Genetic Polymorphism in Cytochrome P450 1B1 in a Spanish Population

Beatriz Pérez-Cadahía[a,b], Vanessa Valdiglesias[a,b], Eduardo Pásaro[a], Josefina Méndez[b], Blanca Laffon[a,b*]

[a] Toxicology Unit, University of A Coruña, Spain
[b] Department of Cell and Molecular Biology, University of A Coruña, Spain

Basic and Clinical Pharmacology and Toxicology, volume 101, issue 1, pages 70–72, July 2007
Received 24 January 2007, accepted 13 March 2007, version of record online 02 June 2007

This is the peer reviewed version of the following article:

Pérez-Cadahía, B., Valdiglesias, V., Pásaro, E., Méndez, J. and Laffon, B. (2007), Genetic Polymorphism in Cytochrome P450 1B1 in a Spanish Population. Basic & Clinical Pharmacology & Toxicology, 101: 70-72. https://doi.org/10.1111/j.1742-7843.2007.00086.x

which has been published in final form at https://doi.org/10.1111/j.1742-7843.2007.00086.x. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions. This article may not be enhanced, enriched or otherwise transformed into a derivative work, without express permission from Wiley or by statutory rights under applicable legislation. Copyright notices must not be removed, obscured or modified. The article must be linked to Wiley's version of record on Wiley Online Library and any embedding, framing or otherwise making available the article or pages thereof by third parties from platforms, services and websites other than Wiley Online Library must be prohibited.

One of the most important groups of metabolic enzymes involved in the detoxification of a wide range of toxic compounds is the cytochrome P450 (CYP) superfamily. This superfamily is subdivided into a number of families and subfamilies, based on nucleotide sequence homology where genes within a family have a minimum of 40% sequence identity [1]. They catalyse a large number of chemical reactions with an almost unlimited number of biologically occurring and xenobiotic compounds and are preferentially expressed in liver, although some isozymes can be tissue-specific [2].

Among this group of enzymes the isozyme CYP1B1 deserves special attention. CYP1B1 gene is located in chromosome 2p22-p21 [3] and encodes a 543-amino acid protein. CYP1B1 promoter contains a xenobiotic response element (XRE: 5′-TNGCGTG-3′) and is regulated by aryl hydrocarbon receptor [1]. This receptor belongs to the helix-loop-helix transcription factors family and constitutes a cytosolic protein that, after binding to some of its ligands, translocates to the nucleus and dimerizes with a nuclear protein [4]. This dimer interacts with the XRE and allows the complex to regulate the gene transcription [5]. Unlike CYP1A1, other important gene of this family, the structure and function of CYP1B1 promoter is similar to the constitutively expressed genes [6], although it has been reported that its expression can also be induced by the binding of several substances as dioxins or polycyclic aromatic hydrocarbons to the aryl hydrocarbon receptor [7].

*blaffon@udc.es

Attending to the fact that many carcinogenic agents must be bioactivated by means of phase I oxidative metabolism and that the CYP family is the predominant source of this metabolic activity, its expression constitutes a determinant toxicological factor [8]. Specifically, it has been shown that the CYP1B1 enzyme induces the metabolic activation of a wide variety of carcinogens as arylamines, nitroaromatic compounds and polycyclic aromatic hydrocarbons [9]. Moreover, CYP1B1 catalyses both the formation of certain pro-carcinogen polycyclic aromatic hydrocarbons dihydrodiols and their additional oxidation to dihydrodiol epoxides, the ultimate carcinogens [7], and is apparently more active than CYP1A1 [10]. So, as in most cases metabolites resulting from its action possess more toxic characteristics than the original compounds, this metabolic pathway constitutes an activation process, and potential quantitative differences will be of special relevance in the damage induced. In fact, CYP1B1 is over-expressed in many tumours, and McFadyen and Murray [11] identified this enzyme as the main CYP present in a wide range of human cancers of different histological types, being considered nowadays a neoplastic phenotype biomarker.

