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IFCC PROFESSIONAL SCIENTIFIC EXCHANGE PROGRAMME (PSEP) REPORT: CYP1A2 AND CYP3A4 GENE POLYMORPHISMS

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Dr. Paloma Oliver Sáez visited the Institute of Psychiatry, Div. Psychological Medicine, Kings College, London, UK for 3 months (February – April 2005). The topic of her training was "Training in Multiplex PCR (Polymorhpisms - Antipsychotics)". Her supervisor at the Institue of Psychiatry in London was Dr. María Jesús Arranz.

4.1 Introduction

4.1.1 CYPs, their function

The cytochrome P450 proteins, CYPs, are mono-oxygenases that catalyze many reactions including epoxidation, N-dealkylation, O-dealkylation, S-oxidation and hydroxylation involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. Many of the CYPs in man are found in the liver but a remarkable amount is also found in the small intestine.

There are lots of CYPs isoforms depending on the particular gene from which they derive. CYP3A4, CYP2D6 and CYP2C9 are examples of these isoenzymes and each one identifies an individual gene. Therefore, they may have differences in enzyme activity, expression in organs…

4.1.2 CYPs polymorphic variants: their effect on metabolism

Differences in DNA sequences occur naturally in a population. A point in the human genome that can exist as two different versions (alleles) is known as polymorphism. Single nucleotide substitutions, insertions and deletions of nucleotides and repetitive sequences (microsatellites) are all examples of polymorphisms. There are upwards of 1,000,000 such SNPs (Single Nucleotide Polymorphisms) in the genome and they are responsible for the unique characteristics of every individual, from hair colour to disease predisposition. Numerous SNPs have been described in CYPs isoenzymes. For instance, within CYP2D6 we can find CYP2D6*1B (3828G>A) (1) or CYP2D6*3A (2549A>del) (2).

These changes in DNA sequences may mean different levels of functioning: CYP2D6*36 has been related to a decreased enzyme activity (3), CYP3A7*2 to an increased activity (4)...

4.1.3 CYPs polymorphic variants: their importance on response to drug treatment

Drugs in the body may go through many stages: absorption, distribution, metabolism and excretion. When drug treatment is required it is important to determine status of patients in order to improve therapeutic response preventing toxic concentrations, drug interactions and side-effects. Consequently, in clinical laboratories we study liver and kidney function, hydroelectrolytic balance…

The involvement of CYPs in drug metabolism have already been mentioned. Many studies have associated CYPs with effects related to drugs. For example, functional mutations in the promoter region of CYP1A2 directly related with reduced activity - movement disorders in psychotropic drug treatment (5) or CYP1A2*1F (-164C>A) which confers an ultrarapid CYP1A2 activity in smokers - nonresponse to clozapine (6).

Therefore, the study about SNPs related to CYPs may be very useful for predicting therapeutic, toxic and side-effects and drug interactions improving clinical outcome.

4.2 Aim of study: Development of easy methods for the identification of common CYP variants

Polymorphisms can be studied analyzing DNA sequences but also protein products because sometimes these SNPs involve a functional change of the protein. It would be interesting for clinical laboratories to have easy techniques in order to determinate CYP variants of patients who are going to be under drug treatment.

The aim of this study was to develop quick and economical methods for identifying frequent mutations in CYP1A2 and CYP3A4 that may have an effect on treatment outcome.

4.3 Plan of investigation

4.3.1 Selection of polymorphisms

CYP1A2 and CYP3A4 are two of the most abundant CYP isoenzymes and both participate in the metabolism of a wide spectrum of drugs used in psychiatric treatment. (7)

The human CYP1A2 gene has the genomic location 72828257-72834505 bp - 72.8 Mb on chromosome 15 (15q22-qter). The gene itself comprises seven exons and six introns. The CYP1A2 types form 13% of liver CYP.

