Intronic polymorphisms of cytochromes P450

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Date received (in revised form): 25th May 2010

Abstract

The cytochrome P450 enzymes active in drug metabolism are highly polymorphic. Most allelic variants have been described for enzymes encoded by the cytochrome P450 family 2 (CYP2) gene family, which has 252 different alleles. The intronic polymorphisms in the cytochrome P450 genes account for only a small number of the important variant alleles; however, the most important ones are *CYP2D6*4* and *CYP2D6*41*, which cause abolished and reduced CYP2D6 activity, respectively, and *CYP3A5*3* and *CYP3A5*5*, common in Caucasian populations, which cause almost null activity. Their discoveries have been based on phenotypic alterations within individuals in a population, and their identification has, in several cases, been difficult and taken a long time. In light of the next-generation sequencing projects, it is anticipated that further alleles with intronic mutations will be identified that can explain the hitherto unidentified genetic basis of inter-individual differences in cytochrome P450-mediated drug and steroid metabolism.

Keywords: *pharmacogenetics, splicing defects, drug response, drug metabolism, POR*

Introduction

The cytochrome P450 (CYP) enzymes are active in the metabolism of xenobiotics, as well as of endogenous compounds. In the human genome, 57 genes coding for active CYP enzymes and 58 pseudogenes have been identified. The CYP enzymes can be divided into two major classes: those that are active in the metabolism of exogenous chemicals, preferentially members of families 1–3, and those that are mainly active in the metabolism of endogenous compounds, in particular steroids, fatty acids, cholesterol and cholesterol derivatives. Many of the active genes are highly polymorphic, as summarised on the Human Cytochrome P450 Allele Nomenclature (CYP-allele) website (http://www.cypalleles.ki.se/), and several hundred different variants have been identified. The most polymorphic gene among CYPs important for the metabolism of exogenous compounds is the *CYP2D6* gene, for which about 80 different allelic variants have been described. Other genes that are highly polymorphic in this gene family are *CYP2C9* and *CYP2C19*, while other genes with important functional polymorphisms are *CYP1A2, CYP1B1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2J2, CYP2R1, CYP2W1, CYP3A4, CYP3A5, CYP3A7, CYP4A22, CYP4B1, CYP4F2, CYP5A1, CYP8A1, CYP19A1, CYP21A2* and *CYP26A1*. The highest number of variant alleles among the cytochromes P450 is seen in *CYP21A2*, which encodes the steroid 21 hydroxylase, for which 119 rare variants have been identified. In addition to the CYPs, NADPH cytochrome P450 reductase (POR), the electron donor for CYP enzymes, has been shown to have important polymorphic variants (http://www.cypalleles.ki.se/por.htm) and, recently, the second electron donor, cytochrome b_5, has also been shown to exhibit functional polymorphisms,[1] although functionally variant alleles are rare.

In the field of pharmacogenetics, emphasis is put on the functionally important polymorphic CYP

variants. This includes about 400 different alleles with non-synonymous mutations or important functional mutations; 79, 253, 34 and 25 variants are present in gene families *CYP1*, *CYP2*, *CYP3* and *CYP4*, respectively (www.cypalleles.ki.se). The majority of the mutations of functional importance cause stop codons, non-synonymous mutations and splice defects. The variant alleles have, to a large extent, been identified based on an altered *in vivo* phenotype within a specific individual. In addition, targeted sequence screening projects have been carried out to search for non-synonymous mutations that have been expressed in heterologous systems and characterised functionally. The *CYP* alleles known so far have generally not been identified through large genomic sequencing projects. Results from such studies are expected to be published in the near future and to yield a high number of novel polymorphic loci. In the authors' laboratory, for example, the Korean genome has been aligned with the Caucasian genome for identification of mutations in the *CYP2C* locus. It is evident that thousands of mutations are localised in introns and gene-flanking regions which are not present in the current databases (Frank Nylén, unpublished observations).

Intronic polymorphisms

Among the *P450* genes, only 15 different alleles with intronic mutations causing functional alterations have been identified so far (see Table 1). The majority are the result of sequencing efforts using genomic DNA from individuals shown to have altered CYP activity *in vivo*. Among these mutations, all but three abolish enzyme activity due to erroneous splicing.

Among the mutations listed in Table 1, several occur in the *CYP21A2* gene, are rare and have been found because of loss of gene function causing congenital adrenal hyperplasia; however, intronic polymorphisms only account for 5 per cent of all functionally important mutations in this gene.

Intron mutations in the *P450* genes encoding drug metabolising enzymes have been found in the *CYP1A2, CYP2D6, CYP2C19* and *CYP3A5* genes. The *CYP1A2**7* allele, which has a mutation disrupting the splice donor site in intron 6, has only been identified in one single individual and not been considered in the literature after the initial identification.[2] The same is also true for *CYP2C19**7*, which contains a single T > A nucleotide transversion in the invariant GT at the 5′ donor splice site of intron 5, causing no functional protein to be expressed. By contrast, the intron mutations in the *CYP2D6* and *CYP3A5* genes are much more important.

CYP2D6

The *CYP2D6*4* allele was the first defective *CYP2D6* variant allele to be identified (in 1990), and constitutes the main explanation for the poor metaboliser (PM) phenotype among Caucasians. The 1846G > A mutation disrupts the splice acceptor site in intron 3. Many of the other mutations present in this allele are also present in the partially defective *CYP2D6*10* allele.[19] The *CYP2D6*10* allele is the most common *CYP2D6* variant in many Asian populations.[20] It is likely that this intronic mutation was introduced into the *CYP2D6*10* allele in the past 10,000 to 20,000 years.

