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Functional characterization of genetic polymorphisms identified in the human cytochrome P450 4F12 (CYP4F12) promoter region

Christelle Cauffiez[a,*], Florian Klinzig[a], Emmanuel Rat[a], Gilles Tournel[a], Delphine Allorge[a], Dany Chevalier[a], Tonio Lovecchio[a], Nicolas Pottier[a], Jean-Frédéric Colombel[b], Michel Lhermitte[a], Jean-Claude D'Halluin[c], Franck Broly[a], Jean-Marc Lo-Guidice[a]

[a]*Equipe d'accueil 2679, Faculté de Médecine de Lille, Pôle Recherche, Lille, France*
[b]*Département de Gastro-entérologie, Hôpital Huriez, CHRU, Lille, France*
[c]*Unité INSERM U524, IRCL, Lille, France*

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Abstract

The human cytochrome CYP4F12 has been shown to be active toward inflammatory mediators and exogenous compounds such as antihistaminic drugs. In the present study, we report the first investigation of polymorphisms in the human *CYP4F12* gene. A screening for sequence variations in the 5′-flanking region was performed by a Polymerase Chain Reaction–Single Strand Conformational Polymorphism (PCR–SSCP) strategy, using DNA samples from 53 unrelated French individuals of Caucasian origin. Several polymorphisms were identified, comprising a large deletion located in intron 1 (*CYP4F12*v1*), two isolated substitutions −402G > A (*CYP4F12*v3*) and −188 T > C (*CYP4F12*v4*) and nine combined mutations, −474T > C, −279A > C, −224A > G, −173G > A, −145C > G, −140T > C, −126T > C, −56T > C, and −21T > G (*CYP4F12*v2*). Considering the nature and location of the polymorphisms characterizing the *CYP4F12*v1* and **v2*, the functional relevance of those two allelic variants was further examined by transfecting different cell lines with constructs of the related region of the *CYP4F12*/luciferase reporter gene. Both alleles lead to a significant decrease of *CYP4F12* gene expression in HepG2 cell line and, therefore, are likely to determine interindividual differences in CYP4F12 gene expression.

Keywords: CYP4F12; Genetic polymorphism; Promoter region; Regulation

1. Introduction

The cytochromes P450 (CYPs) constitute a superfamily of haem-thiolate proteins which catalyze the oxidation of a wide variety of substrates, including endogenous lipids and xenobiotics [1]. Currently, 57 cytochromes P450, classified into families and subfamilies based on deduced-protein sequences homology, have been characterized in humans [2]. The CYP4Fs belong to a growing P450 subfamily that, to date, comprises six members, namely the CYP4F2 [3], the CYP4F3 [4], the CYP4F8 [5], the CYP4F11 [6], the CYP4F12 [7,8], and the CYP4F22 whose amino acid sequence was deduced from genomic DNA databases [2].

Most of the human CYP4F isoforms were originally discovered to be involved in vitro in the biosynthesis and catabolism of structurally different eicosanoids and lipoxins [3,5,9,10], as well as arachidonic acid metabolites [9]. Consequently, by modulating the level of potent inflammatory mediators, the CYP4F enzymes may play a significant physiological role in inflammatory processes [11].

CYP4F12 is catalytically active toward arachidonic acid and PGH2 analogs and, to a lesser extent, toward PGE2, PGF2α, and LTB4 [7]. However, in contrast to other CYP4F members, CYP4F12 is also involved in xenobiotic metabolism [12]. In particular, it has been shown to be highly efficient toward antihistaminic compounds, such as

* Corresponding author. Tel.: +33-3-20-62-68-18; fax: +33-3-20-44-47-29.
E-mail address: christelle.cauffiez@caramail.com (C. Cauffiez).

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ebastine (4′terbutyl-4-[4(diphenylmethoxy)piperidino]butyrophenone) and terfenadine [13,14].

At the molecular level, the CYP4F12 gene or *CYP4F12* is located in a cluster spanning 320 kb on chromosome 19p13.1, along with the other *CYP4F* members and eight *CYP4F* pseudogenes. *CYP4F12* encompasses about 25 kb and contains 13 exons (1694 nucleotides) with an open reading frame being encoded by exons 2–13. The gene product is roughly a 60 kDa protein [8], with an amino acid sequence showing 78–83% identity with other CYP4F enzymes [7]. CYP4F12 is mainly expressed in the liver and was also detected in kidney, colon, heart and, in contrast to other CYP4F members, in small intestine [7,12].

