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RAPID COMMUNICATION

Genotype phenotype correlation in Wilson's disease within families-a report on four south Indian families

S Santhosh, RV Shaji, CE Eapen, V Jayanthi, S Malathi, P Finny, N Thomas, M Chandy, G Kurian, GM Chandy

S Santhosh, CE Eapen, G Kurian, GM Chandy, Department of GI Sciences, Christian Medical College, Vellore, Tamil Nadu, India
RV Shaji, M Chandy, Department of Haematology, Christian Medical College, Vellore, Tamil Nadu, India
V Jayanthi, Department of Medical Gastroenterology, Stanley Medical College, Chennai, Tamil Nadu, India
S Malathi, Department of Pediatrics, Institute of Child Health, Chennai, Tamil Nadu, India
P Finny, N Thomas, Department of Endocrinology, Christian Medical College, Vellore, Tamil Nadu, India
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Correspondence to: S Santhosh, Department of Gastrointestinal Sciences Christian Medical College, Vellore, Tamil Nadu, India. santhoshcmc@yahoo.com
Telephone: +91-416-2222102-2148 Fax: +91-416-2232035
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Abstract

AIM: To study the genotype phenotype correlation in Wilson's disease (WD) patients within families.

METHODS: We report four unrelated families from South India with nine members affected with WD. Phenotype was classified as per international consensus phenotypic classification of WD. DNA was extracted from peripheral blood and 21 exons of ATP7B gene and flanking introns were amplified by polymerase chain reaction (PCR). The PCR products were screened for mutations and the aberrant products noted on screening were sequenced.

RESULTS: Four separate ATP7B mutations were found in the four families. ATP7B mutations were identical amongst affected members within each family. Three families had homozygous mutations of ATP7B gene while one family had compound heterozygous mutation, of which only one mutation was identified. We noted concordance between ATP7B gene mutation and Wilson's disease phenotype amongst members within each family. The age of onset of symptoms or of detection of asymptomatic disease, baseline serum ceruloplasmin and baseline urinary copper levels were also similar in affected members of each family. Minor differences in phenotype and baseline serum ceruloplasmin level were noted in one family.

CONCLUSION: We report concordance between ATP7B mutation and WD phenotype within each family with > 1 member affected with WD. Homozygous ATP7B mutation was present in 3 of the 4 families studied. Our report supports allelic dominance as a determinant of WD phenotype. However, in one family with compound heterozygous mutation, there was a similar WD phenotype which suggests that there may be other factors determining the phenotype.

Key words: Wilson's disease; Genotype phenotype correlation

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INTRODUCTION

Wilson's disease (WD) is an autosomal recessive disorder characterized by excess hepatic copper accumulation and impaired biliary copper excretion. In 1993, the gene encoding WD protein (ATP7B) was cloned and was found to encode a copper transporting P-type ATPase required for biliary copper excretion. However, although the characterization of the molecular genetic basis of this disease has provided insight into the mechanisms of copper homeostasis, clinical studies of specific patients have not been useful in elucidating the mechanism of hepatic copper metabolism[1]. One intriguing question in WD is how closely the genotype determines the phenotype. While over 300 mutations of ATP7B gene have been reported in WD, over one-half of these occur rarely in any given population. Most patients are compound heterozygotes, possessing alleles with two different mutations. This degree of allelic heterogeneity has been a major hindrance in studying genotype phenotype correlation in WD[2,3].

In such a situation, one approach is to study the

phenotypes of a single predominant genotype in a population. The results of this approach have shown poor genotype phenotype correlation in the different populations studied[4-6]. Another approach is to compare genotype and phenotype in WD occurring within members of a family. In a report of two Japanese families, the affected family members had similar compound heterozygous mutations of ATP7B gene but had different phenotypes and different ages of onset of disease suggesting allelic dominance as a factor determining the phenotype[7]. We report four families wherein the affected members within each family had identical mutations of ATP7B. We compared the phenotypes within these families to see if the phenotypes were true to the index case.

MATERIALS AND METHODS

Subjects

We report four unrelated families from the states of Tamil Nadu and Andhra Pradesh in South India with nine documented cases of WD. The basis of the diagnosis of WD was the presence of Kayser Fleischer rings in the cornea on slit lamp examination and the presence of a low serum ceruloplasmin level (measured by copper oxidase method). Further investigations were carried out as clinically indicated. WD phenotype was classified as per the international consensus classification[8]. Neurological involvement was determined by clinical examination, while liver involvement was assessed by liver function tests, ultrasonography and liver biopsy, if indicated, in addition to clinical examination.

Genetic studies

Peripheral blood was collected from the patients after obtaining an informed consent. The genomic DNA was extracted from white blood cells by standard phenol-chloroform method. The 21 exons of ATP7B gene and their flanking intronic sequences were amplified by polymerase chain reaction (PCR) using primers described earlier[5]. PCR amplified products were subjected to conformation sensitive gel electrophoresis (CSGE) for mutation screening[9]; those exhibiting aberrant patterns in CSGE were sequenced using automated sequencer (ABI 310, Applied Biosystems, CA).

