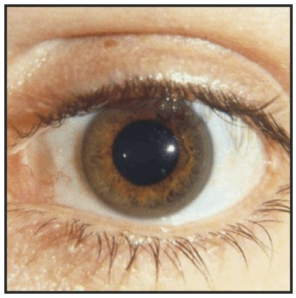
ORIGINAL PROF-984

WILSON DISEASE



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ABSTRACT ... hinaayesha62@yahoo.co.uk **Objectives:** 1). To study the genotypic differences, if any, between Pakistani children suffering from Wilson's disease from those in the west and to correlate phenotype with genotype. 2). To find out the most frequent mutations present in our patients and screen out asymptomatic siblings of the index cases. **Setting:** Department of Pediatrics, Allied Hospital, Punjab Medical College, Faisalabad. **Duration:** May 1997 to June 2005. **Materials and methods:** 41 patients ranging from 5-18 years were diagnosed based on clinical and laboratory data. 13 patients and 6 asymptomatic siblings along with their parents were subjected to mutation analysis. at University of Vienna, Austria. **Results:** None of the patients had His1069Gln, the commonest European mutation. R969Q and I1102T detected in our patients have previously been described. Four novel mutations were found. Asymptomatic siblings screened were either heterozygote or normal. R969Q appears to be associated with sub-acute liver disease with hepatosplenomegaly. I1102T was seen in children with chronic liver disease and L1071W, C1079Y and E583R-fs (insA) with early onset of neurological disease. **Conclusion:** Our Patients are phenotypicaly as well as genotypicaly different. Different genotype could be responsible for the phenotype. Further studies are needed with a larger sample size so that molecular genetic tests be devised for early diagnosis and family screening.

Key Words: Wilson's disease, Children, Genotype Phenotype, Neurological disease, Liver disease, Mutation analysis.

INTRODUCTION

Wilson's disease is an inherited disorder of copper metabolism of Wilson's disease. Gene ATP7B is located on long arm of chromosome 13[1]. Varied clinical manifestations (hepatic, neurological, hematological, psychiatric) and inconsistent laboratory tests make the diagnosis difficult. This is particular so in purely hepatic presentation & in asymptomatic patients[2,3]. It is important to diagnose the patients in pre symptomatic period as the prognosis is excellent if treatment is started at this stage. This is only possible by screening of asymptomatic siblings. But no single test is able to identify affected sibling or heterozygote carriers of the gene of Wilson's disease. Mutation analysis is a useful adjunct to

diagnosis of difficult cases and is the only reliable tool for family screening but it can be problematic; More than 200 mutations have been detected so far in Wilson disease gene[4]. Direct mutation analysis using allele specific probes can be helpful if a mutation occurs with a reasonable frequency in a population. The commonest mutation in European patients, His1069Gln is associated with late neurological presentation[5]. Our patients present earlier as noted in previous study[6]. This phenotypic difference could be due to difference in type of mutations. The objectives of the study were to make an attempt to find commonest mutation in our patients, correlate phenotype with genotype and screen asymptomatic siblings.

PATIENTS AND METHODS

The study was conducted at the Department of Pediatrics Punjab Medical College/Allied Hospital Faisalabad from May 1997 to June 2005. 41 patients were diagnosed. 20 with neurological, 17 with hepatic manifestations and 04 were asymptomatic. Their ages ranged between 5 to 18 Yrs.16 patients were lost, 12 patients expired and 13 are being followed up.

Table-I

Patient Id	Clinical Presentation	Family History	Age of Onset of Symptom	Serum Ceruloplasm in (mg/L)	K-F rings	Mutation
R.O Male 10 yrs	Hepatic-only elevated Transaminases Hepatomegaly	Three Sibs died of Liver disease within 6 months of onset	8 yrs	0.20 0.089 0.360	Negative	R969Q-True homozygote Exon 14
R.S Male 5 yrs	Asymptomatic Sibling of RO	Positive		0.34	Negative	R969Q-True homozygote
R.F Female 3 yrs	Asymptomatic Sibling	Positive		0.25	Negative	Wt/Wt
As A Male 12 yrs	Chronic Hepatitis Hemolytic anemia	Positive	11 yrs	<0.097	Positive	-I1102T-Exon 15 (WD gene) Missense mutation True homozygote
Zub Male 7 yrs	Asymptomatic Sib of As A	Positive		0.34	Absent	-I1102T-Ex 15 (WD gene) Missense True homozygote
Female- 5 yrs	Asymptomatic Sib of As A	Positive		0.29	Negative	-I1102T-Ex 15 (WD gene) Missense True homozygote
SA	Chronic Hepatitis&Subtle neurologic signs	Positive	12 yrs	<.097	Positive	-I1102T-Ex 15 (WD gene) Missense True homozygote
Jw. H Male 11 yrs	Neurologic presentation-Dystonia tremors	Negative	10 yrs	<.097	Positive	E 1201 K Exon 17 (WD gene) Compound Heterozygote. 2nd mutation unknown

Table-II.

