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Wilson Disease in Iceland: A Clinical and Genetic Study

Gordon R. Thomas,[1] Ólafur Jensson,[3] Gunnar Gudmundsson,[4] Leifur Thorsteinsson,[3] and Diane W. Cox[1,2]

[1]Research Institute, The Hospital for Sick Children and Department of Molecular and Medical Genetics and [2]Department of Paediatrics, University of Toronto, Toronto; [3]Department of Medical Genetics, The Blood Bank, and [4]Department of Neurology, National University Hospital, Reykjavik

Summary

A survey of Wilson disease in Iceland has revealed two large kindreds with affected individuals. We have carried out studies of haplotypes of dinucleotide repeat polymorphisms (CA repeats) flanking the Wilson disease gene. The same mutation, a 7-bp deletion, is present in both families, and the clinical features are similar. The haplotype data and nature of the mutation support the existence of a founder chromosome carrying the mutation. This Icelandic mutation was not found in patients of Irish or Scottish origins, who could share some of the Icelandic ancestral genes. Although the protein function is predicted to be completely abolished by the deletion, predicting early-onset liver disease, we find that the patients present with later-onset neurological and psychiatric symptoms. We show that alternative splicing of the transcript in the region of the deletion could contribute to later onset, suggesting that alternative isoforms of the protein might have some functional significance.

Introduction

Wilson disease is an autosomal recessive disorder of copper transport, manifesting as chronic liver disease and/or neurological impairment due to accumulation of copper in several tissues, principally the liver and brain. The defect lies in reduced incorporation of copper into the plasma protein ceruloplasmin and reduced excretion via the bile. Treatment involves removal of excess copper by a chelating agent such as penicillamine or by blocking intestinal copper absorption with zinc salts (Hoogenraad et al. 1979).

The Wilson disease locus (WND) was originally assigned to chromosome 13 by close linkage with the esterase D locus in a large Israeli-Arab kindred (Frydman et al. 1985). Recently, the gene defective in Wilson disease was cloned and coded for a putative copper-transporting P-type ATPase (Bull et al. 1993; Tanzi et al. 1993). Haplotypes of highly polymorphic microsatellite markers associated with the disease locus have been described elsewhere (Petrukhin et al. 1993; Thomas et al. 1994, and in press), and we have also determined the exon/intron structure and screened 58 WND patients for mutations (Thomas et al. 1995). The exon/intron structure and alternative splicing of the transcript of *ATP7B* have also been described by others (Petrukhin et al. 1994).

Iceland, an island in the North Atlantic between Norway and Greenland, 103,000 square km in size, has a population of 265,000. Iceland has been inhabited for >11 centuries and was initially settled by peoples mainly from Norway, Ireland, and Scotland (Bjarnason et al. 1973). From the time of settlement, immigration and emigration has been low, except during the period 1860–90, when a significant number of Icelanders emigrated to Canada and the United States. Urbanization took place rapidly following the First and Second World Wars, and more than half the population of Iceland lives in and around the capital, Reykjavik. Genealogy has been indispensable for Icelanders since the time of settlement, and tracing of family histories is relatively easy, with computerized registration extending back four or five generations. In many instances, family origin can be traced to the first general census in 1703.

During the past 40 years, a total of eight patients with Wilson disease, in two kindreds, have been diagnosed in Iceland. The extensive genealogical data available for each kindred has made possible the tracing of their histories back to common ancestors. DNA of patients from both families was screened for mutations in the Wilson disease gene. The mutation in both families was identified in our initial search for mutations, when we first identified a candidate gene for Wilson disease (Bull et al. 1993). Haplotypes of closely linked markers were also constructed for each family. We present the clinical data for the patients, haplotype evidence for a common origin for the mutation in both families, and reverse transcription (RT)–PCR results, which suggest that alternative splicing of the *ATP7B* transcript could have a

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Address for correspondence and reprints: Dr. D. W. Cox, Department of Genetics, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. E-mail: dcox@sickkids.on.ca