Genotype phenotype correlation

Mutations of ATP7B gene and WD phenotype in each of the affected family member was compared with other affected members within that family. Similarly, other indices like age of onset of symptoms/age of detection of asymptomatic disease, baseline serum ceruloplasmin and baseline urinary copper levels were also compared within symptomatic members of each family.

RESULTS

All the nine patients had Kayser Fleischer rings and low serum ceruloplasmin levels. Three of the nine patients were asymptomatic and were detected to have WD on family screening, after the index patient was diagnosed within the family. All nine patients had abnormal liver function tests and/or abnormal liver on ultrasound. Only one patient (family 4, Table 1) underwent liver biopsy, which showed cirrhosis of liver. The affected members in families 1 to 3 had identical homozygous mutations of ATP7B gene within each family (Table 1). These three mutations of ATP7B gene are novel mutations. The ATP7B gene mutations identified were A2623G (Arg-Gly) in family 1, G4021A intronic mutation in family 2, and a point mutation A3029G (Lys-Arg) in family 3. The two siblings in family 4 were compound heterozygotes, in whom the only mutation identified, G3282A (Phe1094Leu), was identical in both the siblings. This mutation has been recently reported from Brazil[10].

In addition to the mutations, 2-5 polymorphisms of ATP7B gene were also identified in each of the nine patients studied. Similar to the mutations, the polymorphisms of ATP7B gene were also identical in members within the same family (Table 1). In Family 1, 4 of 6 children born to partners in a consanguineous marriage, had WD. DNA was available for mutation analysis from three of the four affected children. While all three had hepatic involvement, the elder brother also had arthritis involving the right knee (Table 1). In Family 2, of the 4 children-products of a consanguineous marriage-one son and one daughter had neurological and neuro-psychiatric involvement respectively. In Family 3, (non-consanguineous marriage), the father presented at age 45 years with WD accompanied with a metabolic bone disorder and renal tubular acidosis. On screening the family, his son was detected to have WD (diagnosed on the basis of low serum ceruloplasmin level and KF rings on slit lamp examination). He also had elevated urinary copper level. However, his liver function tests were normal and the liver appeared normal on ultrasonography. A liver biopsy was refused. Since this subject did not have neurological involvement, it was assumed that he had sub-clinical liver disease. Unlike the father, the son did not have renal tubular acidosis. The mother and daughter did not have WD. In family 4, four children (4 sons) were born to parents in a consanguineous marriage. One son died of liver disease (age of onset of symptoms: 13 years), however he had not been evaluated for WD. Another son aged 17 years, who was healthy, had no clinical, biochemical, and radiological signs of liver disease. Of the two affected sons who were alive, one had overt and the other had asymptomatic hepatic involvement.

The WD phenotype, age of onset of symptoms/age of detection of WD, baseline serum ceruloplasmin and baseline urinary copper levels were similar among members of a family, except for some differences in family 3 (Table 1). In family 3, there were marked differences in the age of onset of symptoms/age of detection of WD and baseline serum ceruloplasmin levels (difference of 50 U/L) between the father and

Table 1 Detailed genotype and phenotype in four families with Wilson's disease

Family	WD patient	Age at onset (yr)	ATP7B mutation	ATP7B gene polymorphisms	Clinical phenotype	Baseline serum ceruloplasmin[5] (U/L)	Baseline urinary copper[6] (μg/24 h)
1	Brother	12	Ex[7] 11: A2623G	Ex[7] 2: T1216G Ex[7] 3: G1366C Ex[7] 12: G2855A Ex[7] 13: G3009A	H2[1]	8	Not available
	Sister[4]	14				4	430
	Brother	16				13	Not available
2	Brother	14	In[8] 19: G4021A	Ex[7] 2: T1216G Ex[7] 3: G1366C Ex[7] 10: T2495C Ex[7] 12: G2855A Ex[7] 13: G3009A	N1[3]	6.2	166
	Sister	12				1.9	112
3	Father	45	Ex[7] 13: A3029G	Ex[7] 2: T1216G	H2[1], O[2]	6	340
	Son[4]	16		Ex[7] 10: C2495A	H2[1]	56	983
4	Brother	11	Ex[7] 15: G3282A/?	Ex[7] 10: T2495C Ex[7] 12: G2855A	H2[1]	32	1067
	Brother[4]	10				26	630
	Brother[9]						

H2[1]: Hepatic; O[2]: Other; N1[3]: Neurological phenotypes of Wilson's disease; [4]Asymptomatic subject, WD detected on family screening; [5]Serum ceruloplasmin: (normal range) 62-140 U/L; [6]Urine copper: normal upto 150 μg/24 h; [7]Ex: denotes Exon; In[8]: Denoted Intron; [9]Died of liver disease (age of onset of symptoms: 13 years), was not tested for Wilson's disease.

son. In addition, renal tubular acidosis was present only in the father.

