Chapter 14

Practical Pharmacogenetics and Single Nucleotide Polymorphisms (SNPs) in Renal Transplantation

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1. Introduction

Optimizing balance between therapeutic efficacy and the occurrence of adverse events is the main goal of individualized medicine. This takes even more importance in narrow therapeutic index drugs such as immunosuppressants. These drugs are highly effective in preventing acute graft rejection but tacrolimus, cyclosporine and mycophenolic acid show highly variable pharmacokinetics and pharmacodynamics. Still nowadays the fragile equilibrium between the risks and benefits of immunosuppression makes the management of immunosuppressive pharmacotherapy a challenge.

Therapeutic drug monitoring (TDM) is an essential and indispensable instrument for calcineurin inhibitors dosing, reducing the pharmacokinetic component of variability by controlling drug blood concentrations. But TDM is only possible once the drug is administered and steady state and patient's compliance are achieved, so complementary strategies are needed. Moreover, despite correct TDM, it may take several days or even weeks to reach target blood concentrations. For many patients this time periods are not appropriate in order to achieve sufficiently high concentrations to prevent graft rejection or adverse reactions or, on the other hand, without exposing the patient to excessive toxicity. In this sense, Pharmacogenetics is an interesting approach, helpful to manage immunosuppressant drugs. Changes in expression or function of proteins and enzymes involved in drug transport, metabolism or mechanism of action will cause changes in drug's absorption, metabolism and distribution and, therefore, can lead to changes in the response and toxicity of the treatment. Characterization of these genetic variants, mainly Single Nucleotide Polymorphisms (SNPs), can help to establish

effective doses and to minimize adverse effects. Many publications, including our own, have found statistically significant correlations between (SNPs) and tacrolimus and/or cyclosporine dose-corrected blood levels. There are also works correlating certain variants in SNPs with safety and efficacy of the treatment. Even some researchers, working groups and consortia recommend guidelines for initial dosing adjust regarding this SNPs.

Pharmacogenetic tests are becoming cheaper every day, so the cost of performing these assays is getting more assumable, especially when clinically relevant complications are demonstrated. The incorporation of pharmacogenetic studies to the real clinical practice will depend on the creation of well-designed sets of SNPs that, in a cost-effectiveness manner, could correlate clinical complications with genotypes, taking into consideration the whole and complicated treatment in polymedicated patients. Many results contribute to highlight the need of prospective controlled studies, with pharmacogenetic analysis prior to transplantation. This will probably be the critical point for the regulatory agencies to settle the most relevant polymorphisms as validated biomarkers to be widely used in the clinical transplantation setting.

For all this reasons, our aim in this chapter is to provide an easy explanation about what a polymorphism is and an updated view of the most relevant SNPs with evidence of their implication in safety and efficacy of immunosuppressive treatment in renal transplantation. The final goal is to give a summary from basic knowledge to concrete examples that help to improve the medical doctors' knowledge of the clinical impact of Pharmacogenetics in their daily practice.

2. Personalized medicine and pharmacogenetics

The term "Personalized Medicine" was not long ago some "scifi" concept, just expressing the best wishes of the scientific community with an aim of adjusting the pharmacotherapy as best as possible to each single patient. However, in the last years we have seen real advances in this area that have brought to the real clinical practice in most of the "first world" countries, a set of new analysis under the same principle: offering an individualized therapy to each different patient.

In order to understand this new approach in medicine and put it into practice, we necessarily have to take genetics in consideration, and particularly, we have to pay attention to the individual differences that make each patient respond in a different way to a given pharmacological treatment. Here, we arrive to the concepts of Pharmacogenetics and Pharmacogenomics, that can be heard in more and more places each day. They are, and for sure will be, components to be considered in the medical practice. We can define them in many ways, and traditionally they have been employed interchangeably although there are differences between them. They are different but complementary disciplines. The European Medicines Agency, EMA, takes their definitions from The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), this is a project with regulatory authorities from Europe, Japan and USA, together with experts from pharmaceut-

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icals that discusses technical and scientific aspects about the products registries. One of its aims is to reach a better harmonization in the interpretation and application of technical guides and requirements for the registries. ICH defines Pharmacogenomics as the study of variations in DNA and RNA characteristics regarding the response to drugs and it defines Pharmacogenetics as a subset inside pharmacogenomics, that studies the variations in the DNA sequence regarding the response to drugs [1-5].

There is another term that is also frequently found: biomarker, genetic or genomic biomarker, whose definition is "a measurable characteristic of DNA and/or RNA which is an indicator of a biological process that can be normal, pathogenic and/or a response to a therapeutic (or other kind) intervention. A genomic biomarker could be, for instance, the measuremente of a gene expression or of its regulation. It can consist in one or more DNA and/or RNA characteristics, as for instance, in DNA, its single nucleotide polymorphisms (SNPs); the variability in the repetition of short sequences; the haplotypes; DNA modifications as methilation; the deletions or insertions of a single nucleotide; the copy number variation; or the cytogenetic rearrangements as translocations, duplications, deletions or inversions. Regarding RNA, it could be a particular trait of its sequence; the levels of expression; the processing (as splicing and editing); or the levels of microRNAs. A deeper explanation of some of these terms will be done in the next paragraph.