Several polymorphisms were identified in CYP1B1 gene; four of them are single nucleotide polymorphisms and give rise to amino acidic substitutions. CYP1B1*3 allele comes from a C-to-G transversion at position 1666 in exon 3 (codon 432) generating an amino acid change from Leu to Val (NCBI database number rs1056836). In general, variant CYP1B1 isozymes are more active (2.4- to 3.4-fold) than the wild types [9,12]. Specifically, Li et al. [13] described a three-fold higher 4-hydroxylase activity for the CYP1B1*3 allele. On the other hand, Aklillu et al. [14] did not find differential activities between variants. Due to the fact that many works link the presence of the variant allele of this gene to the occurrence of a wide variety of cancers, the frequency of this polymorphism has been described in several studies in Caucasian populations (table 1), however, never in Spanish individuals.

Table 1. Frequencies of CYP1B1*3 in different healthy Caucasian populations.

Reference	Numbers of participants	Frequency
Brüning et al. (1999) [15]	300	0.402
Rylander-Rudqvist et al. (2003) [17]	434	0.444
Sasaki et al. (2003) [18]	112	0.299
Pesch et al. (2004) [19]	294	0.600
Landi et al. (2005) [20]	121	0.396

In this study, we have analysed the frequency of CYP1B1 codon 432 polymorphism in a population of 235 healthy Spanish individuals (114 men and 121 women, mean age 29.99 ± 11.09 years, range 17–59). DNA was extracted from 300 µl of whole peripheral blood using Puregene™ DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). The genomic DNA sequence of CYP1B1 referred to as accession number AY393988 (NCBI) was used as a reference sequence. C-to-G substitution in codon 432 was determined by means of the analysis of the melting curve after a real-time polymerase chain reaction (PCR) process using resonance energy transfer probes following Brüning et al. [15], with minor modifications. Briefly, the reaction mix consisted of 1 µM primers (5′-CAGCTTTGTGCCTGTCACTAT-3′ and 5′-CTTAGAAAGTTCTTCGCCAATG-3′), 0.1 µM hybridization probes (5′-LCR-AACTTTGATCCAGCTCG TTCTTGGACAA-3′ and 5′-ATGACCCACTGAAGTGACCTAACCC-FL-3′), 30 ng of DNA and the recommended quantity of LightCycler® FastStart Reaction Mix (LightCycler® FastStart DNA Master^{PLUS} HybProbe, Roche, Germany). In every set of reactions, a negative control was included. Forty-five rounds of amplification preceded by an initial 10 min. 95°C denaturation were undertaken in a LightCycler® according to the following reaction conditions: 3 sec. at 95°C, 10 sec. at 55°C and 25 sec. at

72°C. Melting curves were achieved following a denaturation period of 3 sec. at 95°C at a temperature increase of 0.4°C/sec. from 45 to 80°C. PCR and melting procedure were detected online with the LightCycler® instrument. The melting curve analysis showed a single melting maximum [–(dF2/F1)/dT] of 65°C for homozygous individuals C/C and 56°C for homozygotes G/G. In the case of heterozygous individuals C/G, the two melting maxima were present (fig. 1).

