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Chromosomal Localization of the 5-HT_{1F} Receptor Gene: No Evidence for Involvement in Response to Sumatriptan in Migraine Patients

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The 5-HT_{1F} receptor, which is present in both human vascular and neuronal tissue, may mediate the therapeutic effect and/or side-effects of sumatriptan. We investigated the chromosomal localization of the 5-HT_{1F} receptor gene and the relation between eventually existing polymorphisms and the clinical response to sumatriptan in migraine patients. The 5-HT_{1F} receptor gene was localized using a monochromosomal mapping panel, followed by a radiation-reduced hybrid mapping and fluorescent in situ hybridization. The results of these techniques show that the 5-HT_{1F} receptor gene is localized at 3p12. We investigated the presence of polymorphisms by single strand conformation polymorphism analysis in 14 migraine patients who consistently responded well to sumatriptan, 12 patients who consistently experienced recurrence of the headache after initial relief, 12 patients with no response to sumatriptan, and in 13 patients who consistently experienced chest symptoms after use of sumatriptan. No polymorphisms were detected in any of the patients. We therefore conclude that genetic diversity of the 5-HT_{1F} receptor gene is most probably not responsible for the variable clinical response to sumatriptan. Am. J. Med. Genet. 77:415–420, 1998.

KEY WORDS: migraine; sumatriptan; 5-HT_{1F} receptor; 5-HT_{1F} receptor gene

INTRODUCTION

Migraine is a common paroxysmal neurological disorder, which consists of attacks of moderate to severe headache, nausea, vomiting, and photo- and phonophobia [Headache Classification Committee of the International Headache Society, 1988]. Sumatriptan, a 5-HT_1 (5-HT = 5-hydroxytryptamine = serotonin) receptor agonist, is highly effective in the acute treatment of migraine attacks [Cady et al., 1991; Ferrari, 1991; Plosker and McTavish, 1994]. However, up to 15% of patients consistently do not respond to subcutaneous sumatriptan, and up to 40% of responders consistently experience recurrence of the headache within 24 hours after initial headache relief [Ferrari and Saxena, 1993, 1995; Plosker and McTavish, 1994; Visser et al., 1996c]. In addition, about 40% of patients always experience one or more chest symptoms, including chest pressure or chest pain, heaviness in arms, and shortness of breath, shortly after the use of subcutaneous sumatriptan [Visser et al., 1996c, 1996d].

Comparing clinical, demographic, and pharmacokinetic characteristics of consistent responders and nonresponders, as well as of patients who always experience headache recurrence or chest symptoms and those who never do, yielded only few and relatively insignificant differences. For example, patients with chest symptoms were slightly younger than patients without chest symptoms, and more often female than male. Also, headache recurrence was more frequent in patients with more severe attacks and longer untreated attack duration [Visser et al., 1996a,b,c,e]. Although it was not confirmed by the study mentioned above [Visser et al., 1996d], others suggested determinants such as young age, hypertension, general complaints of abdominal pain, and a family history of myocardial infarction for the development of chest symptoms [Ottervanger et al., 1995].

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Besides demographic, clinical, and pharmacokinetic characteristics, genetic factors may contribute to interpatient differences in clinical responses to drugs or hormones [Arranz et al., 1995; Smith et al., 1994; Widen et al., 1995]. For sumatriptan, differences in clinical responses may well be explained by different alleles of the 5-HT_1 receptor subtypes, the presumed clinically relevant pharmacological target of sumatriptan [Hoyer et al., 1994]. Sumatriptan displays high affinity for the 5-HT_{1B} (former 5-$HT_{1D\beta}$), 5-HT_{1D} (former 5-HT_{1D_α}), and, although tenfold less, for the 5-HT_{1F} receptor [Hartig et al., 1996; Hoyer et al., 1994]. Recently, we have shown that genetic variation in the gene encoding the 5-HT_{1B} receptor, one of the receptors possibly mediating the therapeutic action and coronary side effects of sumatriptan, is not associated with the variation in the clinical effects of this drug [MaassenVanDenBrink et al., 1998]. Another candidate receptor for the mediation of the clinical effects of sumatriptan is the 5-HT_{1F} receptor [Adham et al., 1993]. This receptor is present in human neuronal and vascular tissue [Bouchelet et al., 1996], which are both considered possible targets for sumatriptan [Johnson et al., 1996]. Presently, a selective 5-HT_{1F} receptor agonist is under investigation as a potential antimigraine compound [Phebus et al., 1997]. On the other hand, the compound alniditan, which is effective in the acute treatment of migraine attacks [Goldstein et al., 1996], displays only a low affinity for the 5-HT_{1F} receptor [Leysen et al., 1996], arguing against the fact that the 5-HT_{1F} receptor necessarily should be involved in the therapeutic action of antimigraine drugs.