The aim of pharmacogenomics is to identify the most important genetic elements in the instauration and/or evolution of a pathological process in order to create new strategies for drug evaluation and optimization of the drug development process. These are usually high throughput studies, regarding the simple number and statistical signification but also very exigent with the study subject: the final goal is finding correlation with the disease at a genomic level, not with one single nucleotide but with genes or groups of related genes instead. On the other side, pharmacogenetics studies the influence of genetic factors on the activity of a drug, making attention in concrete changes inside a gene that somehow has already been postulated as a candidate gene, by previous knowledge or by pharmacogenomic studies. Subject of pharmacogenetic studies are especially, the variants in genes related with transport and metabolism of drugs, with the aim that specific drugs can be given to specific groups of genetically defined (or "stratified") patients [6, 7].

To summarize, pharmacogenetics has to be considered as one of the mainstays of personalized medicine, which will let us correlate good or bad response to a drug in a specific population with genetic aspects. It will also let us know which drugs will offer greater therapeutic benefit or lower risk of adverse reactions development for a given population.

3. What are genetic polymorphisms?

We also must review some other basic concepts in genetics and, extensively, pharmacogenetics in order to understand the following information. The most relevant one is Polymorphism, which is defined as a mendelian monogenic character that appears in the population with the presence of more than one allele in the same genetic locus. Applying the term to pharmaco-

genetics, it makes reference to the different alleles or variants of a gene related to a drug interaction with the body. The frequency of the less common allele in the population must not be higher than 1%. The two main groups of genetic polymorphisms are Single Nucleotide Polymorphisms (SNPs) and Lenght Polymorphisms (repetitions of nucleotide groups). The first group represents 90% of genetic variability in our genome, and each nucleotide change appears approximately in 1 every 1000 nucleotides. Length polymorphisms represent more extensive changes in the DNA sequence and approximately are the remaining 10% of polymorphic variability in our genomes.

The NCBI SNP database (www.ncbi.nlm.nih.gov/snp) contains all the SNPs described, arranged by their Reference number, which names all SNPs starting with the letters “rs“, followed by a number code, but also including some classical names that had already been given to some SNPs. By clicking on a SNP code, one can get more information and several links, one of them is called “diversity“ and shows the different allele frequencies found depending on the study and especially, depending on the sample’s ethnicity. There are polymorphic sites with allelic frequencies quite well conserved amongst different ethnicities, but others have relevant differences and we must always pay attention to this point.

The exact biological difference in meaning between “polymorphism” and “mutation” is not always clearly defined. The term "mutation" is classically associated with pathological significance, while "polymorphism" usually refers to a genetic change without health consequences. The problem is that “polymorphism” has also been employed to describe mostly any newly described genetic variant, without having studied it enough to know if it has a pathological consequence or not. The international research project 1000 genomes (www.1000genomes.org) has been a great effort to sequence the whole genome of a thousand different people, so we are still attending to well quantified frequencies of genetic variants, that in some cases will still be measured in not sufficient people and so, knowing exactly the population frequencies of all our genome variants is still a challenge, moreover due to the fact that the frequencies vary amongst different human ethnicities. In conclusion, we must be cautious when interpreting the term “polymorphism” and not assume that it is just a genetic change without any biological consequences, as it may has not been well characterized yet.

The genetic variants that can influence the behavior of a drug in the body, are mainly related to the interaction of the drug with the receptor/ligand involved in their pharmacological action and/or with the systems involved in its pharmacokinetic process of absorption, distribution, metabolism and excretion. So, transport, metabolism and drug target genes are the three groups of genes whose polymorphisms are of interest in pharmacogenetics. In a very simplistic way, an individual carrying a significant polymorphic variant will suffer from different effects from those suffered by the individuals carrying the “normal” variant at the same polymorphic site, but just in the case of being treated with the particular drug affected by that variant. If that individual is not treated with that drug, he may not manifest any effects related to that polymorphism.

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Other relevant concepts to understand pharmacogenetics are Haplotipe and Linkage Disequilibrium (LD). Haplotype refers to those alleles of a chromosome, or part thereof, which are physically close and that tend to be inherited together. In our field, it is especially important that more and more frequently the research is focused not on single SNPs, but on combinations of them, forming haplotypes. Although many research has been simplified, studying SNPs analyzed one by one, the real biological significance of these genetic changes must be seen in the resulting effect of groups of SNPs, since the individual effects of each one can be enhanced, reduced or offset by the effects of others. In addition, the linkage disequilibrium, is the situation in which some alleles are present together in a higher frequency than expected, due to its close location in the chromosomes. This is important in SNPs research, since one can study a SNP that is well know and easy to determine, instead of studying another SNP linked to the first, that is more difficult to assess, and the results can be correlated. For instance, in some cases one SNP, with not known biological significance, is correlated with certain clinical consequence, and after a deeper research it is found that actually that first SNP is in fact in linkage disequilibrium with another SNP, unknown or non studied before, that is directly related to that clinical consequence.

In relation to these concepts, we can now understand that SNPs that have not got a clearly studied functional meaning, for example they do not alter the amino acid sequence or are not regulatory in intronic regions, are usually included in research projects. Maybe these SNPs are linked to others that are not taken into consideration but that do produce a direct effect on the gene product. These studies will be completed when information of LD blocks, provided for instance in public consultation databases as HapMap (www.hapmap.org), would be included. These final integrative approaches require powerful statistical and *in silico* analysis, correlating the large amount of information obtained.

