

INVESTIGATIVE REPORT

A Study of the Mu Opioid Receptor Gene Polymorphism A118G in Patients with Primary Biliary Cirrhosis with and without Pruritus

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The increase in opioidergic tone by the central administration of morphine, which binds to the mu opioid receptor, is associated with pruritus. Pruritus is a symptom of cholestasis, which appears to result, in part, from increased opioidergic tone; a central mechanism has been proposed. The single nucleotide polymorphism A118G in exon 1 of the opioid receptor mu 1 (OPRM1) gene, which codes for the mu opioid receptor, has been associated with alterations in functions mediated by the endogenous opioid system. In this study we found A118G in heterozygosity in 29% of the DNA samples from patients with primary biliary cirrhosis from the USA and from Italy with and without pruritus. A118G was 1.5 times more frequent in the samples from patients without pruritus from the USA than in the rest of the samples. The possibility of protection from pruritus associated with A118G supports the study of genetic polymorphisms of the OPRM1 gene in patients with cholestasis. Key words: pruritus; mu opioid receptor; primary biliary cirrhosis; A118G.

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Pruritus is a complication of cholestasis, including that secondary to primary biliary cirrhosis (PBC) (1). Fifty-five to 70% of patients with PBC are reported to experience pruritus, which can be severe (2, 3). Some patients with PBC, however, do not report pruritus over the course of their disease, suggesting that a patient-specific characteristic (i.e. genetic make-up) may protect them from pruritus.

Increased central neurotransmission via the endogenous opioid system can result in pruritus. The classic example of this phenomenon is the pruritus that results from the central (e.g. intrathecal) administration of morphine (4–6). Morphine binds to opioid receptors, in particular to the mu opioid receptor, to exert its ef-

fect (7, 8). Opiate antagonists (e.g. naloxone) can effectively relieve morphine-induced pruritus, supporting the theory that the pruritus is opioid receptor-mediated (4–6). In laboratory animals the central administration of morphine is associated with scratching behavior, which has been interpreted to result from pruritus in the animals (9–11). Behavioral studies have revealed that the morphine-induced scratching behavior in animals is mediated by the activation of the mu opioid receptor (11–13). In addition, studies from single-unit recordings in superficial lumbar dorsal horn neurons in rats have revealed that the intrathecal administration of morphine facilitated or depressed the response of some units to the intracutaneous injection of histamine, a known pruritogenic substance, and in some, the effect was naloxone-reversible, suggesting an involvement of opioid receptors in a pruritus-inducing stimulus (14).

There is evidence to suggest that the pruritus of cholestasis is mediated, at least in part, by increased opioidergic tone (15). Patients with cholestasis, including those with PBC, can experience an opiate withdrawal-like syndrome after the administration of opiate antagonists; this reaction suggests that in cholestasis there is increased central opioidergic tone (16–18). It was hypothesized that increased opioidergic tone in cholestasis mediates the pruritus; a central mechanism was proposed (15). The amelioration of the pruritus of cholestasis by opiate antagonists supports this hypothesis (16–24). In this hypothesis, the pruritus would be analogous to the pruritus that results from the pharmacological increase in opioidergic tone by the central administration of morphine, which is mediated by the activation of the mu opioid receptor (4–6). Accordingly, there is a rationale to study any changes that may alter the activation of this receptor by its ligand(s).

In this study, we explored the presence of a single nucleotide polymorphism (SNP), A118G, in exon 1 of the OPRM1 gene, which predicts an Asn (asparagine) to Asp (aspartic acid) change in amino acid residue 40 in the extracellular domain of the receptor (25), at a putative N-glycosylation site.

MATERIALS AND METHODS

Patients

The question of itch was addressed as a yes or no answer. The degree of pruritus, as measured subjectively, was not recorded. Samples from 101 patients with PBC from Italy, 40 (63.5%) of which were from patients with pruritus, and samples from 74 patients with PBC from the USA, 47 (63.5%) of which came from patients who had pruritus, were studied.

