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SMN Dosage Analysis and Risk Assessment for Spinal Muscular Atrophy

To the Editor:

Feldkötter et al. (2002) recently reported a new method to determine, on the basis of real-time, quantitative PCR, copy numbers of *SMN1* (MIM 600354) and *SMN2* (MIM 601627). Their method allows a greater degree of automation and a faster turnaround time than do methods that have been described elsewhere (McAndrew et al. 1997; Chen et al. 1999; Wirth et al. 1999; Gérard et al. 2000; Scheffer et al. 2000; Ogino et al. 2001). Using their new method, they demonstrated that the copy number of *SMN2*—which is the centromeric homologue of *SMN1,* the disease gene for spinal muscular atrophy (SMA [MIM 253300 for type I; MIM 253550 for type II; and MIM 253400 for type III])—influences the severity of SMA in affected individuals with homozygous deletions of *SMN1*. They found that, the greater the copy number of *SMN2* was, the greater the likelihood was of a milder SMA type. Because this correlation is not absolute, they used Bayesian-type analyses to determine the posterior probabilities of developing each SMA type, with both a homozygous deletion of *SMN1* and a given copy number of *SMN2*. We discuss below several important ethical, prognostic, and technical issues raised in their article.

In table 6, Feldkötter et al. report "Probabilities That an Unaffected Who Has Been Tested after Birth and Has Been Found to Carry a Homozygous Absence of SMN1 Will Develop Type I, II, or III SMA, on the Basis of Number of SMN2 Copies." SMA is usually a childhood-onset disease, and testing of unaffected children is ethically problematic. We agree with the American Society of Human Genetics and the American College of Medical Genetics that "Timely medical benefit to the child should be the primary justification for genetic testing in children and adolescents" (American Society of Human Genetics Board of Directors and American College of Medical Genetics Board of Directors 1995, p. 1233). Since there are currently no effective treatments, presymptomatic or otherwise, for SMA, the timely medical benefit of the testing of unaffected children is unclear.

For the purpose of predicting SMA type from the *SMN2* copy number in unaffected children who lack *SMN1,* Feldkötter et al. perform Bayesian-type analyses by use of odds ratios, rather than conventional conditional probabilities. For the prior probabilities, they use the distribution of types of SMA among individuals affected with SMA: .51, for type I; .32, for type II; and .17, for type III. Even if one were to test unaffected children in this way, for this purpose, these prior probabilities would not be the correct ones to use for Bayesian or Bayesian-type analyses. If a child is asymptomatic at age 10 mo, for example, he or she is much less likely to have type I SMA than to have one of the other types (Zerres and Rudnik-Schöneborn 1995). One would have to incorporate the conditional probabilities of being asymptomatic at a particular age, for the hypothesis of each SMA type.

The data on *SMN2* copy number given by Feldkötter et al. could be used in prenatal testing, to predict SMA type. However, the prior probabilities that they use would be applicable only if the family history of SMA is of an unknown type. Although families with more than one type of SMA have been described—and are far from rare—knowing the type of SMA in an affected family member increases the prior probability of that type of SMA in a relative who is at risk of developing SMA. If the type of SMA in that affected family member is unknown, then the distribution of SMA types among all individuals with SMA would be relevant to the assignment of prior probabilities.

On the basis of all reported data, Feldkötter et al. state that, because two *SMN1* copies were found on 20/834 (2.4%) healthy chromosomes, "4.8% of normal individuals would be misinterpreted as noncarriers on the basis of the direct SMN1 test" (p. 365). Actually, these data imply that ~4.8% of noncarriers would have three copies of *SMN1* and that ~2.4% of carriers with an *SMN1* deletion on one chromosome 5 would have two *SMN1* copies on the other chromosome 5. We have referred to the latter as the "2 + 0" genotype (Chen et al. 1999). Taking into account the ~1.7% of carriers who

have an intragenic mutation undetectable as an *SMN1* exon 7 deletion, Feldkötter et al. state that this "reduces the sensitivity of the test to 93.5% for a person from the general population" (p. 365). Combining the ~1.7% of carriers who have an intragenic mutation with the ~2.4% (i.e., 0.024 × [1 − 0.017]) of carriers who have the 2 + 0 genotype gives the overall sensitivity of *SMN* dosage analysis for the detection of SMA carriers in the general population as ~95.9%. If an affected family member were known to have a homozygous deletion of *SMN1,* then the sensitivity of *SMN* dosage analysis for the detection of carriers among unaffected family members would be ~97.6% (i.e., 0.959/[1 − 0.017]). This is because the probability of an intragenic-mutation carrier in this family is greatly decreased relative to the probability of a 2 + 0 carrier (Ogino et al., in press).

Updating our combined data (McAndrew et al. 1997; Ogino et al. 2002, in press) gives 23 of 590 normal chromosomes 5 that have two copies of *SMN1*. Combining these data with those of Feldkötter et al. gives a total of 37 of 1,120 (3.3%) normal chromosomes 5 that have two copies of *SMN1*. We excluded other data in the literature (Wirth et al. 1999; Gérard et al. 2000; Scheffer et al. 2000), for reasons described elsewhere (Ogino et al., in press). On the basis of these numbers, ~3.2% (i.e., 0.033 × [1–0.017]) of carriers would have the 2 + 0 genotype. Therefore, the sensitivity of *SMN* dosage analysis for the detection of carriers in the general population would be ~95.1%, and that for the detection of carriers in a family with an affected individual lacking *SMN1* would be ~96.7% (i.e., 0.951/[1 − 0.017]).