4. Genes and drugs

After understanding the basic concepts, we can now enter the approach to the best known gene-drug relationships. There are currently different reference sources that help us in this welter of information, such as the aforementioned HapMap project, the SNP database of NCBI and, to our knowledge, the best pharmacogenetics website which is the Pharmacogenomics Knowledge Base, PharmGKB (www.pharmgkb.org). This latter website, is a very intuitive way of learning and consulting about gene-drug relationships, by performing searches based on gene, SNP, drug or disease; with research and clinical information, and lots of links to external related sites. There we can find a table of the "well-known drug-gene pharmacogenomics associations" which represents the drugs whose relationship with some polymorphic gene has been clearly defined in the literature and is academically accepted, based on extensive reviews of all available information.

The United States Food and Drug Administration (FDA, www.fda.gov) also publishes a list of drugs where a genetic test is recommended or mandatory for the drug administration, explaining which section of the drug label has the genetic-related information.

DRUG	BIOMARKER	DRUG	BIOMARKER
Abacavir	HLA-B*5701	Irinotecan	UGT1A1
Aripiprazole	CYP2D6	Isosorbide and Hydra-lazine62	NAT1, NAT2
Arsenic Trioxide	PML/RARα	Ivacaftor	CFTR
Atomoxetine	CYP2D6	Lapatinib	Her2/neu
Atorvastatin	LDL receptor	Lenalidomide	Chromosome 5q
Azathioprine	TPMT	Letrozole	ER &/ PgR receptor
Boceprevir	IL28B	Maraviroc	CCR5
Brentuximab Vedotin	CD30	Mercaptopurine	TPMT
Busulfan	Ph Chromosome	Metoprolol	CYP2D6
Capecitabine	DPD	Modafinil	CYP2D6
Carbamazepine	HLA-B*1502	Nilotinib	Ph Chromosome, UGT1A1
Carisoprodol	CYP2C19	Nortriptyline	CYP2D6
Carvedilol	CYP2D6	Omeprazole	CYP2C19
Celecoxib	CYP2C9	Panitumumab	EGFR, KRAS
Cetuximab	EGFR, KRAS	Pantoprazole	CYP2C19
Cevimeline	CYP2D6	Paroxetine	CYP2D6
Chlordiazepoxide and Amitriptyline	CYP2D6	Peginterferon alfa-2b	IL28B
Chloroquine	G6PD	Perphenazine	CYP2D6
Cisplatin	TPMT	Pertuzumab	Her2/neu
Citalopram	CYP2C19, CYP2D6	Phenytoin	HLA-B*1502
Clobazam	CYP2C19	Pimozide	CYP2D6
Clomiphene	Rh genotype	Prasugrel	CYP2C19
Clomipramine	CYP2D6	Pravastatin	ApoE2
Clopidogrel	CYP2C19	Propafenone	CYP2D6
Clozapine	CYP2D6	Propranolol	CYP2D6
Codeine	CYP2D6	Protriptyline	CYP2D6
Crizotinib	ALK	Quinidine	CYP2D6
Dapsone	G6PD	Rabeprazole	CYP2C19
Dasatinib	Ph Chromosome	Rasburicase	G6PD
Denileukin Diftitox	CD25	Rifampin, Isoniazid, and Pyrazinamide	NAT1; NAT2
Desipramine	CYP2D6	Risperidone	CYP2D6
Dexlansoprazole	CYP2C19, CYP1A2	Sodium Phenylacetate and Sodium Benzoate	UCD (NAGS; CPS; ASS; OTC; ASL; ARG)
Dextromethorphan and Quinidine	CYP2D6	Sodium Phenylbutyrate	UCD (NAGS; CPS; ASS; OTC; ASL; ARG)
Diazepam	CYP2C19	Tamoxifen	ER receptor
Doxepin	CYP2D6	Telaprevir	IL28B
Drospirenone and Ethinyl Estradiol	CYP2C19	Terbinafine	CYP2D6
Erlotinib	EGFR	Tetrabenazine	CYP2D6
Esomeprazole	CYP2C19	Thioguanine	TPMT
Everolimus	Her2/neu	Thioridazine	CYP2D6

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DRUG	BIOMARKER	DRUG	BIOMARKER
Exemestane	ER &/ PgR receptor	Ticagrelor	CYP2C19
Fluorouracil	DPD	Tolterodine	CYP2D6
Fluoxetine	CYP2D6	Tositumomab	CD20 antigen
Fluoxetine and Olanzapine	CYP2D6	Tramadol and Acetaminophen	CYP2D6
Flurbiprofen	CYP2C9	Trastuzumab	Her2/neu
Fluvoxamine	CYP2D6	Tretinoin	PML/RARα
Fulvestrant	ER receptor	Trimipramine	CYP2D6
Galantamine	CYP2D6	Valproic Acid	UCD (NAGS; CPS; ASS; OTC; ASL; ARG)
Gefitinib	EGFR	Vemurafenib	BRAF
Iloperidone	CYP2D6	Venlafaxine	CYP2D6
Imatinib	C-Kit, Ph Chromosome, PDGFR, FIP1L1-PDGFRα	Voriconazole	CYP2C19
Imipramine	CYP2D6	Warfarin	CYP2C9, VKORC1
Indacaterol	UGT1A1		

Table 1. FDA Pharmacogenomic biomarkers in drug labels (adapted from www.fda.gov)

There is certainly a lot of work done, but there is still much to do. Today, there are many publications and many research articles in the area, and the field is growing exponentially, but most of these studies reflect data from very specific conditions, where sets of patients with convenient features and sometimes far from the clinical reality, where included. It is necessary to validate the actual utility of pharmacogenetics in routine medical practice with serious, well-designed studies [8].

