Clinical significance of tri-nucleotide repeats in Fragile X testing: A clarification of American College of Medical Genetics guidelines

To the Editor:

The purpose of this letter is to reconcile a discrepancy between two documents issued by the American College of Medical Genetics: the Technical Standards and Guidelines for Frag-

ile X testing published in 2001[1] and updated in 2005[2] and the Genetics Practice Guidelines statement on diagnostic and carrier testing for Fragile X syndrome published in 2005.[3] In the Practice Guidelines, a broad range of 41–60 trinucleotide repeats was described for the intermediate or "gray zone" in Fragile X syndrome based on a research context. That is, research groups used this broader range to identify high-risk alleles. More relevant to the clinical setting, a range of 45–54 trinucleotide repeats was quoted for the gray zone in the Technical Standards and Guidelines publication. For a summary of these ranges please see Table 1.

Differences in the intermediate range then led to discrepancies in the reported ranges for Fragile X premutations. In the Practice Guidelines, the premutation range is characterized as 61–200 repeats, whereas in the Technical Standards and Guidelines, the premutation range is defined as 55–200 repeats. The American College of Obstetricians and Gynecologists based their committee opinion on the ACMG Practice Guidelines, leading to confusion among physicians in interpretation of Fragile X test reports. The ranges for intermediate and premutation Fragile X alleles quoted in the 2005 Practice Guidelines have never been used in laboratory practice. After an extensive review of the literature in 2005, the Quality Assurance Committee of the ACMG determined that no changes were required to the ranges originally published in 2001.

In a recent article summarizing two multidisciplinary workshops focused on reproductive counseling for FMR1 premutation carriers, Wittenberger et al.[5] defined the four allelic forms of *FMR1* with respect to CGG repeat size. They stated that consensus has been reached, both in the literature and in the workshops regarding the size of the premutation at 55–200 repeats, and the full mutation at >200 repeats and these ranges agree with those in the Technical Standards and Guidelines as summarized in Table 1. Wittenberger et al. also stated that consensus has not yet been reached for the lower limit of the intermediate or gray zone (i.e., 45–54 repeats or 40–54 repeats).

The clinical significance of intermediate and low premutation size alleles is 3-fold. First, it is the extent to which they may be prone to instability, particularly expansion, in future generations. At the present time, the smallest repeat known to expand to a full mutation in one generation is 59 CGGs.[6,7] Recognizing this and the fact that there is variation between laboratories and between laboratory methods when determining the exact CGG repeat number, the Laboratory Technical Standards and Guidelines place the boundaries of the premutation range at 55 and 200 CGG repeats. Quality Assurance challenges through the College of American Pathologists have shown that repeat lengths sized using polymerase chain reaction–based techniques can vary by ±3–4 repeats. The Technical Standards and Guidelines allowed for this variation in choosing 55 repeats as the lower limit of the premutation range to avoid missing any women at risk for having a child with the Fragile X syndrome.

Second, the clinical significance is the extent to which these repeat size alleles increase the risk for premutation-associated Fragile X tremor ataxia syndrome (FXTAS). FXTAS is a late-onset neurodegenerative disorder with predominant features of cerebellar ataxia and intention tremor. Onset is usually in persons older than 50 years. The risk and/or severity of the disorder is associated with repeat size, the highest risk being associated with larger repeats. Among individuals with late-onset cerebellar ataxia, the prevalence of premutation alleles was 13 times greater than expected based on its prevalence in the general population as assessed by a recent meta-analysis.[8]

Lastly, the clinical significance of intermediate/low premutation repeat size alleles is the extent to which they impose a risk for premutation-associated ovarian insufficiency. The prevalence of premature ovarian failure (POF) or cessation of menses before 40 years of age is about 20%, although it is highly associated with repeat size: the risk seems to increase with increasing premutation repeat size between 59 and 99, thereafter the risk of POF plateaus or even decreases for women with repeat sizes over 100.[9] Premutation carriers have been identified in about 3% of women with sporadic POF and in about 12% of women with familial POF.[10]

Thus, at this point, the risk and/or severity of all three disorders associated with premutation alleles (i.e., instability during transmission, FXTAS and POF) is established for alleles 55–200 repeats. The risk among the alleles in the lower part of this range, 55–70 is significantly lower than that in the upper range, 70–200, for all three disorders.

Table 2 shows the distribution of repeats among the allelic forms of *FMR1* between 41 repeats and 200 repeats as defined in the two conflicting ACMG publications. The table demonstrates that, were genetic counseling to be based on the Practice

Table 1
Comparison of the CGG repeat length ranges for each allelic class as defined by the four reports

Interpretation	Technical standards[2]	Practice guidelines[3]	ACOG committee opinion[4]	Wittenberger et al. [5]
Unaffected	<45	<41	<41	<45
Intermediate, gray zone	45–54	41–60	41–60	45–54
Premutation	55–200	61–200	61–200	55–200
Full mutation	>200	>200	>200	>200

Table 2
Comparison of the clinical interpretation of each allelic class by the two sets of guidelines

No. repeats	Interpretation according to technical standards and guidelines	Interpretation according to practice guidelines
41–44	Unaffected	Intermediate, grayzone
45–54	Intermediate, grayzone	Intermediate, grayzone
55–60	Premutation	Intermediate, grayzone
61–200	Premutation	Premutation

Guidelines, individuals with 59 and 60 repeats, who are at risk to have an affected child in the next generation, would not be counseled appropriately. Furthermore, a greater number of patients would be identified to have intermediate or gray zone alleles. As stated above, carrying the label of intermediate or gray zone currently has no established clinical significance and may cause unwarranted concern to families.

In conclusion, the Quality Assurance Committee and the Professional Practice and Guidelines Committee of the ACMG have determined that no changes are required to the ranges published originally in 2001[1] and restated in 2005 in the Technical Standards and Guidelines for Fragile X testing.[2] The ACMG Quality Assurance Committee and the Professional Practice and Guidelines Committee recommend that the following ranges for CGG repeat size be used in the laboratory as well as in clinical practice:

Unaffected: <45
Intermediate: 45–54
Premutation: 55–200
Full mutation: >200

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