The study was approved by the local ethics committee in Italy and by the Western Institutional Review Board in the USA.

Analysis of MOR1

Genomic DNA extraction. DNA was prepared from blood samples using a DNA Isolation Kit from Gentra Systems (Minneapolis, MN, USA) following manufacturer's protocols.

Exon 1 amplification

Exon 1 sequences of the human mu opioid receptor gene were PCR amplified with forward and reverse primers specific to exon 1. The following oligonucleotide primers: (forward, 5'-GTC-AGT-ACC-ATG-GAC-AGC-AG-3'; and reverse, 5'-GTA-GAG-GGC-CAT-GAT-CGT-GAT-3') were used to amplify exon 1 sequences of OPRM1 (GenBank® No. L25119) (29). The PCR reaction mixtures contained 100 ng of genomic DNA, 200 nM exon 1 forward and reverse primer each, and Platinum PCR SuperMix High Fidelity solution from Invitrogen Corp., Carlsbad, CA (Cat. No. 12532-016). The PCR conditions followed the manufacturer's protocols. Tubes containing the reaction mixture were incubated at 94°C for 30 sec on GeneAmp PCR System 9700 from PE Applied Biosystems (Foster City, CA), to denature the template and to activate the enzymes. Thirty-five cycles of PCR amplification were performed as follows: denaturing at 94°C for 30 sec annealing at 55°C for 30 sec, and extension of the amplified product at 68°C for 1 min.

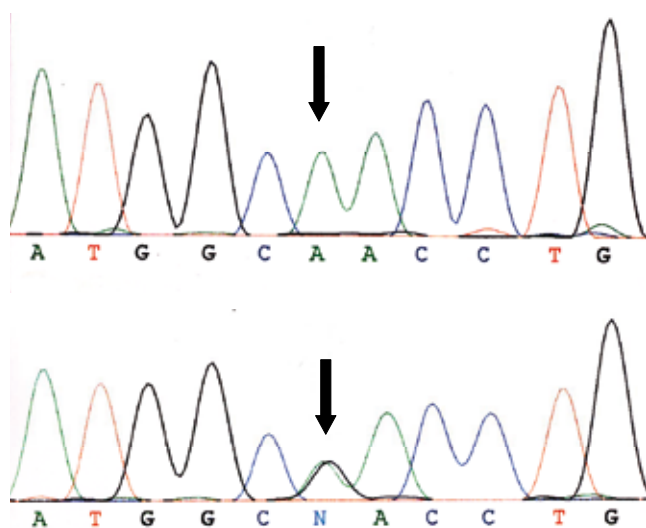


Fig. 1. Expected sequence of exon 1, codon 118, of the opioid receptor mu 1 (OPRM1) gene (top) and the heterozygous nucleotide substitution (bottom) (arrows). The sequencing of exon 1 of the OPRM1 gene revealed a substitution of A118G in codon 118. The A118G nucleotide exchange predicts an Asn (asparagine) to Asp (aspartic acid) change in amino acid residue 40 of the receptor.

Exon 1 sequencing

Exon 1 PCR amplification product's purity was determined by resolution on an ethidium bromide stained 2% agarose gel. The pure exon 1 PCR products were sent to Genewiz, Inc. (NJ, USA) for clean up with ExoSAP-IT Kit (USB Corp., Cleveland, OH) and DNA sequencing with ABI BigDye Kit (Applied Biosystems). The sequencing data were analyzed with Vector NTI® Advance TM 10 Software and the User's Manual from Invitrogen Corp.

Statistical analysis

The percentage of samples displaying the mutation was calculated. Fisher's exact and χ^2 test were used, as appropriate, to determine statistical significance.

