 J Life Sci, 3(2): 87-91 (2011)

Association of Genetic Markers with Mental Retardation

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KEYWORDS Red Cell and Plasma Genetic Markers. Mental Retardation. Association. North Coastal Andhra Pradesh.

ABSTRACT Five red cell genetic markers namely, acid phosphatase locus 1 (ACP1), esterase D (ESD), glyoxalase locus 1 (GLO1), superoxide dismutase (SOD) and haemoglobin (HB) and five plasma proteins namely haptoglobin (HP), ceruloplasmin (CP), group specific component (GC), transferrin (TF) and albumin (ALB) were studied in mentally retarded individuals from Visakhapatnam area of north coastal Andhra Pradesh, South India. The results were compared with the data obtained from controls. Out of studied ten genetic markers, two namely ACP1 and HP showed significant differences between patients and controls. The risk estimates were also showing a significant association.

INTRODUCTION

The studies on the association of genetic markers with diseases are considered useful since they are likely to provide clues for the involvement of genetic or physiological factors in the disease process. It may be possible to confirm the genetic basis of certain diseases with pleiotropic effects which can only be recognized by the study of associated genetic, epidemiological and other factors. Several diseases have been studied for their association with different genetic markers (Mourant et al. 1978), for example, biochemical genetic markers such as genetically determined polymorphisms of red cell enzymes. Blood protein hemoglobin (HB) and plasma proteins are of considerable importance in disease association studies.

Mental Retardation (MR) is a common form of cognitive impairment affecting between 1 and 3% of the population of industrialized countries (Roeleveld et al. 1997; Aicardi 1998). There is debate over the definition and classification of MR (Leonard and Wen 2002). It is often defined by an intelligence quotient (IQ) of < 70, with deficits in adaptive skills included as diagnostic criteria (Luckasson et al. 1992; Dialy et al. 2000). Behavioral and cognitive therapies can help mentally retarded patients reach their maximum potential (Bathaee 2001; Butler et al. 2001), but they are not curative and often focus on treating habit disorders, aggression or self-injurious behavior that can accompany MR (Long and Miltenberger 1998; Dosen and Day 2001).

Many environmental and genetic factors can cause MR, including premature birth, prenatal infections, chromosomal abnormalities (Fragile X syndrome, Down syndrome), and single-gene mutations (Phenylketonuria) (Kinsbourne and Graf 2000). An etiology can be established in 60-75% of cases of severe MR, but only in 38-55% of mild cases. Estimates of genetic causes of severe MR range from 25 to 50% (Mc Laren and Bryson 1987). In India, the incidence of mental retardation is reported to be 2-3%.

Down Syndrome (DS) or trisomy 21, is the most frequently observed autosomal disorder manifested in newborns worldwide, with an incidence of about 1 in 700 live births. This is the most common form of genetic disorder in humans. Individuals with DS have characteristic physical features that are widely recognized. Eighty features are described in Down syndrome. However, not all features are observed in an individual with DS. Usually they are characterized by generalized growth, mild to severe form of mental retardation, heart, eyesight and hearing problems.

In India, the incidence of Down syndrome is 0.88 –1.09 per 1000 births, and every hour three children are reported to be born (Rajangam and Thomas 1992; Verma 2000; Malini and Ramachandra 2006). Studies revealed three genetic mechanisms to cause Down syndrome viz., free trisomy 21 (92-95%), translocation (3-4%) and mosaics (1-2%) (Nussbaum et al. 2001). The critical region for the Down syndrome phenotype is in the region of bands 21q21.3-21q22 (Epstein et al. 1991).

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The main objective of the present study is to observe any association, if any, between various blood genetic markers viz., acid phosphatase (ACP1), esterase D (ESD), glyoxalase1 (GLO1), superoxide dismutase (SOD), like haptoglobin (HP), caeruloplasmin (CP), group specific component (GC), transferrin (TF) and albumin (ALB) and mental retardation in individuals who are affected with Down syndrome in Visakhapatnam city in north coastal region of Andhra Pradesh, South India.