To date, even if no systematic analysis for variations in the nucleotide sequence has been undergone, several polymorphisms in the CYP4F12 gene have been reported. Two variant cDNAs were isolated by Bylund et al. [7] and Hashizume et al. [8]. In particular, compared to the wild-type, one cDNA harbours four substitutions located in exon 3 ($Val^{76}Asn$ and $Val^{90}Ile$), exon 4 ($Arg^{188}Cys$), and exon 13 ($Gly^{522}Ser$).

In the present study, we examined parts of the *CYP4F12* regulatory sequences in genomic DNA from 53 healthy French volunteers of Caucasian origin, using a Polymerase Chain Reaction–Single Strand Conformational Polymorphism (PCR–SSCP) strategy. The various *CYP4F12* variants identified were then functionally characterized by transient transfection assay.

2. Material and methods

2.1. Subjects

Fifty-three unrelated French subjects of Caucasian origin have been involved in the study after ethical committee approval and informed consent had been obtained. Genomic DNA was isolated from peripheral blood leucocytes using the Nucleon BACC3 Kit (Amersham Biosciences), according to manufacturer's instructions.

2.2. PCR–SSCP analysis

Fig. 1 depicts the strategy we developed for PCR–SSCP analysis of *CYP4F12* in genomic DNA from 53 individuals. In a first step, for selective amplification, a large fragment F1 (1333 bp), encompassing the proximal promoter region, exon 1, intron 1, and exon 2 of the *CYP4F12* gene from nucleotide −1049 to +284, was generated by PCR. In a second step, F1 was used as a template in order to generate five fragments, named A–E, by separate amplification reactions, each using a set of nested primers listed in Table 1.

The reaction mixtures contained, in a total volume of 25 μL, 200 ng of genomic DNA or 0.6 μL of F1, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 0.01% gelatine, 0.1 mM of each dNTP, 0.25 μM of each primer and 0.2 U of Taq polymerase (Invitrogen). The $MgCl_2$ concentration was optimized for each primer pair (Table 1). In addition, for nested PCR, 0.7 μCi of [α-^{32}P]dCTP (3000 Ci mmol^{-1}, Amersham Biosciences) were added to label the amplified fragments.

For F1 amplification, after an initial 2-min denaturation at 94 °C, products were generated for 35 cycles with a 1-min denaturation at 94 °C, 1-min annealing at 68 °C, and a 3-min extension at 72 °C followed by a 7-min final extension step at 72 °C. For the nested PCRs, after an initial 2-min denaturation at 94 °C, the reaction was carried out for 12 cycles with a 1-min denaturation at 94 °C, a 30-s annealing at an optimized temperature (Table 1), and a 30-s extension at 72 °C. The reaction was terminated with a 7-min final extension. Size and specificity of PCR

