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Reply to Croes et al.

To the Editor:

Croes et al. (2000 [in this issue]) make the point that genetic testing as a diagnostic tool shows poor performance in differential diagnosis in general medical practice. We fully agree with this comment. Therefore, one of the goals of our study (Finckh et al. 2000) was to establish criteria that would increase the chance of identifying a pathogenic mutation in the setting of a specialized clinic. Indeed, among patients who had both onset at an early age and positive family history for early-onset dementia (EOD), diagnostic sequencing identified disease-relevant mutations in >50% of the patients analyzed by us. Another notable result of our study was the finding of four prion mutations among the 36 EOD patients, which suggested that atypical forms of prion disease may remain underdiagnosed. This assumption is supported by independent observations, such as those made by two coauthors of the letter by Croes et al. (2000), who found a *PRNP* insertion mutation in a patient with both prion disease and ante mortem diagnosis of familial Alzheimer disease (FAD) (Dermaut et al. 1998).

We agree with Croes et al. that assessment of the relevance of previously unknown mutations is a difficult issue. Nonetheless, in recent screening studies of FAD, 72%–83% of the sequence changes corresponded to pathogenic mutations already reported (Kamimura et al. 1998; Campion et al. 1999). In our study, 58% of the mutations had been previously described by others. Repeated identification of any given rare mutation in a rare disorder, together with the absence of the mutation in control probands, significantly increases the likelihood that it has causative effects.

We were pleased to see that Croes et al. agree with our conclusion that E318G in PS1 is a nonpathogenic polymorphism and that they reemphasize the importance of a careful and critical analysis of the literature. The importance of early and disease-specific diagnosis of EOD as a way of identifying treatable forms of dementia is an issue separate from our assertion that diagnostic sequencing of the four known EOD genes may provide important information for proper clinical and genetic counseling in the early phases of the disorder.

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The Efficiency of Pooling in the Detection of Rare Mutations

To the Editor:

After citing a variety of uses of pooled testing in genetic studies, Amos et al. (2000) suggested that mutations in individual patients could be detected more efficiently by being tested in pools. A typical mutation-detection protocol requires that many segments of the gene—for example, an amplicon consisting of one or a few close exons—need to be evaluated for detection of a mutation. Thus, even if the mutation has a prevalence of ~2%, as in the case of *BRCA1* or *BRCA2* in Ashkenazim (Hartge et al. 1999), the probability that any segment will contain a mutation is much smaller, perhaps on the order of .0005–.005. The use of pools or groups of samples to identify individuals or to estimate the prevalence of such a rare characteristic has been extensively studied in the statistical literature (Dorfman 1943; Sobel and Elashoff 1972; Gastwirth and Hammick 1989; Tu et al. 1995; Brookmeyer 1999). Using the corrected formula (see the erratum by Amos et al. [in this issue]) for the number of runs or tests needed to identify individuals with a mutation, one can fully appreciate the potential of pooling methods. A variant of the grouping procedure is described that in some circumstances leads to greater gains in efficiency when grouped testing is utilized.

The sensitivity of an assay—that is, the probability that a mutation will be detected, given that at least one member of the pool has it—is a potential limiting factor in practice. For screening of individuals to determine their carrier status, the sensitivity should be as close as possible to 100%. For detection of mutations by multiplex single-nucleotide primer extension, 100% sensitivity was achieved in pools of size 10–20 but dropped to 80% in pools of 30 (Krook et al. 1992). When denaturing high-performance liquid chromatography was used to identify *BRCA* mutations, 100% sensitivity was observed for several amplicons studied in groups of size five to nine (J. Rutter, personal communication). Thus, for the largest pool size for which a mutation detector is 100% sensitive, it is helpful to know the largest mutation prevalence for which pooling is efficient.

Suppose that the prevalence of a mutation in a single unit (exon or amplicon) being studied is π and that n individuals donate samples. For pools of size r, the probability, γ, that at least one member of the pool has a mutation is $1 - (1 - \pi)^r$. Assume that the test is 100% accurate in classifying a pool as having or not having a mutation. Since Y, the number of runs or tests that need to be done without pooling is n, for any pooling protocol in which the ratio of the expected value (y) of $Y{:}n < 1$, the strategy saves runs. We denote this ratio by F, for fraction of tests required relative to individual testing; and the efficiency of a pooling method is $1 - F$, the fraction of tests saved. When the classical single-stage pooling method (Dorfman 1943), which retests, one at a time, the individuals in a positive pool, is used, the expected number of runs needed to completely identify all the mutations in the segment under study in the sample of n individuals is

$$E(Y) = \left(\frac{n}{r}\right) + n\gamma \ . \qquad (1)$$

The derivation follows. The probability that a pool contains a mutation, which implies that it will test positive, is γ. Since all r individuals in the pool will be tested, a positive pool receives a total of $r + 1$ tests. The probability that a pool is negative is $(1 - \gamma)$. Those pools are classified with one test, so the expected number of tests per pool is $(r + 1)\gamma + (1 - \gamma) = 1 + \gamma r$. Since there are $\frac{n}{r}$ pools, the expected number of tests is given by equation (1). Note that the prevalence, π, enters into equation (1) because it determines the probability, γ, that a pool is positive.

Amos et al. (2000) also considered the situation in which there is a probability β, of a false-positive result in a pool—that is, $1 - \beta$ is the specificity of the mutation-detection process while the sensitivity remains perfect. The same reasoning that led to equation (1) shows that the expected number, y, of runs or tests is given by

$$y = n\left\{\frac{1}{r} + [1 - (1 - \beta)(1 - \pi)^r]\right\} . \qquad (2)$$

From equations (1) and (2), we can calculate the range of values of π for which the ratio of the expected number, y, of tests or runs (Y) to the total sample size, n, is <1, which implies that pooling is at least as efficient as individual testing. We also present the largest π value, $\pi_{.5}$, for which $\frac{y}{n} < .5$, which indicates that pooling will result in a substantial savings in the ex-