DISCUSSION

The present study, examined the genotype phenotype correlation in WD occurring within members of each family, whereas previous studies examined the question with respect to a single common mutation in a given population. While the previous studies gave answers related to that particular mutation, we investigated the impact of four different mutations in members of four unrelated families with more than one member affected with WD. Although the number of subjects studied was small, our report highlights some aspects of interest in genotype phenotype correlation in WD. In the present report, the affected members of each family shared identical mutations of ATP7B gene within each family (homozygous mutations in three families and compound heterozygous mutation in one family). We found strong genotype phenotype concordance in members of each family. The two minor differences in phenotype within members of a family were the following: in the first family, one sibling had monoarticular arthritis; a known association with WD, which was not present in the other two siblings, while in family number 3, the father had renal tubular acidosis and metabolic bone disease where as the son did not have these abnormalities.

The three additional phenotypic indices studied (age of onset of symptoms or age at detection of asymptomatic disease, baseline serum ceruloplasmin and baseline urine copper levels) were similar in members of each family, except for family 3 (Table 1). While the father in family 3 became symptomatic with disabling metabolic bone disease and liver involvement only at the age of 45, WD was detected at an early asymptomatic stage by screening in the son at the age of 16. It is possible that some of the differences in phenotype between father and son in this family could be due to longstanding untreated copper overload state in the father. Defining disease onset accurately in WD is difficult and this could be a confounding factor in genotype phenotype correlation in WD[11].

In summary, we found genotype phenotype concordance in four families with different types of ATP7B mutations in each family: homozygous exonic mutation in two families, homozygous intronic mutation (Family 3) and compound heterozygous mutation (Family 4). It is of interest that such a concordance of genotype and phenotype was seen in different phenotypic manifestations, including neurological involvement in childhood in family 2, an uncommon presentation of WD.

The occurrence of identical homozygous mutations within members of a family could reflect genetic inbreeding in the population perpetuated by consanguineous marriages. Consanguineous marriages are common in South India (28% in one study)[12]. Analysis of data from the 1992-1993 National Family Health Survey to assess trends in consanguinity in the South Indian states of Andhra Pradesh, Karnataka, Kerala and Tamil Nadu showed that in Kerala the frequency of consanguineous marriages was very low and one type of preferred marriage of the Dravidian marriage system-uncle niece marriage-was conspicuously absent, whereas in the other states of South India, consanguinity and the coefficient of inbreeding were high[13]. In a study of 407 infants and children in Karnataka, of a total of 35 genetic diseases detected, autosomal recessive disorders formed the largest single disease category diagnosed[14].

Differences in phenotypic classification used in different studies could affect the interpretation of results of genotype phenotype correlation in WD. Although

the Japanese used a phenotypic classification different from the international consensus classification which we have applied in our report, the distinction between hepatic versus neuropsychiatric phenotypes in both the classification systems appears to be similar[7]. While the Japanese study of two families showed that within each family, members with different mutations of ATP7B gene had different WD phenotypes; we report four families wherein affected members of each family had identical mutations of ATP7B gene and similar phenotypes. Thus, both these studies suggest that within families, a particular ATP7B genotype is associated with a particular WD phenotype thereby supporting the concept of allelic dominance as a significant determinant of WD phenotype.

In a previous report, we did not find symptomatic WD in >1 member in each family. On studying the phenotype of presumed WD in deceased siblings of index patients with WD, we found concordance of WD phenotype within each family in 6 of 8 families studied. The WD phenotype in these 6 families with concordant phenotype within each family was hepatic (4 families), hepatic and neurological (1 family) and neuro-psychiatric (1 family)[15].

By contrast, studies on WD patients with a common mutation in a given population have not shown this degree of concordance between genotype and phenotype. In a study on patients mainly from Austria, neurological presentation was significantly more common than hepatic presentation in H1069Q homozygotes compared to H1069Q compound heterozygotes or H1069Q negative patients[11]. However, these findings were not borne out by studies from other parts of Europe and North America[4,16]. Some studies have reported later age of onset of symptoms in H1069Q homozygotes compared to H1069Q compound heterozygotes[4,17].

Apolipoprotein E genotype, intestinal metallothionine inducibility and the individual's capacity to withstand "copper stress" by utilizing glutathione, superoxide dismutase and heat shock proteins are some epigenetic factors that may affect the WD phenotype[11,18]. It is possible that common epigenetic or environmental factors within families accentuate the genotype phenotype concordance seen in family members affected with WD.

Further studies of WD within families in different populations are needed to assess whether the concept of allelic dominance determining the phenotype holds true. It would also be important to see if the phenotypes associated with a particular mutation of ATP7B gene in WD within families are the same as in WD patients with the same mutations, but in a different family or a different population[19]. Application of a standard phenotypic classification like the international consensus phenotypic classification for WD[8] in future studies will enable valid comparison of the results.

In conclusion, we report 4 families with WD wherein affected members had identical mutations of the ATP7B gene and showed genotype phenotype concordance amongst members within each family. This finding supports allelic dominance as a determinant of WD phenotype and is at variance with previous studies of WD conducted in a given population. Further studies of genotype phenotype correlation in WD occurring in families are needed.

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