Prevalence of the Fragile X Syndrome Among Estonian Mentally Retarded and the Entire Children's Population
Helen Puusepp, Tiina Kahre, Hiljar Sibul, Viljo Soo, Ilona Lind, Elve Raukas and Katrin Õunap
J Child Neurol 2008; 23: 1400
DOI: 10.1177/0883073808319071

The online version of this article can be found at:
http://jcn.sagepub.com/cgi/content/abstract/23/12/1400
Prevalence of the Fragile X Syndrome Among Estonian Mentally Retarded and the Entire Children’s Population

Helen Puusepp, MD, Tiina Kahre, MD, PhD, Hiljar Sibul, MD, MSc, Viljo Soo, MSc, Ilona Lind, MD, Elve Raukas, MSc, and Katrin Õunap, MD, PhD

The aim of this study is to establish the prevalence of fragile X syndrome among Estonian mentally retarded and also among the entire children’s population born during the years 1984-2005. The study group consisted of 516 patients (448 boys and 68 girls) who were screened for full mutations in the FMR1 gene during the period 1997-2006. Fourteen boys (2.7%) were found with full mutations of the total mentally retarded individuals tested (3.1% of mentally retarded boys); the full mutation was not detected among girls. The live-birth prevalence of full mutation among boys was 1:13 947. The overall live-birth prevalence of fragile X syndrome was 1:27 115. It was found that the prevalence of fragile X syndrome among mentally retarded individuals in Estonia was the same as in previous studies, but the live-birth prevalence of fragile X syndrome among boys was significantly lower.

Keywords: fragile X syndrome; live-birth prevalence

Fragile X syndrome is an X-linked mental retardation disorder caused by an unstable CGG trinucleotide repeat in the fragile X mental retardation gene at Xq27 (FMR1—MIM 309550).1-3 The assumption at the beginning of the 1990s was that fragile X syndrome is the most common form of inherited mental retardation associated with a wide range of developmental disabilities and behavioral and physical features in both males and females.4 The cloning of the FMR1 gene in 19912-3 enabled an accurate molecular diagnosis. The first estimates of prevalence of the fragile X syndrome were 1:1000 to 1:2600 for males,5,6 based on cytogenetic testing, but have proved to be less specific than expected. Turner et al7 realized in 1996 that a more realistic figure based on molecular analysis was 1:4000. Large, more recent studies suggest that prevalence of the full mutation ranges from 1:3717 to 1:8918 Caucasian males in the general population.8

The aim of this study is to estimate the prevalence of the fragile X syndrome in children with mental retardation of unknown cause and to establish the live-birth prevalence of fragile X syndrome among children (boys and girls separately).

Methods

Patients and Samples

Study area. The population-based descriptive retrospective epidemiological study was performed involving the whole of Estonia (1.342 million inhabitants on January 1, 2007, www.stat.ee).7 According to medical practice guidelines for Estonian family physicians, all children below 18 years of age with developmental problems should be referred for evaluation to one of 2 tertiary children's hospitals—Tallinn Children's Hospital for Northern Estonia and Tartu University Hospital for Southern Estonia—at least every 2 years if the cause of mental retardation is not confirmed.

Methods. This study is part of an ongoing genetic screening program for X-linked mental retardation in the Estonian population. All genetic tests for fragile X syndrome from
1997 to 2006 were analyzed in the Department of Genetics of United Laboratories at Tartu University Hospital. This is the only laboratory in Estonia in which screening for fragile-X has been performed since 1997 and has been available to all physicians requiring DNA analysis of fragile X syndrome. Two tertiary children's hospitals (Tallinn Children's Hospital and Tartu University Hospital) ordered most of the DNA analysis for fragile X syndrome. This study was approved by the Ethics Committee on Human Research at the University of Tartu. Informed consent was obtained from the parents or legal guardians of the children; 3 families refused to participate in the additional clinical evaluation.

**Patients.** The study group comprised of 516 patients (448 boys and 68 girls) who were born between 1984 and 2005 and were investigated for fragile X syndrome because of mental retardation, behavioral problems, muscular hypotonia, speech delay, epilepsy, autistic behavior, and the characteristic features of fragile X syndrome—for example, large or prominent ears, large testicles, hyperextensible finger joints, and a long narrow face. Only individuals with a genetically proven diagnosis of fragile X syndrome were included into the study.