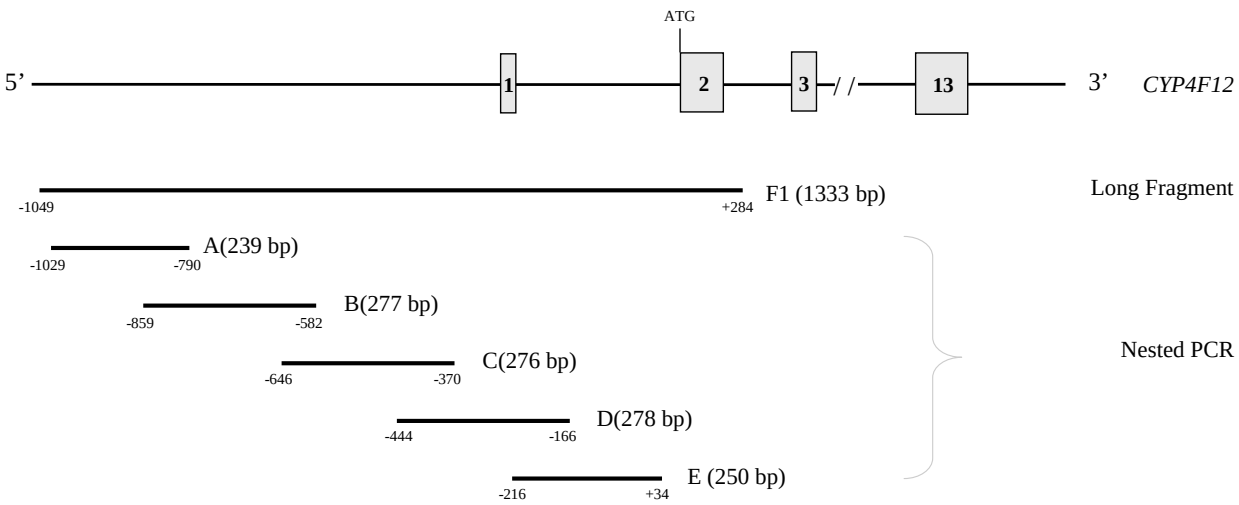

Fig. 1. Outline of the strategy for PCR–SSCP analysis of the *CYP4F12* gene in genomic DNA. In a first step, amplification of a 1333-bp fragment (F1) of the *CYP4F12* gene, spanning nucleotides −1049 to + 284 was performed. In a second step, F1 was used as a template for five parallel PCRs using nested primers. Some exons of the *CYP4F12* are illustrated with solid boxes.

Table 1
Details of the primers used for PCR–SSCP analysis of the *CYP4F12* gene

	Primer	Primer sequence (5′ → 3′)	Location[a]	Size (bp)[b]	*T* (°C)[c]	$MgCl_2$ (mM)
F1	Long1F	GCTGGCTCTTTATCCCCAGAATCTGACGA	−1049	1333	68	1
	Long1R	CAATACCTAGCTCACTGCGCCCCATTCCTG	+284			
A	UTR1F	AATCTGACGAAGGTAATCCTC	−1029	239	60	2
	UTR1R	ACCCTTGTCCCAGATCATCT	−790			
B	UTR2F	CCAAGATGTAGTGCCTGACA	−859	277	60	2
	UTR2R	CTGGGTGCAGCTCAGGGT	−582			
C	UTR3F	TTCCAAGAAGGTACTGAGACC	−646	276	60	1
	UTR3R	AGCCCTGCCACCCCCAG	−370			
D	Exon1F	CAGCAGAAGAGGAGAAGAG	−444	278	60	1
	Exon1R	CAACAACCAAGTGGCCTCC	−166			
E	Intron1F	TCTTTCCCTTTCTGTGTGGTT	−216	250	60	2
	Intron1R	CTGAGGCCCAGCCAGGG	+34			

[a] Location of primers referred to the ATG translation start site.
[b] Size of amplified fragments.
[c] Optimized annealing temperature for each set of primers.

fragments were evaluated in ethidium bromide-stained agarose gels.

For the SSCP electrophoresis, 3 μL of each nested amplicon were mixed with 3 μL of denaturing dye (10 mM NaOH, 20 mM EDTA, 95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol), heated for 2 min at 92 °C and rapidly chilled on ice. Three microliters were then loaded on a non-denaturing MDE (Cambrex Biosciences) polyacrylamide gel (40 cm × 30 cm × 0.4 mm) containing 10% glycerol. Gels were allowed to run for 16–18 h at 6 W constant power. After electrophoresis, gels were transferred to filter paper, dried, and submitted to autoradiography at −70 °C for 24–48 h.

For samples displaying a change in electrophoretic mobility compared to a reference sample with a homozygous wild-type genotype, the PCR–SSCP procedure was repeated to eliminate any detection of mutations due to errors of amplification generated by the DNA polymerase.

2.3. Nucleotide sequence analysis

For nucleotide sequence analysis, amplicons generated by PCR were purified with Qiaquick spin columns (Qiagen) and sequenced on both strands with the primers used for the PCR–SSCP analysis, the ABIprism Dye Terminator Cycle Sequencing Reaction FS Kit (Applied Biosystem Inc.) and an automated DNA sequencer (Model 373 A, Applied Biosystem Inc.).

2.4. Characterization of the CYP4F12 alleles

Genomic DNA samples from wild-type homozygotes or heterozygotes for the identified mutations were amplified to generate a 906-bp fragment (−877 to +29) using the forward primer PlucF (5′-CCC TAA G**CT** TGA ACC AAG ATG TAG TGC CTG ACA-3′), the reverse primer PlucR (5′-CCC AGC CA**T** GGC AGG CTC AGC AGC GAC TTC CTG CAG GG-3′) and the *Pfx* DNA polymerase (Invitrogen), following manufacturer's instructions. Bold nucleotides were voluntary mismatched in order to create a *Hin*dIII site at the 5′-end and a *Nco*I site at the 3′-end of the amplicon. PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) and subsequently cloned between the *Hin*dIII and *Nco*I sites of the promoterless eukaryotic expression vector pGL3-Basic (Promega). Plasmids were purified with a QIAprep Spin Miniprep Kit (Qiagen) and then sequenced, as described above, in order to determine the sequence of each *CYP4F12* allele.