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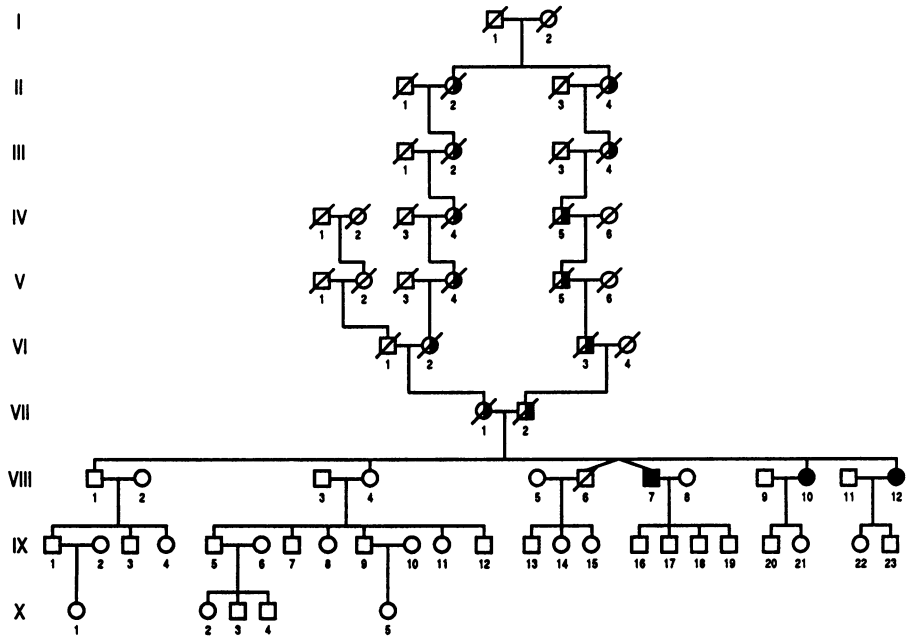

Figure 1 Pedigree of Icelandic WND kindred 1. Clinically affected individuals are shown as filled squares for males and filled circles for females. Obligate heterozygotes (parents) and assumed heterozygotes are shown as half-filled symbols.

direct effect on the severity of the disease symptoms. We also examine the possibilities of the mutation being either unique to Iceland or having originated in Europe.

Patients, Material, and Methods

Patients

A total of eight patients, from three sibships of two kindreds, were ascertained with Wilson disease from the central health registry in Reykjavik, where referrals are made from all parts of Iceland. The kindreds were from the northwest and southeast parts of the island, respectively. Pedigrees of both families are given in figures 1 and 2. Diagnosis of patients was done initially by clinical symptomology. The clinical investigation of patients and their sibs was, in most cases, supplemented by laboratory studies of copper levels in serum and urine, measurement of ceruloplasmin levels, and biopsy specimens of liver tissue. DNA was extracted from whole blood collected in EDTA by a salt-precipitation method (Miller et al. 1988).

Haplotype Analysis

Haplotypes were derived by using the microsatellite markers D13S301, D13S314, and D13S316, which flank the Wilson disease gene (Petrukhin et al. 1993; Thomas et al. 1994). The amplification of CA repeats was as described by Thomas et al. (1994). The relative locations of the markers with respect to the WND gene are given in figure 3.

Direct Detection of 2010del7

The name of the 7-bp deletion (1950del7), originally described by Bull et al. (1993), has been changed to 2010del7 to be consistent with the recently identified ATG start codon (Petrukhin et al. 1994). DNA from all samples in both families was screened by amplification of exon 7 in the presence of 0.2 μCi of [α^{35}S]-dATP and 20 μM of cold dATP for 35 cycles, followed by electrophoresis through 6% denaturing acrylamide gels for 5 h at 75 w. Primers used to amplify exon 7 were 5′-TGTAATCCAGGTGACAAGCAG-3′ and 5′-CACAGCATGGAAGGGAGAG-3′.