The numbers of annual live births during the period 1984-2005 were obtained from the Statistical Office of Estonia.

**Laboratory Methods**

DNA was extracted from peripheral blood leukocytes using the standard salting out method. During the period 1997-2001, testing for the fragile X expansion mutation was carried out using the standard Southern blot method. A brief summary of this method is that DNA was simultaneously digested with EcoRI and the methylation-sensitive restriction nuclease EagI and electrophoresed in a 1% agarose gel. Blots were incubated with a 32P-labeled StB12.3 probe. DNA patterns were analyzed in terms of expansion site and methylation status as previously reported.

Since 2001, assessment of the mutation was based on PCR amplification of the CGG repeat region of the FMR1 gene. PCR conditions and amplification were performed according to the manufacturer's suggestions (Fragile X Size Polymorphism Assay kit, PE Corporation, Foster City, California). Electrophoresis of PCR products was carried out on an ABI PRISM 377 DNA Sequencer. Fragment analysis was performed using GeneScan 2.1 Analysis Software and FRAXA Genotyper 2.0 Software (PE Corporation). To avoid false negatives, the PCR analysis was followed up by Southern blot for any samples that fail to amplify by PCR and any female who appears to be homozygous.

**Statistical Analysis**

We estimated the live-birth prevalence of fragile X syndrome among boys and the entire children's population from the 1984-2005 annual live-birth data using generalized linear model analysis of the GENMOD procedure from the SAS system, version 8.2 (SAS Institute, Cary, North Carolina). Poisson distribution was assumed for the prevalence cases, which is a good model for rare events. The default logarithmic link function was used. The only factor in the model was the observation year. The mean (expected) prevalence rate for a given year and the corresponding 95% confidence limits were predicted with the OUTPUT statement of the GENMOD procedure.

**Results**

The full mutation in the FMR1 gene was found in 14 of 516 patients (2.7%) of the total mentally retarded individuals tested (14 boys); 2 males were mosaic for a pre-mutation and a full mutation in the FMR1 gene (Figure 1). We found that 3.1% (14 of 448) of boys with mental retardation had the fragile X syndrome. It was possible to evaluate retrospectively the age of patients at the time of fragile X molecular analysis in most of the cases (509 of 516 patients, 98.6%). In 286 of the 509 (56.2%) the analyses were performed at less than 7 years of age while the remaining 223 (43.8%) were of school age. The main indication for DNA analysis in boys was mental retardation had the fragile X syndrome. It was possible to evaluate retrospectively the age of patients at the time of fragile X molecular analysis in most of the cases (509 of 516 patients, 98.6%). In 286 of the 509 (56.2%) the analyses were performed at less than 7 years of age while the remaining 223 (43.8%) were of school age. The main indication for DNA analysis in boys was mental retardation in all patients, developmental delay only in 11 patients, autism with developmental delay in 2 patients, and the characteristic facial phenotype with developmental delay (long jaw, high and wide forehead, large prominent ears, long narrow face) was documented in only 1 patient. Additional clinical data on the male patients with...
Table 1. Main Clinical Features of the Boys With Fragile X Full Mutation

