 Anthropologist, 6(4): 235-238 (2004)

A Study of Red Cell Genetic Markers in Myopia

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INTRODUCTION

Myopia or short-sightedness is a common complex disorder. High myopia (>6.00 diopter) predisposes individuals to retinal detachment, glaucoma, macular degeneration and premature cataract.

Review of literature shows that Garg and Pahwa (1965), Deshmukh et al. (1983) and Jindal and Bansal (1983) first attempted to find association between various eye diseases and dermatoglyphic traits in Indian populations. However, no information on any biochemical blood genetic marker in these diseases is available in them. It is therefore that this pilot study was planned to fill this void by providing phenotype and allele frequency distribution data on as many as 10 different red cell genetic markers comprising 2 blood groups (A_1A_2BO, RhD), 7 red cell enzyme polymorphisms (ADA, AK1, ESD, PGM1, GLO1, ACP1, GPI) and haemoglobin (Hb) variants in myopia patients and controls from Patiala district of Punjab, North-West India.

MATERIALS AND METHODS

The subjects of this study were 53 unrelated mixed (both low and high myopia) patients attending Govt. Rajendra Hospital and various private eye hospitals in Patiala city and 50 matched controls. From each individual about 1 ml blood sample was drawn using a disposable blood lancet and collected in a microcentrifuge vial containing E.D.T.A.K_2 as an anticoagulant. Samples were processed for the preparation of the red cell suspensions and haemolysates, following standard methods (Bhasin and Chahal, 1996).

A_1A_2BO and RhD blood groupings of the red cells were done serologically by the tube method using antisera anti-A, -B and -D and lectin anti-A_1. Haemolysates were typed biochemically for a battery of seven different polymorphic red cell enzymes following standard horizontal electrophoretic techniques. Isozymes of erythrocyte enzymes Esterase D (ESD) and Phosphoglucomutase locus 1 (PGM1) were electrophoresed together on one agarose gel and stained in the same order (Wraxall and Stolorow, 1986). Similarly, those of Adenosine Deaminase (ADA) and Adenylate Kinase locus 1 (AK1) enzymes were separated together on the same agarose gel and simultaneously stained as described by Murch et al. (1986). The method of Wraxall and Emes (1976) was followed for typing red cell Acid Phosphatase locus 1 (ACP1), albeit the medium of separation was agarose instead of starch. Mixed agarose/starch gel electrophoretic technique of Scott and Fowler (1982) was employed for Glyoxalase locus 1 (GLO1) typing. Screening of variants of Glucose Phosphate Isomerase (GPI) was basically by the original method of Detter et al. (1968) with some modifications i.e. electrophoresis was carried out in agarose rather than starch and staining procedure of Papiha and Chahal (1984) was followed; for Haemoglobin (Hb) variants it was done in conjunction with GLO1 typing (Divall and Greenhalgh, 1983).

The allele frequencies in the A_1A_2BO blood group system were calculated by the formulae of Yasuda (1984) yielding maximum likelihood estimates; in the Rh(D) system the square root method was used. In enzyme polymorphisms and Hb system these were obtained by the gene counting method. The Chi-square test was employed to assess significance of deviations of observed phenotype numbers from those expected under the Hardy-Weinberg equilibrium. To determine the overall differences in allele frequencies between the patients and controls, heterogeneity Chi-square analysis following Workman and Niswander (1970) was performed.

RESULTS AND DISCUSSION

Phenotypes of various blood groups, red cell enzymes and haemoglobins observed in myopia patients alongwith controls are presented in Table 1. In both the series, their distributions were found to be in Hardy-Weinberg equilibrium in each genetic system studied. Allele frequencies calculated from them are listed in Table 2.