RESULTS

The sequencing the exon 1 of the MOR1 gene revealed the SNP A118G in 51 of 175 samples (29%) in heterozygosity (Fig. 1, bottom) and in homozygosity, in one sample from a patient without pruritus from Italy (0.5%). All other samples revealed homozygosity for the common wild type sequence (Fig. 1, top). A118G was found in 24 (27.6%) samples from the group of patients with pruritus, and in 27 (30.6%) from the group of patients without pruritus ($p=0.7$). In the samples from Italy, 11 of the 40 (27.5%) from patients with pruritus and 16 of the 61 (26.2%) from patients without pruritus revealed A118G ($p=0.9$). In the samples from the USA, A118G was found in 13 of the 47 (27.6%) samples from the patients with pruritus and in 11 of the 27 (40.7%) from the patients without pruritus ($p=0.3$) (Table I).

The proportion of samples that had A118G was similar in the samples from the group of patients with and without pruritus; A118G was more common, although not significantly, in the samples from the patients without pruritus from the USA.

DISCUSSION

A SNP at position 118 (A118G), predicting an amino acid change from Asn to Asp was found in 29% of samples from a group of patients with PBC. The frequency of A118G was similar in the samples from patients with and without pruritus; however, A118G was more prevalent in the group of samples from patients without pruritus from the USA.

Genetic polymorphisms in association with changes in the behavioral effects that result from the stimulation of the mu opioid receptor have been reported. In this context, in subjects of Asian extraction, the SNP A118G of the mu opioid receptor gene was found to be more prevalent in patients with heroin dependence than in control subjects (26). Treatment with naltrexone in alcoholic subjects who displayed the SNP A118G was

associated with prolonged abstinence from alcohol as compared to those who did not have this SNP (27). Accordingly, it is conceivable that A118G has an impact on whether pruritus is perceived in patients with PBC.

In cholestasis, it is believed that increased opioidergic neurotransmission contributes to the pruritus (15). Plasma concentration of some endogenous opioids are increased in cholestasis (30, 31); furthermore, at least one endogenous opioid, Met-enkephalin, is expressed in the liver in cholestasis and, indeed, there are data that support the theory that the cholestatic liver can make endogenous opioids *de novo* (32, 33). In addition, transporters, of which opioid peptides are substrates, are expressed in the hepatocyte (34) and in the brain (35), supporting the existence of a pathway through which peripherally derived opioids can reach the brain, thus increasing opioidergic tone. These possibilities would require an interaction between pruritogenic substances (e.g. opioids) and the mu opioid receptor, through which it has been shown that opiate-induced itch is mediated. In addition, central sensitization for pruritus, which would not allow nociceptive stimuli to inhibit itch but to facilitate itch, would also require an interaction between endogenous opioid ligands, and the mu receptor. Thus, a SNP that may alter the effect resulting from the activation of the mu opioid receptor may be relevant. In this context, studies on the binding of beta-endorphin, one of the endogenous opioid peptides, were conducted in a cell line transfected with the OPMR1 with the Asn40Asp or with the wild-type sequence. It was reported from these studies that the affinity of beta-endorphin for the A118G variant receptor was 3 times higher than that for the wild-type, and that the binding of beta-endorphin to the A118G variant receptor was also 3 times stronger than the binding to the common variant. In support of the idea that A118G changes the effect of receptor activation is the report of a patient heterozygote for A118G who did not respond as expected to the effects of morphine (36). Furthermore, patients expressing A118G had a heightened activation of the hypothalamic pituitary adrenal axis in association with an opiate antagonist than the subjects carrying the wild-type (37). Taken together, these data tend to suggest that A118G may have an impact on the behavioral expression of functions mediated by opioid neurotransmission. Thus, it can be speculated that the activation of the mu opioid receptor containing the Asn40Asp sequence by

endogenous ligands (e.g. an endogenous pruritogen, or in opioid-mediated central sensitization to itch) may not be associated with pruritus, as suggested by the higher frequency of this SNP in the samples from patients with PBC without pruritus from the USA.

The sample size of this study limits the interpretation of these findings, as it may not have allowed for the detection of the true prevalence of this SNP in patients with PBC; however, these results suggest that A118G in the mu opioid receptor gene may protect patients with PBC from pruritus. A large sample size is necessary to explore further the frequency of A118G in patients with PBC with and without pruritus; these studies may provide some insight into the reason for the absence or presence of pruritus in patients with PBC.