MATERIALS AND METHODS

A total of 104 blood samples were collected from mentally retarded individuals who are affected with Down syndrome from local mentally retarded schools and 104 normal healthy age and sex matched controls from Visakhapatnam. Samples were collected intravenously in sterile glass tubes containing ACD solution as an anticoagulant. Plasma was separated and kept at –20°C. Fresh and clear hemolysates were prepared according to standard procedures. The genetic markers investigated included acid phosphatase locus 1 (ACP1) by horizontal agarose gel electrophoresis using the method of Wraxall and Emes (1976), esterase D (ESD) was typed by agarose gel electrophoresis technique described by Wraxall and Stolorow (1986) and glyoxalase locus 1 (GLO1) was typed using starch-agarose gel electrophoresis method following Scott and Fowler (1982). Hemoglobin types were determined by standard cellulose acetate membrane electrophoresis (Kate et al. 1976). The plasma samples were typed using standard acrylamide gel electrophoresis following Kitchin and Bearn (1966) for group specific component (GC), transferrin (TF) and albumin (ALB) systems and following (Clark 1964) for haptoglobin (HP) and caeruloplasmin (CP) systems. The allele frequencies were estimated by maximum likelihood method (Balakrishnan 1988) and the statistical significance of differences between patients and controls were tested by the χ^2 test (Taylor and Prior 1938).

RESULTS AND DISCUSSION

Distribution of phenotype frequencies for red cell enzymes and plasma proteins in mental retardation are shown in Tables 1 and 2 and allele frequencies are shown in Tables 3 and 4. Out of the ten biochemical markers studied, no variation was found with respect to SOD, CP, TF and ALB loci markers in the present study patients of mental retardation and controls.

Table 1: Red cell enzyme and hemoglobin phenotypes in mentally retarded and controls

System	*Phenotype*	*Patients*		*Controls*	
		Obs.	*Exp.*	*Obs.*	*Exp.*
ACP1	A	4	14.62	7	9.54
	AB	70	48.76	49	43.92
	B	30	40.62	48	50.54
	Total	104	104.00	104	104.00
		χ^2_1 = 19.7432 (p>0.001)		χ^2_1 = 19.7432 (0.30>p>0.20)	
ESD	1-1	55	56.25	56	58.50
	2-1	43	40.45	44	39.00
	2-2	6	7.30	4	6.50
	Total	104	104.00	104	104.00
		χ^2_1 = 3.1248 (0.10>p>0.05)		χ^2_1 = 1.7093 (0.20>p>0.10)	
GLO-I	1-1	19	16.96	15	19.47
	2-1	46	50.07	60	60.51
	2-2	39	36.97	29	33.47
	Total	104	104.00	104	104.00
		χ^2_1 = 0.6875 (0.50>p>0.30)		χ^2_1 = 2.6884 (0.20>p>0.10)	
HB	AA	100	100.02	104	104.00
	AS	4	3.93	0	0.00
	SS	0	0.03	0	0.00
	Total	104	104.00	104	104.00
		χ^2_1 = 0.0312 (0.90>p>0.80)			
SOD	1-1	104		104	
	Total	104		104	

In red cell acid phosphatase system, a significant differences in their distribution among patients was observed, as compared to controls with an increase of AB phenotype and a corresponding decrease of A and B phenotypes in the patients group (χ^2 = 8.6778; d.f.= 2; 0.02>p>0.01). As a result of the disease association, a highly significant deviation from the Hardy-Weinberg equilibrium was found in the patients with mental retardation (χ^2 = 19.7432; d.f.= 1; p<0.001). For the ESD system, no heterogeneity was found between patients and controls (χ^2= 0.4204; d.f.=2; 0.90>p>0.80) and frequency of *ESD*2* was recorded 0.2645 in mentally retarded and 0.2500 in controls. For the GLO1 system, both the examined groups

Table 2: Plasma protein phenotypes in mentally retarted and controls

System	*Phenotype*	*Patients*		*Controls*	
		Obs.	*Exp.*	*Obs.*	*Exp.*
HP	1-1	0	3.18	2	1.16
	2-1	36	30.03	18	19.66
	2-2	68	70.79	84	83.18
	Total	104	104.00	104	104.00
		$\chi^2 = 4.4800$ (0.05>p>0.02)		$\chi^2 = 0.7566$ (0.50>p>0.30)	
CP	B	104		104	
	Total	104		104	
GC	1-1	56	53.16	50	50.24
	2-1	36	42.39	45	44.09
	2-2	12	8.45	9	9.67
	Total	104	104.00	104	104.00
		$\chi^2 = 2.6068$ (0.20>p>0.010)		$\chi^2 = 0.0568$ (0.95>p>0.90)	
TF	C	104		104	
	Total	104		104	
ALB	N	104		104	
	Total	104		104	

were in Hardy-Weinberg equilibrium but the chi-square test for heterogeneity between patients and controls was again found to be non-significant (χ^2:= 3.7900; d.f. =2; 0.20>p>0.10). The frequency of *GLO1*1* allele in mental retardation patients was found to be 0.4038 while in controls it was 0.4327.

