

# Association of erythrocyte acid phosphatase phenotypes with myopia

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Acid phosphatase is a polymorphic nonspecific orthophosphate monoesterase which catalyses the cleaving of phosphoric acid and subsequent breakdown of several monophosphoric esters under acidic pH conditions. Acid phosphatase has a physiologic function as a flavin mononucleotide phosphatase (FMN) and regulates the intracellular concentrations of flavin coenzymes that are electron carriers in the oxidative phosphorylation pathway. Myopia or nearsightedness is caused by both environmental and genetic factors. Myopic eyes when subjected to excessive oxidative stress results in retinal detachments. In the present study there is a significant elevation of AA phenotype in myopes when compared to controls. The AA phenotype is more susceptible to oxidative stress and its lower enzyme activity is known to be associated with increased intrauterine growth that further results in increased axial length in progressive myopia. The AA phenotype also confers risk for myopia development in males, early age group and cases with parental consanguinity.

**Key Words:** Myopia, acid phosphatase, oxidative stress, cellulose acetate electrophoresis, Phenotype

## Introduction

Acid phosphatase is a polymorphic nonspecific orthophosphate monoesterase which catalyses the cleaving of phosphoric acid and subsequent breakdown of several monophosphoric esters under acidic pH conditions. Erythrocyte acid phosphatase (ACP1/EAP) is an enzyme prevalent in several tissues such as bone, kidney, spleen, liver, breast, and pancreas with the most concentrated sources being the human prostrate gland.

Mapped to the locus 2p25, erythrocyte acid phosphatase (ACP1) is encoded by four alleles, namely, ACP1\*A, ACP1\*B, ACP1\*C and ACP1\*R.<sup>[1]</sup> The combinations of these four alleles give rise to 6 phenotypes AA, BB, AB, CC, AC, BC, and RC. Of these, the most commonly encountered phenotypes are type AA, BB and AB in Caucasian and Indian populations.<sup>[2]</sup> Acid phosphatase has physiologic function as a flavin mononucleotide phosphatase (FMN) and regulates the intracellular concentrations of flavin coenzymes that are electron carriers in the oxidative phosphorylation pathway. Each allele of ACP1 encodes 2 isoenzymes, f and s that show fast and slow electrophoretic mobility respectively. These two isoenzymes exhibit structural differences that result in different catalytic sites and cellular substrates. The ACP1 phenotypes AA and AC have same concentration of 'f' isoform but different f/s ratio and the susceptibility to favism is more for AC as compared to AA. The AA phenotype has lower f/s ratio compared to BB phenotype. Experimental evidence had shown A phenotype is more susceptible to oxidative damage. The reduced concentration of f isoform of AA phenotype may result in enhanced tyrosine phosphorylation of B3P protein resulting in enhanced rate of glycolysis and subsequent oxidative stress.<sup>[3]</sup>

Myopia or nearsightedness is a condition whereby images are focused in front of retina. Genetic and several environmental factors have been implicated in development of myopia. Myopia development involves

variation in corneal structure or increase in axial length. Refractive error is measured in diopters, which is the combined power of the cornea and the lens that is needed to focus distant objects correctly on the retina. There are etiologically distinct forms of myopia. High myopia or pathological myopia causes progressive elongation of the globe and stretching of the scleral wall leading to a high refractive error of more than 6.0 diopters. The simple or less severe form is known as physiological myopia, occurs as a result of correlative effect of refractive components of the eye and has refractive error upto 6.0 Diopters.

Myopic eyes when subjected to excessive oxidative stress may result in retinal detachments. Children or adolescents with progressive myopia and retinal detachments are known to have impaired oxidant and antioxidant balance.<sup>[4-6]</sup>

Materials and Methods

Blood samples were collected in EDTA vacuatainer from 213 myopia patients reported at Sarojini Devi Eye Hospital, Kanchan Eye Hospital and Jagadamba Nursing Home. Each of these hospitals was visited twice a week for a period of 20 months. The information regarding age at onset, sex, Para, maternal reproductive history, nutritional status, socio-economic status, familial incidence and parental consanguinity were collected from the patients by personally interviewing them on the basis of selected proforma All the patients under study were clinically examined by ophthalmologist accurately for spherical error of refraction, retinal changes, fundus and macula changes. Age and sex matched controls (n = 126) examined by the ophthalmologist without any history of myopia and any other genetic disease were collected randomly from hostels, schools, colleges and various institutions for the purpose of comparison. RBC's were separated from blood sample and hemolysate was prepared using chilled distilled water. The haemolysate was analyzed for acid phosphate phenotypes by cellulose acetate electrophoresis using phosphate citrate buffer of pH 5.9 [Figure 1]. The staining was done using fluorescence Stain Methyl umbelliferyl phosphate.<sup>[7]</sup>

