Anesthesiology 2007; 107:168–9

Basic Genetic Statistics Are Necessary in Studies of Functional Associations in Anesthesiology

To the Editor:—After several years of enthusiastic exploration of pharmacogenetics in anesthesia, the area may have come to a point where general standards of writing and statistic assessment are due to be adhered to.

Recently, significantly increased postoperative morphine requirements by Chinese female carriers of the μ-opioid receptor variant N40D (genetic polymorphism *OPRM1* 118A>G) have been reported in ANESTHESIOLOGY.[1] Among 80 women, patient-controlled analgesia morphine consumption in the first 24 h after abdominal hysterectomy was 27 ± 10 mg in the *OPRM1* 118AA group (n = 43), 29 ± 9 mg in the AG group (n = 19), and 33 ± 10 mg in the *OPRM1* 118GG group (n = 18), respectively.

Because no genetic selection criterion was specified,[1] the reader may assume a random sample of subjects enrolled by criteria other than the *OPRM1* 118A>G polymorphism. A genetic association study performed in a random sample provides information about both the magnitude of the genetic effect and the frequency at which this genetic modulation may be expected to occur in a particular patient population.

However, a random genetic sample has a distribution of homozygous and heterozygous carriers of the variant and wild-type alleles that corresponds to the Hardy-Weinberg equilibrium. That is, on the basis of the observed allelic frequency, the expected number of homozygous and heterozygous carriers of the alleles is given by $p^2 + 2pq + q^2 = 1$, where p and q are defined as the probabilities of occurrence for the dominant and mutated alleles, respectively.[2] In this study population,[1] the allelic frequency of the variant *OPRM1* 118G allele can be calculated from the number of variant alleles and the total number of alleles, *i.e.*,

$$\text{allelic frequency} = \frac{\text{number of variant alleles}}{\text{total number of alleles}}.$$

With 43 nonmutated subjects (having zero copies of the mutated 118G allele), 19 heterozygous subjects (each having one copy of the 118G allele), and 18 homozygous subjects (each having two copies of the 118G allele), amounting to a total of 80 subjects (with two alleles per person, regardless of whether these alleles are 118A or G), this leads to

$$\text{allelic frequency} = \frac{0 \times 43 + 1 \times 19 + 2 \times 18}{2 \times 80} = 0.344.$$

The frequency of the dominant 118A allele was thus $1 - 0.344 = 0.656$. Using the Hardy-Weinberg equation,[2] the expected frequency of homozygous carriers of the wild-type *OPRM1* 118A allele was $p^2 = 0.431$, that of heterozygous carriers was $2pq = 0.451$, and that of homozygous carriers of the variant *OPRM1* 118G allele was $q^2 = 0.118$. Comparing this expected frequency distribution with the observed frequencies by means of a chi-square goodness-of-fit test[3] shows that the observations differ from the expectations to a statistically highly significant degree ($\chi^2 = 17.95$, $df = 2$, $P = 0.000126$). According to the Hardy-Weinberg law, 34 homozygous carriers of the dominant 118A allele, 36 heterozygous carriers of the 118A and G alleles, and 9 homozygous carriers of the variant 118G allele should be expected, the numbers resulting from the calculated fractions for each genotype ($0.431 + 0.451 + 0.118 = 1$) and the total count of 80 patients.

Assuming correct genetic diagnosis of each DNA sample and its origin from nonrelated subjects, a violation of the Hardy-Weinberg equilibrium may be the result of inbreeding, assortative mating, or a small size of the population from which the sample has been drawn. If these reasons are unlikely, selection of the subjects by genotype is a possible explanation for the noncorrespondence with the Hardy-Weinberg law.

While increasing the number of subjects carrying the rare genotype is often applied to reach enough statistical power to study a genetic functional association, the distribution of the variant and dominant alleles in those studies may not be used for comparisons with other populations. Therefore, by selecting subjects for their genotype, the comparability of the sample with allelic frequencies in other populations is sacrificed for increased statistical power to detect a genetic functional association. Allelic frequencies can only be compared among populations if the samples from which they are obtained comply with the Hardy-Weinberg law as a basic statistical property of genetic random samples.

For example, in an association study of the *OPRM1* 118A>G SNP with the effects of morphine in Caucasians,[4] the number of 10 noncarriers, 4 heterozygous carriers, and 6 homozygous carriers of the variant 118G allele would correspond to an allelic frequency of the variant of 0.4. This is in disagreement with the observed allelic frequencies in Caucasians of 12.1%[5] or 18.8%[6] and would correspond to the allelic frequency in Thai and Malay populations.[7] However, because this was a selected sample as opposed to a random sample, comparison with other populations is invalid. Moreover, the distribution of noncarriers, heterozygous carriers, and homozygous carriers of the 118G allele[4] does not correspond to the Hardy-Weinberg law ($\chi^2 = 6.81$, $df = 2$, $P = 0.033$).