The intronless 5-HT_{1F} receptor gene encodes for a receptor protein consisting of 366 amino acids, containing 7 membrane-spanning domains [Adham et al., 1993]. The chromosomal localization of the 5-HT_{1F} receptor gene has not been reported until now. The 5-HT_{1F} receptor gene was screened for mutations in a population consisting of schizophrenic patients, bipolar patients, and healthy controls [Shimron-Abarbanell et al., 1996]. The gene contains three rare sequence variants, namely a silent A→T transversion at bp 785 (third position of codon 261), a silent C→T transition at bp 530 (third position of codon 176), and a C→T transition in position 78, upstream from the start codon. The frequency of these sequence variants ranged from 1 to 4% [Shimron-Abarbanell et al., 1996]. These sequence variants were not related to the presence of the diseases [Shimron-Abarbanell et al., 1996]. Nevertheless, it is feasible that sequence variants of the 5-HT_{1F} receptor gene are involved in the clinical response to drugs such as sumatriptan.

In the present study, we established the chromosomal localization of the 5-HT_{1F} receptor gene. Furthermore, we investigated the presence of mutations in the 5-HT_{1F} receptor gene in subgroups of migraine patients who display different clinical responses (improvement–no improvement, recurrence–no recurrence, chest symptoms–no chest symptoms) to sumatriptan.

SUBJECTS AND METHODS

Patients

In the present study, we included 40 unrelated migraine patients (35 female, 5 male; age 20–69 years), all fulfilling the criteria of the International Headache Society [Headache Classification Committee of the International Headache Society, 1988], from the outpatient clinic database of the Department of Neurology of the Leiden University Hospital. Patients were divided into five groups according to their clinical response to 6 mg subcutaneous sumatriptan: i) Responders were defined as patients who had headache relief within 2 hours after sumatriptan in at least four out of five migraine attacks and who experienced headache recurrence within 24 hours in less than one out of five successfully treated attacks. ii) Patients with headache recurrence responded to subcutaneous sumatriptan in at least four out of five migraine attacks, followed by recurrence of the headache within 24 hours in at least four out of five successfully treated attacks. iii) Nonresponders were defined as patients who had headache relief in not more than one out of five migraine attacks treated with subcutaneous sumatriptan, or in none of three consecutively treated migraine attacks. iv) Patients with chest symptoms had at least three migraine attacks treated with subcutaneous sumatriptan and had experienced one or more chest symptoms in each of these attacks. The use of the minimal number of three rather than five attacks in the latter groups emerged from practical reasons; patients with severe adverse events or no response usually refrained from continuing sumatriptan use. v) Patients without chest symptoms, derived from the other groups, were compared with patients with chest symptoms. Clinical and demographic data on the patients are listed in Table I.

Chromosomal Localization of the 5-HT_{1F} Receptor Gene

Somatic cell hybrid mapping. A polymerase chain reaction (PCR) amplifying the whole coding sequence of the 5-HT_{1F} receptor gene was performed on a human monochromosomal mapping panel (U.K. HGMP Resource Centre) and on a panel of somatic cell hybrids previously defined [Leach et al., 1994; Naylor et al., 1996]. The monochromosomal panel and the somatic cell hybrids were used as a template for a PCR

TABLE I. Demographic Data of the Migraine Patients With Various Responses to Sumatriptan

	N	Gender	Age (range)[a]
Responders	14	1M, 13F	49 (23–69)
Recurrence	12	1M, 11F	50 (34–66)
Nonresponders	12	3M, 9F	44 (20–60)
Chest symptoms	13	13F	48 (23–59)
No chest symptoms	27	5M, 22F	47 (20–69)
Total (all patients)[b]	40	5M, 35F	47 (20–69)

[a]Age is expressed as the mean age in years, followed by the minimum and maximum age in each group.
[b]Same patients may be included in different groups.

reaction using the primers $5HT_{1F}$1F and $5HT_{1F}$4R (Table II), resulting in a 1.3-kb fragment. The PCR was performed in a total volume of 30 μl containing 15 pmol of each primer, 1 U of AmpliTaq (Perkin Elmer Cetus), 0.2 mM of each dNTP, and a reaction buffer with 60 mM Tris-HCl, 15 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, pH 10 (30 seconds 94°C, 30 seconds 59°C, and 1 minute 72°C, for 33 cycles).

