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INVITED EDITORIAL
The FRAXE Syndrome: Is It Time for Routine Screening?

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The accompanying article by Knight et al. (1996) presents important new information on the apparently low prevalence of the FRAXE syndrome. Their overall conclusion—that the syndrome was relatively rare among the developmentally delayed population studied—is consistent with results from several other smaller studies. Knight et al. found 1 case of a FRAXE mutation in 896 males tested. This yields a prevalence of 0.11% for this developmentally delayed population, whose prior cytogenetic fragility status was either unknown (60%) or negative (40%). Among an additional 15 samples (8 female and 7 male) that were known to be cytogenetically positive but negative by DNA for fragile X (FRAXA), one additional FRAXE family was also identified. A previous report in the *Journal* (Allingham-Hawkins and Ray 1995) found 0/300 FRAXE mutations among a similarly referred developmentally delayed male population. Holden et al. (in press) screened 298 institutionalized severely affected males and also 115 noninstitutionalized mildly affected males (J. J. A. Holden, personal communication), finding none positive for FRAXE. A similar screening of 150 males, by Murgia, identified one FRAXE case (Tranebjaerg et al., in press). Another screening, by Wang et al. (1993), of 425 males identified 1 FRAXE and 12 FRAXA mutations. Combining these figures gives a FRAXE prevalence of 3/2,184 (0.14%) among males referred for screening because of developmental disability and suspicion of fragile X. With the exception of the report by Wang et al. (1993), the FRAXA prevalence in the populations studied has not been noted. To help determine accurately the expected ratio of the two mutations, future screening studies should report both when they are done on the same populations. We have found the prevalence of fragile X among similarly referred populations by DNA testing to be ~4% (33/806), but we have not routinely screened for FRAXE (Brown et al. 1993, and in press). This 4% figure is similar to previous results of 14 cytogenetic-based prevalence studies (Sherman, in press), where the overall prevalence of fragile X had a mean of 3.7% among 3,971 unselected retarded males. Overall, these studies imply that the expected ratio of FRAXE to FRAXA is ~0.14/3.7 (3.8%). In other words, the expected prevalence of FRAXE is likely to be <4% of the fragile X syndrome prevalence. Thus, the clinical diagnostic laboratory might expect to identify <1 FRAXE case for every 25 fragile X cases identified, if all referred samples are tested for both mutations.

The true prevalence of fragile X in the general population is still somewhat uncertain. We previously estimated that the prevalence figure for affected males was ~1/1,250 (Brown 1990; Brown and Jenkins 1992), on the basis of two large epidemiological studies with complete ascertainment and with correction for those who refused testing (Gustavson et al. 1986; Webb et al. 1986). Turner and Webb have revised their estimated prevalence downward, to a minimum of ~1/4,000—or ~1/3,300, after correcting for those who refused testing—since they have found that nearly 50% of the subjects previously diagnosed as positive by cytogenetics are negative by DNA (Turner et al., in press). This indicates that cytogenetic testing with a threshold of 2%–3% is likely to be overly sensitive, because, in addition to FRAXA, it likely detects nonspecific fragile sites, as well as FRAXE and FRAXF (a fragile site with no associated phenotype). However, their corrected estimate of 1/3,300 is likely to underestimate as well, since some molecularly positive subjects are cytogenetically negative (Sklower Brooks et al. 1991). Rousseau et al. (1995) recently estimated the expected prevalence of full-mutation fragile X males to be a minimum of 1/1,500–1/2,500, on the basis of screening for premutation-only carrier women in Quebec and finding a prevalence of 1/259. Since founding-chromosome effects were clearly present in Quebec and are likely present in many populations, as is perhaps most clearly apparent in Finland (Oudet et al. 1993; Haataja et al. 1994; Zhong et al., in press-*b*), the true prevalence is likely to vary somewhat from population to population. However, if the prevalence of full-mutation males in the general population is ~1/2,000, which also is consistent with the gen-

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eral impression that, among males, Down syndrome is perhaps two- to threefold more common than fragile X, then we might anticipate that the prevalence of FRAXE would be ~4% of FRAXA, or 1/50,000 males.