4.1. Genes and drugs in transplantation

In the pharmacogenetics of transplantation, as in other therapeutic areas, three groups of genes specifically involved in the response to immunosuppressive therapy have been identified: the genes encoding drug transporter proteins, inward or outward of the cells; the genes encoding metabolic enzymes involved in drug biotransformation and, finally; those encoding receptors or drug targets. Although the great majority of immunosuppressive drugs are transported and metabolized by a limited set of enzymes which mostly are known genes, the interpretation of the results observed in transplanted patients is complicated in many times. One reason for this is that these patients are highly subjected to polytherapy, and so interactions, both pharmacokinetic and pharmacodynamic, may have great significance and may condition the response to treatment. Another important aspect to consider when interpreting the observed response is the fact that each patient actually contains two different genetic entities: the donor and the recipient. This phenomenon is particularly relevant when the transplanted organs are the liver or the kidney. In these types of transplantation, it must be considered that the drugs administered to the recipient will be metabolized or excreted by the transplanted organ from the donor. In fact, more and more studies in transplantation pharmacogenetics consider both the donor and recipient genotypes to evaluate the response to treatment [9-12].

Moreover, one of the main problems of pharmacogenetic studies is the difficulty to recruit the number of patients needed to achieve sufficient statistical power and demonstrate conclusively the existence of significant clinically relevant differences according the different genotypes, that is, according to the different alleles or variants of a polymorphic site. This is due to the uneven distribution of allele frequencies in the population, which makes it difficult to collect a large enough number of individuals to study minor genotypes. Furthermore, the distribution of allelic frequencies in some genes varies according to ethnicity so, for instance, the expected frequencies of each allele of a polymorphic site in the Caucasian population are not the same as in the Asian. The expected effects of each allelic variant are presumably the same, but the ease for recruiting different genotype patients is not.

Figure 1 shows, in summary, an integrator scheme with the best known genes encoding transporter proteins and enzymes involved in the metabolism of several drugs commonly used in transplantation.

