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Identification of a Locus for Progressive Familial Intrahepatic Cholestasis *PFIC2* on Chromosome 2q24

Sandra S. Strautnieks,[1] Amir F. Kagalwalla,[2] M. Stuart Tanner,[3] A. S. Knisely,[4] Laura Bull,[5] Nelson Freimer,[5] Sam A. Kocoshis,[6] R. Mark Gardiner,[1] and Richard J. Thompson[1]

[1]Department of Pediatrics, University College London Medical School, Rayne Institute, London; [2]King Fahad Hospital, Riyadh; [3]The Sheffield Children's Hospital, Sheffield, United Kingdom; [4]Denver-Aurora Pathology Associates, Denver; [5]Department of Psychiatry, University of California San Francisco, San Francisco; and [6]Children's Hospital of Pittsburgh, Pittsburgh

Summary

Progressive familial intrahepatic cholestasis (PFIC; OMIM 211600) is the second most common familial cholestatic syndrome presenting in infancy. A locus has previously been mapped to chromosome 18q21-22 in the original Byler pedigree. This chromosomal region also harbors the locus for benign recurrent intrahepatic cholestasis (BRIC) a related phenotype. Linkage analysis in six consanguineous PFIC pedigrees from the Middle East has previously excluded linkage to chromosome 18q21-22, indicating the existence of locus heterogeneity within the PFIC phenotype. By use of homozygosity mapping and a genome scan in these pedigrees, a locus designated "*PFIC2*" has been mapped to chromosome 2q24. A maximum LOD score of 8.5 was obtained in the interval between marker loci *D2S306* and *D2S124*, with all families linked.

Introduction

The designation "progressive familial intrahepatic cholestasis" (PFIC) has been used in a broad, descriptive sense to encompass a number of inherited disorders in which there is failure of bile production or excretion and a progressive clinical course in the absence of evidence of a primary hepatitis. It is also used more specifically to refer to a disease first described in an Old Order Amish pedigree descended from a Jacob Byler (Clayton et al. 1965). This variety of PFIC is sometimes referred to as "Byler disease" (OMIM 211600) or, in patients with similar features but no Amish ancestry, as "Byler syndrome" (Riely 1994). Its biochemical hallmark is the presence of normal levels of both serum cholesterol and plasma γ-glutamyl transpeptidase (γGT) (Whitington et al. 1994). This is a distinctive feature, since, in most diseases associated with cholestasis, γGT is released into the circulation, from the canalicular membrane, by the detergent effects of bile acids. Benign recurrent intrahepatic cholestasis (BRIC; OMIM 243300) is a phenotype similar to PFIC, with a relapsing course but no progressive derangement of liver function and in which, between attacks, the biochemical parameters return to normal.

Recent linkage studies have demonstrated that the genetic heterogeneity underlying these diseases does not correspond to the existing phenotypic or eponymous classification. A locus for BRIC was mapped to chromosome 18q21-22 by detection of a shared haplotype in an inbred Dutch population (Houwen et al. 1994). A locus for PFIC (*PFIC1*) was subsequently mapped to the same region by detection of a preserved haplotype in affected members of the original Byler pedigree, suggesting that the two phenotypes are allelic variants (Carlton et al. 1995). However, analysis of three separately ascertained groups of families segregating a similar clinical phenotype, including the group from the Middle East that was analyzed in the present study, excluded linkage to the region on chromosome 18 (Anneren et al. 1996; Strautnieks et al. 1996; Bull et al. 1997). These observations indicated the existence of locus heterogeneity.

A genomewide search was therefore undertaken in a group of six consanguineous families of Middle Eastern origin, using homozygosity mapping. Evidence was obtained for a second locus on chromosome 2, designated "*PFIC2*."

Subjects and Methods

Patients and Families

The six pedigrees included in this study are shown in figure 1. All families are consanguineous, the parents being first cousins in each case. The families are not known to be interrelated. Families 1–5 are as numbered in the study by Strautnieks et al. (1996), and family 6 is the same as family 2 in the study by Bull et al. (1997). The diagnosis was made by use of clinical and biochemical criteria as described above and as reported elsewhere (Whitington et al. 1994; Kagalwalla et al. 1995).

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Address for correspondence and reprints: Dr. Richard Thompson, Department of Pediatrics, University College London Medical School, Rayne Institute, University Street, London WC1E 6JJ, United Kingdom. E-mail: richard.thompson@ucl.ac.uk

