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To the editor:

Leu208Val and Ile181Leu variants of cytochrome P450 *CYP2C9* are not related to the acenocoumarol dose requirement in a Spanish population

Cytochrome P450 CYP2C9 is the principal catalyst of warfarin and acenocoumarol hydroxilation reactions in human liver microsomes.[1] There is a growing interest in the identification of genetic variants of cytochrome P450 *CYP2C9*, which can modify its ability to inactivate warfarin and acenocoumarol, since a reduced CYP2C9 activity on these drugs would put patients at risk of over anticoagulation and subsequent bleeding complications.[2] *2C9*2* (Arg144Cys) and *2C9*3* (Ile359Leu) variant alleles of this cytochrome have been associated with increased sensitivity to warfarin and acenocoumarol.[3-6] But the allelic frequencies for these variants differ considerably among different ethnic groups: Caucasians carry the *2C9*2* and *2C9*3* variants (8% to 20% and 6% to 10%, respectively) more frequently than Asians do (0% and 2% to 5%, respectively).[7] Recently, Leung et al[8] have identified several genetic polymorphisms of cytochrome P450 *CYP2C9* in a Chinese population. Two of them, Leu208Val and Ile181Leu, could have importance in the sensitivity to oral anticoagulant treatment in Chinese patients. Allele 208Val is more frequent than the Caucasian wild-type Leu208 in the Chinese population (75% heterozygotes and 19% homozygotes) and is associated with a lower warfarin dose requirement, which could explain why the Chinese population is more sensitive to warfarin than the Caucasian one. The Ile181Leu allelic variant was present in 9% of studied patients in heterozygous form and was associated with a higher warfarin dose requirement in that population.[8]

We have studied Leu208Val and Ile181Leu variants in 106 Spanish anticoagulated patients with a stable requirement for acenocoumarol to keep the international normalized ratio (INR) between 2 and 3.2 (41 patients required no more than 7 mg/wk acenocoumarol; 44 patients required between 7 mg/wk and 28 mg/wk; finally, 21 patients required more than 28 mg/wk). The population is described in detail elsewhere.[6] Genotyping for Leu208Val and Ile181Leu was done by polymerase chain reaction followed by digestion with restriction enzyme. Primers for genetic analysis were TGTGCTCCCTGCAATGTGATCTGGTC (forward) and TGGCCTTACCTGGATCCAGGGGCTGGTC (reverse). A forced mismatch was included in position 3 from the 3′ end of forward primer to create in combination with 527ATT>CTT (polymorphism Ile181Leu) a restriction site for *Nla*IV. The reverse primer also has a forced mismatch in position 3 from the 3′ end to create in combination with 608TTG>GTG (polymorphism Leu208Val) a restriction site for *Tsp*45I.

Neither Leu208Val nor the Ile181Leu variants were detected in any of the studied patients, indicating that neither of these genetic variants is involved in the variability of acenocoumarol requirement in this Spanish population: if these polymorphisms played a significant role in determining the acenocoumarol dosage in this population, we should have found some patients carrying the Leu208Val variant in the group with low acenocoumarol requirement and patients carrying the Ile181Leu variant in the high-dose group.

In conclusion, we demonstrate that the Leu208Val and the Ile181Leu polymorphisms of cytochrome P450 *CYP2C9* do not seem to play an important role in sensitivity to acenocoumarol in the Spanish population. Factors such as *2C9*2* and *2C9*3* variants of *CYP2C9*, age, sex, pharmacologic interactions, or associated diseases do not completely account for the interindividual differences in sensitivity to anticoagulant treatment. Therefore, it is probable that unknown genetic variants influencing the coumarin metabolism, which are perhaps different in different populations, will be described in the near future.

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