Fluorescent in situ hybridization (FISH) mapping. The 1.3-kb PCR fragment ($5HT_{1F}$1F and $5HT_{1F}$4R), cloned into the pCR™ vector (Invitrogen) and the chromosome-specific library, pBS3 [Collins et al., 1991] (kindly provided by Dr. J.W. Gray), were labelled by nick translation [Langer et al., 1981] with biotin-11-dATP (Gibco BRL) and digoxigenin-11-dUTP, respectively. The probes were simultaneously hybridized to metaphase chromosomes from normal human lymphocytes and visualised as previously described [Dauwerse et al., 1992]. Slides were analyzed on a Leitz DM-RBE microscope equipped for fluorescence microscopy and mounted with a Photometrics Series 200, KAF1400 CCD camera. Image acquisition and processing were performed on a Power Macintosh 7100, using the IP Lab Spectrum Multiprobe software.

Mutation Analysis

PCR. A primary PCR was performed in a reaction volume of 30 μl containing 15 pmol of each primer, 1 U of AmpliTaq (Perkin Elmer Cetus), 0.2 mM of each dNTP, and a reaction buffer with 60 mM Tris-HCl, 15 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, pH 8.5; 100 ng of genomic DNA was subjected to 34 cycles of amplification (30 seconds 94°C, 30 seconds 60°C, and 30 seconds 72°C). Four sets of primers were chosen to produce four overlapping fragments (354–414 bp) covering the whole coding region of the 5-HT_{1F} receptor gene (Table II).

Primary PCR products were radioactively labelled by a second PCR performed in a 15-μl reaction volume containing 1 μl primary PCR product, 7.5 pmol of each primer, 0.2 mM of dTTP, dGTP, and dATP, 0.002 mM of dCTP, and 0.7 μCi of [α-^{32}P]-dCTP (3000 Ci/mmol; Amersham), 0.5 U AmpliTaq (Perkin Elmer Cetus), 10% DMSO, and buffer as for the primary PCR. Denaturation was performed at 94°C for 45 seconds, annealing at 62°C for 45 seconds, followed by elongation at 72°C for 1 minute and 30 seconds for a total of 13 cycles.

Single-strand conformation polymorphism analysis. Subsequent to the secondary PCR, the four fragments were subjected to restriction enzyme digestions to yield shorter fragments (≤300 bp) suitable for single-strand conformation polymorphism (SSCP) analysis. Digestion was performed by adding 5 μl of mixture containing a specific buffer and 3 U of restriction enzyme directly to the secondary PCR product (15 μl) for 3 hours at a temperature optimal for the restriction enzyme (Table II).

The digested fragments were diluted 1 to 15 in loading buffer (47% formamide, 0.05% SDS, 15 mM EDTA, 0.005% bromophenol blue, and 0.005% xylene cyanol) and denatured at 94°C for 5 minutes. Three microliters of the denatured DNA was loaded per lane on a 8% polyacrylamide gel with 10% glycerol and 1 × TBE. Electrophoresis was carried out for 7 hours at 27 W constant power at room temperature. Gels were dried and exposed to X-ray film (Kodak X-AR).

RESULTS

Localization of the 5-HT_{1F} Receptor Gene

PCR mapping. Based on the PCR with the human monochromosomal mapping panel, the 5-HT_{1F} receptor gene was found to be localized on chromosome 3 (results not shown). Further investigation was performed using a radiation-reduced hybrid mapping panel containing fragments of chromosome 3. One of the cell lines in this panel, GM11750, resulted in a discordant, negative PCR result (Fig. 1). However, we have occasionally detected deletions in this cell line before, and considering the results of the other cell lines, the gene should be localized on the short arm of chromosome 3, region H (region 3p14.1 to 3p12), which is located close to the centromere (Fig. 1; for detailed description of regions, see Leach et al., 1994).

FISH mapping. The 1.3-kb fragment ($5HT_{1F}$1F–$5HT_{1F}$4R) was hybridized in situ to human metaphase chromosomes. Analysis of chromosomes with a positive hybridization signal showed the fragment of the 5-HT_{1F} receptor gene to be localized on region p12.1 of chromosome 3 (Fig. 2).