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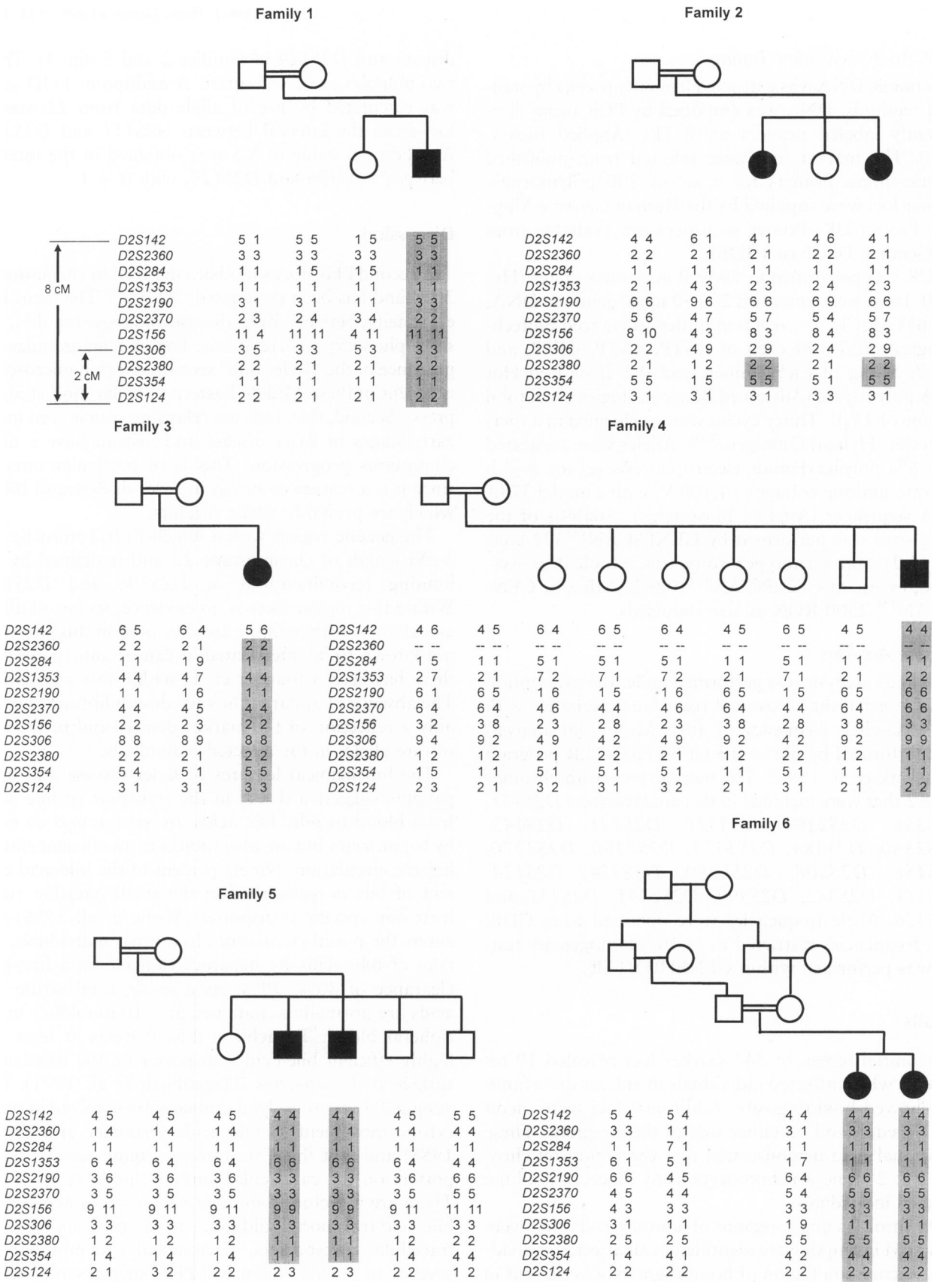

Figure 1 Pedigrees and allele data of PFIC families. Consanguineous marriages are all between first cousins. Allele sizes for the marker loci used are shown under each individual available for marker typing. The numbers are alleles of each individual at each locus and correspond to the allele numbers used in the Genome Database. Genotypes unavailable are depicted as dual pairs of dashes (-- --). The regions of homozygosity in the affected individuals are gray shaded. The limiting recombinations are confirmed by other markers not shown. The relative positions of loci are based on a number of published maps (Collins et al. 1996; Dib et al. 1996).

DNA Analysis/Marker Typing

Genomic DNA was extracted from white cells by standard methods. DNA was amplified by PCR using fluorescently labeled primers or dUTPs (Applied Biosystems). The marker loci were selected from published genetic maps. Primers for a set of 200 polymorphic marker loci were supplied by the Human Genome Mapping Project UK. Primer sequences are available from the Genome Database (GDB).

PCR was performed in 96-well microtiter plates (Hybaid). Each well contained 20–50 ng of genomic DNA; 1.5 mM $MgCl_2$; 1 × reaction buffer (Advanced Biotechnologies); 200 mM each of dGTP, dATP, dTTP, and dCTP; 50 ng of each primer, and 0.2 U of Red Hot DNA polymerase (Advanced Biotechnologies), in a total volume of 15 μl. Thirty cycles were performed in a thermocycler (Hybaid Omnigene™). Alleles were separated on a 6% polyacrylamide electrophoresis gel for 3–4 h at a rate-limiting voltage of 1,100 V, with a model 373A DNA sequencer (Applied Biosystems). Analysis of the allele sizes was performed by GENESCAN™672 (version 1.2) and Genotyper softwares (Applied Biosystems), by use of GENESCAN™-500 TAMRA or GENESCAN™-2500 ROX as size standards.