RT-PCR

Total RNA was isolated from lymphoblast cultures for two Icelandic patients (individuals VIII-7 from kindred 1 and IX-15 from kindred 2) and an unrelated WND patient, using the RNeasy kit (Qiagen). Total RNA samples from normal brain, liver, kidney, and lymphoblast were obtained from Dr. Roderick McInnis.

RT-PCR was carried out with the GeneAmp EZ *rTth* RNA PCR kit (Perkin Elmer) according to the manufacturers instructions. Primers used were AS1 (located in exon 5) 5′-TCCACGGGATATTATCAAAA-3′ and AS2 (located in exon 9) 5′-GCCAGGGCTTCTGAGGTT-3′. One hundred nanograms of primer AS1 were end-labeled with [γ^{32}P]-dATP to a specific activity of 4×10^5 counts per min (cpm) μl^{-1}. RT-PCR was carried out in 50-μl volumes with 1 μg of total RNA, as per manufacturer's instructions, with 1×10^6 cpm of labeled primer.

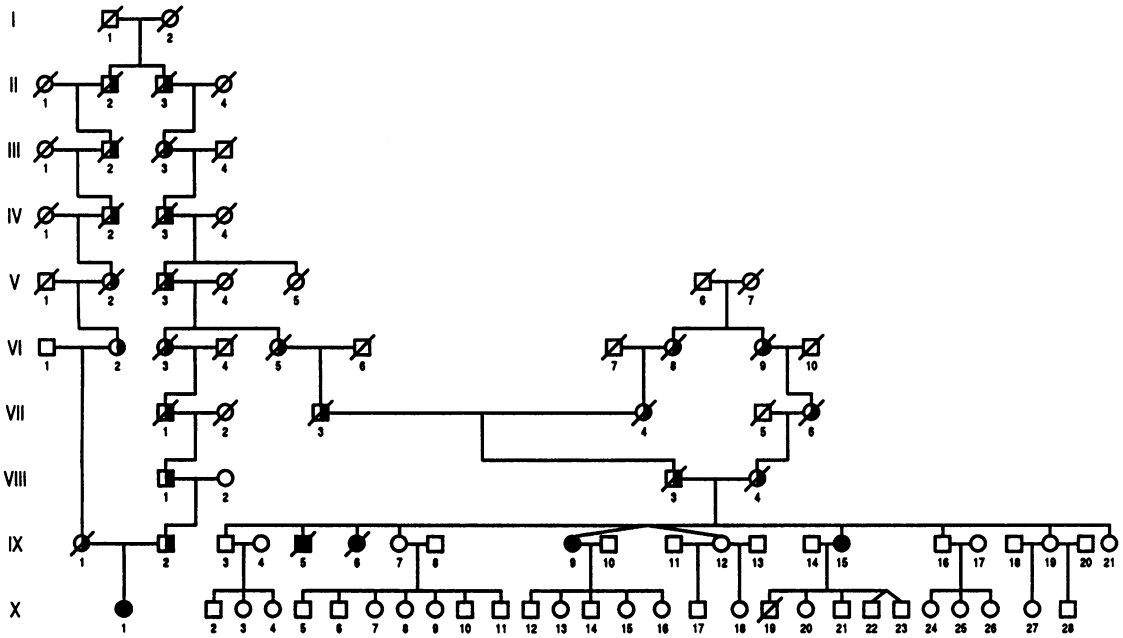

Figure 2 Pedigree of Icelandic WND kindred 2. Symbols are as in figure 1.

Five-microliter aliquots of the reactions were taken at 30, 32, 34, 36, 38, and 40 cycles and were electrophoresed in 6% denaturing acrylamide gels for 2 h at 75 W. The gels were dried and exposed overnight to BioMax film (Kodak). The relative amount of RNA in each sample was determined by RT-PCR with the control primers (IL-1α) provided with the kit. These samples were electrophoresed in 3% agarose for 20 min at 80 V.

