

Short Communication

## Cyp3A5\*3 is Not a Pharmacogenetic Marker for Cyclosporine Treatment Response in Psoriasis

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### Abstract

Psoriasis is a chronic skin disease that affects 2-3% of the population worldwide, causing significant morbidity and high financial burden. Cyclosporine is considered to be first-line systemic therapy for moderate to severe psoriasis. However, patients exhibit heterogeneity in their response to therapy, which could be due to genetic factors. Recently, we have shown that the use of a genetic marker in ABCB1, the gene that encodes for P-glycoprotein which is responsible for the influx-efflux of active drug (cyclosporine) into target cells alone or synergistically with the drug-metabolizing enzyme CYP3A4, can predict the response of a 7% of the patients in treatment with cyclosporine. With the scope of increasing this prediction efficiency, in this report we have studied two well-known functional polymorphisms in CYP3A4 and CYP3A5 genes, which contribute around 40-90% of the metabolism of cyclosporine. While the frequencies of these polymorphisms agree with the ones reported in the literature, our results showed no statistically significant association between the polymorphisms rs2740574 and rs776746 and the response of psoriatic patients to treatment with cyclosporine. Thereby we suggest that these two polymorphisms should be further analysed in larger groups prior to their exclusion for use as pharmacogenetic markers for psoriasis-cyclosporine, under a standard dose of treatment, in the Greek/caucasian population.

**Keywords:** Psoriasis; Pharmacogenetics; Cyp3A; Cyclosporine; Polymorphisms

### Introduction

Cyclosporine is one of the most commonly used immunosuppressant in the treatment of moderate-to-severe psoriasis [1]. Cyclosporine suppresses the immune system and acts selec-

tively on T- cells by slowing down their growth [2]. At a dose of 3mg/kg/day, cyclosporine rapidly produces a PASI- 75 response in up to 70% of the patients. Moreover, cyclosporine has a narrow therapeutic window, with significant variability in its pharmacokinetic activity [3]. The oral bioavailabili-

ty and systemic clearance cyclosporine are controlled by the cytochrome P450 (CYP) isoenzymes CYP3A4 and CYP3A5 and by the efflux P-glycoprotein (P-gp), the protein product of the *ABCB1* gene. Many SNPs in *CYP3A4*, *CYP3A5* and *ABCB1* genes have been identified to account for the variability in the pharmacokinetics of cyclosporine [4]. Recently, we have reported that the use of a polymorphism in *ABCB1* can predict the response of a 7% of the patients in treatment with cyclosporine [5]. Aiming to increase this rate, we studied functional polymorphisms in *CYP3A4* and *CYP3A5* genes which contribute to 40-90% of the metabolism of cyclosporine.

## Recent Reports

CYP3A4\*1B and CYP3A5\*3 are the most recently reported CYP3A4 and CYP3A5 alleles to be associated with cyclosporine response. CYP3A4\*1B (rs2740574) is known to be the polymorphism that increases expression by changing the transcription factor binding affinity, whereas CYP3A5\*3 (rs776746) is well known as the polymorphism that causes severe decrease of enzyme activity by a splicing defect [6]. It has been reported that individuals with CYP3A4\*1B and CYP3A5\*3 show a higher and lower metabolic rate of cyclosporine, respectively. Therefore, the aim of this study was to investigate the possible association between CYP3A4 and CYP3A5 genes rs2740574 and rs776746 polymorphisms and response to treatment with cyclosporine.

## Materials and Methods

A total of 63 patients from General University Hospital of Larissa and Papageorgiou General Hospital of Thessaloniki, who agreed to donate blood samples for genetic testing, were included in our study. All patients included in this study were under a stable cyclosporine dose (3mg/kg/day). For genotype determination, genomic DNA was extracted from peripheral blood samples. To genotype the polymorphisms in CYP3A4 and CYP3A5 genes, the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was used according to Drogemoller and Rahsaz, respectively [7,8].