The human CYP3A4 gene is located on chromosome 7, at 7q21, with 98999255-99026459 bp - 99.0 Mb. It consists of 13 exons and 12 introns. It is by far the most important in oxidative metabolism since it forms 30% of liver CYP and 70% of small intestinal CYP (60% of the CYP activity).

I revised CYP1A2 and CYP3A4 polymorphisms described to date and we selected some of them according to their frequency, location and influence over enzyme activity. These polymorphisms are shown in the following tables.

Table 1. CYP1A2 polymorphisms

ALLELE	POLYMORPHISM	ENZYME ACTIVITY	FREQUENCIES
CYP1A2*1C	-3860 G>A (5')	Decreased	• Japanese: 23% • Egyptians: 7%
CYP1A2*1D	-2464 delT		• Japanese: 42% • Egyptians: 40% • Caucasians: 4.82%
CYP1A2*1F	-164 C>A (intron 1)	Higher inducibility	• Caucasians: 46% / 33.3% (AA) 44% (AC) 10% (CC) • Zimbabweans: 57% • Tanzanians: 49% • Ethiopians: 49.6% • Egyptians: 68%
CYP1A2*1J	-740 T>G (intron 1) -164 C>A		• Ethiopians : 7.5% • Saudi Arabians : 5.9% • Spaniards: 1.3%
CYP1A2*1B CYP1A2*1G CYP1A2*1H CYP1A2*3	1545 C>T		• Caucasians: 38.2%

Table 2. CYP3A4 polymorphisms

ALLELE	POLYMORPHISM	ENZYME ACTIVITY	FREQUENCIES
CYP3A4*1B	-392 A>G (5')		• Whites: 4.2% / 5.5% • Blacks: 66.7% • Chinese: 0% • Portuguese: 4% • GuineaBissau: 72%
CYP3A4*2	1713 T>C (Ser222Pro)	Lower clearance substrate dependent	• Whites: 2.7% • Blacks & Chinese: 0% • Portuguese: 4.5%
CYP3A4*3	1334 T>C (Met445Thr)		• Caucasians: 4%

4.4 Development of PCR protocols

Protocols for the rapid genotyping of the selected CYP1A2 and CYP3A4 were developed by means of polymerase chain reaction and restriction fragment length polymorphism analysis (PCR-RFLP) with electrophoresis in agarose gel. Materials and methods used are described in tables 3 and 4. All PCR reactions were run with each mastermix comprising 10mM dNTPs, 20µM of each of the primers, 1 U of Tag polymerase, the appropriate concentration of MgCl2 (see table 3) and PCR buffer, purchased from Abgene, Bioline and Proligo.

Analysis of restriction fragment patterns was performed on agarose gels. Digestion mix consisted of restriction enzyme and buffer, both from New England Biolabs. Results were documented using a digital gel-analysis system and AlphaImager v5.5 software.

Table 3. Primers and PCR conditions

ALLELE	POLYMORPHISM	PRIMER - F PRIMER - R	PCR program	Mg^{2+} concentration	FRAGMENT (bp)
CYP1A2*1C	-3860 G>A (5')	ttgctctgtcaccca ggctg gaggtgggaggat cacttga	35x (35s 96°C-60s 63°C-60s 72°C)	3.8 mM	233
CYP1A2*1D	-2464 delT	tgagccatgattgtg gcata aggagtctttaatat ggacccag	35x (35s 96°C-60s 63°C-60s 72°C)	3.8 mM	167
CYP1A2*1F	-164 C>A (intron 1)	gcatgcatgctgtgc caggg tctgtggccgagaa gggaac	35x (35s 96°C-60s 61°C-60s 72°C)	1.9 mM	401
YP1A2*1J	-740 T>G (intron 1) -164 C>A	caagcacctgcctct acagg cccttgtgctaaggg ggaag	35x (35s 96°C-60s 63°C-60s 72°C)	3.8 mM	222
CYP1A2*1B	1545 C>T	agcccttgagtgag aagatg ggtcttgctctgtca ctca	35x (35s 96°C-60s 61°C-60s 72°C)	1.9 mM	479
CYP3A4*1B	-392 A>G (5')	ctgcagttggaaga ggcttc gtgtaggagtcttct agggg	35x (35s 96°C-60s 59°C-60s 72°C)	1.0 mM	321
CYP3A4*3	1334 T>C (Met445Thr)	gagttagtctctgga gctcc caaccacatgactgt cctgtag	35x (35s 96°C-60s 61°C-60s 72°C)	1.9 mM	421