In the A_1A_2BO system, blood group B predominated in the patient series with highest frequency (35.84%), followed by groups A_1 and O (both with an identical value of 24.52%) and A_1B (5.66%). In controls also group B was most common (40%), followed by 0 (30%), A_1 (22%) and A_1B (6%). The Chi-square comparison of the two series for this serological marker did not reveal any statistically significant difference (χ^2 = 3.188, d.f. 3, 0.40 >p> 0.30), suggesting perhaps little association between the A_1A_2BO blood groups and myopia. Regarding the Rhesus blood group system, the frequency of Rh(D) negatives was recorded 11.32% in patients and 6% in controls; albeit the difference was again found to be statistically non-significant (χ^2 =0.917, d.f. 1, 0.40 >p> 0.30).

Table 1: Phenotype distribution of genetic markers in myopia patients an d controls

Genetic marker	*Phenotype*	*Number observed*	
		Myopia patients	*Controls*
A_1A_2BO	A_1	13 (24.52)	11 (22)
	A_2	2 (3.77)	0
	B	19 (35.84)	20 (40)
	A_1B	3 (5.66)	3 (6)
	A_2B	1 (1.88)	1 (2)
	O	13 (24.52)	15 (30)
Rh(D)	D+	47 (88.67)	47 (94)
	D -	6 (11.32)	3 (6)
ADA	1	41 (77.36)	34 (68)
	1,2	12 (22.64)	15 (30)
	2	0	1 (2)
AK1	1	40 (75.47)	43 (86)
	1,2	13 (24.53)	5 (10)
	2	0	2 (4)
ESD	1	34 (64.15)	34 (68)
	1,2	18 (33.96)	15 (30)
	2	1 (1.89)	1 (2)
PGM1	1	26 (49.05)	26 (52)
	1,2	20 (37.73)	19 (38)
	2	7 (13.20)	3 (6)
	1,6	0	1 (2)
	1,7	0	1 (2)
GLO1	1	2 (3.77)	6 (12)
	1,2	19 (35.84)	19 (38)
	2	32 (60.37)	25 (50)
ACP1	A	11 (20.75)	8 (16)
	A,B	23 (43.49)	23 (46)
	B	19 (35.84)	17 (34)
	B,C	0	2 (4)
GPI	1	52 (98.11)	49 (98)
	1,3	1 (1.88)	0
	1,5	0	1 (2)
Hb	A	53 (100)	49 (98)
	A,V*	0	1(2)

Figures in parantheses are percentages
* Variant

As for the ABO blood groups, Giannantoni (1928) worked on myopia patients of Italy and found a relative incidence (RI) of 0.422 for A/O and 0.743 for B/O. Another study from Italy (Scialdone and Pantalone, 1963) reported RI to be 2.938 and 3.150, respectively while one from Poland (Wilk et al., 1969) recorded the values of 1.563 and 1.321, respectively. Pooling the data on a total of 519 myopia patients available from these three studies Mourant et al. (1978) calculated a combined incidence of A/O to be 2.552 and that of B/O to be 2.620, both with very high values of χ^2 for difference from unity. In the present study these values were found to be 1.5734 and 1.0962, respectively.

Padma and Murty (1983) while seeking association of genetic markers with eye diseases like cataract, corneal dystrophy, retinal detachment, primary glaucoma, myopia and strabismus found that blood group O individuals have high risk for myopia, nuclear cataract and convergent squint. Brooks and Gillies (1988) assessed a series of 474 mixed cases of glaucoma for the antigenic systems ABO, Rh, ABH