2.5. Transient transcription assay

The plasmid constructs pGL3-Wt, pGL3-v1, and pGL3-v2 correspond, respectively, to the wild-type *CYP4F12* allele, the 192 bp deleted variant (*CYP4F12*v1*), and the variant carrying nine combined mutations (*CYP4F12*v2*). In order to analyse the effect of these allelic variants on the promoter activity of the luciferase reporter gene, the human renal (HEK) and hepatic (HepG2) cell lines (American Type Culture Collection) were transfected with pGL3-Wt, pGL3-v1, pGL3-v2, and pGL3-Basic (control plasmid without insert), using lipofectamine 2000 (Invitrogen). Thirty-six hours after transfection, cells were lyzed with Reporter Lysis buffer (Promega) and the luciferase activity was assayed by using a Berthold Lumat LB 9501 luminometer (Berthold Technologies). A pSV-β-galactosidase control vector (Promega) was co-transfected with each pGL3 construct to normalize the transfection efficiency. The β-galactosidase activity was assayed by chemiluminescence, using the β-Gal Reporter Gene Assay Kit (Roche). Transfection and reporter assays were repeated in two independent experiments, each experiment comprising three replicates.

Putative binding sites for transcription factors analysis was performed with Alibaba2 [15] and TRANSFAC [16] database search.

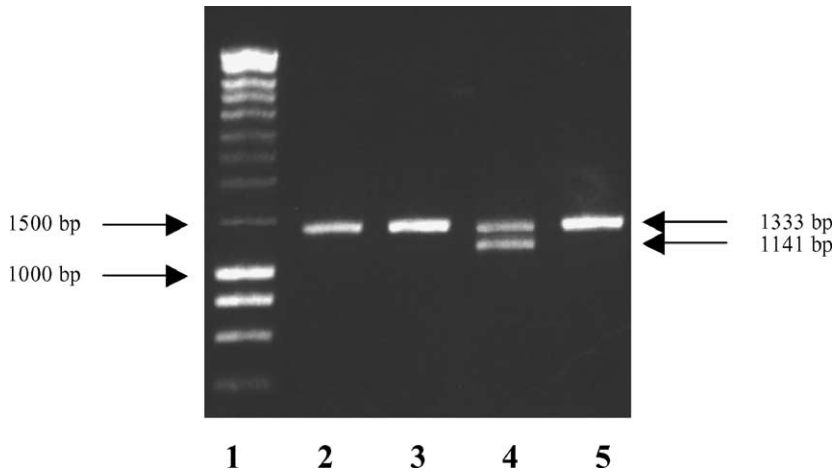

Fig. 2. Agarose gel electrophoresis of fragment F1 (1333 bp) encompassing part of the 5′-untranslated region (exon 1) and intron 1 of the *CYP4F12* gene. PCR was performed as described under Section 2, with the use of long1F and long1R primers (see Table 1 for sequences). PCR products were visualized by staining with ethidium bromide. Lane 1: mass molecular ladder. Lanes 2, 3, and 5: PCR products from genomic DNA samples from three individuals with a wild-type genotype. Lane 4, PCR product from genomic DNA of an individual heterozygote for the 192 bp deletion (*CYP4F12*v1*).

3. Results

3.1. Genetic polymorphism of CYP4F12

To detect any mutation in the promoter sequence of the cytochrome P450 4F12 gene (*CYP4F12*), a strategy based on PCR–SSCP analysis was developed (Fig. 1) and applied to genomic DNAs of 53 unrelated volunteers.

In a first step, a large fragment (F1), spanning the proximal promoter region to exon 2 was generated and used as a template for the amplification of five nested fragments (A–E). Interestingly, for 4 out of the 53 individuals analysed, F1 amplification led to the visualization of two distinct fragments after agarose gel electrophoresis. One of them exhibited a 1300 bp length, as expected, whereas the second band was 1100 bp long (Fig. 2). Gel purification and sequencing of the shorter fragment allowed the identification of a 192 bp deletion (IVS1-229-37del) located in intron 1 from nucleotide −229 to −37 (Fig. 5).