TABLE II. Primer Sequences, Fragment Sizes, and Used Restriction Enzymes

Primer	Primer sequence	Fragment size (bp)	Nucleotide position (5′–3′)[a]	Restriction enzyme	Fragment size after digestion (bp)
$5HT_{1F}$1F	5′-AAAACCTTCAATCTGAACCTCA-3′	358	–186–172	*Bst* XI	99, 259
$5HT_{1F}$1R	5′-GCTGGATGGTGCAGCTTCCG-3′				
$5HT_{1F}$2F	5′-TCGCTGCAATTATTGTGACC-3′	414	134–547	*Hinf* I	142, 272
$5HT_{1F}$2R	5′-TGGTGGAAACAATGTGGTCG-3′				
$5HT_{1F}$3F	5′-TCCTCTATTCTGGAGGCACC-3′	354	471–825	*Rsa* I	86, 286
$5HT_{1F}$3R	5′-CTCATGCTTGAATTCAGACC-3′				
$5HT_{1F}$4F	5′-GCACAGTGAGAAGTCTCAGG-3′	366	788–1153	*Rsa* I	69, 297
$5HT_{1F}$4R	5′-GTTATTCCTCCCCTCAAAAACC-3′				

[a]Numbering of nucleotide position starts with the first nucleotide of the initiating methionine codon (+1).

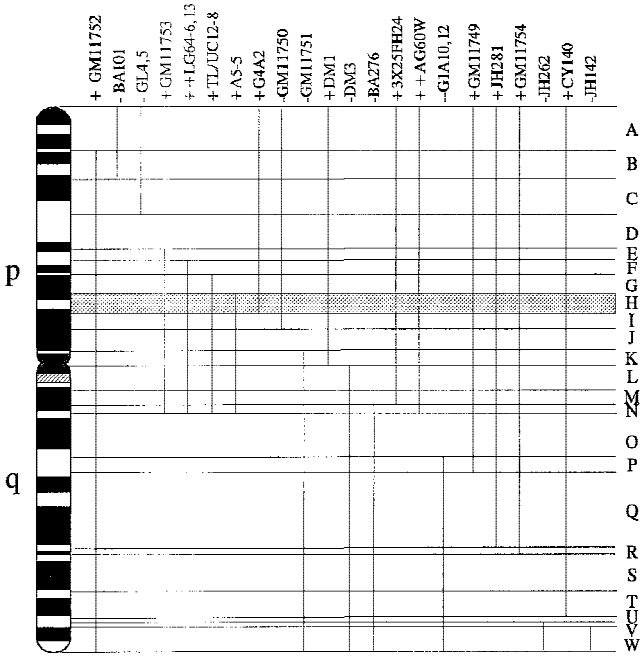

Fig. 1. Map of chromosome 3, obtained after a PCR covering the whole coding region of the 5-HT_{1F} receptor gene on a panel of somatic cell hybrids. The fragments of the hybrids are represented by the vertical lines. Positive PCRs are indicated by plus signs and negative PCRs with minus signs preceding the hybrid names. From the above results, it is concluded that the 5-HT_{1F} receptor gene is located in region H, corresponding to region 3p14.1 to 3p12. For details about this method, see Leach et al., 1994.

SSCP Analysis

The coding region of the gene was screened for sequence variation by SSCP. To achieve high sensitivity, the four overlapping fragments were further reduced in size by restriction enzyme digestion prior to SSCP. In all eight DNA fragments, no polymorphism was observed, neither in any of the migraine patient groups nor in the control subjects.

DISCUSSION

In the present study, we investigated the localization and the presence of polymorphisms of the 5-HT_{1F} receptor gene. The precise physiological role of the 5-HT_{1F} receptor is not known yet, but it is conceivable that this receptor, which is present in human neuronal and vascular tissue [Bouchelet et al., 1996], is involved in the pathophysiology or therapeutics of migraine. The therapeutic action and the coronary side effects of the antimigraine drug sumatriptan could also be mediated via the 5-HT_{1F} receptor [Phebus et al., 1997]; sumatriptan has a high affinity for the 5-HT_{1F} receptor [Adham et al., 1993], and has been shown to bind to putative 5-HT_{1F} receptor binding sites [Rhodes et al., 1995].