Table 2: Allele frequencies of genetic markers in myopia patients and controls

Locus	*Allele*	*Allele frequency*	
		Myopia patients	*Controls*
A_1A_2BO	*ABO*A1*	0.194	0.155
	*ABO*A2*	0.038	0.000
	*ABO*B*	0.256	0.292
	*ABO*O*	0.512	0.553
Rh(D)	*D*	0.664	0.755
	d	0.337	0.245
ADA	*ADA*1*	0.887	0.830
	*ADA*2*	0.113	0.170
AK1	*AK1*1*	0.877	0.910
	*AK1*2*	0.122	0.090
ESD	*ESD*1*	0.811	0.830
	*ESD*2*	0.188	0.170
PGM1	*PGM1*1*	0.679	0.730
	*PGM1*2*	0.320	0.250
	*PGM1*6*	0.000	0.010
	*PGM1*7*	0.000	0.010
GLO1	*GLO1*1*	0.216	0.310
	*GLO1*2*	0.783	0.690
ACP1	*ACP1*A*	0.424	0.390
	*ACP1*B*	0.575	0.570
	*ACP1*C*	0.000	0.020
GPI	*GPI*1*	0.990	0.990
	*GPI*3*	0.010	0.000
	*GPI*5*	0.000	0.010
Hb	*Hb*A*	1.000	0.990
	*Hb*V***	0.000	0.010

** Variant

secretion and PTC tasting ability and reported a significant decrease in Rh negative patients from normals in chronic closed angle glaucoma ($p < 0.05$).

As far various red cell enzyme polymorphisms viz., ADA, AK1, ESD, PGM1, GLO1, ACP1, GPI and Hb types investigated here in myopia patients and controls, similar distribution patterns of phenotypes and allele frequencies were observed in both the series. However, some differences were apparent, as for instance, a raised frequency of the *PGM1*2* allele (0.320) in patients compared to controls (0.250). Similarly, in ACP1 enzyme system, the frequency of *ACP1*A* allele was comparatively higher in the patient series (Table 2). However, in ADA and GLO1 systems, the incidences of the *ADA*2* and *GLO1*1* alleles were lower in the patients (0.113 and 0.216, respectively) as compared to the controls (0.170 and 0.310 respectively). However, individually as well as overall the allele frequencies of the 10 red cell genetic systems considered in this study between the myopia patients and controls did not reveal any statistically significant difference (Table 3).

Zygulska-Mach et al. (1994) attempted to analyze the relationship between various serological markers (ABO, MN, Rh, Gm), ACP1, ESD and Hp and congenital cataract. It was revealed that the frequency of Hp heterozygote phenotype 2-1 was higher in families with congenital cataract with simultaneous decrease in frequency of homozygote Hp 2-2.

In the present work, the frequencies of genetic markers RhD, PGM1, ADA, GLO1 and ACP1, located on chromosomes 9, 1, 20, 6 and 2, respectively, are different from controls in myopia patients. On the other hand, various genes controlling myopia have been assigned to chromosomes 12, 18 and X. Since myopia is not a condition due to a single gene with fully penetrant expression, functional connection between myopia and any of the ten genetic markers investigated has not been indicated by this study.

Table 3: Chi-square analysis of allele frequency differences between myopia patients and controls

Locus	χ^2	*d.f.*	*Significance*
A_1A_2BO	*3.188*	3	0.40>p>0.30
Rh(D)	0.917	1	0.40>p>0.30
ADA	0.788	1	0.40>p>0.30
AK1	0.575	1	0.50>p>0.40
ESD	0.122	1	0.80>p>0.70
PGM1	2.972	3	0.40>p>0.30
GLO1	1.368	1	0.30>p>0.20
ACP1	*2.052*	2	0.40>p>0.30
GPI	2.034	2	0.40>p>0.30
Hb	0.971	1	0.40>p>0.30
All loci	14.986	16	0.60>p>0.50

ACKNOWLEDGEMENTS

N.S. acknowledges gratefully the financial support provided to her by the Council of Scientific and Industrial Research (New Delhi) in the form of a Research Associateship. Thanks are also due to the subjects who volunteered to provide their blood samples for this study.

KEYWORDS Red Cell Genetic Markers. Myopia. Association.

ABSTRACT Two blood groups (A_1A_2BO, RhD), 7 red cell enzyme polymorphisms (ADA, AK1, ESD, PGM1, GLO1, ACP1, GPI) and haemoglobin (Hb) types were investigated in patients suffering from myopia alongwith a control sample. The present results did not show any statistically significant differences between the patient and control series in the distribution of these genetic markers.

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