Patient Id	Clinical Presentation	Family History	Age of Onset of Symptom	Serum Ceruloplasm in (mg/L)	K-F rings	Mutation
Has 13 yrs male	Neurologic-Dysarth & Writing difficulty	Negative	8 yrs	<.097	Positive	L 1071 W EX 14 (WD gene) True Homozugote
Ah Al 14 yrs male	Neurologic	Negative	11 yrs	<.097	Positive	C 1079Y EX 14 (WD gene) True Homozugote
Saad.Z 19 yrs female	Neurologic presentation-Dystonia tremors	Sister died of WD Cirrhosis	5 yrs	<.097	Positive	E 583R-fs (ins A) Exon 5 (WD gene) True Homozugote
Nadi 7 yrs female	Asymptomatic Sib of Saad	Positive		.33	Negative	Normal
Irf 7 yrs male	Asymptomatic Sib of Saad			0.29	Absent	True Homozugote
Uj 12 yrs female	Chronic Hepatitis	Negative	8 yrs	<.081	Positive	Negative for His 1069 Gln Mutation not detected
Soh 21 yrs male	Neurologic	S Sister Expired & WD Neurologic	12 yrs	<.097	Positive	Negative for His 1069 Gln Mutation not detected
Rb Nw 12 yrs male	Neurologic	Negative	11 yrs	<.097	Positive	Negative for His 1069 Gln Mutation not detected
Rabi 19 yrs female	Neurologic	Positive	11 yrs male	<.097		Negative for His 1069 Gln Mutation not detected
Rob 20 yrs female	Neurologic	Positive	12 yrs	<.097		Negative for His 1069 Gln Mutation not detected

DIAGNOSTIC CRITERIA

For those with neurological onset: Typical clinical findings, presence of Kayser-Fleischer corneal rings on Slit Lamp examination and low serum ceruloplasmin (<20 mg/dl) was used. For Hepatic and asymptomatic patients, in addition to the above, Free serum copper (>10ug/dl), 24-hrs urinary copper (>100ug/24hrs), Liver histology & staining (where feasible) were done in addition.

GENETIC TESTING

25 samples of 5ml Edta contained whole blood were sent by DHL courier to Professor Peter Ferenci University of Vienna, Austria Vienna.13 were patients and 06

asymptomatic siblings (along with parents). H1069Q mutation was tested by a PCR-based Restriction Fragment Length Polymorphism assay. All negatives were sequenced exon by exon by direct mutation analysis.

RESULTS

All patients tested were negative for his1069Gln, the commonest European mutation. Patients' history, clinical manifestations and laboratory data is given in the tables I and II.

DISCUSSION

In our previous study, it was noticed that our patients were phenotypicaly different from those mentioned in literature. They presented at an earlier age[6]. The mutation analysis showed that different phenotype is possibly due to the differences in the mutations. All the children were negative for the commonest European mutation His 1069Gln.This mutation was seen in 42% of German patients in a study by Duc H H et al[7].

R969Q was detected in one family. It appears to be related to hepatic onset and rapid progression. The family in whom this mutation was detected lost three kids because of unexplained liver disease. All three sibs presented with hepatosplenomegaly and progressed to death within 6 months. All were not having K F rings. Serum ceruloplasmin and 24 hours urinary copper were inconclusive.

The disease started at 8-9 years of age in all. Last of the three had undergone Liver biopsy that revealed copper stores on staining. He expired within a month of starting chelation therapy. Fourth alive sib was screened and found to have raised transaminases. Rest of the diagnostic tests were again normal. Chelation therapy was started and mutation analysis sent. R969Q mutation was detected. The other two living sibs were screened and were found to be heterozygote and normal respectively. R969Q mutation has been detected previously in Italian and Turkish population[8].

I1102 was seen in two sibs presenting with chronic liver disease at the age of 11 and 12 years respectively. One of them, who was having no compliant, developed neurological symptoms later. This mutation has been previously detected in Pakistani, Indian and Saudi patients[10].Other mutations like 15821-fs and G711W reported in Pakistani population by various workers were not seen in our patients[9,10].

Four Novel mutations were seen in early onset neurological manifestations i.e E583R-fs (insA) in Exon 5, C1079Y in Exon 14, L1071W in Exon14 and E1201K in Exon17. C1079Y was detected in a patient who presented at 8 years of age with dysarthria, dystonia and poor school performance. C1079Y appears to be related to early onset neurological disease.

E583R-fs (insA) was detected in a girl who presented with neurological symptoms at the age of 5 yrs, showed a good response to D- Penecillamin and is now 19 years old. Her elder sister developed Wilsonian Hepatitis, later died of cirrhosis. Mutation analysis could not be done. Both the sisters are expected to have the same mutation but presented differently; making genotype phenotype correlation difficult. Okada, T analyzed 41 Japanese patients and found no correlation of phenotype with genotype.[11]

The mutation spectrum of Wilson disease may indicate a population dependant pattern as noticed by Norikazu et al among Japanese patients. The commonest mutations detected in 23 Japanese patients were 2871 del C in exon 13 and Arg 778 Leu in exon 8 that were different from those found in Europe patients[12].

Most of our patients were true homozygote making it possible to correlate phenotype with genotype. One of our patients presenting with neurologic symptoms at the age of 10 years was compound heterozygote for E1201T mutation in Exon 17.

The second mutation remained undetected. Compound heterozygotes constituted a large majority of Mediterranean cohort, making this co-relation difficult[13]. Based on population dependant manner of occurrence of ATP7B mutations, it may be possible to establish a molecular diagnosis system. A molecular diagnosis

system is considered to be very effective for making a definitive diagnosis in very young patients and for detecting carriers

All asymptomatic siblings tested were luckily either heterozygote or normal

CONCLUSIONS

The variation in mode of presentation patient with Wilson's disease is possibly due to differences in the type of mutations. Our patients presenting at an earlier age are genotypicaly different. Mutation analysis is a useful tool in the definite diagnosis of asymptomatic siblings. Further studies are needed to find the commonest mutation in our population and for genotype-phenotype correlation.

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When it is dark enough;
you can the see the stars.

Charles A. Beard