Additional mutations were detected by SSCP analysis of the nested PCR products. For 20 samples, electrophoretic patterns did not differ from that of the reference sample for any of the five investigated fragments (Fig. 3; lanes 1, 4, 10; wt/wt). From the analysis of the 33 remaining subjects, no mutation was found for both fragments A and B and at least one alternative profile was found in fragments C–E (Fig. 3), suggesting the presence of mutations. Sequencing of the corresponding regions allowed the characterization of eleven different mutations (Table 2).

For fragment C, two SSCP patterns differing from that of the wild-type were observed. They correspond to two heterozygous base-pair changes, −474T > C (Fig. 3, lane 2) and −402G > A (Fig. 3, lane 3).

For fragment D, five abnormal electrophoretic profiles were identified. The first one (Fig. 3, lane 5) corresponds to the −402G > A mutation. Sequencing of DNA samples displaying the two following types of abnormal SSCP profile (Fig. 3b, lanes 6 and 9) allowed the identification of a −188T > C mutation either in a homozygous or in a heterozygous state. The next profile corresponds to two combined mutations, −279A > C and −224A > G, both in a heterozygous state (Fig. 3, lane 7). The last profile (Fig. 3, lane 8) results from a compound heterozygous genotype, with one allele carrying the −188T > C mutation and the other allele harbouring the −279A > C and −224A > G mutations.

For fragment E, four SSCP patterns differing from that of the wild-type were observed. Sequencing of the two first ones (Fig. 3, lanes 11 and 12) confirmed the presence of the −188T > C mutation in a homozygous and in a heterozygous state. The third one (Fig. 3, lane 13) corresponds to a sample containing six mutations, −173G > A, −145C > G, −140T > C, −126T > C, −56T > C, and −21T > G, combined on the same allele. Finally, the last profile (Fig. 3, lane 14) corresponds to a compound heterozygous

Table 2
Distribution of *CYP4F12* mutations identified in 53 French individuals

Nucleotide changes	n^a	Frequency (%)	Allele
−474T > C	9	8.5	*CYP4F12*v2*
−402G > A	2	1.9	*CYP4F12*v3*
−279A > C	9	8.5	*CYP4F12*v2*
−224A > G	9	8.5	*CYP4F12*v2*
−188T > C	22	20.8	*CYP4F12*v4*
−173G > A	9	8.5	*CYP4F12*v2*
−145C > G	9	8.5	*CYP4F12*v2*
−140T > C	9	8.5	*CYP4F12*v2*
−126T > C	9	8.5	*CYP4F12*v2*
−56T > C	9	8.5	*CYP4F12*v2*
−21T > G	9	8.5	*CYP4F12*v2*
192 bp deletion	4	3.8	*CYP4F12*v1*

[a] Number of mutated alleles out of 106 alleles.