Perhaps screening of populations with certain special phenotypes would yield a higher frequency of FRAXE mutations. But what phenotype should be screened? Compared with FRAXA, the phenotype of FRAXE seems relatively mild and lacks dysmorphic features. Within large FRAXE families, there does appear to be an excess of nonspecific mental retardation after removal of index cases (Knight et al. 1994; Mulley et al. 1995). Most IQ tests of identified males have been reported to be low normal to mildly retarded, with an average of ~70 (Mulley et al. 1995). But the range is broad, and many are apparently normal, which could reflect tissue mosaicism or the lack of accurate psychometric testing. Speech delay, along with learning, behavioral, and some psychiatric problems, appears to be the most common psychological profile (Mulley et al. 1995). Females with the full expansion usually appear unaffected or mildly affected, but they may have a higher incidence of anxiety disorders or psychiatric disabilities.

Most FRAXE subjects have been identified by follow-up testing of cytogenetically positive, molecularly negative individuals such as Knight et al. (1996) report. We have identified 2 FRAXE families in this way, in a total of ~350 identified FRAXA families. The proband in the first family was speech delayed and hyperactive but now is in a normal school. The affected individual in the second family was only mildly retarded. We have screened for FRAXE mutations in 459 normal controls and in 206 with FRAXA mutations and have found none among the controls. Among those with FRAXA mutations, we found one with both a FRAXA expansion and a FRAXE microdeletion (Zhong et al., in press-*a;* N. Zhong, personal communication). Other studies of control populations have not identified FRAXE mutations either (Knight et al. 1994). Perhaps it will be necessary to screen a very large number of random blood samples for FRAXE, as was done for FRAXA by Rousseau et al. (1995). However, as the above analysis indicates, the number would indeed have to be very large to obtain reliability. Wang et al. (1995) have proposed a method for PCR screening that detects only normal sized alleles but that is fairly cost effective. Perhaps such approaches might reveal higher prevalence in certain populations, such as those with speech delay. However, speech delay is very common, and, because of the low frequency of FRAXE mutations in controls, it seems unlikely to be a common finding in this population. Perhaps a better definition of the FRAXE phenotype is needed in order to select a population with a higher likelihood of mutations.

Toward this end it will be important to understand the function of the missing FRAXE gene product. Recently progress has been made in this regard. A CpG island was initially associated with the expanded CGG-triplet repeat (Knight et al. 1993), indicating that a gene was probably nearby. A candidate FRAXE gene, *FMR2,* has recently been cloned independently by the laboratories of Davies, Sutherland, and Nelson (Chakrabarti et al. 1996; Gecz et al., in press; Gu et al., in press). The 3-year delay in finding this gene resulted from difficulties in identifying a transcribed region, since the first intron is >150 kb! The *FMR2* gene is the closest distal gene to *FMR1,* located some 600 kb away. Because of its expanded CGG-triplet repeat and its position adjacent to *FMR1,* an attractive hypothesis was that it might have arisen by a gene-duplication event. However, this does not appear to be the case. It is still surprising that two genes with CGG repeats are located next to each other, while the closest identified gene proximal to *FMR1,* designated *CDR34* (Chen et al. 1990), is ~10 Mbp away! Perhaps, as we have suggested, this implies that there is a commonly acting *cis* factor near FRAXA and FRAXE, creating localized repeat instability (Brown et al. 1996). The *FMR2* coding sequence does not resemble the *FMR1* family of genes, which currently includes two other highly homologous but autosomal genes, *FXR1* and *FXR2,* all with highly conserved RNA-binding-function motifs (Coy et al. 1995; Siomi et al. 1995; Zhang et al. 1995). Rather, it is most similar to a presumptive DNA transcription factor, *MLL* or *AF*-4, which has ubiquitous expression but unknown function (McCabe et al. 1992). *FMR2* expression appears to be highest in placenta, lung, and the brain amygdala and hippocampus (Chakrabarti et al. 1996). The site of highest brain expression is intriguing, since it may link the phenotype to memory and behavioral problems.

For the present, since most all FRAXE mutations have been found among subjects who have had a positive fragile X chromosome test but a negative FRAXA DNA analysis, a few reference or research laboratories with a specific interest in this gene can probably handle the demand for this type of follow-up testing. Thus, in view of its low apparent prevalence and mild phenotype, for the present it seems that there is no need to undertake routine FRAXE screening.

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