<table>
<thead>
<tr>
<th>Main Clinical Features</th>
<th>1</th>
<th>2</th>
<th>3a</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7a</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>Positive Findings/ Total Number of Investigated Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>3 y</td>
<td>17 y</td>
<td>15 mo</td>
<td>3 y</td>
<td>3.5 y</td>
<td>10 mo</td>
<td>6 y</td>
<td>2.5 y</td>
<td>1 y</td>
<td>5.5 y</td>
<td>3 y</td>
<td>3.5 y</td>
<td>3.5 y</td>
<td>9 y</td>
<td></td>
</tr>
<tr>
<td>Age of last clinical evaluation</td>
<td>19 y</td>
<td>23 y</td>
<td>17 y</td>
<td>14 y</td>
<td>8 y</td>
<td>16 y</td>
<td>8 y</td>
<td>20 y</td>
<td>3 y</td>
<td>7.5 y</td>
<td>6 y</td>
<td>3.5 y</td>
<td>6 y</td>
<td>9 y</td>
<td></td>
</tr>
<tr>
<td>Height +2 SD or over</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>n.i.</td>
<td>3/13 (23%)</td>
<td></td>
</tr>
<tr>
<td>Weight +2 SD or over</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>n.i.</td>
<td>8/13 (61%)</td>
<td></td>
</tr>
<tr>
<td>Head circumference +2 SD or over</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>n.i.</td>
<td>2/13 (15%)</td>
<td></td>
</tr>
<tr>
<td>Mental retardation (moderate to severe)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>14/14 (100%)</td>
<td></td>
</tr>
<tr>
<td>Behavioral problems/autism</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+/</td>
<td>+</td>
<td>+</td>
<td>+/</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>13/14 (93%)</td>
<td></td>
</tr>
<tr>
<td>Long narrow face</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>12/14 (86%)</td>
<td></td>
</tr>
<tr>
<td>High and wide forehead</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>11/14 (79%)</td>
<td></td>
</tr>
<tr>
<td>Long jaw</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>7/14 (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prominent ears</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>13/14 (93%)</td>
<td></td>
</tr>
<tr>
<td>Mild ptosis</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5/14 (36%)</td>
<td></td>
</tr>
<tr>
<td>High arched palate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>n.i</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>n.i</td>
<td>6/12 (50%)</td>
<td></td>
</tr>
<tr>
<td>Hyperextensible finger joints</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>14/14 (100%)</td>
<td></td>
</tr>
<tr>
<td>Flat feet</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n.i</td>
<td>11/12 (92%)</td>
</tr>
<tr>
<td>Muscular hypotonia</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>12/14 (86%)</td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>At 3 y</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>At 2 y</td>
<td>At 5.5 y</td>
<td>-</td>
<td>At 4.5 y</td>
<td>4/14 (29%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: n.i., not investigated

*aPatients with mosaicism for a premutation and a full mutation in the FMR1 gene.
full mutation in the \textit{FMR1} gene are listed in Table 1. The median age of diagnosis was 4.5 years (ranging from 10 months to 17 years). Two boys with fragile X syndrome were institutionalized; 1 patient was deceased at the age of 6 years due to epileptic status and overweight. The full mutation in the \textit{FMR1} gene was not detected among girls. Two girls with premutation in the \textit{FMR1} gene were identified. One had a clinical indication for fragile X testing—mild mental retardation and primary ovarian failure. Additionally, she had macrocephaly, obesity, generalized tonic seizures (since 1 year of age), hypoplastic uterus, bradydactyly, and high palate. The second girl apparently only had attention deficit syndrome, which caused her problems at school, but was tested because her mother was carrying a premutation.

In our study, 323 males and 43 females were analyzed in the period 2001-2006 with the PCR-based method, which enabled the detection of the exact number of repeats. The highest incidences of CGG repeats in the \textit{FMR1} gene in Estonia were 30 and 29 repeats, 29.3\% and 16.4\%, respectively, of all detected repeats. The distribution of all investigated \textit{FMR1} alleles is seen in Figure 2.

A total of 379 616 live births (195 184 boys and 184 432 girls) were recorded from 1984 to 2005 in Estonia (www.stat.ee).\textsuperscript{9} This indicates a minimum live-birth prevalence for fragile X syndrome among boys of 1:13947 (95\% confidence interval 1:8264 to 1:23 529). The live-birth prevalence showed a slight increase among boys (Fig. 3), but was not statistically significant ($ P = .89$). The prevalence of fragile X syndrome was not calculated separately for girls, as we did not find any girls with the full mutation. The overall live-birth prevalence of fragile X syndrome among Estonian children was 1:27 115 (95\% confidence interval 1:16 059 to 1:45 787).

**Discussion**

We found full mutation causing fragile X syndrome in 2.7\% (14 of the total 516) of the individuals tested (3.1\% of mentally retarded boys) (Fig. 1), which is in accordance with previous studies on series of mentally retarded individuals. Screening studies of Caucasian males diagnosed with non-specific mental retardation have yielded frequencies from 2.6\% to 8.7\%.\textsuperscript{14} Usually 1\% to 2\% of samples referred for molecular testing for the fragile X syndrome have actually been positive for the syndrome.\textsuperscript{15,16}