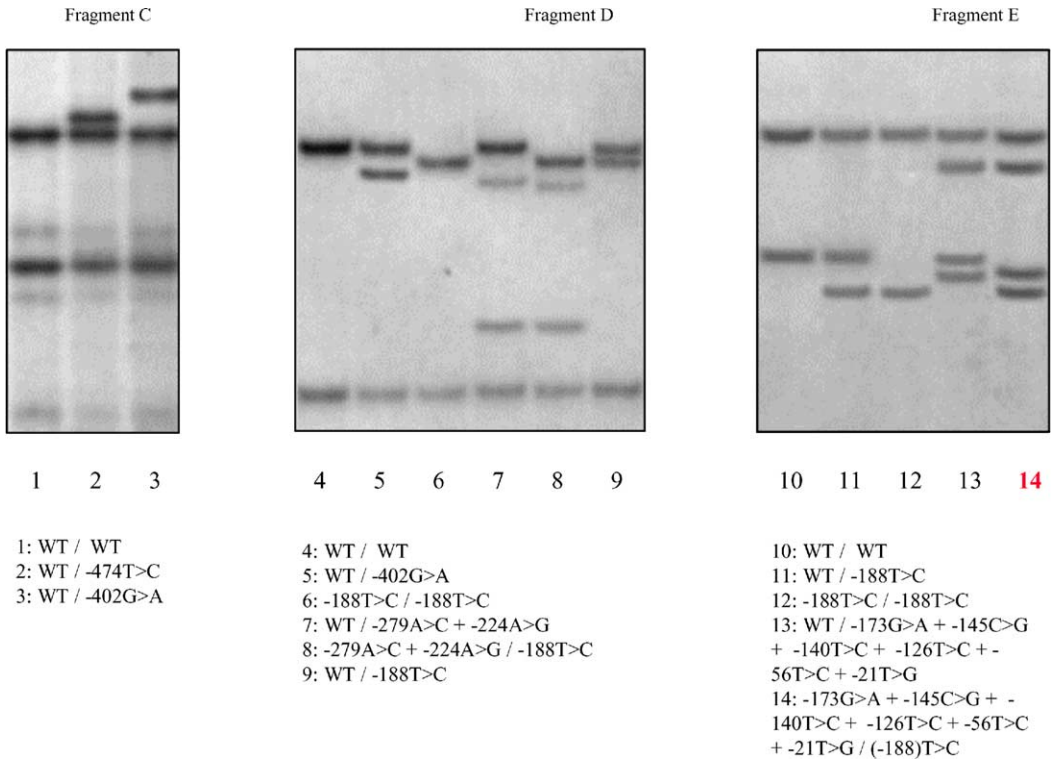

Fig. 3. PCR–SSCP analysis of the human *CYP4F12* gene. Five nested fragments (A–E) were separately amplified from 53 unrelated French individuals, using the specific fragment F1 as a template. The single-stranded PCR products were separated by non-denaturing electrophoresis. One sample with a wild-type sequence (lane 1, wt) was used as a reference sample for SSCP analysis. Abnormal SSCP patterns were observed with fragments C–E. Results from sequencing of the corresponding fragments are reported at the bottom of the gels.

genotype, with the −188T > C mutation on one allele and the six combined mutations on the other allele. Frequencies of all mutations are listed in Table 2.

Cloning of the mutated regions and analysis of the distribution of mutations described above allowed the characterization of five different *CYP4F12** allelic variants in our French Caucasian population. They comprised the wild-type allele, which is the most frequent (67%), the *CYP4F12*v1* allele, which harbours the 192 bp deletion located in intron 1, the *CYP4F12*v2* allele, which carries a combination of nine mutations (−474T > C, −279A > C, −224A > G, −173G > A, −145C > G, −140T > C, −126T > C, −56T > C, and −21T > G) located in the 5′-untranslated region and in intron 1, and the *CYP4F12*v3* and **v4* alleles, which harbour a unique mutation, −402G > A and −188T > C, respectively. Since the coding sequence was not investigated in this study, complete haplotypes could not be characterized and the official nomenclature for CYP alleles could not be used. All these novel alleles have been submitted to GenBank with accession numbers AY544782-5.

3.2. Functional consequences of CYP4F12 variants

The promoter activities of the wild-type sequence and of the *CYP4F12*v1* and **v2* alleles, which correspond, respectively, to fragments harbouring the 192 bp deletion and the nine combined mutations, have been analysed by measuring the luciferase expression from the lysate of transfected HEK and HepG2 cell lines. Results of the transient transfection assay are shown in Fig. 4. The promoterless pGL3-Basic vector was used as the baseline

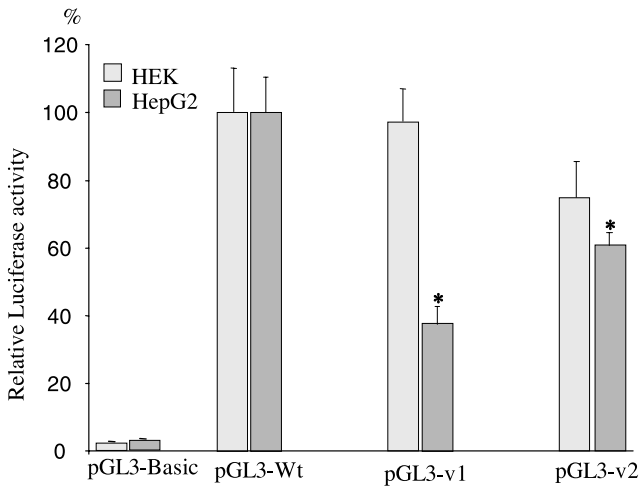

Fig. 4. Promoter activity of the wild-type sequence, *CYP4F12*v1* and **v2* alleles in HEK and HepG2 cell lines. The promoterless pGL3-Basic was used as the baseline control. A pSV-β-galactosidase control vector was co-transfected with each construct to normalize the transfection efficiency. PGL3-Wt luciferase activity was used as a reference (100%). Values represent the mean of two independent experiments performed in triplicate. Relative luciferase activity and standard errors are shown for each construct. Values that differ significantly from those of the wild-type construct are indicated : *($P < 0.05$).