Results

A survey of Wilson disease in Iceland revealed a total of eight patients in three sibships of two kindreds. Their clinical symptoms and signs and biochemical and histological investigation (table 1) confirmed the diagnosis in all cases. Two affected members—a 52-year-old male (VIII-7) and a 25-year-old female (VIII-10), in family 1—suffered serious symptoms and signs of the disease, before treatment was initiated. Their sister (VIII-12) had signs (Kayser-Fleischer rings) but no symptoms of the disease before she was diagnosed at the age of 22 years, when therapy started. Two patients in family 2 died, a 23-year-old male (IX-5) and a 30-year-old female (IX-6). Three affected members in family 2 (IX-9, IX-15, and X-1) have been treated successfully with penicillamine, although the latter (X-1) had serious symptoms of the disease at the age of 18 years, before diagnosis and treatment. Three of the eight patients showed psychiatric symptoms, less common manifestations of the disease, involving organic dementia with apathy, loss of memory, disorientation, and anxiety. These psychiatric manifestations disappeared permanently on treatment. In all but one case, individual IX-9 of kindred 2, liver disease was found secondarily on closer examination of patients with neurological disease.

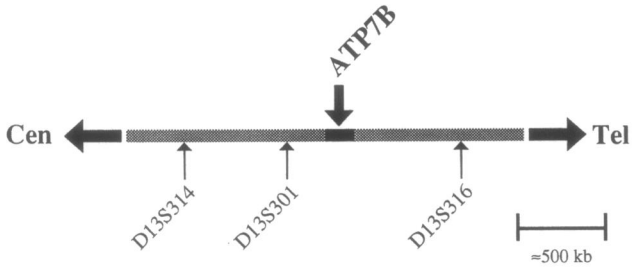

Figure 3 Diagram of the *ATP7B* region. The relative locations of the CA repeat markers used in this study are shown (Thomas et al., in press).

Highly polymorphic microsatellite polymorphisms (CA repeats) were typed in selected members of all three sibships to determine the possible relation of the mutations in both families. Figure 4 shows the results of the marker typing. Two haplotypes are present on the Wilson disease chromosomes in these families. Both families share one of the haplotypes (11-5-7), and a second is present in both sibships of family 2 (11-5-4). Analyses of normal and WND chromosomes from other populations have shown haplotype 1 to be present in other northern European populations, while haplotype 2 appears to be unique to WND chromosomes in Iceland (Thomas et al., in press).

Table 1

Clinical and Laboratory Results of Wilson Disease Patients in Iceland

	Family 1			Family 2				
	VIII-7	VIII-10	VIII-12	IX-5	IX-6	IX-9	IX-15	X-1
Sex	Male	Female	Female	Male	Female	Female	Female	Female
Year of birth	1939	1945	1951	1933	1935	1940	1944	1974
Clinical:								
Age at onset	24	25	22	14	18	18	16	18
Age at diagnosis	52	25	22	18	24	20	18	19
Age at death	...	...	...	23	30	...	...	...
Ataxia	+	+	−	+	+	+	+	+
Tremor	+	+	−	+	+	+	+	+
Psychiatric	−	+	−	−	+	+	−	−
K.F. rings[a]	+	+	+	−	−	+	+	+
Laboratory:[b]								
Fatty liver	+	−	−	−	−	−	−	−
Liver cirrhosis	−	+	+	−	−	+	+	−
Ceruloplasmin	.3	.3	.2	...	...	n/a	n/a	1.0
Serum copper	4	5	8	...	...	n/a	9	7
Urine copper	3.5	7.1	4.3	...	...	n/a	6.1	4.7
Treatment[c]	Tri	Pen	Pen	...	...	Pen	Pen	Pen

NOTE.—Plus sign (+) indicates that the condition is present; minus sign (−) indicates that the condition is absent.

[a] Kayser-Fleischer rings.

[b] Normal values: ceruloplasmin, 1.0–2.3 μmol/L; serum copper, 11–25 μmol/L; urine copper, .1–.3 μmol/L.