Our results show that the 5-HT_{1F} receptor gene is located at chromosome region 3p12. We defined this localization using three different approaches, which resulted in concordant conclusions. The coding region of

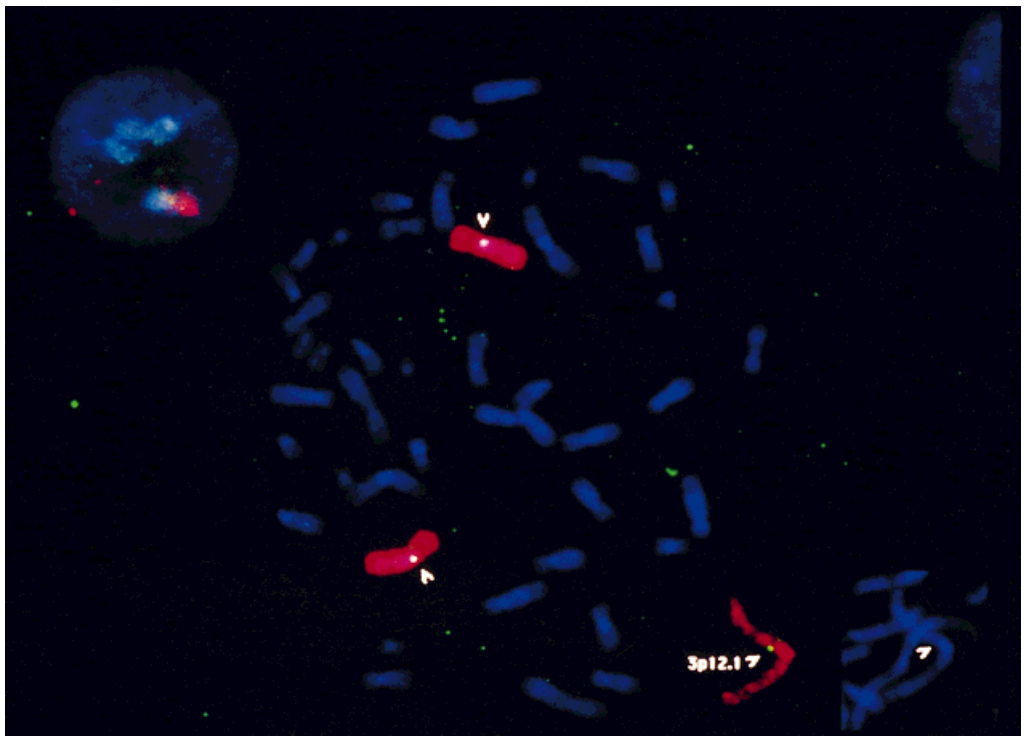

Fig. 2. Metaphase chromosome spread from human lymphocytes simultaneously hybridized with a 1.3-kb fragment covering the whole coding region of the 5-HT_{1F} receptor gene labelled with digoxigenin and visualized with FITC (green) and a chromosome 3 specific library labelled with biotin and visualized with Texan Red (red), showing hybridization signals on both chromosomes 3, on the long arms, close to the centromere. Chromosomes were counterstained with DAPI (blue). More precise localization was obtained on extended chromosomes and by making use of DAPI banding (shown on extended chromosome, bottom right hand corner) to 3p12.1.

the 5-HT_{1F} receptor was screened by SSCP analysis. The sensitivity of this method is not fully 100% [Hayashi and Yandell, 1993], but this limitation was partially overcome by the use of overlapping PCR fragments, and by increasing the SSCP sensitivity. Nevertheless, we did not find polymorphisms among the groups of migraine patients with different clinical responses to sumatriptan, arguing against genetic diversity of the 5-HT_{1F} receptor gene as a major determinant of the clinical response to sumatriptan. Admittedly, our relatively small number of subjects could have allowed overlooking sporadic mutations like those reported by Shimron-Abarbanell et al. [1996], which had a frequency of 1 to 4%. However, taking into account that we did not detect any mutation in any of the patients in our study, it is highly unlikely that rare mutations play a pivotal role in the clinical response to sumatriptan. Although genetic variation surrounding the coding region of the 5-HT_{1F} receptor gene such as promotor or enhancer sequences may be associated with the clinical response to sumatriptan via regulation of expression of the 5-HT_{1F} receptor, we think, in conclusion, that genetic diversity of the 5-HT_{1F} receptor is not responsible for the variable clinical response to sumatriptan.

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