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SHORT REPORT

Endemic Tyrolean infantile cirrhosis is not an allelic variant of Wilson's disease

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Recently, 138 cases of infantile cirrhosis originating in several families in the Austrian province of the Tyrol were reported. This endemic Tyrolean infantile cirrhosis (ETIC) is indistinguishable from Indian childhood cirrhosis (ICC), idiopathic copper toxicosis (ICT), and resembles the early forms of Wilson's disease (WND). It has been argued that ETIC might represent an allelic variant of the WND gene, which is a copper transporting P-type ATPase (*ATP7B*). Assuming that ETIC results from a founder effect, a possible role for *ATP7B* in ETIC was investigated by association studies and haplotype sharing. Because of its lethality, the mapping of ETIC was focused on obligate gene carriers, i.e. the patients' parents. Our data indicate that ETIC is a separate genetic entity, distinct from WND.

Keywords: childhood cirrhosis; copper; founder populations; Wilson's disease; linkage disequilibrium; chromosome 13

Introduction

Wilson's disease (WND) is an autosomal recessive disorder characterised by accumulation of copper in the liver and several other tissues. Mutations in the *ATP7B* gene located on 13q14.3 cause WND.[1,2] Other copper overload syndromes have been described. Until recently, the majority of non-Wilsonian copper toxicosis patients were observed in India (ICC).[3] The rare genetic copper-overload diseases occurring outside India include ICT[4] and ETIC.[5] Recently, 138 ETIC cases were reported[5] which are clinically and pathologically indistinguishable from ICC, ICT, and the early-onset forms of WND. Although ETIC, ICC and ICT require copper-enriched diets to become manifest,[6,7] it is thought that these disorders might represent allelic variants of WND.[4,8–10]

Given that ETIC also has an autosomal recessive inheritance, we investigated whether *ATP7B* mutations could cause ETIC. In contrast to the 30 isolated cases described worldwide,[6] the high frequency of ETIC in the Tyrol suggested a founder effect, ie the diseased individuals have a common ancestor. The lethality of the disease[5] means no living children were available but 8 pairs of parents were identified with at least one ETIC child. These parents must be obligate carriers for the same mutation and share relatively large regions of DNA around the ETIC locus.[11–13] The size of the shared region depends on the degree of relationship. This

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would result in a strong association between the ETIC locus and alleles of markers in the surrounding chromosome region.

Patients and Methods

Patients

Blood was taken from six nuclear families, consisting of both parents and one or more normal children (C, I, M, N, L and O), selected from the ETIC pedigree.[5] The families A and B were later linked to this pedigree. For families A, B, C, and I, a common ancestor could be identified 10 generations ago (Figure 1). For families M, N, L and O a common ancestor has not yet been identified but they clearly belong to the overall pedigree. In total 32 individuals were studied (15 parents and 17 siblings). Controls were randomly chosen from a blood donor trial performed in the same area of the Tyrol.

Marker and Haplotype Analysis

DNA was extracted from blood using standard procedures.[14] PCR reactions and marker analysis are described in Giltay *et al*.[15] D13S301[16] resides within intron 1 of *ATP7B* (D Cox, 1997 personal communication). D13S16[16] and D13S133[17] are close to *ATP7B*. D13S126, D13S164, D13S321, D13S119, and D13S788 span a 20 cM region around *ATP7B*.

Haplotypes were constructed including all markers except D13S788; the position of D13S788 with respect to D13S301 is currently unknown.

Statistical Analysis

Terwilliger[18] described a method of quantifying the strength of association between multi-allelic markers and a disease mutation, without a priori knowledge of which marker allele might be over-represented on disease chromosomes. This method requires the patients' genotypes. In our analyses, genotypes were only available for the parents and at least one sibling for each of the patients. We incorporated the Terwilliger statistic[19] into the ILINK program of the LINKAGE package[20] to perform association studies using patients' relatives. Analyses were carried out