[c] Tri = trientine; Pen = penicillamine; n/a = not available.

The mutation present in both families has been described elsewhere and is a 7-bp deletion in exon 7 of the gene (Bull et al. 1993). In order to test the reliability of mutation detection and to confirm the genotype of other individuals within each family, we amplified exon 7 from genomic DNA by PCR. The PCR was carried out in the presence of ^{35}S-dATP, and the products were visualized on polyacrylamide gels (fig. 5). Family members heterozygous or homozygous for the deletion were easily detected, and these results match the clinical predictions.

RT-PCR was carried out on patients from both families in order to determine the amount of *ATP7B* RNA being produced as well as the alternative isoforms present in these patients. To ensure approximately equal loading of RNA, IL-1α primers were used to amplify total RNA from normal liver tissue, normal lymphoblast culture, two Icelandic patient lymphoblast cultures, and an unrelated WND patient lymphoblast culture. As shown in figure 6, all five samples have approximately equal amounts of RT-PCR product.

RT-PCR, using end-labeled AS-1 primer, was carried out across the region of the gene, which includes the mutation and several alternatively spliced exons. Figure 7 shows the products obtained from normal brain, kidney, liver, and lymphoblast RNA, compared with those from the Icelandic patients and an unrelated WND patient. There are differences in the alternative isoforms present in the normal tissues, as has been described elsewhere (Petrukhin et al. 1993). Full-length product (containing exons 5–9) was not detected under these conditions but was observed on agarose gels for all samples except the Icelandic patients (data not shown). There is a reduced amount of product from all isoforms in both Icelandic patients.

Discussion

Three sibships in two Icelandic kindreds contain eight members with Wilson disease. The prevalence is ~1/30,000, similar to incidence figures suggested for other populations (Houwen et al. 1993). Ascertainment from the disease registry is believed to be complete for all patients diagnosed, including those with liver disease (Gudmundsson 1969). The kindreds are not reliably connected genealogically, and they originate from widely separated parts of Iceland. However, given the small founding population of the island, these two families are likely descendants of a common progenitor over six generations ago. The idea of a common ancestor for these families is strongly supported by the fact that all of the Icelandic patients are homozygous for 2010del7, which has not been found in other patients of different populations (Thomas et al. 1995). These two families are unlikely to represent two different occurrences of this specific mutation.

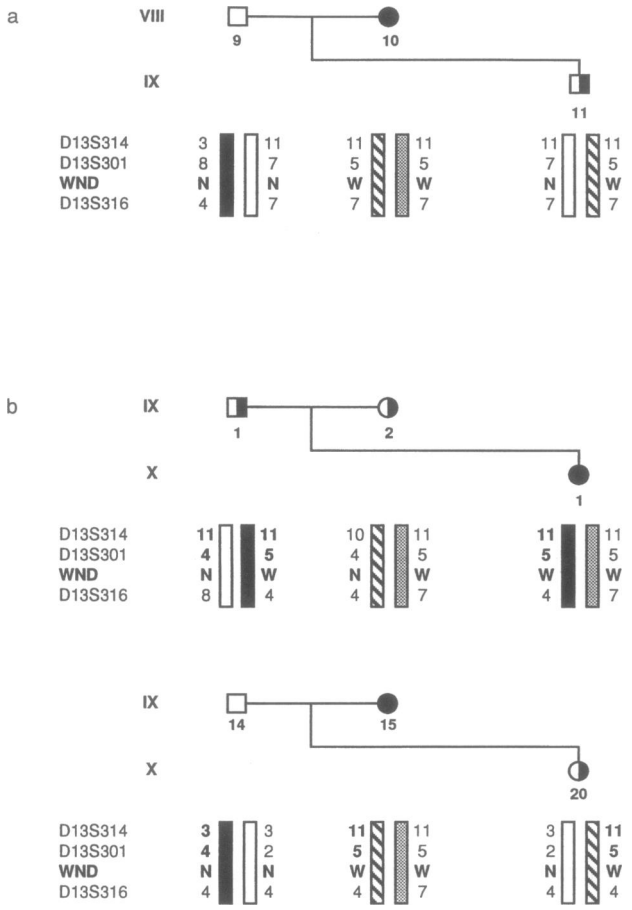

Figure 4 Haplotype analysis within families. Markers are arranged vertically in order with the centromere at the top and telomere at the bottom. Individuals are numbered as in figures 1 and 2. Patients are shown as filled symbols and heterozygotes as half-filled symbols. *a*, Patient, spouse and child from kindred 1. *b*, Two sibships from kindred 2.