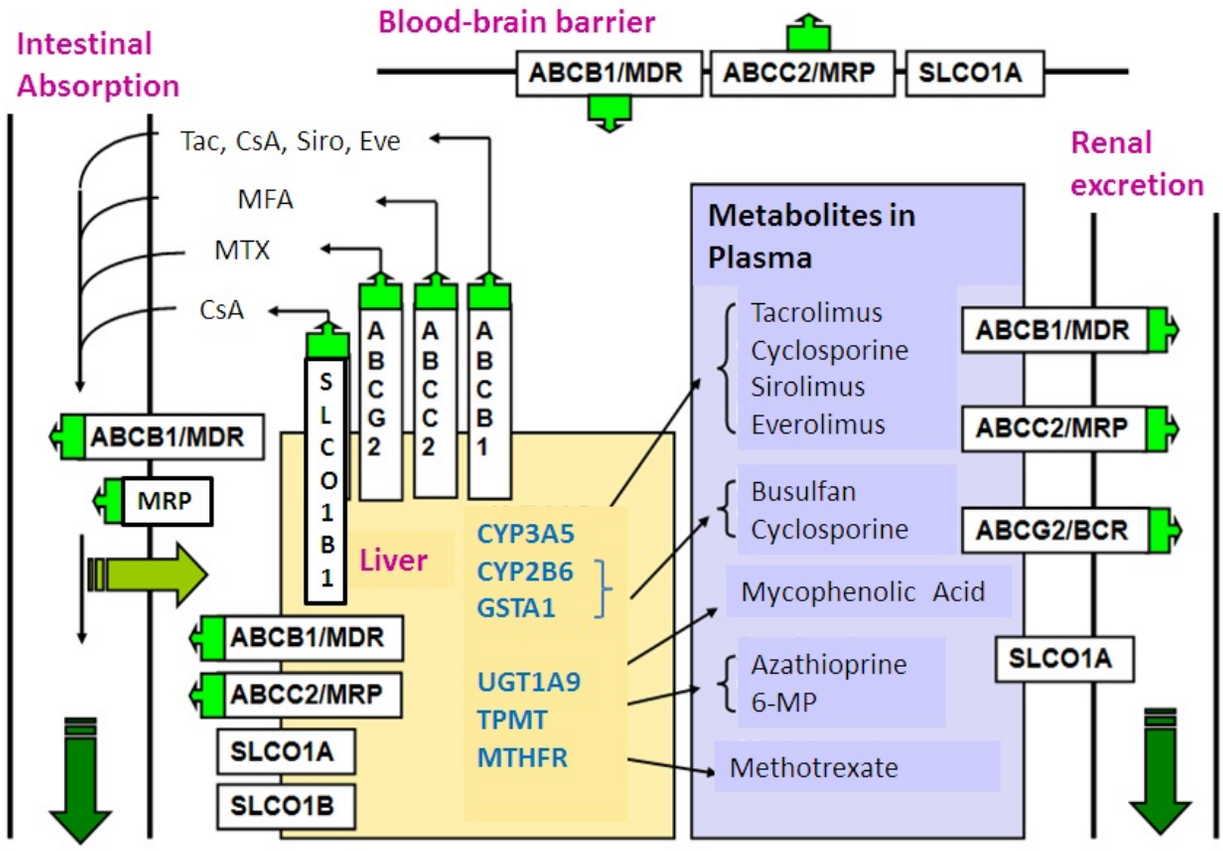

Figure 1. Integrative scheme of pharmacogenetic genes related to transport and metabolism of immunosuppressive drugs for transplantation. (Adapted from ref. 13, ©Astellas Pharma S.A. y Master Line & Prodigio S.L.). The drawing shows a broad view of the location and influence of metabolic enzymes and transporters on the main immunosuppressive drugs used in transplantation. The integrated view of many of the genetic factors that influence the achievement of therapeutic and stationary blood levels, should allow a better interpretation of pharmacogenetic data and also, help improve the safe and effective use of medication.

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4.2. Pharmacogenetic examples in renal transplantation

Pharmacogenetic information of immunosuppressants in renal transplantation is mainly related to Tacrolimus, Cyclosporine and Mycophenolic Acid. Sirolimus, Everolimus and Corticoids are also being studied but to a much lesser extent, so we will focus here on the most consolidated conclusion about the first three drugs.

The first two, being both Calcineurin Inhibitors (CNI), share their mechanism of action and so, share transporters, metabolism enzymes and targets and therefore, they also share pharmacogenetic results in most of the cases. The fact that they are both subject of a controlled therapeutic drug monitoring, with "in some way" standardized blood measuring methods, has allowed the publication of many works dealing with correlations between drug levels and polymorphisms [14-23]. To a lesser extent, there are also many works correlating drug adverse effects with SNPs [24-28]. The therapeutic drug monitoring of mycophenolic acid is not as followed as for CNIs, as there is not such a clear consensus about the effects of different blood levels in the possible drug related toxicity. However, many efforts have also been done in the pharmacogenetic studies of this drug [29-36], as it is widely employed in combination with tacrolimus or cyclosporine.

The most consensuated genes regarding polymorphic effects on these three immunosuppressants are shown in table 2.

DRUG	GENE	SNP	Effect
Tacrolimus Cyclosporine	ABCB1 *transport*	rs1045642 C>T; 3435 C>T	C: higher transporter activity, less drug absorption T: lower transporter activity, more drug absorption
	CYP3A5 *metabolism*	rs776746 A>G; *1 (A), *3 (G)	Allele *1 carriers have functional enzyme and require higher drug doses to reach target levels. Allele *3 carriers have nonfunctional allele, the enzyme is not metabolizing the drug, so they need lower doses
	CYP3A4 *metabolism*		Implications not clearly defined
Mycophenolic Acid	UGT1A9 *metabolism*	-275 T>A -2152 C>T	-275A and -2152T: Increased gene expression, lower exposition to MFA and acute rejection in patients with fixed dose MFA+Tac
	ABCC2 *transport*	C-24T C3972T	Implications not clearly defined
	IMPDH1 *target*	rs2278293	Higher risk of leucopenia, lower risk of BPAR
	IMPDH2 *target*	3757 T>C	C: higher IMPDH activity, higher incidence of BPAR (biopsy-confirmed acute rejection)
	SLCO1B1 *transport*	*5	Implications not clearly defined

Table 2. Most studied SNPs related to Tacrolimus, Cyclosporine and Mycophenolic Acid in Renal Transplantation.

As shown in the table, even in these SNPs that are the most extensively studied, the clinical implications are not always well established. Many more other SNPs are currently under

research consideration, being what we can call "candidates" to have a clinical meaning. Virtually, every polymorphism of a gene implicated in a drug's route of transport, metabolism or mechanism of action is a potential candidate to be investigated. Especially if the polymorphism is known to have a biological consequence on the gene product as for instance, if it is a polymorphism producing a premature STOP codon or a relevant aminoacid change.

Returning to the SNPs in table 2, we will pay attention now to ABCB1 and CYP3A5 most relevant results. In Figure 2, we can see a schematic example of what happens in the intestine epithelial cells, according to the SNP rs1045642 C>T (also known as 3435 C>T) in ABCB1 gene. This gene codes for glicoprotein P (gp-P)which is an adenosine triphosphate-dependent transporter, that pumps many endogenous substances and also xenobiotics, as drugs, outside of the cell. It is specifically expressed in the intestine, liver and kidney, amongst others, and also in several types of leukocytes so it is postulated to function as a protective barrier by actively extruding different compounds out of the cell, into the gut lumen, bile or urine. The expression of ABCB1 in the kidney plays an important role in the renal elimination of metabolic waste products and toxins. It seems like after renal injury, ABCB1 expression is upregulated, which may represent an adpative response in the renal regeneration process [37]. Parallelly, it has been shown that treatment with CNI induces ABCB1 expression both *in vivo* and *in vitro*, which could serve to protect the kidney from the injurious effects of CNIs by facilitating their extrusion. If we add to this, the polymorphic influence shown in figure 2, we can better understand that a failure to adequately upregulate ABCB1 expression or a constitutively low expression in renal cells (as for instance due to 3435 TT variant), could lead to intrarenal accumulation of CNIs and predispose patients to the occurrence of CNI-related nephrotoxicity [38].