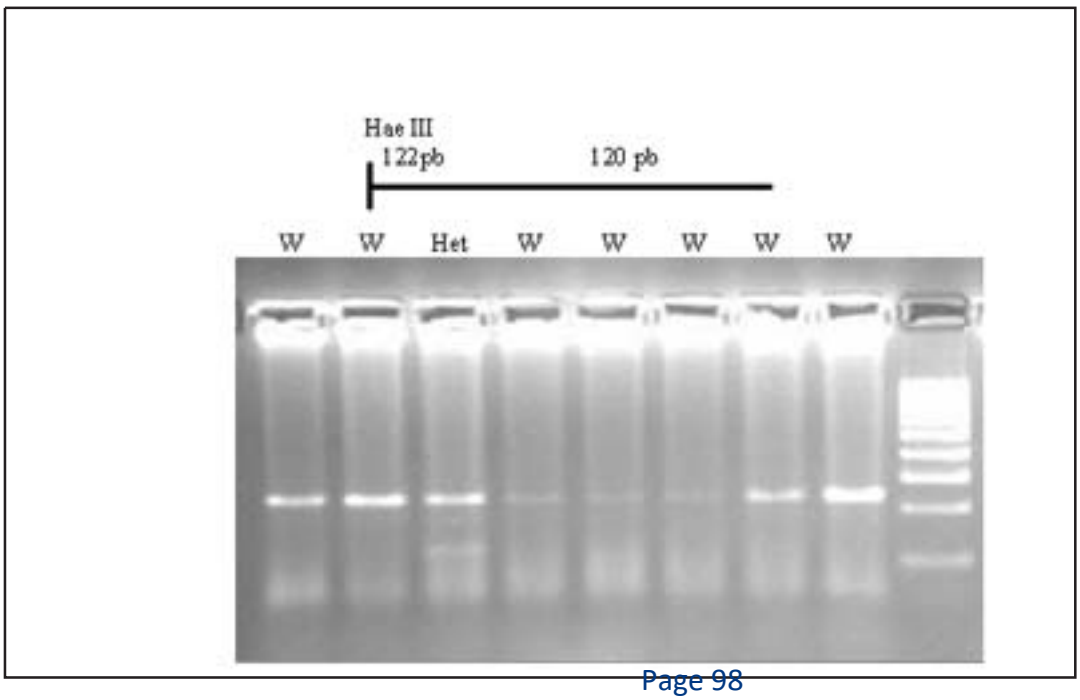
Table 4. Restriction enzymes and digestion conditionstumours

ALLELE	POLYMORPHISM	RESTRICTION ENZYME	INCUBATION TEMPERATURE	BAND PATTERNS
CYP1A2 *1C	-3860 G>A (5')	BsI I	55°C	G: 64 - 169 A: 233
CYP1A2 *1D	-2464 delT	Nde I	37°C	T: 18 - 149 delT: 167
CYP1A2 *1F	-164 C>A (intron 1)	Hae III	37°C	A: 394 C: 199 -195 - 7
CYP1A2 *1J	-740 T>G (intron 1) -164 C>A	Hae III	37°C	T: 222 G: 122 - 120
CYP1A2 *1B	1545 C>T	Tsp509 I	65°C	T: 218 - 175 - 52 - 34 C: 393 - 52 - 34
CYP3A4 *3	1334 T>C (Met445Thr)	BsI I	55°C	T: 346 - 75 C: 327 - 19 - 75

4.5 Current state of research (methods developed)

Now we are having the first results in order to see the allele frequencies of our patients. In figure 1 an example of CYP1A2*1J is shown.