The haplotype data also support a single origin for this mutation in both families, as haplotype 1 (11-5-7) is found on WND chromosomes in both families. However, the existence of haplotype 2 (11-5-4) within both sibships of family 2 is interesting. Both chromosomes carry the 2010del7 mutation, as well as the same alleles, at the centromeric markers D13S314 and D13S301 but differ at the marker located on the telomeric side of the gene, D13S316. This suggests that a recombination event has occurred within this pedigree, which has resulted in a derivative haplotype related to the original. Such a recombination event is predicted to have occurred between individuals II-3 and VI-3 in the pedigree and is supported by the fact that IX-2 carries this variant chromosome. It was not possible to determine whether VIII-3 also carries this chromosome. The fact that both kindreds 1 and 2 share haplotype 1 indicates that this is the ancestral chromosome on which this mutation occurred. Most of the normal chromosomes within both kindreds carry allele 4 at D13S316, indicating that this allele may be common in the population and supports the idea that the variant WND haplotype derives from an ancestral recombination event between haplotype 1 and a normal chromosome.

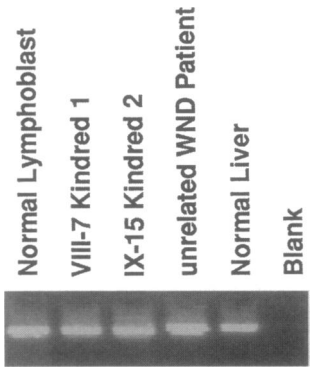

Figure 6 RT-PCR of total RNA with IL-1α control primers

Haplotype 1 and related variants are also found in patients and normal individuals from other northern European populations, including Scotland and Ireland (Thomas et al., in press). Since none of these chromosomes carries the 2010del7 mutation, they probably represent a common haplotype on which a number of mutations have occurred, including that found in Icelandic patients.

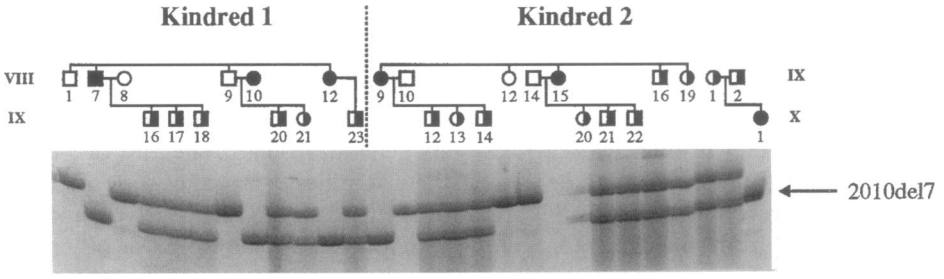

Figure 5 Direct PCR detection of the Icelandic mutation. The deletion is indicated with an arrow. Individuals are numbered as in figures 1 and 2. Patients are shown as filled symbols and heterozygotes as half-filled symbols.