The phenotypic distribution of acid phosphatase in

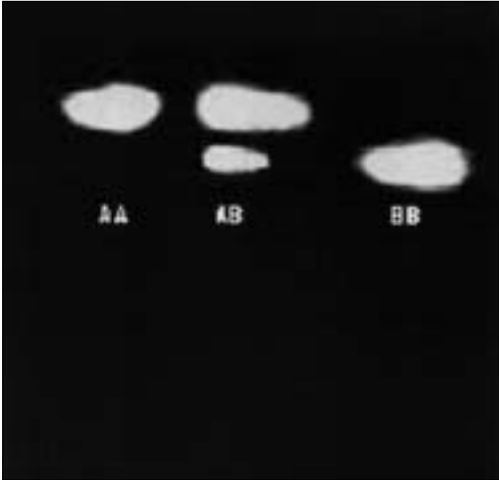


Figure 1: Cellulose acetate electrophoresis showing different ACP1 phenotypes in myopia cases

myopia was analyzed in comparison to control using appropriate statistical test such as  $\chi^2$  test and Woolfs test. The level of significance was taken as 5% i.e ( $P<0.05$ ).

Results and Discussion

In the present study of acid phosphatase polymorphism, the AA, BB and AB phenotypes were observed in the both control and disease population. Previous studies of acid phosphatase in Caucasian population and Indian population showed only these three phenotypes. Table 1 shows the frequency distribution of ACP1 phenotypes in myopia (AA; 17.3%, AB; 26.76%, BB; 55.8%) and controls (AA; 6.3%, AB; 28.6%, BB; 65.1%) along with allele frequencies. It is evident that AA phenotype has significantly increased in myopia with corresponding increase in allele frequency of 'A' as compared

Table 1: Frequency distribution of erthrocyte acid phosphatase phenotypes in myopia and control groups

	Phenotypes								
	AA		AB		BB		Total	Allele frequencies	
	N	%	N	%	N	%		P	Q
Myopia	37	17.3	57	26.76	119	55.8	213	0.307	0.69
Controls	8	6.3	36	28.61	82	65.1	126	0.206	0.79

As Compared to Controls:  
Genotype distribution:  $\chi^2 = 8.437$   
Relative incidence as compared to heterozygotes:  
AA versus AB;  $\chi^2= 5.822$ ; Odds ratio = 2.921  
AA versus BB;  $\chi^2 = 7.783$ ; Odds ratio = 3.187  
AB versus BB;  $\chi^2 = 0.024$ ; Odds ratio = 1.091  
\* $P<0.05$  (Chi-Square Distribution)  
Departure from Hardy – Weinberg equilibrium:  
Myopia;  $\chi^2 = 29.43$ , Control  $\chi^2 = 2.061$

to controls. The phenotype distribution of ACP1 in myopia group had shown significant departure ( $\chi^2=29.432$ ) from Hardy Weinberg equilibrium, whereas the control did not show any such deviation ( $\chi^2=2.06$ ). The relative risk calculated for AA vs AB ( $\chi^2=25.822$ ) and AA vs BB ( $\chi^2= 7.78$ ) revealed that individuals carrying A allele are at higher risk to develop myopia.

The distribution of ACP phenotypes [Table 2] with respect to the sex of proband revealed that males (19.6%) had higher frequency of AA phenotype as compared to females (15.5%). The occurrence of myopia in males could be attributed to the difference in the growth pattern of males and females. Myopia generally develops during adolescence as the postnatal growth of the eye starts during this period. The BMI (body mass index) of males is lower as they experience a rapid growth spurt in puberty, thus leading to an uncoordinated growth for refractive elements of eye resulting in increased myopia.<sup>[8,9]</sup> The AA phenotype in males might be conferring higher risk for myopia development.

When the ACP phenotype distribution was studied in different age groups [Table 3], an elevation of AA phenotype was observed in early onset myopia cases of age 0-10 years (40.5%; 15/37 of AA phenotype.) as compared to late onset myopia cases of age 16-20 years

(21.65%; 8/37). Not much difference was seen in genotype frequencies of BB with respect to age at onset. A slight elevation of the AB phenotype frequency was also seen among early onset cases (35.08%; 20/57) as compared to late onset cases (24.56%14/57). These results indicate that the younger age groups with AA phenotype are at greater risk to develop myopia early, which is known to progress into pathological or degenerative myopia.