Interestingly phenotype HB AS (Sickle cell Trait) records the lowest incidence (3.85%) in mental retardation compared with controls. The chi-square test for goodness of fit between observed and expected phenotype numbers was statistically non- significant in patients (χ^2: 0.0312; d.f.=1; 0.90>p>0.80), indicating no association was found between mental retardation and hemoglobin's. Regarding abnormal hemoglobin's Grant Steen et al. (1999) and Schatz and McClellan (2006), observed subtle brain abnormalities in children with sickle cell disease stating that this condition was mainly associated with a 23 fold increase in the risk of mild mental deficiency. In the present study, no sickle cell disease individual was observed.

For serum protein haptoglobin, a significant difference in its distribution among patients was observed, compared to the control group. In patients, an increase of HP 2-1 phenotype and a corresponding decrease of HP 2-2 phenotype was observed compared to the control group (χ^2=7.8421; d.f.=2;0.02>p>0.01). Thus due to the association, a significant deviation from the Hardy-Weinberg equilibrium was found in the mental retardation patients (χ^2= 4.4800; d.f.= 1; 0.05>p>0.02). For the group specific component system, no significant differences were observed between patients and controls (χ^2= 1.7680; d.f.=2; 0.20>p>0.10), and both the examined groups were in Hardy-Weinberg equilibrium indicating no association between mental retardation and this protein marker

Table 3: Allele frequencies of red cell enzymes and hemoglobin in mentally retarded and controls

Allele	*Patients*	*Controls*	*Inter group heterogeneity*	*d.f*
ACP1 A*	0.3750 ± 0.0328	0.3029 ± 0.0319	8.6778	2
*ACP1*B*	0.6250 ± 0.0328	0.6971 + 0.0319		
*ESD*1*	0.7355 ± 0.0306	0.7500 ± 0.0300	0.4204	2
*ESD*2*	0.2645 ± 0.0306	0.2500 + 0.0300		
*GLO1*1*	0.4038 ± 0.0340	0.4327 ± 0.0343	3.7900	2
*GLO1*2*	0.5962 ± 0.0340	0.5673 + 0.0343		
*SOD*1*	1.0000 ± 0.0000	1.0000 ± 0.0000		
*HB*A*	0.9807 ± 0.0095	1.0000 ± 0.0000		
*HB*S*	0.0193 ± 0.0095	0.0000 + 0.0000		

Table 4 : Allele frequecies of serum proteins in mentally retarded and controls

Allele	*Patients*	*Controls*	*Inter group heterogeneity*	*d.f*
*HP*1*	0.1750 ± 0.0243	0.1057 ± 0.0253	7.8421	2
*HP*2*	0.8250 ± 0.0243	0.8943 + 0.0253		
*GC*1*	0.7500 ± 0.0250	0.6750 ± 0.0271	1.7680	2
*GC*2*	0.2500 ± 0.0250	0.3250 + 0.0271		
*CP*B*	1.0000 ± 0.0000	1.0000 ± 0.0000		
*TF*C*	1.0000 ± 0.0000	1.0000 ± 0.0000		
*ALB*N*	1.0000 ± 0.0000	1.0000 ± 0.0000		

The test of association of red cell acid phosphatase phenotypes with the disease condition compared to the control group is presented in Table 5. An increased predisposition of heterozygous ACP1 AB phenotypic individuals (χ^2= 7.4715) was observed. Relative risk estimates of ACP1 phenotypes in disease and control group is presented in Table 6. A significant association of ACP1 AB phenotype with mental retardation was observed. (χ^2 = 7.4715).