REFERENCES

1. Sherlock S, Dooley J, editors. Cholestasis. In: Diseases of the liver and biliary system. 9th edn. London: Blackwell, 1991: p. 214–235.
2. Heathcote J. The clinical expression of primary biliary cirrhosis. *Semin Liver Dis* 1997; 17: 23–33.
3. Talwalkar JA, Souto E, Beigley CS, Jorgensen RA, Lindor KD. Natural history of pruritus in primary biliary cirrhosis. *J Clin Gastroenterol Hepatol* 2003; 1: 297–302.
4. Ballantyne JC, Loach AB, Carr DB. Itching after epidural and spinal opiates. *Pain* 1988; 33: 149–160.
5. Baraka A, Noueihid R, Hajj S. Intrathecal injection of morphine for obstetric analgesia. *Anesthesiology* 1981; 54: 136–140.
6. Slappendel R, Weber EW, Benraad B, van Limbeek J, Dirksen R. Itching after intrathecal morphine. Incidence and treatment. *Eur J Anaesthesiol* 2000; 17: 616–621.
7. Fukuda K, Kato S, Mori K. Location of regions of the opioid receptor involved in selective agonist binding. *J Biol Chem* 1995; 270: 6702–6709.
8. Yu L. The mu opioid receptor: from molecular cloning to functional studies. *Addict Biol* 1996; 1: 19–30.
9. Thomas DA, Hammond DL. Microinjection of morphine into the rat medullary dorsal horn produces a dose-dependent increase in facial scratching. *Brain Res* 1995; 695: 267–270.
10. Ko MC, Naughton NN. An experimental itch model in monkeys: characterization of intrathecal morphine-induced scratching and antinociception. *Anesthesiology* 2000; 92: 795–805.
11. Umeuchi H, Togashi Y, Honda T, Nakao K, Okano K, Tanaka T, Nagase H. Involvement of central mu-opioid system in the scratching behavior in mice, and the suppression of it by the activation of kappa-opioid system. *Eur J Pharmacol* 2003; 477: 29–35.
12. Kuraishi Y, Yamaguchi T, Miyamoto T. Itch-scratch responses induced by opioids through central mu opioid receptors in mice. *J Biomed Sci* 2000; 7: 248–252.
13. Ko MC, Song MS, Edwards T, Lee H, Naughton NN. The role of central mu opioid receptors in opioid-induced itch in primates. *J Pharmacol Exp Ther* 2004; 310: 169–176.
14. Jinks SL, Carstens E. Superficial dorsal horn neurons identified by intracutaneous histamine: chemonociceptive responses and modulation by morphine. *J Neurophysiol* 2000; 84: 616–627.
15. Jones EA, Bergasa NV. The pruritus of cholestasis: from bile

Table I. Proportion of samples (%) in which A118G was detected according to the country of origin and the presence or absence of pruritus

Italy		USA	
With pruritus	Without pruritus	With pruritus	Without pruritus
11/40 (27.5)	16/61 (26.2)	13/47 (27.6)	11/27 (40.7)

