

LETTERS

## Comment on Gialluisi *et al*

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As authors of the first paper describing the methodology used by Gialluisi *et al* in their paper on the high allele frequency for Wilson disease in the Sardinian population, we want to congratulate them with their result.<sup>1,2</sup> The paper clearly shows the strength of this methodology. We were also particularly impressed by their conscientious approach to determine the inbreeding coefficient in this special population.

Still we want to draw attention to some inaccuracies in their paper, which—in our opinion—should be avoided by future users of this method, as they may result in underestimation of the gene frequency – ie, the total pathogenic allele frequency—and birth prevalence of the disorder. First of all, the authors disregard 14 mutations with relative frequencies below 1%. By including these, the gene frequency becomes 0.0195, instead of 0.0191. Second, the authors included only patients with unambiguous genotype and detailed geographical provenance of parents. However, leaving out seemingly heterozygous patients with a second, unidentified mutation will have the same effect on the estimation of the gene frequency as disregarding known mutations. Finally, the authors seem to have calculated the birth frequency of the disorder in Sardinia by simply squaring the gene

frequency. This can be justified in random mating populations but not in the Sardinian population where the inbreeding coefficient is higher than zero. For instance in the mountains, where the inbreeding coefficient is 0.00112344, and using the gene frequency estimate of Gialluisi *et al*<sup>2</sup> the birth prevalence of Wilson disease will be 1:2585 instead of the 1:2732 calculated by these authors. Using the gene frequency estimate that includes the 14 rare mutations, the prevalence estimate in the mountains even becomes 1:2499, – 11% higher than the original estimate.

We admit that each of our proposed corrections separately only has a small effect for the final estimate, but these effects are additive, and thus together not always insignificant. Therefore, we hope that by pointing to these inaccuracies future users of the methodology will be forewarned.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Ten Kate LP, Teeuw M, Henneman L, Cornel MC: Autosomal recessive disease in children of consanguineous parents: inferences from the proportion of compound heterozygotes. *J Comm Genet* 2010; **1**: 37–40.

2 Gialluisi A, Incollu S, Pippucci T *et al*: The homozygosity index (HI) reveals high allele frequency for Wilson disease in the Sardinian population. *Eur J Hum Genet* 2013; **21**: 1308–1311.

## Reply to ten Kate *et al*

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We thank Prof ten Kate *et al*<sup>1</sup> for the useful observations, which will allow us to clarify some aspects of the HI method.<sup>2,3</sup>

With regard to the inclusion of mutations with relative frequency below 1% in the formula, we may notice how these negligibly affect the final result, with a gene frequency increasing from 1.91 to 1.95%. This corresponds to an even smaller increase in the prevalence of the disease (from  $3.65 \times 10^{-4}$  to  $3.8 \times 10^{-4}$ , if we assume  $P = q^2$  to simplify).

With reference to the exclusion of heterozygous patients from the formula, this is a conservative choice that we made under the basic assumption of strict recessive inheritance in the model, as including them would mean to (erroneously) assume that they are all compound heterozygotes. We also underline that, in this specific study, every sample was first investigated for the six most common reported mutations in Sardinia, and then, if no mutation was found, by single-strand conformation polymorphisms and Sanger sequencing of all exons and of the flanking intronic regions in the *ATP7B* gene. This makes it highly unlikely that a mutation already found in the Sardinian population was undetected.

Finally, we agree that in a non-random mating population  $q \rightarrow P$  (instead of  $P = q^2$ , as per Hardy–Weinberg equilibrium). However, the current Sardinian population is characterized by high endogamy more than by high frequency of consanguineous matings. Under this scenario, also known as ‘random inbreeding’,<sup>4</sup> the chance that an individual will mate with a genetically related one will be higher, and will finally lead to  $P \approx q^2$ , as already discussed in a recent letter published in this Journal.<sup>5</sup>

Nonetheless, we acknowledge that this is an unavoidable approximation and we always mentioned the gene frequency ( $q$ ) of a recessive disorder instead of the Prevalence ( $P$ ) as the main output of the HI method, inferring  $P$  only when required for a comparison with other epidemiological methods.<sup>2,3</sup>

In summary, we agree with ten Kate *et al*<sup>1</sup> that all of these details only slightly affect the final result but we disagree that they will jointly create a big discrepancy between our  $q$  estimate and the real one, as also demonstrated by the factual equivalence between our  $q$  and the one estimated by Zappu *et al*<sup>6</sup> through a classical and reliable neonatal molecular screening. What is more important, the main purpose of the HI method is not to make a precise inference of the prevalence of a given recessive disorder in a population (as variables like the inbreeding coefficient can still be a source of error), but to produce ‘a ranking order of the prevalence of autosomal recessive disorders, thus establishing priorities for genetic testing at the population level’.<sup>2,3</sup>

Our wish is that the HI method becomes of common use by public health institutions, especially in those country characterized by highly