control and pGL3-Wt luciferase activity was used as a reference (100%). In the two cell lines, HEK and HepG2, the plasmid containing the wild-type fragment yielded luciferase activity which was about 100-fold higher than that of the empty vector. In HEK cell line, there was no statistically significant difference in promoter activity of constructs pGL3-Wt, pGL3-v1, and pGl3-v2. In contrast, in HepG2 cell line, a significant decrease in promoter activity of 60 and 40%, compared to the wild-type construct, was observed for pGL3-v1 and pGl3-v2, respectively,

4. Discussion

To carry out a screening for sequence variations in the proximal promoter region of the *CYP4F12* gene, a PCR–SSCP strategy was developed and applied to genomic DNA samples from 53 unrelated French individuals of Caucasian origin. This method has been successfully used for the detection of mutations in several other cytochrome P450 genes [17–22]. Since the sensitivity of the method is closely related to electrophoresis, the SSCP analysis was performed after optimization of several factors affecting single strands separation (time, temperature of migration, and gel matrix). However, despite many precautions, some additional mutations might not have been detected under the chosen SSCP conditions. In addition, as highly homologous *CYP4F* genes are present in the *CYP4F12* containing gene cluster, a first amplification step using *CYP4F12*-specific primers has been included to the PCR–SSCP strategy.

This study has allowed the identification and characterization of eleven novel single nucleotide polymorphisms located in the 5′-untranslated region ($-474T > C$), exon 1 ($-402G > A$) and intron 1 ($-279A > C$, $-224A > G$, $-188T > C$, $-173G > A$, $-145C > G$, $-140T > C$, $-126T > C$, $-56T > C$, and $-21T > G$) (Table 2). The mutations $-402G > A$ and $-188T > C$ have been identified as isolated SNPs and define two allelic variants, *CYP4F12*v3* and **v4*, respectively. The other mutations have been found in combination. The linkage between the nine mutations ($-474T > C$, $-279A > C$, $-224A > G$, $-173G > A$, $-145C > G$, $-140T > C$, $-126T > C$, $-56T > C$, and $-21T > G$) harboured by the *CYP4F12*v2* variant has been subsequently confirmed by cloning and sequencing a 900-bp PCR fragment, encompassing these nine mutations and corresponding to a single chromosome. In addition to these SNPs, a more deleterious abnormality, which consists in a large deletion of 192 nucleotides located in intron 1 straight upstream the ATG start codon, was identified in four individuals and defines to the *CYP4F12*v1* allele.

In order to analyse the functional effect of the *CYP4F12*v1* and **v2* alleles, we first evaluated the potential in vitro promoter activity of a *CYP4F12* region spanning nucleotides -877 to $+29$ (relative to the translation start site) and encompassing part of the 5′-flanking region, exon 1, intron 1, and exon 2, using a transient transfection assay in HEK and HepG2 cell lines. The wild-type 900-bp fragment exhibits high promoter activity when coupled to the luciferase reporter gene in both cell lines. These results therefore suggest that the corresponding sequence likely contains regulatory elements for CYP4F12 expression whatever the cell type. To date, although the *CYP4F12* promoter region has never been investigated and the regulation of CYP4F12 expression has not been elucidated, data from the studies of other *CYP4F* members could provide some insights into the mechanism of *CYP4F12* regulation. In particular, Cui et al. have shown that the 134 bp sequence before the untranslated exon 1 contains the promoter for constitutive expression of the rat *CYP4F5* gene, whereas two negative regulatory regions seem to be located upstream [23].

Compared to the wild-type allele, the *CYP4F12*v1* and **v2* variants exhibited about 50% reduction in luciferase activity in the HepG2 cell line. In order to determine if this reduced promoter activity was due to nucleotide differences in putative transcription factors binding sites, we used TRANSFAC [16] and Alibaba2 [15] computer tools. Several major putative transcriptional element binding sites, including retinoic acid receptor (RAR)/retinoic acid X receptor (RXR), nuclear factor-κB (NFκB), Sp1 or hepatocyte nuclear factor (HNF) sites, appear to be located in the upstream promoter region and intron 1 of *CYP4F12*. In addition, several of these elements likely for nuclear receptors RXR, RAR, NFκB, HNF, or GATA are located in the 192-bp fragment which is deleted in *CYP4F12*v1* (Fig. 5). Moreover, we found that the $-126T > C$, $-224A > G$, and $-145C > G$ mutations present in *CYP4F12*v2* are responsible for the loss of putative HNF, C/EBPα site and GATA-1 binding sites, respectively (Fig. 5). Nevertheless, the mechanism by which the sequence alterations in *CYP4F12*v1* and **v2* decrease the transcription rate of the reporter gene remains to be clarified.