Table 5: Test of association of ACP1 phenotypes in mentally retarded and controls

ACP1 Phenotype(s)	*Controls*	*Patients*	*χ^2 Values*
B	48	30	-
B X AB	49	70	7.8256**
B X A	7	4	0.0175
B X AB/A	56	74	6.6460**
AB X B	7	4	3.1610

**p<0.01

Table 6: Relative risk estimates of ACP1 phenotypes in mentally retarded and control groups

ACP1 Phenotype(s)	*Controls*	*Patients*		
	n	*n*	*RR*	*χ^2 Values*
B	48	30	-	-
B X AB	49	70	2.2858	7.8256**
B X A	7	4	0.9135	0.0175
B X AB/A	56	56	2.1142	6.6460**
AB X A	7	4	0.3998	3.1610

RR = Relative risk ** p<0.01

Similarly, association of haptoglobin phenotypes with the disease condition compared to the control group is presented in Table 7, indicating that an increased predisposition of heterozygous HP 2-1 phenotype individuals for mental retardation was observed. Risk estimates are also showing a significant association of HP 2-1 phenotype with mental retardation (χ^2= 9.6690) (Table 8).

CONCLUSION

To conclude, it may be said that to evaluate genetic markers for their association with mental retardation, out of ten markers, only two genetic markers, namely, acid phosphotase and haptoglobin were showing significant associations with mental retardation. Acid phosphatase (ACP1) mainly interacts with EPH (Ephrin) receptor A2 (Kikawa et al. 2002) and EPH receptor B1 (Stein et al. 1998). Both EPH and EPH related receptors and their ligands have been implicated in mediating developmental events, particularly in the nervous system.

Table 7: Test of association of HP phenotypes in mentally retarded and controls

Hp Pheno-type(s)	*Controls*	*Patients*	*χ^2 Values*
	n	*n*	
2-2	84	68	-
2-2 X 2-1	18	36	7.6690**
2-2 X 1-1	2	0	1.5920
2-2 X 2-1/1-1	20	36	5.2396*
2-1 X 1-1	2	0	3.3796

* p<0.02 ** p<0.01

Table 8: Relative risk estimates of HP phenotypes in mentally retarded and controls

Hp Pheno-type(s)	*Controls*	*Patients*		
	n	*n*	*RR*	*χ^2 Values*
2-2	84	68	-	-
2-2 X 2-1	18	36	2.4706	7.6690**
2-2 X 1-1	2	0	0.0000	1.5920
2-2 X 2-1/1-1	20	36	2.2243	5.2396*
2-1 X 1-1	2	0	0.0000	3.3796

RR = Relative Risk * p<0.02 ** p<0.01

The role of iron and its oxidative capabilities in tissue damage is well -documented (Thompson et al. 2001) and iron-containing proteins such as hemoglobin can initiate or enhance oxidative processes (Sadrzadeh et al. 1984). Increased accumulation of iron in the brain and defective antioxidant defenses have been linked to both Parkinson and Alzheimer diseases. Defective haptoglobin mediated clearance of free hemoglobin from the central nervous system could lead to hemoglobin-dependent central nervous system damage. Most of the neurological disorders are mainly associated with HP 2-2 phenotype. Our data clearly showed an association of HP 2-1 with mental retardation. At present we do not know the mechanism for this phenomenon. The sample size of the present study was relatively small but for better understanding of the role of haptoglobin in the pathophysiology of mental retardation further large scale surveys are desirable.

REFERENCES

Aicardi J 1998. The etiology of developmental delay. *Semin Pediatr Neurol*, 5: 15-20.

Balakrishnan V 1988. Hardy-Weinberg equilibrium and allele frequency estimation. In: KC Malhotra (Ed.): *Statistical Methods in Human Population Genetics*. Calcutta: Indian Statistical Institute and Indian Society of Human Genetics, pp. 39-93.

Bat-haee MA 2001. A longitudinal study of active treatment of adaptive skills of individuals with profound mental retardation. *Psychol Rep*, 89: 345-354.

Butler FM, Miller SP, Lee KH, Pierce T 2001. Teaching mathematics to students with mild-to-moderate mental retardation: A review of the literature. *Ment Retard*, 39: 20-31.

Clark JT 1964. Simplified "Disc" (Polyacrylamide) electrophoresis. *Ann NY Acad Sci*, 121: 428 - 436.

Daily DK, Ardinger HH, Holmes GE 2000. Identification and evaluation of mental retardation. *Am Fam Physician*, 61: 1059-1067.

