Editorial

Pharmacogenomics of Methotrexate in Rheumatoid Arthritis: Does Race Make a Difference?

An increasing number of original reports suggest that common polymorphisms in the folate gene pathway are associated with methotrexate (MTX) effects in rheumatoid arthritis (RA)[1-5]. In this issue of *The Journal*, our respected colleagues from Washington University and University of North Carolina identified novel genetic risk factors for MTX induced toxicity in RA[6]. For this, Ranganathan and colleagues used a candidate-gene approach and measured by pyrosequencing a comprehensive set of 25 single nucleotide polymorphisms (SNP) in 6 genes associated with MTX pharmacokinetics [folylpolyglutamate synthase; ATP-binding cassette (ABC) transporters] and pharmacodynamics [methylene tetrahydrofolate reductase (MTHFR), and thymidylate synthase]. The authors should be acknowledged for the large number of genotypes measured in this large cohort of RA individuals. Interestingly, the authors employed a two-stage approach in which potential genetic associations were initially screened in a training cohort[7] and subsequently tested in a validation cohort. In addition, the authors (a priori) stratified their analysis by race, given that allele frequencies in SNP differed between Caucasian and African Americans. The conclusion that associations were race specific should be debated in detail.

Racial differences in folate status (and thus potentially MTX effects) have been investigated. One report from Perry and colleagues[8] suggested that African American women present with a lower folate status than their Caucasian counterparts, a finding consistent with a higher frequency of low birth weight and preterm births in Blacks versus Whites[9]. Another study from Gerhard and colleagues[10] suggested that the increased risk for coronary artery disease in Black women compared to White women was the result of increased homocysteine levels in the former group. However, population differences in folate status as they relate to MTX adverse events are potentially dependent on a variety of factors such as diet, lifestyle, education, and socioeconomic status, which may differ between African and Caucasian descent, and are independent of genetic background. Yet another consideration is that 85% of genetic variations between humans are found within individuals from the same population, with the remaining 15% of the total genetic diversity occurring between individuals from different populations[11]. Finally, it is important to remember that a simple phenotype such as skin color is controlled by a very limited number of genetic variants[12], while dozens if not hundreds of other genetic variants may contribute to an adverse event to MTX. In the present study, the authors justify the stratification between Africans and Caucasians, a priori, because there were racial differences in allele frequencies of SNP measured. However, the candidate SNP passing the screening step in the training cohort exhibited only minor or negligible differences in allelic frequency between Blacks and Whites (12% vs 33% for MTHFR C677T, 18% vs 49% for ABCB1 1236 C→T; 73% vs 62% for ABCC2 IVS 23+56 T→C, 2% vs 0% for ABCC2 1058 G→A). In addition, the adverse events data presented do not highlight major differences between ethnicities (the overall incidence of toxicity was 62% in African Americans vs 56% in Caucasians when the training and validation cohorts were combined), and MTHFR C677T was associated with some signs of toxicity in both Caucasians and African Americans. Thus, the stratification by race a priori and the conclusion that pharmacogenetic associations are race-specific are questionable.

However, some arguments in favor of a racial stratification should be discussed. First, given that African Americans have a lower frequency of thermolabile 677T variant than Caucasians, it would follow that African Americans should present a lower incidence of toxicity than Caucasians (if one leaves out potential confounding factors such as lifestyles). Thus, one cannot rule out the notion that genetic variants that are over or under represented in Blacks

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versus Whites may explain the lack of differential in the incidence of toxicity presented here, and the recent observation[13] that a T→C variant in exon 7 of MTHFR resulted in an increased rate of side effects in Blacks versus Whites concurs with the notion conveyed by authors. In addition, the concept that an adverse event to MTX is polygenic and far more complex than a more simple phenotype such as skin color (which is mostly controlled by variants in SLC24A5) does not take into account the possibility that genetic variants controlling folate homoeostasis and skin color may have co-segregated during evolution[14].

