CONCISE COMMUNICATION

A Novel Multidrug-Resistance Protein 2 Gene Mutation Identifies a Subgroup of Patients With Primary Biliary Cirrhosis and Pruritus

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A single nucleotide polymorphism characterized by the substitution of valine for glutamate (V1188E) in exon 25 of the multidrug resistance protein 2 gene was found in a group of patients with primary biliary cirrhosis. This heterozygous mutation was significantly associated with the presence of pruritus. (HEPATOLOGY 2006;43:1152-1154.)

Pruritus is the most distressing symptom experienced by patients with primary biliary cirrhosis (PBC); however, not all patients with PBC experience this symptom, even when profoundly cholestatic.[1] It is inferred that the pruritogen(s) of cholestasis is produced in the liver and excreted in bile and, as a consequence of cholestasis, it accumulates in tissues.[1] Bile acids have been considered the pruritogens in cholestasis.[1] Increased opioidergic tone presumably from increased availability of endogenous opioids at opioid receptors also has been proposed to mediate the pruritus of cholestasis.[1]

MRP2 (ABCC2) is a member of the family of adenosine triphosphate–binding cassette transporters expressed in various organs, including the liver and the blood–brain barrier.[2,3] In the hepatocyte, MRP2 mediates the transport of several organic anions, especially conjugated compounds, including dianionic conjugated bile salts into the bile.[4] Data suggesting that opioid ligands are substrates of MRP2 have been published.[5] Accordingly, there is a rationale to examine the presence of genetic polymorphisms in the gene that codes for MRP2. Exon 25 encodes for a conserved protein region and, according to previous studies, is one of the regions more frequently associated with MRP2 mutations.[6]

Abbreviations: PBC, primary biliary cirrhosis; PCR, polymerase chain reaction; TM, transmembrane.

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Material and Methods

Patients. One hundred and one patients with PBC from Italy, 76 patients with PBC who were residents of the United States, and 101 blood donors matched for sex, age, and geographical area were studied, with the Italian patients serving as controls.

Blood samples and clinical data were available from the patients from Italy, who were followed prospectively at the Gastroenterology Department of the University of Padua. Patients from the United States were divided into "itch" or "no itch" group according to whether they had experienced pruritus in the course of their disease at the time of enrollment into the study.

The study was approved by the local Ethical Committee in Italy and by the Western Institutional Review Board in the United States.

Analysis of MRP2. Genomic DNA was extracted from peripheral blood leukocytes with a QUIAmp Blood Midi Kit (Quiagen S.p.a., Milan, Italy) and DNA concentration quantified by spectrophotometry. A primary polymerase chain reaction (PCR) was carried out in 50 μL reaction medium containing 19 μL distilled water, 25 μL Master Mix (2×) (Promega, Milan, Italy), 5 μL DNA, 0.5 μL forward primer (10 μmol/L), and 0.5 μL reverse primer (10 μmol/L).

The PCR forward primer for the amplification of the exon 25 of MRP2 gene was 5′-GGAGCCTCTCATCATTCTGC-3′ and the reverse primer was 5′-TTTCACACCACTAGCCATGC-3′).[7]

The extended fragments of the primary PCR product were purified from non-incorporated nucleotides and primers through Microcon Centrifugal Filter Devices (Millipore, Molsheim France) and sequenced with the Big Dye Terminator cycle Sequencing Reading Reaction kit (PE, Applied Biosystems, Applera, Milan, Italy). The second PCR amplification was performed by adding 4.5

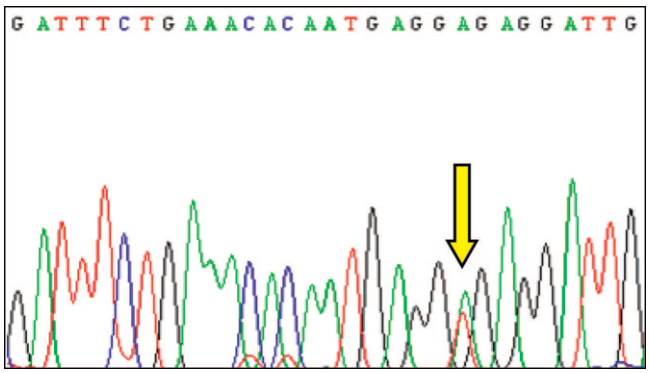

Fig. 1. Electropherogram of heterozygous MRP2 gene mutations in exon 25 at codons 1188 (arrow). The sequencing of exon 25 of MRP2 gene showed a novel mutation (t3563a) in codon 1188. This mutation led to the substitution of valine by glutamate (V1188E).

μL purified primary PCR product to a secondary PCR mix (final volume 10 μL). The PCR Mix contained 1 μL BigDye Terminator × 3.1, 1.5 μL buffer 5×, 3 μL forward or reverse primers (1 μmol/L), and 4.5 μL purified DNA. PCR conditions included a denaturation step at 94°C for 4 minutes, followed by 35 cycles of denaturation at 96°C for 10 seconds, annealing at 50°C for 5 seconds, and extension at 60°C for 4 minutes.