*Figure 1. CYP1A2*1J - W, homozygous wild-type; Het, heterozygous mutated allele*



At this moment we are going on genotyping all samples. For that we use techniques such as authomatic genotyper (ABI 3100), authomatic sequencer (MEGABASE)…

The present study allows us to know the prevalence of different polymorphisms in our population and their association with drug effects but also providing a method of PCR and enzyme digestion affordable for almost any clinical laboratory.

4.6 Future work: Calculation of allele frequencies in Spanish population

There are relatively few studies that have investigated the frequencies of CYP1A2 variants in Spanish population. Consequently, in La Paz Hospital we are planning to study these polymorphisms together with Medicine College of Autónoma University of Madrid. At the beginning we will work with healthy volunteers and next with patients under different treatments. Moreover, we expect to study new polymorphisms related to CYP1A2. The aim is to know the allele frequencies of these polymorphisms in our population and to study the association between each polymorphism and different treatments (pharmacokinetics, pharmacodynamics, therapeutic and side effects...). Therefore, we would have the possibility of obtaining a common technique which allows us to predict responses to specific treatments improving assistance for patients.

4.7 Acknowledgements

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4.8 Footnotes

(1) - Marez et al., 1997

2) - Kagimoto et al., 1990

(3) - Wang, 1992; Johansson et al., 1994; Leathart et al., 1998

(4) - Rodríguez-Antona et al., 2005

(5) - Basile et al. 2000; Ozdemir et al. 2001

(6) - Eap, CD et al., "Nonresponse to clozapine and ultrarapid CYP1A2 activity – clinical data and analysis of CYP1A2 gene", J Clin Psychopharmacology, Apr 2004, Vol 24, (214-219)

(7) - Bertilsson et al., 2002; Coutts & Urichuk 1999; Eichelbaum & Evert 1996; Prior et al., 1999

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References

Arranz M. J., Mancama D. T., Kerwin R.W., "Pharmacogenetic and Pharmacogenomic Research in Psychiatry: Current Advances and Clinical Applications", Current Pharmacogenomics, 2003, Vol 1, No 3

Aklillu E., Carrillo JA., Makonnen E., "Genetic polymorphism of CYP1A2 in Ethiopians affecting induction and expression", Molecular Pharmacology, 2003, Vol 64.

Dandara C., Basvi PT., "Frequency of -163 C>A and 63 C>G single nucleotide polymorphism of cytochrome P450 1A2 in two African populations", Clinical Chemistry and Laboratory Medicine, 2004, Vol 42.

Hamdy SI., Hiratsuka M., "Genotyping of four genetic polymorphisms in the CYP1A2 gene in the Egyptian population", British Journal of Clinical Pharmacology, 2003, Vol 55.

Sachse C., Bhambra U., "Polymorphisms in the cytochrome P450 CYP1A2 gene in colorectal cancer patients and controls: allele frequencies, linkage disequilibrium and influence on caffeine metabolism", British Journal of Clinical Pharmacology, 2003, Vol 55.

Michihiro Chida, Tsuyoshi Yokoi, "Detection of Three Genetic Polymorphisms in the 5'-Flanking Region and Intron 1 of Human CYP1A2 in the Japanese Population", Jpn. J. Cancer Res., 1999, Vol 90.

Fumihiro Sata MD., Andrea Sapone MD., "CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: Evidence for an allelic variant with altered catalytic activity", Clinical Pharmacology & Therapeutics, 1999, Vol 67

Licinio J., Wong M., "Pharmacogenomics", Wiley-VCH 2002.

National Centre for Biotechnology Information, http://www.ncbi.nlm.nih.gov/

Sanger Centre, http://www.sanger.ac.uk

Sanger Institute, http://www.ensembl.org/

Human Cytochrome P450 (CYP) Allele Nomenclature Committee, http://www.imm.ki.se/CYPalleles/

Children's Hospital Informatics Program, http://snpper.chip.org/