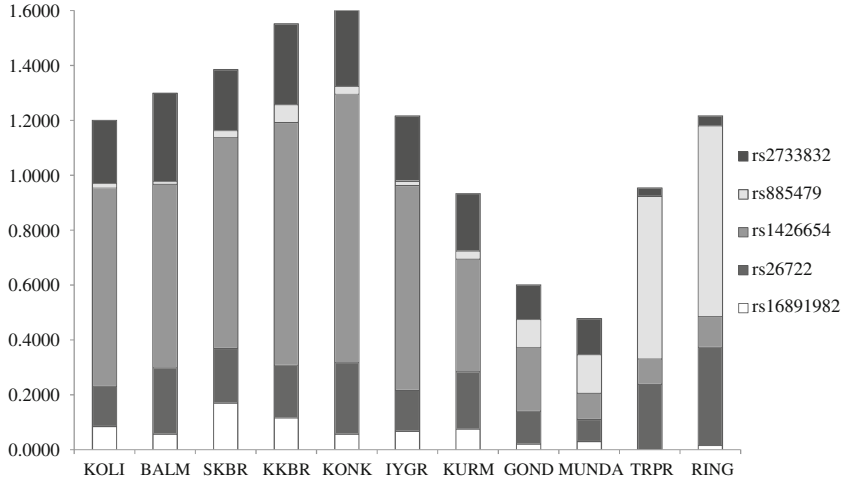

Figure 1. The distribution of minor allele frequencies of the studied SNPs among the studied populations.

Table 4. Genetic differentiation among studied populations based on five SNPs.

	Number of groups	Among groups (%)	Among populations within groups (%)	Within populations (%)
Linguistic groups (IE, DR, TB, AA)	4	25.48	4.00	70.52
Social groups (castes and tribes)	2	19.73	11.59	68.68
Geographic groups (north, central, east, west, northeast, south)	6	9.88	15.38	74.74

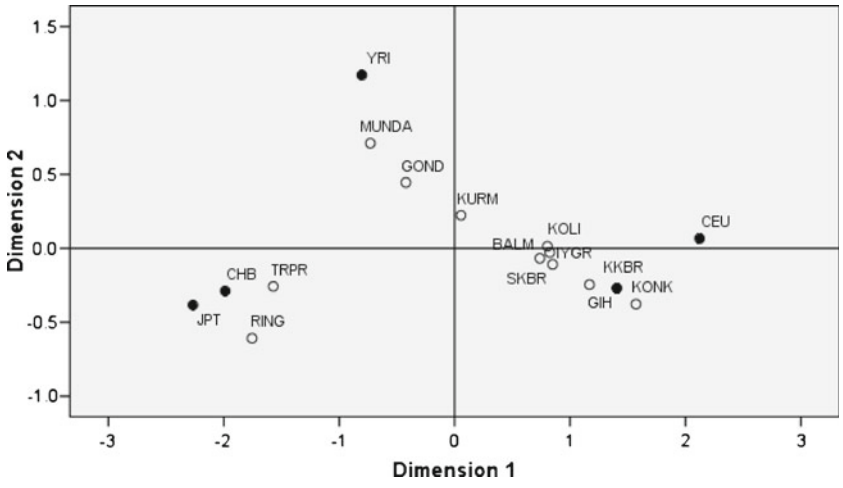

Figure 2. MDS plot depicting genetic affinities among the studied Indian populations with HapMap populations.

European populations (Jackson 2006). Due to large allele frequency differences across major human populations of the world, rs1426654 and rs16891982 were evaluated as ancestry informative markers (AIMs) (Soejima and Koda 2007). The Munda and Gond populations showed closeness to the YRI population. Regardless of different linguistic affiliations, the caste populations of IE and DR families were observed in close proximity. This corroborates that different events of migrations and admixture in the prehistoric era may have contributed to the gene pool of the contemporary Indian populations. Despite their clear genetic differentiation based on a study of Y chromosomal short tandem repeats (Y-STRs) (Ghosh *et al.* 2011), Munda and Gond populations appeared to be closer in MDS plot based on SNPs associated with the pigmentation. This may indicate the role of common genetic determinants shaping normal pigmentation variation among them.

The high degree of genetic differentiation observed among linguistic and social groups cannot rule out the probable affects of geography (environmental influences) on the genes widely associated with skin pigmentation across the Indian sub-continent. Since the present study has been conducted on male individuals, the results must be validated in a larger set of samples consisting of male and female individuals to explore the influence of sexual dimorphism with respect to skin pigmentation. Due to lack of pigmentation data, association of these markers with the pigmentation phenotype could not be evaluated. Nevertheless the noticeable allele frequency differences for rs1426654, rs885479 and rs2733832 located in *SLC24A5*, *MC1R* and *TYRP1* genes respectively, suggest further study to investigate the possible correlation between pigmentation phenotype and variant alleles of these three and some additional genes in the Indian subcontinent.

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References

Bennett D. C. and Lamoreux M. L. 2003 The color loci of mice- a genetic century. *Pigment Cell Res.* **16**, 333–344.

Excoffier L., Laval G. and Schneider S. 2005 Arlequin ver 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* **1**, 47–50.

Ghosh T., Kalpana D., Mukerjee S., Mukherjee M., Sharma A. K., Nath S. *et al.* 2011 Genetic diversity of 17 Y-short tandem repeats in Indian population. *Forensic Sci. Int. Genet.* **5**, 363–367.

Graf J., Hodgson R. and Van Daal A. 2005 Single nucleotide polymorphisms in the MATP gene are associated with normal human pigmentation variation. *Hum. Mutat.* **25**, 278–284.

Jackson I. J. 2006 Identifying the genes causing human diversity. *Eur. J. Hum. Genet.* **14**, 979–980.

Lamason R. L., Mohideen M. A., Mest J. R., Wong A. C., Norton H. L., Aros M. C. *et al.* 2005 *SLC24A5*, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science* **16**, 1782–1786.

Majumder P. P. 2008 Genomic inferences on peopling of south Asia. *Curr. Opin. Genet. Dev.* **18**, 280–284.

Makova K. and Norton H. 2005 Worldwide polymorphism at the *MC1R* locus and normal pigmentation variation in humans. *Peptides* **26**, 1901–1908.

Nei M., Tajima F. and Tateno Y. 1983 Accuracy of estimated phylogenetic tree from molecular data. II. Gene frequency data. *J. Mol. Evol.* **19**, 153–170.

Norton H. L., Kittles R. A., Parra E. J., McKeigue P., Mao X., Cheng K. *et al.* 2007 Genetic evidence for the convergent evolution of light skin in Europeans and East Asians. *Mol. Biol. Evol.* **24**, 710–722.

Ota T. 1993 DISPAN: computer program for genetic distance and phylogenetic analysis. Institute of Molecular and Evolutionary Genetics. Pennsylvania State University, Pennsylvania, USA (http://www.softsea.com/download/DISPAN-Genetic-Distance-and-Phylogenetic-Analysis.html).

Parra E. J., Kittles R. A. and Shriver M. D. 2004 Implications of correlations between skin color and genetic ancestry for biomedical research. *Nat. Genet.* **36**, S54–S60.

Rana B. K., Hewett-Emmett D., Jin L., Chang B. H., Sambuughin N., Lin M. *et al.* 1999 High polymorphism at the human melanocortin 1 receptor locus. *Genetics* **151**, 1547–1557.

Soejima M. and Koda Y. 2007 Population differences of two coding SNPs in pigmentation-related genes *SLC24A5* and *SLC45A2*. *Int. J. Legal. Med.* **121**, 36–39.

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