The PCR was carried out in 25µl solution consisting of 100ng DNA, 1.5mM dNTP, 15pmol each primer, 2mM MgCl<sub>2</sub> and 1U Taq polymerase. For the CYP3A4\*1B polymorphism, the following primers were used for amplification: F: 5'-GGA-CAG-CCA-TAG-AGA-CAA-GGG-GA-3'; R: 5'-GGC-TAT-GTG-CAT-GGA-GCT-TT-3'. Polymerase chain reaction amplification conditions were 3 minutes of initial denaturation at 94°C followed by 30 cycles of melting at 94°C for 15 seconds, annealing at 58°C for 15 seconds and elongation at 72°C for 30 seconds. The PCR products were digested with MboII which recognizes and digests when "A" allele is present and visualized on 6% acrylamide gel. For the CYP3A5\*3 polymorphism, the following primers were used for amplification: F: 5'-ATG-GAG-AGT-GGC-ATA-GGA-GAT-A-3'; R: 5'-TGT-GGT-CCA-AAC-GAA-GAA-ATA-3'.

Polymerase chain reaction amplification conditions were 10 minutes of initial denaturation at 94°C followed by 40 cycles of melting at 94°C for 30 seconds, annealing at 57°C for 45 seconds and elongation at 72°C for 30 seconds. The PCR products were digested with SspI which also digests when "A" allele is present and visualized on 3% agarose gel.

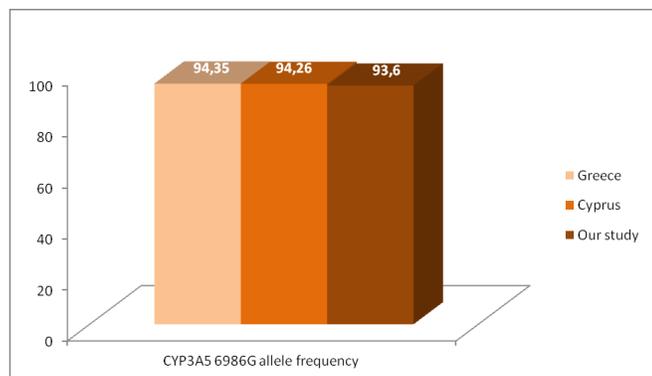
## Analysis

The mean age of the patients was 45.58 years. Among the participants 30 were male and 33 female confirming that the disease appears at the same frequency in both sexes. For the analysis of the polymorphisms, the patients were divided into responders and non-responders. Responders were defined as having a 75% reduction in Psoriasis Area and Severity Index from baseline (PASI 75) within 3 months of treatment. Non-responders were defined as not achieving a 50% reduction in PASI from baseline (PASI 50). Patients were grouped into homozygous and heterozygous for the polymorphisms. For Hardy-Weinberg equilibrium test, chi-square test was used, while for the association of the alleles on the response of the patients to treatment Cochran-Armitage test was used. All data were analyzed using the Statistical Package for Social Sciences (SPSS) version 14. Statistical significance was defined as a two-tailed P-value less than 0.05 (P<0.05). The polymorphism in the *CYP3A4* gene does not follow the Hardy-Weinberg equilibrium. For this reason the results from the study of this polymorphism cannot be statistically analyzed.

For the polymorphism in the CYP3A5 gene, the association between patient's genotype and the allele frequency with response to treatment after 3 months is shown in Table 1. In our study, in the CYP3A5 gene, 1.59% (n=1) of the patients are homozygous for the allele \*1, 88.9% (n=56) are homozygous for the allele \*3 and 9.52% (n=6) are heterozygous (\*1/\*3). The frequency of the allele \*3 in the population study is in agreement (~95%) to that reported in previous studies in the Greek population and in Caucasians [9,10] (Figure 1). This results to the conclusion that our sample is representative of the population.

**Table 1.** Association between patients genotype and response to cyclosporine therapy (n=63).

CYP3A5 Polymorphism	Test	Responders (PASI≥75%)	Non-responders (PASI<50%)	P value
CYP3A5	Genotypic [GG/GA/AA]	36/4/0	20/2/1	0.75
	Cochran-Armitage [G/A]	76/4 (0.95)	42/4 (0.954)	0.62
A6986G (rs776746)	Dominant [(GG+GA)/AA]	40/0	22/1	0.36
	Recessive [GG/(GA+AA)]	36/4	20/3	0.69



**Figure 1.** Frequency of the common allele of CYP3A5 A6986G in our study compared with two studies in the Greek population [9,10].

