



## Letter to the Editor

**Reply to the commentary: Estimating genotype and allele frequencies of the CYP2D6 gene**


Mrs. Mónica Acuña (MT) and Eaton Lafayette commented on our manuscript [1], raising some important questions, which we would like to address.

There appears to be a misinterpretation of Tables 3 and 4. PCR-RFLP does not permit the location of more than one SNP each time, hence haplotypes cannot be directly determined. For example, a heterozygote subject for \*2 and also for \*4, could be \*2/\*4 or heterozygote for \*4K, because \*4K possesses the 2850C>T SNP (marker for \*2) and 1846G>A SNP (characteristic for \*4) [2]. Moreover, CYP2D6\*1 is not genotyped directly, and is assigned when no variants are detected. Variants not tested also default to a CYP2D6\*1 assignment and hence contribute to the frequencies reported for this allele [3]. Therefore, in pharmacogenetics it is common to accept “Not \*X” = \*1 (further elaboration on this issue can be found in Ref. [3], specifically Supplemental Table S1). We therefore presented results separately in Tables 3 and 4 for better understanding.

In Table 3, we do not determine CYP2D6\*1 because the inferred frequency should be calculated as “1 – (sum of variant allele frequencies)” [3]. We did not analyse all “Not \*1” alleles as more than a hundred are described, and it is thus not feasible to estimate the exact allele frequency for \*1 using genotyping techniques for individual variants.

Mrs. Acuña's comment “check the other stains for the same individual to see if she/he have one of both of these alleles” was actually addressed in our work. We analysed CYP2D6\*2 (2850C>T), \*3 (2549A>del), \*4 (1846G>A), \*17 (1023C>T) and the gene duplication in all subjects, and in Table 4 we show those subjects which resulted in more than one allele (volunteers 17–19, 2, 24 and 8). Additionally, our way to assign genotypes in this table was a representation of mixed genotypes, not showing a kind of “tetraploid” subjects, which is certainly not appropriate. In fact we did not use any genetic nomenclature to give these genotypes. This assignment was performed to give the results showing two genotypes determined in separated experiments.

Sample size calculations were performed considering the \*4 allele frequency (see 2.5. Statistical Analyses) [1]. We did not use \*2 allele because this is a default assignment, unless tested and discriminated for CYP2D6\*8, \*11, \*12, \*14, \*17, \*35, \*41 along with other variants [2,3]. This could also explain different frequencies between men and women for \*2/\*2, i.e. many assigned \*2 alleles by default could be other alleles with the same SNP (2850C>T).

Finally, we want to highlight that the goal of our study was not to characterize allele and genotype frequencies of the Chilean population, but to study phenotype-genotype relationships in a sample of Chilean people, not necessarily representative of the population. In this “mestizo” group it was relevant to estimate Amerindian-Caucasian admixture (using ABO blood distribution). The Amerindian component was estimated at 18%, and we considered redundant to report the Caucasian component (82%), as requested for Acuña and Lafayette.

## References

- [1] N. Varela, L.A. Quiñones, J. Stojanova, J. Garay, D. Cáceres, S. Cespedes, J. Sasso, C. Miranda, Characterization of the CYP2D6 drug metabolizing phenotypes of the Chilean mestizo population through polymorphism analyses, *Pharmacol. Res.* 101 (2015) 124–129, <http://dx.doi.org/10.1016/j.phrs.2015.07.020>.
- [2] The Human Cytochrome P450 (CYP) Allele Nomenclature Database. The Human Cytochrome P450 (CYP) Allele Nomenclature Committee. Editors Magnus Ingelman-Sundberg, Ann K Daly, Daniel W Nebert. <http://www.cypalleles.ki.se/cyp2d6.htm> (page was updated 18-Nov-2015).
- [3] J.K. Hicks, J.J. Swen, C.F. Thorn, K. Sangkuhl, E.D. Kharasch, V.L. Ellingrod, T.C. Skaar, D.J. Müller, A. Gaedigk, J.C. Stingl, Clinical Pharmacogenetics Implementation Consortium<sup>1</sup> Guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants, *Clin. Pharmacol. Ther.* 93 (5) (2013) 402–408, Nature publishing group. (Reference No. 8 in [1]). <sup>1</sup>Membership of Dr. L.A. Quiñones from 2015.

Nelson M. Varela<sup>a,b</sup>

Luis A. Quiñones<sup>a,\*</sup>

<sup>a</sup> Laboratory of Chemical Carcinogenesis and Pharmacogenetics, ICBM, Program of Molecular and Clinical Pharmacology, Faculty of Medicine, University of Chile, Chile

<sup>b</sup> Department of Medical Technology, Faculty of Medicine, University of Chile, Chile

\* Corresponding author at: Carlos Schachtebeck 299, Quinta Normal, P.O. Box 70111, Santiago 7, Chile.

E-mail address: [lquinone@med.uchile.cl](mailto:lquinone@med.uchile.cl) (L.A. Quiñones)

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