Dosen A, Day K (Eds.) 2001. *Treating Mental Illness and Behavior Disorders in Children and Adults with Mental Retardation*. Washington, DC: American Psychiatric Press,

Epstein CJ, Korenberg JR, Anneren G, Antonarakis SE, Ayme S, Courchesne E et al. 1991. Protocols to establish genotype-phenotype co-relations in Down syndrome. *Am J Hum Genet*, 49: 207-235.

Kate SL, Khedkar AV, Mukherjee BN 1976. Cellulose acetate membrane electrophoresis- simple, rapid and inexpensive method for detection on hemoglobin variants. *Ind J Phys Anthrop Hum Genet*, 2: 123 - 124.

Kikawa KD, Vidale DR, Van Etten RL, Kinch Michael S 2002. Regulation of the Eph A2 kinase by the low molecular weight tyrosine phosphatase induces transformation. *J Biol Chem*, 277: 39274 -279.

Kinsbourne M, Graf WD 2000. Disorders of mental development. In: JH Menkes, HB Sarnat (Eds.): *Child Neurology.* Philadelphia: Lippincott Williams and Wilkins, pp.1155-1211.

Kitchin FD, Bearn AG 1966. The electrophoretic patterns of normal and variant phenotypes of the Group Specific Components (GC) in human serum. *Amer J Hum Genet*, 18: 201 -214.

Luckasson R, Coulter DL, Polloway EA, Reiss S, Schalock RL et al. 1992. *Mental Retardation: Definition, Classification and Systems of Support.* Washington DC: American Association on Mental Retardation

Malini SS, Ramachandra NB 2006. Influence of advanced age of maternal grandmothers on Down syndrome. *BMC Med Genet*, 147: 4.

McLaren J, Bryson SE 1987. Review of recent epidemiological studies of mental retardation: Prevalence, associated disorders and etiology. *Am J Ment Retard*, 92: 243-254.

Mourant AE, Kopec AC, Domaniewska SK 1978. *Blood Groups and Diseases.* London: Oxford University Press.

Nussbaum RL, McInnes RR, Willard HF 2001. *Thomson and Thomson Genetics in Medicine.* Philadelphia: WB Saubders Company.

Rajangam S, Thomas IM 1992. Down syndrome and birth order. *Man in India*, 72: 239-242.

Roeleveld N, Zielhuis GA, Gabreels F 1997. The prevalence of mental retardation: A critical review of recent literature. *Dev Med Child Neurol*, 39: 125-132.

Sadrzadeh SM, Graf E, Panter SS, Hallaway PE, Eaton JW 1984. Hemoglobin. A biologic fenton reagent. *J Biol Chem*, 259: 14354 –14356.

Schatz J, Mc Clellan CB 2006. Sickle cell disease as a neuro-developmental disorder. *Ment Retard Dev Disabil Res Rev*, 12: 200 -2007.

Scott AC, Fowler JCS 1982. Electrophoretic typing of glyoxalase I (GLO1) isozymes using a mixed starch/agarose gel. *Forens Sci Intern*, 20: 287 - 294.

Steen G, Xioping R, Raymond X, Mulhern K, James W, Langston W, Wang C 1999. Subtle brain abnormalities in children with sickle cell disease: Relationship to blood hematocrit. *Ann Neurol*, 45: 279 – 286.

Stein E, Lane AA, Cerretti DP, Schoecklmann HO, Schroff AD, Van Etten RL, Daniel TO 1998. Eph receptors discriminate specific ligand oligomers to determine alternative signaling complexes, attachment and assembly responses. *Genes Dev*, 12: 667 – 678.

Taylor GL, Prior AM 1938. Blood groups in England II distribution in the population. *Ann Eugen*, 8: 356 – 361.

Thompson KJ, Shoham S, Connor JR 2001. Iron and neurodegenerative disorders. *Brain Res Bull*, 55: 155 –164.

Verma IC 2000. Burden of genetic disorders in India. *Ind J Pediatr*, 67: 893-898.

Wraxall BGD, Emes EG 1976. Erythrocyte acid phosphates in blood stains. *J Forens Sci Soc*, 16: 127 – 132.

Wraxall BGD, Stolorow MD 1986. The simultaneous separation of the enzymes glyoxalase-I , esterse D and phosphoglucomutase. *J Forens Sci*, 31: 1439 – 1449.