Taking advantage of the single nucleotide differences between *SMN1* and *SMN2* in both exon 7 and intron 7, Feldkötter et al. used gene-specific primer pairs to amplify only *SMN1* or only *SMN2*. The primer pairs for each gene were mismatched for the other gene at either the final or the penultimate nucleotide from the 3′ end. These mismatches corresponded to the sequence differences in exon 7 (forward primers) and intron 7 (reverse primers). Gene conversions between *SMN1* and *SMN2,* which have been reviewed elsewhere (Burghes 1997), could potentially complicate this approach. If the *SMN1* exon 7 sequence (C) were converted to the *SMN2* exon 7 sequence (T) but the *SMN1* intron 7 sequence remained the same, the converted gene would presumably function as an *SMN2* gene in vivo. This is because the C→T transition in exon 7 of *SMN2,* although translationally silent, decreases the activity of an exonic splicing enhancer, so that less full-length protein is expressed (Lorson et al. 1999; Monani et al. 1999; Jong et al. 2000). By use of the gene-specific primers given by Feldkötter et al., the converted gene might have a different amplification efficiency from that of the normal *SMN1* or *SMN2* gene. Primers that are allele specific only for the functionally important polymorphism in exon 7 but not for the polymorphism in intron 7 might alleviate this problem.

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Electronic-Database Information

Accession numbers and the URL for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for type I SMA [MIM 253300], type II SMA [MIM 253550], type III SMA [MIM 253400], *SMN1* [MIM 600354], and *SMN2* [MIM 601627])

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Reply to Ogino and Wilson

To the Editor:

Drs. Ogino and Wilson (2002 [in this issue]) raised some issues regarding our paper on quantitative testing of *SMN1* and *SMN2* in spinal muscular atrophy (SMA) (Feldkötter et al. 2002). First, they raised some ethical issues regarding the testing of unaffected children for SMA. We are also aware of the controversial aspects of such testing and, in general, agree with Drs. Ogino and Wilson: the identification, at birth, of homozygous absence of *SMN1* in children, followed by the quantitative analysis of *SMN2,* should be offered as a prognostic tool only when a therapy for SMA is available. In this case, a newborn screening (similar to that in phenylketonuria) could—and possibly should—be considered. Since several drugs that up-regulate full-length *SMN2* have been found (Andreassi et al. 2001; Chang et al. 2001) and since the identification of many more is in progress, the development of a therapy for SMA seems likely to become a reality in the near future. Therefore, the development of a highly sensitive and fast method to determine the number of *SMN2* copies will be an essential prerequisite before starting a therapy. Furthermore, the identification, immediately after birth, of children who carry homozygous absence of *SMN1* will be equally essential, to start the therapy before the motor neurons are degenerated. On the basis of the number of *SMN2* copies, the dosage and starting-point of a therapy may significantly vary.

Since an efficient therapy has to be started early, we calculated the posterior probability that a child with an *SMN1* deletion would develop type I, type II, or type III SMA, under the assumption that the analysis is done immediately after birth. As a consequence, we have used a Bayesian-type analysis that is based on the odds ratios and a priori probabilities as chosen.

We reevaluated the sensitivity calculations, and we agree with Drs. Ogino and Wilson that the sensitivity of the test, for the detection of an SMA carrier from the general population without family history, is 95.9% (i.e., $1 - [0.024 + 0.017]$), since 2.4% of carriers have two *SMN1* copies per chromosome and 1.7% carry intragenic *SMN1* mutations. Therefore, there is a posterior probability of ~1:850 (i.e., $[4.1/100] \times [1/35]$) that a person from the general population who carries two *SMN1* copies is an SMA carrier. The carrier frequency of 1:35 is based on the results presented in our previous article (Feldkötter et al. 2002). The sensitivity of the test for the detection of an SMA carrier from a family with an affected patient who carries a homozygous absence of *SMN1* is 97.6% (i.e., $1 - 0.024$).

With reference to the primers designed to detect either *SMN1* or *SMN2,* the test is based on two nucleotide differences in exon 7 and in intron 7 (position +100). This implies that converted *SMN* genes may amplify with a decreased efficiency. At this point, it is important to mention that, in the large majority (42/44 [~95%]) of converted *SMN* genes, the complete gene, except for the region containing the nucleotide difference in exon 8, is converted (Hahnen et al. 1996). This means that, for most converted *SMN* genes, the two primers that we have applied lie in either *SMN1* or *SMN2* only and will not hamper the efficiency of the PCR. Additionally, the analysis of 20 patients with only homozygous absence of *SMN1* exon 7 showed identical number of *SMN2* copies analyzed with both methods—multiplex competitive PCR (Wirth et al. 1999) and LightCycler PCR (Feldkötter et al. 2002). Nevertheless, the efficiency of the PCR may be reduced for those rarely observed *SMN*