The live-birth prevalence of full mutation in the \textit{FMR1} gene causing fragile X syndrome in Estonia among boys was 1:13 947 with an upper limit 95\% confidence interval of 1:8264 (during 1984-2005), but the overall live-birth prevalence of fragile X syndrome among Estonian children was 1:27 115. We found the live-birth prevalence of the fragile X syndrome among boys in Estonia to be significantly lower than in previous studies. Previously reported population-based studies suggest that the prevalence of the full mutation is about 1:4 000 Caucasian males in the general population,\textsuperscript{7} in the range 1:3 717 to 1:8918,\textsuperscript{8} which is much higher than the live-birth prevalence found in Estonia. However, Crawford et al\textsuperscript{8} stated that most of these studies screened target populations (as also this study), and few of them later extrapolated these results to the general population. Crawford et al also stated that the screening of a large population of consecutive newborns would solve this problem of complete ascertainment of fragile X syndrome in the general population. Presently, there is only one work, by Rousseau et al\textsuperscript{17}, which screened the \textit{FMR1} alleles among 24 446 mother-newborn pairs from the general population.
and reported that the incidence of the full mutation causing fragile X syndrome in the Canadian population is 1:24,446 (upper limit of 95% confidence interval 1:7065), which results are very similar to ours (1:27,115).

We assume that most fragile X syndrome cases in Estonia are included in our study as almost all males with the full mutation exhibit some clinical features of this syndrome, usually by the age of 3 years. However, Crawford et al. suggested that the syndrome is not being diagnosed through the referral system because identification of new milder cases of the fragile X syndrome are made only among school-aged children. Therefore, it is possible that we have missed the mild cases of fragile X syndrome among children who have not reached school-age, but on the other hand, half of our tested children were older than 7 years. Moreover, the results of statistical analysis support that most fragile X syndrome cases should be included, as the prevalence of fragile X syndrome has been stable during the period 1984-2005 (Fig. 3).

We may have missed some fragile X syndrome patients due to the fact that we did not visit all the long-term care institutions and special educational facilities for disabled children in Estonia. However, our health insurance system has been organized so that all children with developmental delay have to refer to 2 main children’s hospitals every 2 to 3 years in order to receive disability support. Our assumption is also based on the observations by Õiglane-Shlik et al. (epidemiological study for Angelman syndrome and Prader-Willi syndrome), who did not find any additional cases in this process. Their study proved that the regular evaluation according to consensus document of children below 18 years of age with developmental problems is very effective in Estonia. Furthermore, there is an ongoing study of X-linked mental retardation families in Estonia, and in all of those families fragile X syndrome has been excluded (more than 50 families with suspicion of X-linked mental retardation). In addition, this study included patients with fragile X syndrome who were institutionalized at the time of investigation but diagnosed during regular evaluations in one of our tertiary hospitals. It is also important that the Department of Genetics of United Laboratories at Tartu University Hospital is the only diagnostic laboratory for all of Estonia and available for all hospitals and institutions. This department has, therefore, tested all patients with a suspicion of fragile X syndrome, and consequently the whole population of Estonia is covered. The molecular study for the diagnosis of fragile X syndrome in Estonia was performed by 2 different techniques: Southern blot analysis and PCR with fluorescently marked primers followed by CGG repeat length detection on the ABI PRISM 377. These are worldwide accepted screening methods.

Brown et al. analyzed 570 pregnant women and found that the most common number of CGG repeats in Caucasians is 30. The same results were reported in Greece, where they found that the most common alleles in the Hellenic population in Greece for FMR1 repeats in females were 29/30 (15.3% for heterozygous) and 30/30 (27.6% for homozygous). We show that the most prevalent CGG repeat number in Estonia is also 30 (Figure 2). In the populations of China, India, and the former Republic of Yugoslavia the most prevalent CGG repeat number has been shown to be 29.

In conclusion, we can say that we found similar prevalence of the fragile X syndrome among mentally retarded individuals in Estonia as in previous studies, but the live-birth prevalence of this syndrome was significantly lower. The precise reason for this is still unknown to us. Our study on fragile X syndrome is the first in the Baltic states and in countries of the former Soviet Union; the nearest country where this research has been performed is Finland. This study has considerably improved the awareness of fragile X syndrome in this region.

Acknowledgments

We thank the participating parents and children, whose cooperation made this study possible. We also thank Mr. Tõnu Möls for his valuable comments and help with the statistical analysis and Prof. Tiina Talvik for helping with manuscript preparation. This work was supported by TARLA 2695.

References