Interestingly, in contrast to HepG2 cell line, no significant decrease of luciferase activity was measured in the HEK cell line for any variant. Such differences in activation and repression of gene expression between various cell lines suggest that cell-specific modulation may be involved, as it has already been described with other P450s [23]. Recently, evidence has been accumulated to support that the expression of the CYP4F enzymes is regulated in a species-, tissue-, isoform-, and even sex-dependent manner [23–26]. For example, a strong activity of the human *CYP4F3* first intronic region suggests that this region may be used as an alternative promoter sequence for a particular tissue [27,28]. Which factors contribute to tissue-specific expression of CYP4F12 still remains to be established.

In conclusion, in order to examine the extent of CYP4F12 genetic polymorphism in a French population,

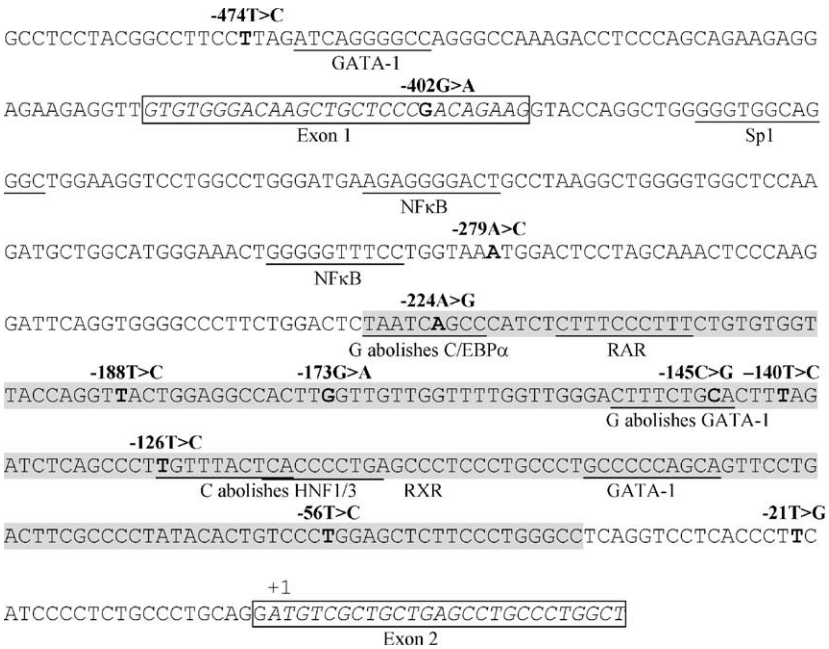

Fig. 5. Partial nucleotide sequence of the fragment F1 encompassing part of the 5′-untranslated region, exon 1, intron 1, and exon 2 of the *CYP4F12* gene. 1333 bp was generated by PCR and only 517 bp is shown. The translation start is indicated by +1. Open boxes correspond to exon 1 and partial exon 2. The potential transcription binding sites searched with TRANSFAC are underlined. Single nucleotide polymorphisms are in bold characters and the 192 bp deletion is highlighted in grey.

we performed a screening of the *CYP4F12* proximal promoter region and 5′-untranslated region by PCR–SSCP. Two major frequent polymorphisms were identified, namely a 192-bp fragment located in intron 1 and nine combined mutations distributed in intron 1 and in the 5′-UTR. Furthermore, their functional impact was evaluated by transient transfection assay in HepG2 and HEK cell lines. As both polymorphisms lead to a 50% decrease of the promoter activity in HepG2 cell lines, they could determine interindividual differences in hepatic gene expression and, thereby, in CYP4F12 protein synthesis. In contrast, no significant variation in the promoter activity has been observed in HEK cell lines. Then, bioactivity of CYP4F12 probably depends on the interplay of cell-specific transcription and genetic factors. Phenotyping studies could help in determining the in vivo functional impact of the identified variants and will provide more definite data on the role of *CYP4F12* genetic polymorphism in certain inflammatory mediators and drugs metabolism.

Acknowledgments

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