## Results

The results of this study showed no statistically significant association between the polymorphisms rs2740574 and rs776746 and the response of psoriatic patients to treatment with cyclosporine. As a result, these two polymorphisms should be taken with caution as pharmacogenetic markers for psoriasis-cyclosporine, under a standard dose of treatment, in the Greek population, while also our result should be replicated in larger samples prior to their exclusion as pharmacogenetic markers. However, one should also not exclude these markers from further studies in the context of dose-response, as in some cases the clinical daily practice includes dose ranges between 3 and 5 mg/kg/daily. An additional parameter for future studies is the inclusion in the analysis of cyclosporine plasma levels between study groups as they are known to influence the efficacy of cyclosporine therapy in other clinical settings. Thus, taking into account all the above one should have a good view of the role of these markers in the prognosis of the therapeutic response.

## References

- Rocío Prieto-Pérez, Teresa Cabaleiro, Esteban Daudén, Dolores Ochoa, et al. Genetics of Psoriasis and Pharmacogenetics of Biological Drugs. *Pharmacogenomics*. 2013, 14: 1623-1634.
- Fantini MC, Becker C, Kiesslich R, Neurath MF. Drug Insight: novel small molecules and drugs for immunosuppression. *Nature Clinical Practice Gastroenterology & Hepatology*. 2006, 3(11): 633-644.
- Moes DJ, Swen JJ, den Hartigh J, van der Straaten T, van der Heide JJ, et al. Effect of CYP3A4\*22, CYP3A5\*3, and CYP3A Combined Genotypes on Cyclosporine, Everolimus, and Tacrolimus Pharmacokinetics in Renal Transplantation. *CPT Pharmacometrics Syst. Pharmacol*. 2014, 12(3): e100.
- Jing Li, Martin H Bluth. Pharmacogenomics of drug metabolizing enzymes and transporters: implications for cancer therapy. *Pharmacogenomics and Personalized Medicine*. 2011, 4: 11-33.
- Vasilopoulos Y, Sarri C, Zafiriou E, Patsatsi A, Stamatis C et al. A pharmacogenetic study of ABCB1 polymorphisms and cyclosporine treatment response in patients with psoriasis in the Greek population. *Pharmacogenomics J*. 2014, 14(6): 523-525.
- Crettol S, Venetz JP, Fontana M, Aubert JD, Pascual M et al. CYP3A7, CYP3A5, CYP3A4, and ABCB1 Genetic Polymorphisms, Cyclosporine Concentration, and Dose Requirement in Transplant Recipients. *Ther Drug Monit*. 2008, 30(6): 689-99.
- Drögemöller B, Plummer M, Korkie L, Agenbag G, Dunaiski A et al. Characterization of the genetic variation present in CYP3A4 in three South African populations. *Front Genet*. 2013, 4: 17.
- Rahsaz M, Azarpira N, Nikeghbalian S, Aghdaie MH, Geramizadeh B et al. Association between tacrolimus concentration and genetic polymorphisms of CYP3A5 and ABCB1 during the early stage after liver transplant in an Iranian population. *Exp-Clin Transplant*. 2012, 10(1): 24-29.
- Arvanitidis K1, Ragia G, Iordanidou M, Kyriaki S, Xanthi A et al. Genetic polymorphisms of drug-metabolizing enzymes CYP2D6, CYP2C9, CYP2C19 and CYP3A5 in the Greek population. *Fundam Clin Pharmacol*. 2007, 21(4): 419-426.
- Despina Hadjipanagi, Stephanie Chrysanthou, Konstantinos Voskarides, Constantinos Deltas. Genetic polymorphisms in warfarin and tacrolimus-related genes VKORC1, CYP2C9 and CYP3A5 in the Greek-Cypriot population. *BMC Research Notes*. 2014, 7: 123.