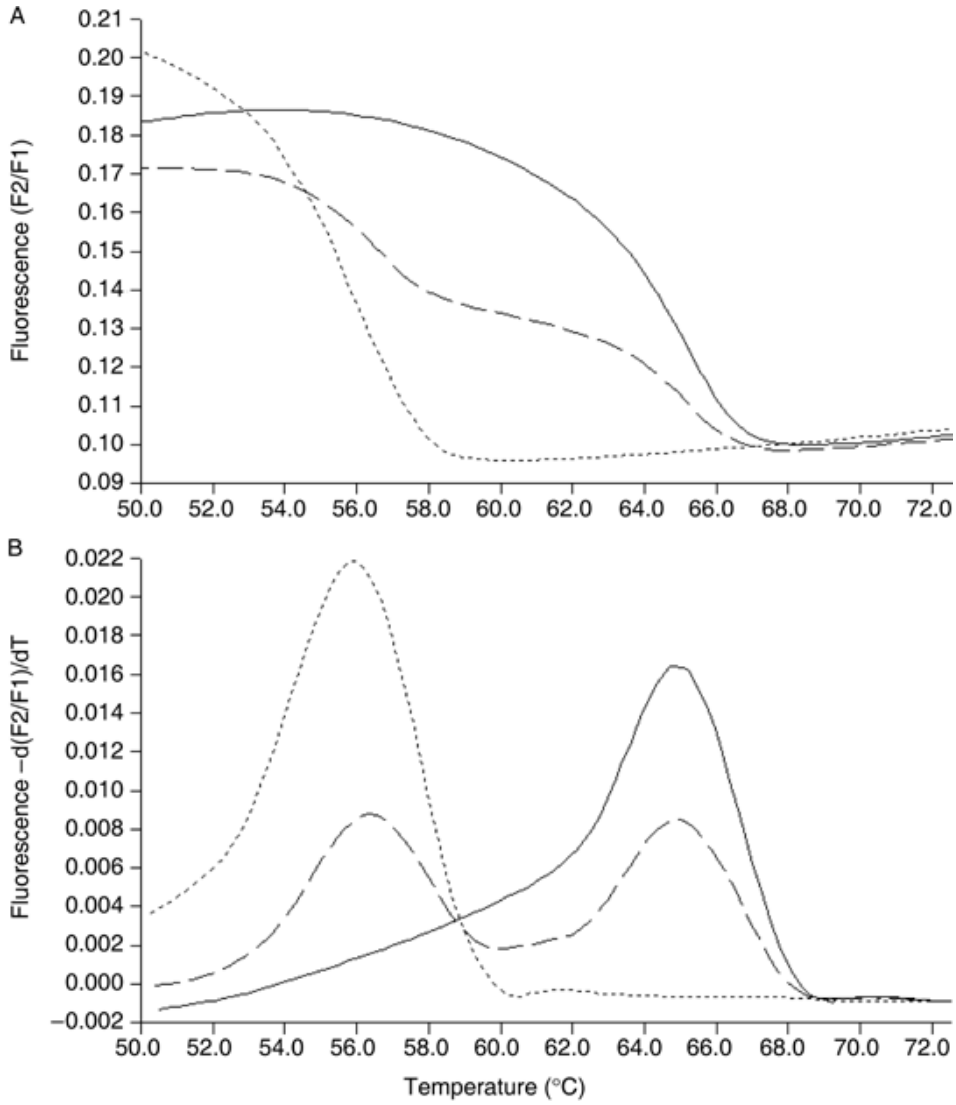

Figure 1. LightCycler® assisted analysis of CYP1B1 codon 432 polymorphism: Homozygous C/C individual (solid line), homozygous G/G individual (dotted line) and heterozygous C/G individual (dashed line). (A) Melting curves (Fluorescence versus Temperature) and (B) melting peaks (–dFluorescence/dTemperature versus Temperature).

Genotype and allele frequencies obtained in the Spanish population analysed can be seen in table 2. Variant allele frequency CYP1B1*3 was 0.432 and Hardy–Weinberg equilibrium was tested and confirmed, as the observed genotype frequency showed no deviation from Hardy–Weinberg equilibrium ($H = 1.882$, $P = 0.170$). This absence of significant deviation is a good indicator for the quality of the single nucleotide polymorphism genotyping method [16]. The frequency of this polymorphism has been previously described [15,17–20] (table 1), being results from most studies similar to our frequency. In addition, data from a recent meta-analysis reported a lower frequency for the variant allele (0.235) [21].

Table 2. Genotype and allele frequencies of CYP1B1 codon 432 in the Spanish population.

		Total numbers of cases	Frequency
Genotype	*1/*1	81	0.345
	*1/*3	105	0.447
	*3/*3	49	0.208
Allele	*1	267	0.568
	*3	203	0.432

The concrete processes that lead to the enzyme activity variation have not yet been studied in depth, but it has been reported that exon 3 (where the studied polymorphism is located) encodes the haem-binding domine, a region critical to the catalytic function of the gene [22].

Further studies are needed to confirm the importance of CYP1B1 genotyping in individuals exposed to xenobiotics that are metabolized and bioactivated by this enzyme, especially polycyclic aromatic hydrocarbons. Nevertheless, we think that such toxicogenetic testing might be helpful in the ascertaining of the factors contributing to the different individual responses to similar exposures.

References

1 Smith G, Stubbins MJ, Harries LW, Wolf CR. Molecular genetics of the human cytochrome P450 monooxygenase superfamily. *Xenobiotica* 1998; **28**: 1129–65.

2 Pavanello S, Clonfero E. Biological indicators of genotoxic risk and metabolic polymorphisms. *Mutat Res* 2000; **463**: 285–308.

3 Aklillu E, Oscarson M, Hidestrand M, Leidvik B, Otter C, Ingelman-Sundberg M. Functional analysis of six different polymorphic CYP1B1 enzyme variants found in an Ethiopian population. *Mol Pharmacol* 2002; **61**: 586–94.