- acids to opiate agonists. *Hepatology* 1990; 11: 884–887.
16. Thornton JR, Losowsky MS. Opioid peptides and primary biliary cirrhosis. *BMJ* 1988; 297: 1501–1504.
 17. Bergasa NV, Alling DW, Talbot TL, Wells M, Jones EA. Oral nalmefene therapy reduces scratching activity due to the pruritus of cholestasis: a controlled study. *J Am Acad Dermatol* 1999; 41: 431–434.
 18. Bergasa NV, Talbot TL, Schmitt JP, Alling DW, Swain MG, Turner M, et al. Open label trial of oral nalmefene therapy for the pruritus of cholestasis. *Hepatology* 1998; 27: 679–684.
 19. Bergasa NV, Talbot TL, Alling DW, Schmitt JM, Walker EC, Baker BL, et al. A controlled trial of naloxone infusions for the pruritus of chronic cholestasis. *Gastroenterology* 1992; 102: 544–549.
 20. Bergasa NV, Alling DW, Talbot TL, Swain MG, Yurdaydin C, Schmitt JM, et al. Naloxone ameliorates the pruritus of cholestasis: results of a double-blind randomized placebo-controlled trial. *Ann Int Med* 1995; 123: 161–167.
 21. Carson KL, Tran TT, Cotton P, Sharara AI, Hunt CM. Pilot study of the use of naltrexone to treat the severe pruritus of cholestatic liver disease. *Am J Gastroenterol* 1996; 91: 1022–1023.
 22. Wolfhagen FHJ, Sternieri E, Hop WCJ, Vitale G, Bertolotti M, van Buuren HR. Oral naltrexone treatment for cholestatic pruritus: a double-blind, placebo-controlled study. *Gastroenterol* 1997; 113: 1264–1269.
 23. Terg R, Coronel E, Sorda J, Munoz AE, Findor J. Efficacy and safety of oral naltrexone treatment for pruritus of cholestasis, a crossover, double blind, placebo-controlled study. *J Hepatol* 2002; 37: 717–722.
 24. Kunzmann S, Kullak-Ublick GA, Greiner A, Jeschke R, Hebestreit H. Effective opiate-receptor antagonist therapy of cholestatic pruritus induced by an oral contraceptive. *J Pediatr Gastroenterol Nutr* 2005; 40: 596–599.
 25. Wendel B, Hoehe MR. The human mu opioid receptor gene: 5' regulatory and intronic sequences. *J Mol Med* 1998; 76: 525–532.
 26. Tan EC, Tan CH, Karupathivan U, Yap EP. Mu opioid receptor gene polymorphisms and heroin dependence in Asian populations. *Neuroreport* 2003; 14: 569–572.
 27. Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, O'Brien CP. A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. *Neuropsychopharmacol* 2003; 28: 1546–1552.
 28. Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, et al. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci USA* 1998; 95: 9608–9613.
 29. Wang JB, Johnson PS, Persico AM, Hawkins AL, Griffin CA, Uhl GR. Human mu opiate receptor. cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. *FEBS Lett* 1994; 338: 217–222.
 30. Thornton JR, Losowsky MS. Plasma methionine enkephalin concentration and prognosis in primary biliary cirrhosis. *BMJ* 1988; 297: 1241–1242.
 31. Spivey J, Jorgensen R, Gores G, Lindor K. Methionine-enkephalin concentrations correlate with stage of disease but not pruritus in patients with primary biliary cirrhosis. *Am J Gastroenterol* 1994; 89: 2018–2032.
 32. Bergasa NV, Sabol SL, Young WSD, Kleiner DE, Jones EA. Cholestasis is associated with preproenkephalin mRNA expression in the adult rat liver. *Am J Physiol* 1995; 268: G346–G354.
 33. Bergasa N. Hepatic Met-enkephalin immunoreactivity is enhanced in primary biliary cirrhosis. *Liver* 2002; 22: 107–113.
 34. Hoffmaster KA, Zamek-Gliszczynski MJ, Pollack GM, Brouwer KL. Multiple transport systems mediate the hepatic uptake and biliary excretion of the metabolically stable opioid peptide [D-penicillamine2,5]enkephalin. *Drug Metab Dispos* 2005; 33: 287–293.
 35. Dagenais C, Ducharme J, Pollack GM. Uptake and efflux of the peptidic delta-opioid receptor agonist. *Neurosci Lett* 2001; 301: 155–158.
 36. Hirota T, Ieiri I, Takane H, Sano H, Kawamoto K, Aono H, et al. Sequence variability and candidate gene analysis in two cancer patients with complex clinical outcomes during morphine therapy. *Drug Metab Dispos* 2003; 31: 677–680.
 37. Wand GS, McCaul M, Yang X, Reynolds J, Gotjen D, Lee S, Ali A. The mu-opioid receptor gene polymorphism (A118G) alters HPA axis activation induced by opioid receptor blockade. *Neuropsychopharmacol* 2002; 26: 106–114.