(i) with and without the genotypes of 30 controls,

(ii) assuming a gene frequency of 0.001, and

(iii) classifying the disease phenotypes of all unaffected relatives as unknown

For each marker we calculated: $LOG_{10}(L_\lambda/L_0)$ where λ is the proportion of disease chromosomes with the associated allele, and L_λ the likelihood computed on the data for different fixed values of λ (from 0.01 to 1), and L_0 the likelihood computed on the data assuming no association ($\lambda = 0$). The expected value of λ can be determined via

$$\lambda = (1-\theta)^n + (1-(1-\theta)^n)f$$

where θ is the recombination frequency (see Table 1), n the number of generations between the patients and their presumed ancestor, and f the frequency of the associated marker allele in the population. Since the value of f is uncertain, the second term in the calculation of λ was ignored, and a conservative expectation of λ was obtained via $\lambda = (1-\theta)^n$.

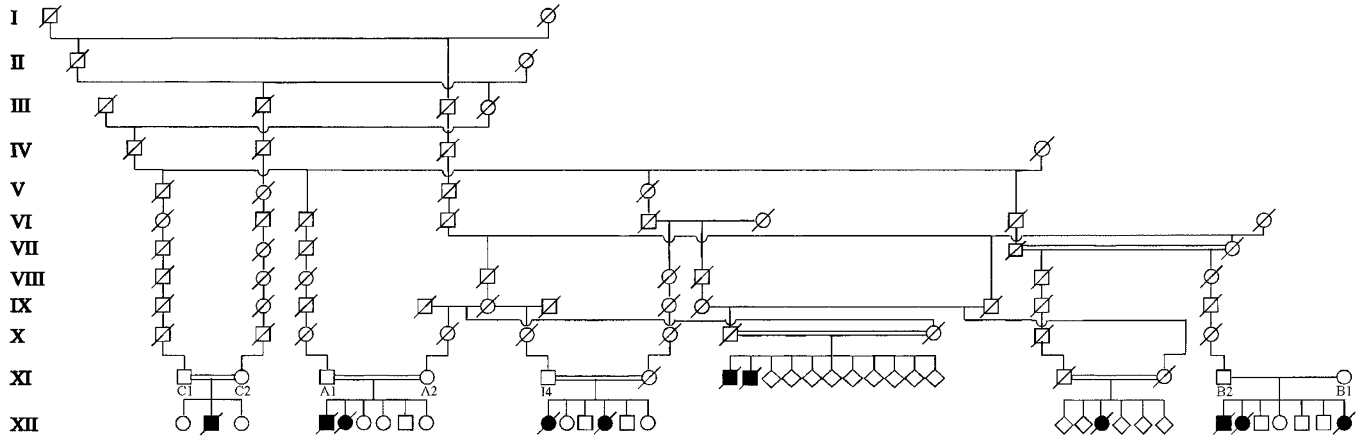

Figure 1 *For families A, B, C, and I, a common ancestor could be identified 10 generations ago. All affected individuals are deceased*

Table 1 Expected proportion of disease chromosomes with an associated allele and the corresponding lod score based on the existence of either 8 or 12 generations between the current ETIC carriers and a common founder

		8 generations			*12 generations*		
Locus	θ[a]	*% expected sharing*	*lod score no controls*	*lod score plus controls*	*% expected sharing*	*lod score no controls*	*lod score plus controls*
D13S301	0.01	92	–6.24	–6.13	85	–4.26	–4.12
D13S133	0.01	92	0.99	0.43	85	0.75	0.36
D13S316	0.01	92	–0.63	0.10	85	–0.41	0.28
D13S788	0.05	66	–2.29	–2.16	44	–1.01	–0.90
D13S164	0.05	66	–0.60	–0.72	44	–0.33	–0.35
D13S119	0.10	43	–0.74	0.00	20	–0.20	0.07
D13S126	0.10	43	–0.24	–0.43	20	–0.13	–0.12
D13S321	0.15	27	–0.19	0.00	7	–0.02	–0.01

[a]Frequency of recombination between the marker and *ATP7B*.

Results

Association studies between ETIC and markers close to, or within, *ATP7B* were carried out using a likelihood-based approach[18] modified to analyse associations using relatives of ETIC patients. The λ value calculated by this modified version indicates the proportion of disease chromosomes still carrying the allele that became associated with the disease at the time of mutation; the expected value of λ was based on the existence of either 8 or 12 generations between the current ETIC carriers and a common founder. The majority of markers yielded statistical evidence against association (Table 1). Only D13S133 showed weak statistical evidence for association. However, D13S133 is hardly informative, since almost every parent is homozygous for allele 1. Allele 1 was also the most common allele in the controls (74%).