The authors appropriately acknowledge that racial stratification significantly decreased statistical power. I would add that the cross-validation design employed might have contributed to the uncertainty of the findings and may have in fact yielded artefactual race specific associations. Racial stratification and data splitting resulted in a very low number of African Americans evaluable in the training cohort (29 patients), thereby inflating the potential for type II error and the risk of missing "true" associations. Alternatively, the sample size in the Caucasian group was much higher (62 patients in the training cohort) than African Americans, and this imbalance might have increased the probability of detecting differences in Caucasians versus African Americans. Thus, the conclusion that those associations were significant in Caucasians but not in African Americans does not necessarily imply that the association was race specific. Clearly, larger cohorts controlling for statistical power and false discovery rate will be necessary to establish race specific contributions, if any.

Noteworthy, this study is the first to evaluate the contribution of germ-line polymorphisms in ABC transporters to low dose MTX effects in RA. MTX is a substrate for ABCC2, a transporter present in the luminal side of renal proximal tubular cells, and a recent report established the contribution of a promoter polymorphism (–24 C/T) in ABCC2 to MTX effects in white European females with acute lymphoblastic leukemia[15]. The polymorphism was associated with higher exposure to high-dose MTX and appeared to have clinical relevance as females with the variant required higher dose intensity of leucovorin to prevent toxicity. In the present study, the authors have not detected an association between the –24C/T variant and low-dose MTX toxicity in the training cohort, but have identified a novel association between MTX related adverse events (in Caucasians) and a transversion at position 56 of the 23rd intron of ABCC2 (IVS 23+56 T→C). In the training cohort, 60% of Caucasian carriers of the homozygous variant genotype presented with alopecia, while the incidence of this side effect was only 3% in those with the wild type or heterozygous genotypes. In the validation cohort, the authors detected an increased incidence of gastrointestinal toxicity (33%) in Caucasian patients with the homozygous variant genotype versus those with the wild type or heterozygous genotype (13%). This suggests that the trait conferred by the variant is recessive, and this was supported by similar drug survival rates in wild type and heterozygous (29 and 23 months) versus homozygous variants (2 months). However, the variant is silent (although possibly in linkage disequilibrium as discussed) and the association of the polymorphism with alopecia in the training cohort (which warranted its selection for further evaluation) was not significant in the validation cohort. Reciprocally, the association of the variant with gastrointestinal toxicity in the validation cohort was not significant in the original training cohort. Thus, the finding is equivocal with respect to the relevance of ABCC2 IVS 23+56 T→C to a specific trait. Whether the genetic association with alopecia, gastrointestinal toxicity, or either side effect holds true when both training and validation cohorts are combined (to increase sample size, and thus power) was not presented by the authors. Moving forward, this novel association needs to be replicated in independent cohorts, and the contribution (if any) of the variant to MTX serum levels, or alternatively, intracellular active MTX polyglutamates should help establish its relevance[16].

There are additional analyses that could be conducted in this large cohort of patients. First, the contribution of genetic polymorphisms in AICAR (aminoimidazole carboxamide ribonucleotide) transformylase has been consistently associated with MTX effects in various populations with autoimmune diseases[2,4,17,18], and it would be worthwhile to establish its relevance in this population. Second, other folate pathway SNP including MS A2756, MTRR A66G, and SHMT C1420T[2,4] could potentially be relevant to MTX toxicity in this cohort.

In conclusion, these novel data complement and expand beyond previous findings and provide evidence that additional common polymorphisms contribute to the overall phenotype of drug response, a finding consistent with the polygenic nature of drug response. However, well powered studies will be necessary before concluding that pharmacogenetic associations are race specific.

THIERRY DERVIEUX, PharmD, PhD,
Proprius Pharmaceuticals/Cypress Bioscience
12264 El Camino Real,
San Diego, California, USA

Address reprint requests to Dr. Dervieux.
E-mail: tdervieux@cypressbio.com

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