Letter to the Editor

Is Fragile X Syndrome a Risk Factor for Dizygotic Twinning?

To the Editor:

Several researchers have looked for an association between fragile X (FRAXA) syndrome and dizygotic (DZ) twinning. Fryns [1986] reported a 4-fold increase in twinning in a study of 134 obligate FRAXA carriers, while Turner et al. [1994] noted a twinning rate of one in 11 in 253 carriers of the FRAXA premutation. On the other hand, Sherman et al. [1988] found no excess of twins in fragile X pedigrees compared to twins in hemophilia pedigrees. In a subsequent study, Sherman et al. [1996] noted only 3 sets of DZ twins from 231 births from female FRAXA premutation carriers.

Analysis of Turner's data suggests that expansion of the FRAXA (CGG)n repeat into the premutation range (50–200 repeats) may account for up to 7% of DZ twins born. This prompted us to investigate the distribution of the (CGG)n repeat size in the FMR1 gene in a sample of mothers of dizygotic twins (MODZTs) to determine whether an increased predisposition for fragile X syndrome existed in DZ twinning families, and to determine how much of the genetic predisposition to DZ twinning could be explained by the triplet repeat polymorphism in FMR1.

In contrast to previous studies where MODZTs were ascertained through offspring or relatives affected with fragile X syndrome, our subjects were identified on the basis of DZ twin conceptions and twinning history. Appeals for sisters, each of whom had a pair of spontaneous DZ twins, were made through the Australian NHMRC Twin Registry, the Australian and New Zealand Multiple Births Associations, and through various national television programs and magazine articles.

DNA was collected a) from 150 pairs of sisters, each of whom had had spontaneous DZ twins, and from 14 cases where 3 sisters each had DZ twins, b) from 92 relatives of these sisters comprising 58 mothers, 25 fathers, and 9 other female relatives who had had DZ twins, c) from 91 other MODZTs including 63 who conceived their twins when less than 30 years and who had at least one female relative, up to 3rd degree, who also had DZ twins and 28 others who did not comply with this strict selection criterion. All participants were questioned to ensure that twins were conceived without hormone induction of multiple ovulation or other assisted reproduction techniques. Zygosity of like-sexed twins was confirmed with multiplexed microsatellite markers as required.

A total of 525 individuals was genotyped with the FRAXA polymerase chain reaction (PCR) [Fu et al., 1991] with Southern analysis follow up on any female samples which displayed only a single PCR product. No FRAXA premutations or full mutations were detected, 49 being the largest (CGG)n repeat number identified.

We used MENDEL 2.2 [Lange et al., 1988] to obtain maximum likelihood estimates for the allele frequencies for our sample, taking account of the relatedness of our subjects, and to estimate an effective sample size, which we rounded to 625 chromosomes. The Pearson-Clopper 95% confidence interval for the frequency of premutations (point estimate of zero) is 0–0.006, large enough to include the point estimates reported by Rousseau et al. [1995] of 0.39% (41/10,624) and Spence et al. [1996] of 0.40% (3/745).

The allele frequencies seen within the range of normal repeat lengths in the Australian sample (see Table I) differed significantly from those reported in 2 large North American population studies when tested by Kolmogorov-Smirnov or chi-square testing. Relative to our results, Fu et al. [1991] found fewer alleles of repeat length less than the mode and more than the mode. Brown et al. [1992], by contrast, found relatively fewer supramodal control group for association analysis of these data. The transmission disequilibrium test [TDT; Spielman et al., 1993] offers one method to deal with this problem.

When the TDT was applied to the family data, the only notable distortions were for the 19 (8 of 23 transmitted), 30 (10 of 14 transmitted), and 31 (8 of 9 transmitted) repeat alleles. However, these were not significant when a Bonferroni correction was applied. A multiallelic TDT [Wang et al., 1996] gave \( \chi^2_{23} = 28.9 \) (empiric \( P = 0.13 \)); no linear correlation between direction of transmission skewing and higher repeat length was detected.

Finally, affected sister-pair linkage analysis was performed using the program ERPA [Curtis and Sham, 1994]. Mean identity by descent (IBD) sharing in 192...
sister pairs was 0.77 (Pearson-Clopper 95% CI = 0.73–0.81), not significantly different from the null expectation for an X-linked gene of 0.75 ($\chi^2 = 1.1, P = 0.30$).

In conclusion, these data exclude a large role for FRAXA in explaining the familial aggregation of DZ twinning. This is not unexpected in that the premutation is too rare to be able to generate a population risk ratio to sisters ($\lambda$s) as large as 2—the value usually seen for this trait [Lewis et al., 1996].

Furthermore, the upper limit on the 95% CI for the premutation frequency in this sample excludes (with 95% power) a greater than 2-fold increase in DZ twinning in carriers, given a population frequency of 0.003 for the premutation. These results are consistent with the lower limits of the 95% CI for our earlier estimate for the recurrence risk of DZ twinning due to FRAXA premutations of 5.6 [95% CI 1.7–29.9, Turner et al., 1994]. In that study, the proportion of total DZ twins due to FRAXA premutations was 7.5% (95% CI 3.4–11.5%). In the current study, the upper limit to the proportion of the total MODZTs which could be due to FRAXA premutations is only 2.7%. In either case, it is clear that FRAXA can play no more than a minor role in the inheritance of the tendency to DZ twinning.

**ACKNOWLEDGMENTS**

We thank the mothers of twins for their cooperation.

**REFERENCES**


**TABLE I. FMR1 Allele (Number of CGG Repeats) Frequency Estimates and Asymptotic Standard Errors From MENDEL Analysis of DZ Twinning Families Plus Unrelated Mothers of DZ Twins**

<table>
<thead>
<tr>
<th>Repeats</th>
<th>Frequency</th>
<th>ASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–20</td>
<td>0.102</td>
<td>0.012</td>
</tr>
<tr>
<td>21–27</td>
<td>0.174</td>
<td>0.015</td>
</tr>
<tr>
<td>28</td>
<td>0.119</td>
<td>0.013</td>
</tr>
<tr>
<td>29</td>
<td>0.321</td>
<td>0.019</td>
</tr>
<tr>
<td>30</td>
<td>0.111</td>
<td>0.012</td>
</tr>
<tr>
<td>31</td>
<td>0.047</td>
<td>0.008</td>
</tr>
<tr>
<td>32</td>
<td>0.020</td>
<td>0.006</td>
</tr>
<tr>
<td>33–39</td>
<td>0.060</td>
<td>0.009</td>
</tr>
<tr>
<td>40–44</td>
<td>0.033</td>
<td>0.007</td>
</tr>
<tr>
<td>45–49</td>
<td>0.008</td>
<td>0.003</td>
</tr>
</tbody>
</table>

S.C. Healey
D.L. Duffy
N.G. Martin*
Queensland Institute of Medical Research
Brisbane, Australia

G. Turner
Newcastle Medical Genetics Unit
Newcastle, Australia