The PCR products were run on ABI 3100 Genetic Analyzer (PE Applied Biosystems) and analyzed by comparison to the Genbank sequence file.

Statistical Analysis. Association between genotypes and presence of PBC in the case series and controls was analyzed by applying Fisher's exact test with a significance level α = 5% and odds ratio calculations. Odds ratio (OR) and Relative Risk (RR), with a 95% confidence interval, were calculated when appropriate. The analysis was performed by using StatXact-5 and LogXact-4 of Cytel Software Corporation (www.cytel.com).

Results

The sequencing the exon 25 of MRP2 gene indicated a novel mutation (t3563a) in codon 1188. This mutation resulted from the substitution of valine by glutamate (V1188E) (Fig. 1).

Table 1. Frequency of the V1188E Polymorphism in Patients With PBC From Italy and From the United States (US) and in Controls

	Wt/Mutation N/Total (%)
US PBC	5/76 (6.6%)*
Italian PBC	19/101 (18.8%)
Italian controls	12/101 (11.9%)

*US PBC vs. Italian PBC: P = .02.

A heterozygous mutation (wt/mutation) was present in 19 of 101 (18.8%) Italian patients with PBC, in 12 of 101 (11.9%) Italian controls (P = .241), and in 5 of 76 (6.6%) patients from the United States. The association between genotypes and the Italian or the United States cases was statistically significant (P = .02, OR = 3.29, 95%CI: 1.17-9.26) (Table 1). All other patients were homozygous wt/wt carriers.

V1188E was identified in 17 of 87 (19.5%) patients who reported pruritus, and in 7 of 90 patients who did not (7.8%) (P = .02, RR = 2.51, 95% CI: 1.13-5.69). In the patients from Italy, the V1188E mutation was present in 13 of 39 (33.3%) patients with pruritus and in 6 of 62 (9.7%) patients without pruritus (P = .003, RR = 3.44, 95% CI: 1.47-8.20). In the group of patients from the United States, the mutation was found in 4 of the 48 patients with pruritus (8.3%) and in 1 of the 28 patients (3.6%) without pruritus (P = .42, RR = 2.33, 95%CI: 0.37-15.4) (Table 2).

Discussion

We identified a novel polymorphism in exon 25, in which valine was substituted by glutamate in position 1188 (V1188E) in the gene that codes for MRP2. This polymorphism was present more frequently in Italian patients with PBC than in the patients from the United

Table 2. Frequency of the V1188E Polymorphism and Pruritus in Patients With PBC

Symptom and Genotype	Total PBC Population (N = 177)	PBC Patients From Italy (N = 101)	PBC Patients From the United States (N = 76)
Pruritus			
wt/m	17 (19.5%)	13 (33.3%)	4 (8.3%)
wt/wt	70 (80.5%)	26 (66.7%)	44 (91.7%)
Total	87 (100%)	39 (100%)	48 (100%)
No pruritus			
wt/m	7 (7.8%)	6 (9.7%)	1 (3.6%)
wt/wt	83 (92.2%)	56 (90.3%)	27 (96.4%)
Total	90 (100%)	62 (100%)	28 (100%)
RR (95%CI)	2.51 (1.13-5.69)	3.44 (1.47-8.20)	2.33 (0.37-15.4)
P value (pruritus vs. no pruritus)	.02	.003	.42

States. V1188E was associated with pruritus in the patients with PBC.

MRP2 is a membrane protein with 1,545 amino acids and contains 17 transmembrane (TM) segments organized into three membrane-spanning domains (MSD0, MSD1, MSD2).[8] The V1188E polymorphism is located in the MSD2 membrane-spanning domain, in the cytoplasmic loop linking TM15 and TM16. This region is suggested to play an important role as a substrate-binding site. Although photoaffinity labeling studies have not as yet been reported for MRP2, inferences about substrate binding sites have been made on the basis of site-directed mutagenesis.[9] These studies indicate the involvement of several TM segments, including T16 in the human protein and in the rat.[10,11] Finally, the novel V1188E mutation located in the cytoplasmic loop between T15 and T16 might reduce the ability of the protein to transport substrates.

A pathogenic role of this mutation alone is doubtful, as the frequency of V1188E in controls is similar to that of patients with PBC in the Italian group of subjects; however, the development of pruritus in any of these control subjects were they to develop cholestasis is unknown.

These data suggest that polymorphisms in MRP2 may be associated with pruritus in PBC. No data suggest that the presence of infrequent SNP in the MRP2 gene may be associated with a decrease in the *in vivo* function of the transporter.[12] Thus, MRP2 may participate in the transport of the pruritogen(s) or its cofactors in cholestasis. Possible mechanisms by which V1188E affects the development of pruritus of cholestasis include alterations in the transport of pruritogen(s) or its cofactors from the hepatocyte into bile or into the central nervous system.

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