Specifically, in renal transplantation, it has been found a correlation between the genotype of donors TT at this SNP and cyclosporine nephrotoxicity [40], while no consistent relationships were found according to the same SNP in the recipient.

Regarding CYP3A5, there is more statistical evidence, especially regarding its impact on CNIs blood levels and these findings have led to some clinical recommendations, as we will see in the next paragraph. Inside the CYP 450 family, the CYP3A subfamily metabolizes more than 50% of all drugs that are currently in use [41]. CYP3A5 is expressed in the small intestine and the liver but also in the kidney.

One of the most relevant studies regarding CYP3A5 SNP rs776746 A>G (*1 (A), *3 (G)) is the one published by Thervet et al. in 2010 [23]. It is a prospective randomized clinical trial that demonstrates the usefulness of this SNP determination before the first tacrolimus dose in renal transplantation. In this study, the pharmacokinetic parameters were correlated with the recipients' genotype and two arms were constructed, one with the classical management of the patients, adjusting tacrolimus doses according to TDM; and the second arm, where the initial dose was chosen according to a previous genetic analysis to include the patients in "CYP3A5 expresser" or "CYP3A5 non-expresser" cathegories. The expressers were given an initial 0.25mg/kg dose and the non-expressers 0.15mg/kg. As a result, the genetically driven dosage was associated with an earlier obtention of tacrolimus concentrations inside the therapeutic range, also with fewer dose adjustments. Also, it was demonstrated that in the first arm, patients with genotype *1 needed double tacrolimus dose to reach the target levels, as

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compared to those patients that were *3. Moreover, there are also consistent data in pediatric renal transplant recipients [42].

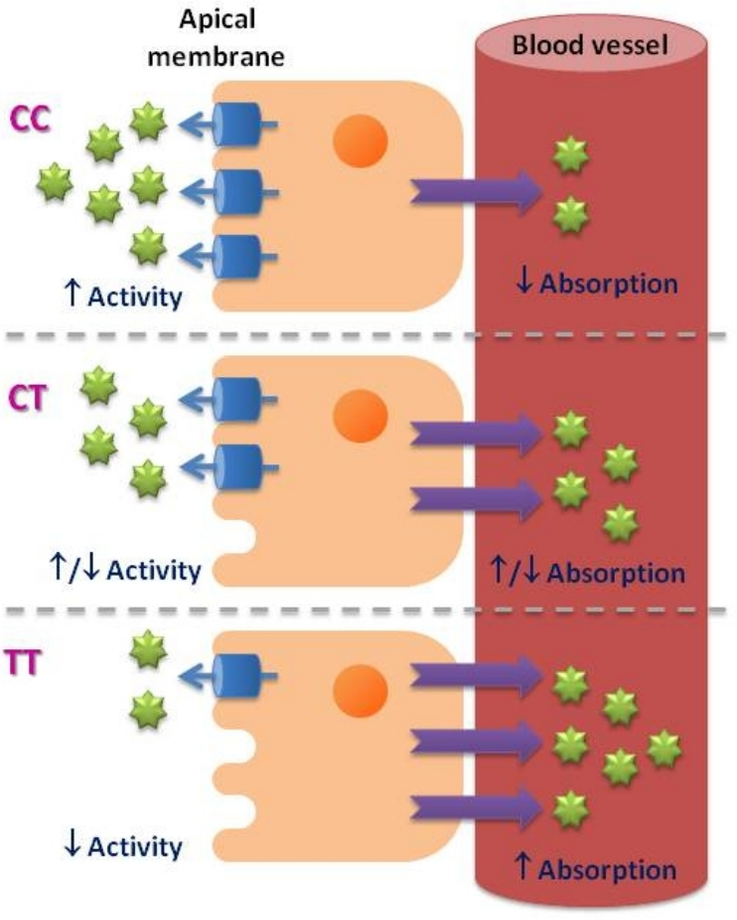

Figure 2. Influence of the functional activity of glycoprotein-P (transporter in apical membrane) in the transport of tacrolimus (green stars) in the intestine epithelium. The diagram shows the different degree of drug absorption due to variations in ABCB1/MDR1 polymorphic site rs1045642. Individuals with TT variant have a decreased transporter activity and hence greater absorption efficiency. CC variant causes more expulsion out of the cell, which decreases absorption. (Figure adapted from ref. 39)

Just as a final remark, we cannot forget to mention the great importance of drug interactions. Although it is not the subject of this chapter, and we are not going to get into it, we just wanted to point here that drug interactions can mask even genetic variations in the clinical practice.

5. Regulatory aspects and final conclusions

5.1. Clinical practice recommendations

We have only seen, with a little bit of detail, two of the SNPs that could actually be influencing the pharmacologic treatment in renal transplantation. And with these two SNPs, only one,

CYP3A5 rs776746, has reached some kind of clinical recommendations. These have not been adopted by any of the regulatory agencies FDA nor EMA, but they already have a strong evidence as to be considered by expert doctors in the area.

The 3rd European Science Foundation- University of Barcelona (ESF-UB) Conference in Biomedicine on Pharmacogenetics and Pharmacogenomics, held in June 2010 in Spain, published a summary of their practical recommendations [43] which include the explained tacrolimus results. They recommend CYP3A5 rs776746 genotyping prior to grafting as it could help to reach steady state plasma tacrolimus concentrations earlier, and therefore prevent overdose (risk of nephrotoxicity) or underdose (risk of acute graft rejection). This recommendation is mainly based on Thervet's publication [23] and suggests the introduction of tacrolimus at 0.15 mg/kg/day when the recpient's genotype is *3/*3, at 0.20 mg/kg/day when it is *3/*1, and at 0.25 mg/kg/day when it is *1/*1; always taking into consideration that the patients will also require the regular TDM.