The ACP distribution comparison between types of myopia [Table 4] also revealed association of AA phenotype with high myopia (22.5%) as compared to low myopes (14.8%). These findings support our earlier contention that AA phenotype might be conferring risk for development and progression of myopia as this phenotype is sensitive to oxidative stress owing to lower concentration of f/s ratio.

The comparison of acid phosphatase phenotype distribution among familial and non-familial cases revealed a decrease in the AA phenotype frequency (14.4%) in familial cases as compared to non-familial cases (23.0%). Whereas increase in the AA (20.5%) phenotype frequency in consanguineous group was seen as compared to non-consanguineous group (16.7%).

Our results suggest that AA phenotype confers risk for the development of myopia in males, early age group and cases with parental consanguinity. Further significant association of AA phenotype with high myopia suggests that the incidence with AA phenotype is more susceptible to oxidative stress. AA has lower enzyme activity compared to other phenotypes and is known to be associated with increased intrauterine growth. It is probable that AA phenotype might be contributing greatly to post natal eye growth resulting in increased axial length in progressive myopia.

**Table 2: Frequency distribution of erythrocyte acid phosphatase phenotypes with respect to sex of the proband**

Sex of the proband	AA		AB		BB		Total	Allele frequencies	
	N	%	N	%	N	%		P	Q
Male	19	19.6	23	23.7	55	56.7	97	0.314	0.685
Female	18	15.5	34	29.3	64	55.2	116	0.302	0.695

$$\chi^2 = 1.145 \text{ 2d.f } P=0.56$$

**Table 3: Frequency distribution of erythrocyte acid phosphatase phenotypes with respect to age at onset of the proband**

Age at onset	AA		AB		BB		Total	Allele frequencies	
	N	%	N	%	N	%		P	Q
0-5	5	21.7	6	26.1	12	52.2	23	0.34	0.65
5-10	10	21.3	14	29.8	23	48.9	47	0.36	0.63
11-15	14	16.9	23	27.7	46	55.4	83	0.30	0.69
16-20	8	20.5	8	20.5	23	59.0	39	0.30	0.69
21-25	-	-	6	28.6	15	71.4	21	0.14	0.85
>25									

$$\chi^2 = 6.775 \text{ 8d.f } P=0.561$$

**Table 4: Frequency distribution of erythrocyte acid phosphatase phenotypes with respect to types of myopia**

Types of myopia	AA		AB		BB		Total	Allele frequencies	
	N	%	N	%	N	%		P	Q
High Myopia	16	22.5	17	23.9	38	53.5	71	0.345	0.658
Low Myopia	21	14.8	40	28.2	81	57.0	142	0.288	0.711

$$\chi^2 = 2.056 \text{ 2d.f } P=0.358$$

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## References

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1. Wakita Y, Narahara K, Takahashi Y, Kikkawa K, Kimura S, Oda M, et al. Duplication of 2p25. Confirmation of the assignment of soluble acid phosphatase (ACP1) locus to 2p25. *Hum Genet* 1985;71:259-66.
2. Dissing J, Johnsen AH. Human red cell acid phosphatase (ACPI): The primary structure of the two pairs of isozymes encoded by the ACPI\*A and ACPI\*C alleles. *Biochim Biophys Acta* 1992;1121:261-8.
3. Bottini F, Borgiani P. ACPI and human adaptability; Association with common diseases: A case-control study. *Hum Genet* 1995;96:629-37.
4. Romero FN, Bosch-Morell F, Romero MJ, Jareno EJ, Romero B, Marin NM, et al. Lipid peroxidation products and antioxidants in human disease, *Environ Health Perspect* 1998;106:1229-34.
5. Florence TM. The role of free radicals in disease. *Aust N Z J Ophthalmol* 1995;23:3-7.
6. Vinetskaia MI, et al. Significance of lacrimal fluid peroxidation and anti-radical defense parameters for prediction and treatment of complicated myopia. *Vestn Oftalmol* 2000;116:54-6.
7. Graumbaum BW. Handbook for forensic individualization of human blood and bloodstains: Göttingen: Sartorius; 1981.
8. Parssinen O, Era P, Leskinen AL. Some physiological and psychological characteristics of myopic and non-myopic young men. *Acta Ophthalmol Suppl* 1985;173:85-7.
9. Curtin BJ. The myopia's Philadelphia: Harper and Row; Human genetics 1985;80:53-8.