Linkage Analysis

Linkage analysis was performed under the assumption of fully penetrant autosomal recessive inheritance with a disease-allele frequency of .001. Multipoint analysis was performed by use of the GENEHUNTER program (Kruglyak et al. 1996). The marker loci from chromosome 2 that were included in this analysis were *D2S151, D2S356, D2S2299, D2S321, D2S141, D2S142, D2S2360, D2S284, D2S1353, D2S2190, D2S2370, D2S156, D2S306, D2S2380, D2S354, D2S124, D2S111, D2S382, D2S399, D2S2345, D2S335* and *D2S326*. Allele frequencies were obtained from GDB, with frequencies restricted to ⩾.10. Homogeneity testing was performed within GENEHUNTER.

Results

An initial screen of 342 marker loci revealed 10 regions at which affected individuals in at least three families showed homozygosity. Additional loci were therefore typed in and on either side of these regions. These additional data demonstrated that the region on chromosome 2 alone was homozygous by descent, in all the affected individuals.

On chromosome 2, regions of homozygosity that were 3–89 cM in length were identified in all affected individuals. A common region of homozygosity was present in the interval encompassed by *D2S2380* and *D2S354*. This interval has a minimum genetic length of 2 cM. Flanking recombinations were detected between the disease locus and *D2S306* in family 2 and between the disease and *D2S124* in families 2 and 5 (fig. 1). These two markers are 2 cM apart. A multipoint LOD score was calculated by use of allele data from 22 marker loci from the interval between *D2S151* and *D2S326*. A maximum value of 8.5 was obtained in the interval between *D2S306* and *D2S124*, with $\alpha = 1$.

Discussion

A second PFIC locus has been mapped to chromosome 2q24 and has been designated "*PFIC2*." The locus heterogeneity, between Byler disease and these families, has some phenotypic correlations. The striking granular appearance of the "Byler bile" seen on electron microscopy is absent in these Middle Eastern patients (Bull et al., in press). Second, they lack the relapsing course seen in the early stages of Byler disease and instead have a more continuous progression. This is of particular interest, since it is a feature common to Byler disease and BRIC, which are probably allelic variants.

The genetic region within which *PFIC2* must lie is a 2-cM length of chromosome 2q and is defined by the limiting recombinations at *D2S306* and *D2S124*. Within this region there is no evidence, so far, of allelic association. Although the families used in this study are not known to be interrelated, it can be anticipated that there has been a founder effect within this population. The absence of apparent linkage disequilibrium is probably a reflection of the marker density and of the lack of rare alleles in the affected individuals.

The biochemical features of Byler disease and these patients suggest a defect in the transport of bile acids from blood to bile. Bile acids are synthesized de novo by hepatocytes but are also subject to an efficient enterohepatic circulation. Ninety percent of the bile-acid content of bile is reabsorbed in the small intestine via at least one specific transporter (Wong et al. 1996) and enters the portal circulation. In normal individuals, uptake of bile acids by hepatocytes achieves a first-pass clearance of 80%–90%. As a result, total serum bile acids are normally maintained at <10 μmol/liter in peripheral blood. To achieve this, there is at least one highly efficient bile-acid transporter on the basolateral surface of hepatocytes (Hagenbuch et al. 1991). It is assumed that cytoskeletal elements are involved in transcytotic movement of bile acids (Frimmer and Ziegler 1988) and that there are multiple, quite distinct transporters on the canalicular surface (Inoue et al. 1984). There are therefore a number of sites at which hepatic bile-acid transport could fail: basolateral membrane, intracellular transporters, or canalicular membrane. The severity of the liver disease in PFIC suggests an intracellular accumulation of bile acids. In contrast, a failure of uptake at the basolateral membrane would be unlikely to lead to this degree of liver disease and cirrhosis, making this an implausible site for the defects responsible

for this type of PFIC. Therefore, the canalicular membrane or the cytoskeleton would seem to be the most likely candidates for the site of the defect. Transmission electronmicroscopy of PFIC liver biopsies shows pericanalicular microfilamental condensation as seen in phalloidin administration (Bull et al., in press). It has been suggested that some forms of cholestasis may be due to abnormalities of the cytoskeleton (Weber et al. 1981). However, similar changes have been noted in extrahepatic biliary obstruction (Adler et al. 1980). This may therefore be a secondary and nonspecific effect of cholestasis. It is therefore anticipated that the site of the defect in these patients will be at the canalicular membrane.

These new data identify the second locus capable of causing critical disruption of bile-acid transport. The region is still genetically large (2 cM), and the corresponding physical distance is ~2 Mb. There are, however, 20 expressed sequence tags (ESTs) already mapped to this region, most with no significant homology. Refinement of the localization of the *PFIC2* gene therefore will be required before detection and analysis of candidate genes. This will involve both the use of further markers, in an attempt to detect linkage disequilibrium, and analysis of other PFIC populations, to find limiting recombinations closer to the gene. Cloning of the genes underlying PFIC will greatly increase our knowledge of normal bile-acid transport mechanisms within the liver and will shed light on mechanisms of cholestasis that may lead to new forms of treatment.

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