The *CYP2D6*41* allele carries a 2988G > A mutation in intron 6, creating an alternate splice donor site. The *CYP2D6*41* allele has a very high sequence homology with *CYP2D6*2*, but early investigations regarding the functional properties of the *CYP2D6*2* allele revealed heterogeneous activity. Much later on, Toscano and collaborators showed the functional properties of the *CYP2D6*41* allele giving rise to reduced enzyme activity expressed heterologously in COS-1 and Huh-7 cells.[8] The extent of the decrease in activity caused by this allele is important, and carriers who are homozygous for *CYP2D6*41* have a reduced activity similar in magnitude to those who are heterozygous for a defective *CYP2D6* allele.[20] In addition to the *CYP2D6*41* allele, Gaedigk *et al.* identified a similar rare variant, named *CYP2D6*69*, carrying an additional non-synonymous mutation.[6]

Table 1. Intronic polymorphism of the cytochrome P450 genes and of NADPH cytochrome P450 reductase (POR)

Gene	Allele	Genomic position*	Intronic position	Effect	Activity *in vivo*	References
CYP1A2	*CYP1A2*7*	3533G > A	IVS6 + 1G > A	Disruption of the splice donor site in intron 6	None	2
CYP2C19	*CYP2C19*7*	19294T > A	IVS5 + 2T > A	Disruption of the splice donor site in intron 5	None	3
CYP2D6	*CYP2D6*4*	1846G > A	IVS3 − 1G > A	Disruption of the splice acceptor site in intron 3	None	4
	*CYP2D6*11*	883G > C	IVS1 − 1G > C	Disruption of the splice acceptor site in intron 1	None	5
	*CYP2D6*41*, *CYP2D6*69*	2988G > A	IVS6 + 39G > A	Insertion of an alternative splice donor site in intron 6	Decreased	6–8
	*CYP2D6*44*	2950G > C	IVS6 + 1G > C	Disruption of the splice donor site in intron 6	None	9
CYP3A5	*CYP3A5*3*	6986A > G	IVS3 − 237A > G	Insertion of an alternative splice acceptor site in intron 3	Decreased	10
	*CYP3A5*5*	12952T > C	IVS5 + 2T > C	Disruption of the splice donor site in intron 5	None	11
CYP21A2	Common I2 splice variant**	655A/C > G	IVS2 − 13A/C > G	Insertion of an alternative splice acceptor site in intron 2	Decreased	12
	*CYP21A2*26*	1779G > C	IVS7 + 1G > C	Disruption of the splice donor site in intron 7	None	13
	*CYP21A2*31*	295A > G	IVS1 − 2A > G	Disruption of the splice acceptor site in intron 1	None	14
	*CYP21A2*39*	387G > A	IVS2 + 1G > A	Disruption of the splice donor site in intron 2	None	15
	*CYP21A2*43*	1780T > G	IVS7 + 2T > G	Disruption of the splice donor site in intron 7	None	16
	*CYP21A2*68*	666A > G	IVS2 − 2A > G	Disruption of the splice acceptor site in intron 2	None	17
POR	*POR*3*	27615G > A	IVS6 + 1G > A	Disruption of the splice donor site in intron 6	Decreased	18

* A in translational start codon ATG is denoted +1.
** The I2 splice variant is found in several different alleles.

CYP3A5

The metabolism of clinically important drugs is primarily carried out by the CYP3A4 and CYP3A5 enzymes. They have very similar substrate specificities, with CYP3A5 being less efficient.[20] The *CYP3A4* gene is very well conserved, and no common functionally important variant allele has been identified. This is not the case regarding the *CYP3A5* gene. Based on phenotype differences between individuals, Kuehl and collaborators sequenced the *CYP3A5* gene and identified the variant allele *CYP3A5*3* with a 6986A > G

mutation creating an alternate splice acceptor site in intron 3.[10] Prior to identifying the real background for the defective *CYP3A5* allele, several mutations in the 5′-region were thought to form the genetic basis for the reduced CYP3A5 expression. Also the *CYP3A5*5* allele with a 12952T > C mutation that results in a disruption of the splice donor site in intron 5 was found in the Chinese.[11] Interestingly, the *CYP3A5*3* and *CYP3A5*5* alleles are much less common in African populations, causing an overall higher capability for CYP3A-dependent hydroxylase activities. Since CYP3A5 is also expressed in the foetal liver, this polymorphism additionally creates a higher detoxifying potential in foetuses of African origin.[21]

In conclusion, the intronic polymorphisms in the *CYP* genes account for only a small number of the important variant alleles. Their discoveries have been based on phenotypic alterations within individuals in a population, and their identification has, in several cases, been difficult and taken a long time. As a result of the next-generation sequencing projects, it is anticipated that new, important information regarding the intron sequence variability among the *CYP* genes will be discovered, and that further alleles will be identified that can explain the hitherto unidentified genetic basis of inter-individual differences in CYP-mediated drug and steroid metabolism.

Acknowledgments

The research in the authorś laboratory is supported by grants from the Swedish Research Council, The Swedish Cancer Fund and by Vinnova.

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