4 Chan NL, Wang H, Wang Y, Leung HY, Leung LK. Polycyclic aromatic hydrocarbon-induced CYP1B1 activity is suppressed by perillyl alcohol in MCF-7 cells. *Toxicol Appl Pharmacol* 2006; **213**: 98–104.

5 Nebert DW, Puga A, Vasiliou V. Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer, and signal transduction. *Ann N Y Acad Sci* 1993; **23**: 624–40.

6 Wo YY, Stewart J, Greenlee WF. Functional analysis of the promoter for the human CYP1B1 gene. *J Biol Chem* 1997; **272**: 26702–7.

7 Ko Y, Abel J, Harth V *et al*. Association of CYP1B1 codon 432 mutant allele in head and neck squamous cell cancer is reflected by somatic mutations of p53 in tumor tissue. *Cancer Res* 2001; **61**: 4398–404.

8 Thomas RD, Green MR, Wilson C *et al*. Cytochrome P450 expression and metabolic activation of cooked food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in MCF10A breast epithelial cells. *Chem Biol Interact* 2006; **160**: 204–16.

9 Shimada T, Watanabe J, Kawajiri K *et al*. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. *Carcinogenesis* 1999; **20**: 1607–13.

10 Shimada T, Hayes CL, Yamazaki H *et al*. Activation of chemically diverse procarcinogens by human cytochrome P450 1B1. *Cancer Res* 1996; **56**: 2979–84.

11 McFadyen MC Murray GI. Cytochrome P450 1B1: a novel anticancer therapeutic target. *Future Oncol* 2005; **1**: 259–63.

12 Hanna IH, Dawling S, Roodi N, Guengerich FP, Parl FF. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. *Cancer Res* 2000; **60**: 3440–4.
13 Li DN, Seidel A, Pritchard MP, Wolf CR, Friedberg T. Polymorphisms in P450 CYP1B1 affect the conversion of estradiol to the potentially carcinogenic metabolic 4-hydroxyestradiol. *Pharmacogenetics* 2000; **10**: 343–53.
14 Aklillu E, Ovrebo S, Botnen IV, Otter C, Ingelman-Sundberg M. Characterization of common CYP1B1 variants with different capacity of benzo[a]pyrene-7,8-dihydrodiol epoxide formation from benzo[a]pyrene. *Cancer Res* 2005; **12**: 5105–11.
15 Brüning T, Abel J, Koch B *et al*. Real-time PCR analysis of the cytochrome P4501B1 codon 432-polymorphism. *Arch Toxicol* 1999; **73**: 427–30.
16 Hosking L, Lumsden S, Lewis K *et al*. Detection of genotyping errors by Hardy-Weinberg equilibrium testing. *Eur J Hum Genet* 2004; **12**: 395–9.
17 Rylander-Rudqvist T, Wedren S, Granah F *et al*. Cytochrome P450 1B1 gene polymorphism and postmenopausal breast cancer risk. *Carcinogenesis* 2003; **24**: 1533–9.
18 Sasaki M, Tanaka Y, Kaneuchi M, Sakuragi, N, Dahiya R. Alleles of polymorphic sites that correspond to hyperactive variants of CYP1B1 protein are significantly less frequent in Japanese as compared to American and German populations. *Hum Mutat* 2003; **21**: 652.
19 Pesch B, Dusing R, Rabstein S *et al*. Polymorphic metabolic susceptibility genes and longevity: a study in octogenarians. *Toxicol Lett* 2004; **15**: 283–90.
20 Landi MT, Bergen AW, Baccarelli A Jr *et al*. CYP1A1 and CYP1B1 genotypes, haplotypes, and TCDD-induced gene expression in subjects from Seveso, Italy. *Toxicology* 2005; **207**: 191–202.
21 Paracchini V, Raimondi S, Gram IT *et al*. Meta- and pooled analysis of the cytochrome P450 1B1 Val432Leu polymorphism and breast cancer: a HuGE-GSEC review. *Am J Epidemiol* 2006; **165**: 115–25.
22 Bailey LR, Roodi N, Dupont WD, Parl FF. Association of cytochrome P450 1B1 (CYP1B1) polymorphism with steroid receptor status in breast cancer. *Cancer Res* 1998; **58**: 5038–41.