For the same reason of nonadherence to the Hardy-Weinberg law, interethnic comparisons of the distribution of the 118G allele based on the sample of 43 noncarriers, 19 heterozygous carriers, and 18 homozygous carriers of the variant 118G allele, respectively,[1] are invalid. This is not undermined by the correspondence of the allelic frequency of 0.344[1] with previous reports. Specifically, the cited comparative allelic frequencies of 0.351 to 0.474 in Malay, Chinese, and Indian populations[7] (the control samples in that report) were obtained from samples that explicitly complied with the Hardy-Weinberg equilibrium. Moreover, the cited comparative allelic frequency in Caucasians of 18.8%[6] was derived from 121, 52, and 8 noncarriers, heterozygous carriers, and homozygous carriers, respectively, of the *OPRM1* 118G allele, which also agreed with the Hardy-Weinberg law ($\chi^2 = 0.64$, $df = 2$, $P = 0.72$). In addition, a previous study reporting a similar observation of higher morphine needs by carriers of the *OPRM1* 118G allele observed in 78 noncarriers, 17 heterozygous carriers, and 4 homozygous carriers of the variant 118G allele, respectively,[8] was also performed in a random sample that agreed with the prediction of the frequencies from the Hardy-Weinberg law ($\chi^2 = 4.87$, $df = 2$, $P = 0.09$). In contrast, a parallel report of increased morphine consumption for analgesia after total knee arthroplasty by *OPRM1* 118G carriers,[9] where 74, 33, and 13 noncarriers, heterozygous carriers, and homozygous carriers of the 118G allele, respectively, did not agree with the expectation of the frequencies from the Hardy-Weinberg law of 0.569, 0.371, and 0.060, respectively ($\chi^2 = 8.13$, $df = 2$, $P = 0.017$), from which 68, 44, and 7 noncarriers, heterozygous carriers, and

homozygous carriers, respectively, of the *OPRM1* 118G allele would have been expected.

On the other hand, the compliance with the predictions from the Hardy-Weinberg equilibrium does not necessarily make a selected sample a random sample. In a study on the consequences of the G118 allele for the effects of morphine, performed in selected Caucasian subjects consisting of six noncarriers of the *OPRM1* 118G allele, four heterozygous carriers, and two homozygous carriers,[10] the distribution of the genotypes corresponded to the Hardy-Weinberg equilibrium ($\chi^2 = 2.3$, $df = 2$, $P = 0.317$). Nevertheless, the allelic frequency of 22.2% cannot be compared with other reported frequencies of the *OPRM1* 118G allele in Caucasians because by recruitment policy, the sample from which it originates was not a random sample.

To ease comparisons between study populations and to not to burden the reader with calculations of basic genetic statistics, studies of genetics in anesthesia should contain standard genetic statistics and precise information about recruitment procedure of the subjects with respect to genotypes. Violations of the Hardy-Weinberg law should be commented on, and preferably, in those cases, the correctness of the genetic screening should be double checked to avoid violations of distribution predictions due to assay error.

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(Accepted for publication February 19, 2007.)

Anesthesiology 2007; 107:169

In Reply:—We thank Dr. Lötsch for his comments regarding our work.[1] Strictly speaking, his comments for the Hardy-Weinberg equilibrium are correct, but they may not apply to our study. For the Hardy-Weinberg equations to apply, several conditions must be met. No mutations can be occurring, no natural selection pressures must be operating, the population of interest is infinitely large, all members of the population must breed and mating is totally random, and there must be no in or out migration of the populations.* There are several situations in regard to our study sample that may violate one or more of these conditions.

First, we worked with a "convenience" (nonrandom), small (80-subject) sample. Because these were surgical patients (and only women), this sample may not be representative of the "infinite population."

Second, because our patients were drawn from a limited geographic area, they may not be representative of the population as a whole. We cannot exclude the possibility that inbreeding (or in/out migration) may have occurred; in small human groups, such factors cannot be controlled nor easily identified.

Third, the locus of interest is autosomal (males and females have similar allele frequency).

Fourth, because we know so little about the evolutionary impact of these alleles, it is impossible to know whether evolutionary pressures exist within our population or our sample. It is entirely possible that different genotypes in the human opioid receptor A118G polymorphism may convey different degrees of "evolutionary fitness."

If the assumptions underlying the Hardy-Weinberg equilibrium are violated, statistical methods using allele frequencies may not be valid, and methods that use genotype frequencies should be preferred.[2]

In summary, although we thank Dr. Lötsch for his thoughtful comments, we do not believe that they invalidate the basic observations contained in our study.

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(Accepted for publication February 19, 2007.)

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