Interestingly, D13S301 is located within the *ATP7B* gene and should show the strongest association if the ETIC mutation were present within *ATP7B*. However, D13S301 shows strong evidence against association (Table 1).

The genotypes were further arranged into haplotypes (Table 2). Based on the location of *ATP7B*, the haplotype containing D13S301–D13S133–D13S316 is potentially the most informative. The most frequent haplotype for D13S301–D13S133–D13S316 is 3–1–1, which is found in individuals A1, A2, B2, C1, M1 and N1 (Table 2). Families A, B, C and I can be connected to a common ancestor 10 generations ago (Figure 1). Given their close relationship, the carriers from this part of the pedigree should share large regions of DNA around

Table 2 Haplotypes around WND locus in ETIC carriers

	Observed alleles per chromosome						
Individual	*S126*	*S164*	*S301[a]*	*S133[a]*	*S316[a]*	*S119*	*S321*
A1[b]	4	4	2	1	1	2	4
	5	1	3	1	1	5	3
A2[c]	1	1	3	1	1	3	2
	4	4	5	1	2	5	4
B1[b]	1	4	3	–	8	5	3
	4	4	2	1	1	5	4
B2	4	1	5	1	4	3	3
	4	4	3	1	1	6	2
C1[b]	4	1	3	1	1	2	3
	5	1	3	1	6	5	3
C2[b]	2	4	5	2	1	3	3
	4	4	5	2	7	4	1
I4[c]	2	4	2	1	5	3	4
	2	4	6	1	1	5	3
L1	4	4	5	1	1	4	3
	4	4	6	1	1	2	3
L2	2	1	4	1	6	4	3
	3	3	4	1	1	1	4
M1	4	4	3	1	1	3	3
	6	4	3	1	1	4	4
M2	4	1	5	1	10	4	3
	4	4	4	1	1	4	3
N1	1	1	1	1	7	3	4
	4	3	3	1	1	3	4
N2	2	4	4	1	1	4	4
	4	2	2	1	9	3	3
O1	4	4	7	1	1	3	3
	2	4	5	1	3	4	3
O2	2	4	2	1	9	4	1
	4	1	4	1	1	2	3

[a]Three markers within a region of approximately 300 kb.
[b]Individuals separated from each other by fewer than 20 meioses (see Figure 1).
[c]Individuals separated from each other by 6 meioses (see Figure 1).

ATP7B. However, the 3–1–1 haplotype is hardly extended towards the centromere or telomere.

Discussion

Given the rare occurrence of 'non-Wilsonian copper overload syndromes' it is likely that the ETIC cases in the Tyrol are all descendants of a common ancestor. Such founder populations can be resources for mapping rare traits, by searching for segments shared by patients, and then demonstrating that sharing is identical-by-descent.

The ETIC carriers originated in an isolated community in the Tyrol. A common ancestor was identified for four of the affected families. The constructed haplotypes in ETIC carriers did *not* demonstrate sharing and the association studies did *not* detect linkage disequilibrium between ETIC and the individual 13q14.3 markers.

We used the modified Terwilliger approach to analyse the strength of association between multi-allelic markers and the ETIC mutation in the absence of patients' genotypes. Each parent was expected to have one copy of a (partly) shared haplotype. In practice, the high frequency of the marker alleles used and the fact that we observed little or no difference between the allele frequencies in the carriers and controls were confounding factors. Fortunately, D13S301 demonstrated a highly negative lod score and excluded *ATP7B* as a candidate gene for ETIC.

Our data indicate that the ETIC phenotype is a separate genetic entity, distinct from WND since it involves another unidentified, genetic locus. A total genome screen performed in the same individuals should define this. The striking phenotypic similarities of ETIC and ICC and ICT raise critical questions of whether the non-Wilsonian hepatic copper toxicosis occurring outside the Tyrol also involves a locus other than *ATP7B*.

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