The Dutch Pharmacogenetics Working Group Guideline from the Royal Dutch Pharmacists Association have also evaluated therapeutic dose recommendations for tacrolimus based on our CYP3A5 SNP [44] and have found evidence to support an interaction between the drug and the gene. However, they do not make dosing recommendations adducing that in dutch transplantation hospitals, the tacrolimus dose is titrated in response to TDM.

5.2. What is a meta-analysis and what is the need of them?

The number of publications is increasing every day in an accelerated manner to limit the ability of researchers and clinicians to assess critically and to assume the results of the studies. This, added to the fact that the knowledge about something is not born due to a single article, but to the integration of many, requires conducting systematic reviews of available evidence, of which there are two types:

- Qualitative systematic reviews, where evidence is presented descriptively
- Quantitative systematic reviews or meta-analyses, which combine the results in a single endpoint and determine the causes of the variations between studies.

Meta-analysis is a summary of different qualitative and quantitative studies (usually randomized controlled trials) that evaluate one aspect whose results are combined using statistic resources to determine the directionality of the effect and the causes of variability between studies. It is the gold standard tool to assess the consistency of an evidence in the effect of a particular intervention, especially when studies are heterogeneous and discordant, when studies evaluating outcomes affect a low number of patients (as it happens when reporting adverse events), when conducting new clinical trials is expensive or when we want to know the existence of patient subgroups responding differently to the intervention analyzed.

- In this way the results allow us to:
- Plan future clinical trials on a related topic.

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- Quantify publication bias (as many studies fail to be published because their results are not significant) [45, 46].
- Provide evidence to generate new hypotheses.
- Decide whether further clinical trials are needed on the subject.
- Help to document the approval of the use of interventions by regulatory agents and also expand the knowledge to academics.

Calculate the sample size needed for future clinical trials about a similar topic.

Conducting such studies is cumbersome, it is really time-consuming, requires complex methodological knowledge and its performance is not free of trouble. The main difficulties are the presence of a small number of previously existing studies, the fact that the selected studies to be analysed are usually very heterogeneous and difficult to be combined, and in many of them the necessary information is absent or with low methodological quality. However, meta-analysis studies are low-cost and have high impact.

However, we must be careful as the name "meta-analysis" does not ensure a quality review and readers should critically evaluate it before accepting its results, for which there are currently accessible guides [47, 48]. Its validity largely depends on the quality of the included studies and the absence of bias in its execution [49]. The studies analyzed in the meta-analysis are mostly randomized trials, which are those that offer the best evidence, but there are scenarios where the information comes only from observational studies [50], as studies on etiological hypotheses or adverse events. This represents a challenge as this type of design has a higher risk of bias and lack of essential information for the integration of studies [51]. Furthermore, the inclusion of studies with a large heterogeneity or variability between them, hinders the results interpretation [52], requires the knowledge of statistical tools for proper interpretation [53] and one must know that it is a limitation for the applicability of the results. Meta-analysis is a retrospective process, so it is susceptible to errors of this type of design. It could have biases in any of its stages: in the search and selection of studies, analysis and synthesis of information.

The meta-analysis is the highest level of evidence and summarizes the studies available about a particular matter in a reliable way. Its implementation has its difficulties and limitations, so methodological rigor is required to help reduce the risk of bias and a critical and cautious view of its results.

As far as we know, two meta-analyses have been published regarding clinical implications of CYP3A5 and CNIs in renal transplantation. One is about tacrolimus [54] and its conclusion agrees with the data explained about CYP3A5 expressers/non-expressers dose requirements. The other one deals with cyclosporine [55], and also concludes that there is an association between our SNP and cyclosporine dose-adjusted concentration, where patients carrying *3/*3 genotype will require a lower dose of the drug to reach target levels, compared with *1/*1 or *1/*3 carriers.

5.3. Barriers for the clinical application of Pharmacogenetics

The introduction in the medical practice of new strategies is always difficult, among other reasons due to economic factors and the inertia of much of the professional sector, which is typically conservative. But in the particular case of pharmacogenetics, and genomics in general, there are other social factors that we will now comment, that hinder the implementation of these new techniques.

Contrary to what has usually happened in other fields of biomedicine, in this one we have the paradox that technological progress has gone faster than the advancement of knowledge. Today's technology platforms can just bring in a few days the data that used to take months or even years to achieve. The advances in knowledge of the human genome sequence have been really quick, especially since in February 2001 Nature and Science published simultaneously the results of the Human Genome Project. The enormous progress in data collection through technology could not be accompanied by a corresponding advance in the association of the data with biological effects or implications for medical treatments [56]. A great amount of research investment is still necessary in order to understand and take advantage of this huge avalanche of data.

Clearly, every great discovery is preceded by circumstances that make it possible, and for the deciphering of the human genome, and overall progress of genomics, including Pharmacogenetics-omics, milestones were achieved with the confluence of three fundamental aspects: the opportunity of high performance technologies (high throughput), the multidisciplinary working groups and the development of bioinformatics.