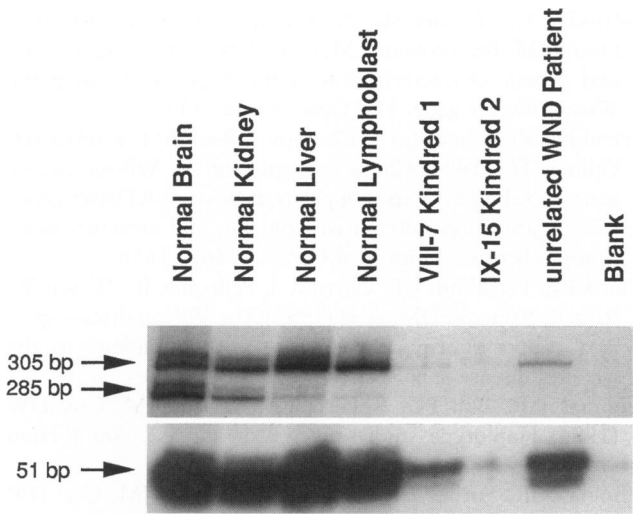

Figure 7 RT-PCR detection of alternative splicing. The upper band represents the 303-bp product missing exon 8, the middle 285-bp band is derived from transcripts missing exons 6 and 7, and the 51-bp product is missing exons 6–8.

The direct PCR detection method is suitable for identification of this mutation. The results are clear, and the analysis quick and easy to carry out. The same type of analysis can also be carried out by using primers that closely flank the deletion point and by analyzing the products on agarose gels. This would greatly facilitate testing of larger numbers of samples and makes direct diagnosis of WND at the DNA level possible in Iceland. We have not detected this mutation in the Irish and Scottish samples available to us (Thomas et al. 1995, and in press), but it is still possible that the 2010del7 mutation is present in a small number of patients from these populations. DNA from Norwegian WND patients has not been available for analysis, and the deletion could be present in this founder group. The direct PCR test for this mutation will allow additional patients from these groups to be screened.

Wilson disease has two main presentations, a hepatic form, frequently with early onset, and a neurological form, often with later onset. The patients in Iceland have primarily neurological symptoms, although four of the eight were secondarily discovered to have cirrhosis. Onset in late teens and early twenties is somewhat unexpected, in view of the severity of the mutation, as we have demonstrated that mutations that are predicted to completely disrupt the protein result in earlier-onset liver disease than do missense mutations (Thomas et al. 1995). The deletion of 7 bp in exon 7 will truncate the protein after the first transmembrane region, removing regions of the protein that are critical for copper-transporting function. Long-Evans Cinnamon rats, with a deletion that removes a smaller region of the 3′ portion of the protein (Wu et al. 1994), have an early adult onset (4 mo of age) but present with liver disease, which is frequently fatal. Yet the Icelandic patients escape early liver failure and can remain undiagnosed for many years (table 1).

A possible explanation for the later onset in Iceland is that alternative splicing preserves the reading frame, bypassing the mutation and allowing the production of some partially functional protein. Alternative splicing that removes exons 6, 7, and/or exon 8 has been described elsewhere (Petrukhin et al. 1994), and we also observe these isoforms in several tissues (G. R. Thomas and D. W. Cox, unpublished data). RT-PCR of the lymphoblast RNA from patients from both kindreds has shown reduced levels of *ATP7B* mRNA. However, we were able to detect the 51-nt transcript lacking exons 6–8 in the lymphoblast RNA of at least one patient, which would remove the mutation and preserve the reading frame. The 285-nt transcript, which lacks exons 6 and 7 and would also maintain the reading frame, was not detected, as this transcript is present at low levels in normal lymphoblast. Normal liver has greater amounts of this transcript, and we would expect that the 285-bp fragment would be detectable in Icelandic patient liver RNA. These alternate isoforms may be able to provide enough function to prevent early liver failure but not enough to avoid eventual brain dysfunction. There is evidence that production of small amounts of functional protein in patients with splicing defects in the Menkes disease gene result in the milder *cutis laxa* rather than full-blown Menkes disease (Kaler et al. 1994; Das et al. 1995). Alternative explanations for the reduced severity of this mutation and the symptoms of the disease might include low levels of copper in the Icelandic diet or other genetic factors that confer some resistance to copper toxicity in the liver, such as increased activity of *ATP7A*, the Menkes disease gene, or liver metallothionein inducibility.

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