Investment in technology and the big bet of different private companies have been crucial for the rapid performance rate of genetic sequences data collection. In fact, as we have already mentioned, more and more individual human sequences have been obtained, demonstrating the variability of our genome and even small errors in the initial sequencing generations. Anyway, such data cannot give us more information than little white dots on a blackboard, with sometimes very specific information on diseases or even just some predispositions, but little conclusive information for the moment. This is mainly due to two major keys in genetics and biology: the first is that rarely a single gene is responsible for a disease, usually diseases result from the interaction of many genes, with particular variants or defects. The second key is that our phenotype is not an exclusive product of the expression of our genes, instead it is the gene-environment combination. In most cases, the weight of each of the two components in a given disease is difficult to decipher.

Moreover, not only the gene-environment relationship offers serious knowledge gaps, but also the relationships between genes. Everyone knows that life is the result of Systems Biology, waterfalls of activation or repression of components that influence each other. That is the kind of approach that we have to tend to, once we have more experience and results in reductionist studies. Biological systems are complex networks of thousands of routes, many of which are interconnected, biosynthetic pathways, signal transduction pathways, routes of regulating the expression of genes. The integration, representation and modeling of the interconnections of biological information analysis require global, systemic analyses. This is how we enter the era

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of "omics" referring to global studies, "whole set", where we pass from the analysis of specific phenomena to the search of the interrelationship of phenomena, where we must integrate not only genomics but also proteomics (the sequences and expression patterns of all proteins), metabolomics (identification and quantification of all metabolites) and even transcriptomics (sequences and expression patterns of all transcripts) and to close the circle, reach the interactome (full set of physical interactions between proteins, DNA sequences and RNA). The review of T. Manolio [57] is very useful for understanding the current situation of genomic studies.

In relation to this need for training and knowledge, we will introduce one of the biggest problems facing the Pharmacogenetics and Pharmacogenomics application in our society: as in any area of knowledge that directly affects Health and Drugs, clinical applications arising from Pharmacogenetics should be well regulated and should be given proper use. Both the patient and the doctor must be well informed of the scope and meaning of the data that can be obtained. It is crucial to know what to expect of a pharmacogenetic analysis, realistically, without creating false hopes.

It is said that in a couple of years, the cost for sequencing a complete human genome will be about 1,000$. It is not difficult to imagine that there will soon be many patients who will consult their physicians with their genome sequence in their hand, asking whether they will have cancer or Alzheimer's or not, according to what is written in their genes. Are we prepared to deal with these situations? The New England Journal of Medicine published a series of articles and editorials addressing these issues on the occasion of the first decade of the publication of the human genome, with very interesting articles written by experts in the field, as the great review of Collins and coworkers [58].

And, if we are not prepared for this new tool yet, how will we discriminate between reliable and fraudulent information? Who should be responsible for setting common guidelines to drive us in making decisions about which tests are acceptable and which are not? We think that the key are not only the regulatory agencies, which do not always agree with each other when defining whether a marker is valid or not. However, other agents, Industry, and especially the scientific societies, should be the ones to influence education on these issues and serve as a reference under the most rigorous scientific method.

At the academic level, many efforts have been demanded to these disciplines because they generated great expectations that were not met as fast as expected. Now, regulatory agencies require a greater statistical significance than that of many other types of studies, to accept the validity of a new marker. That is why well designed clinical studies and meta-analyses are necessary for the agencies to accept new validated markers. We must also be aware of the alarm triggered in relation to commercial proposals that are clearly misleading the consumer. Just a quick search on the Internet to realize that they are on sale genotyping chips that offer scientifically implausible predictions, such as predicting vulnerability to sudden death in athletes, obesity, the ability to succeed at school, etc. The U.S. committee SACGHS (the Secretary's Advisory Committee on Genetics, Health and Society) has already issued several reports concerning with the issue and stressing the need to regulate this area of biomedicine in order to not leave the consumer completely unprotected. There are two excellent publications from Dr. JP Evans, illustrating this problem [59, 60].

5.4. The economical impact

Pharmacogenetics must allow not only the money saving through the prevention of a large number of side effects derived from the use of unsuitable drugs, but also it has to reduce the money spending on unnecessary drugs. In the U.S. there is an incidence of adverse effects of 6.2-6.7% of hospitalized patients, representing two million adverse drug reactions per year [61]. Of these, 0.15 to 0.3% are fatal, leading to about 100,000 deaths annually [62]. In Europe, the data are similar.

Today, there are still few studies on the cost-effectiveness of pharmacogenetic studies, although considerable efforts have been made, including regulatory authorities [63-65]. We cannot forget that everyday genotyping platforms are more compact and economical and, as mentioned, even the whole genome sequencing of a patient will have an assumable cost, so it is not difficult to imagine that the benefits will overcome the costs and that the balance will be tilted towards the realization of these studies [66]. A practical example is found in the studies of mutations in the K-ras gene in colorectal cancer patients to decide about treatment with Cetuximab, which are rendering large amounts of data regarding the savings thanks to the genotyping of patients, avoiding ineffective treatment in 40% of cases.

In conclusion, the economical and clinical benefits of pharmacogenetics are day by day, clearly surpassing its costs. We need to have especialized personnel, to help us know how to interpret the pharmacogenetic information, always in close contact with clinicians and research advances. We cannot obviate this new and real tool for the benefit of our patients' health.

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