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Short Communication

FRAGILE X SYNDROME IN MENTALLY RETARDED PATIENTS FROM LATVIA

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The aim of this study was to estimate the prevalence of FXS in Latvia and characterise the FMR1 CGG-repeat structure in Latvian patients exhibiting mental retardation. A group of 352 unrelated patients with mental retardation (MR) referred from clinical geneticists was screened by PCR for the normal allele. In a sample of 245 chromosomes the CGG repeat number was determined by Applied Biosystems protocol on ABI Prism 310. Prevalence of 29, 30, and 31 CGG repeats was found for the normal allele. Five affected patients were detected (detection rate 2.56%). AGG interspersion pattern analysis showed stability of transmission to the next generation for 12 intermediate alleles. The found detection rate of FXS in our survey among MR patients was similar to the detection rate reported in literature. Taking into account the number of confirmed FXS cases we suggest that FXS is still clinically unrecognized in paediatrician practice.

Key words: *fragile X, FRAXA, FMR1, mental retardation.*

Fragile X syndrome (FXS – MIM 300624) accounts for 10%–12% of mental retardation (MR) cases and is the second most common cause of mental impairment after trisomy 21 (Ropers and Hamel, 2005). The prevalence of the full mutation is approximately 1 in 4,000–6,000 males and 1 in 8,000–10,000 females (Crawford, 2001; Turner, 1996). The most common mutation at *FRAXA* locus is expansion of the CGG triplet repeat located in the 5'-untranslated region of the *fragile X mental retardation-1* (*FMR1*- MIM 309550) gene. The expanded CGG triplet repeats are hypermethylated and the expression of the *FMR1* gene is repressed in patients with fragile X syndrome (FXS), which leads to the absence of FMR1 protein (FMRP) and subsequent mental retardation (MR). Normal alleles vary from 6 to 50 (52) CGG repeats. Intermediate alleles vary from 39 to 55 repeats, premutation alleles up to 200 repeats, full mutation greater than approximately 200 repeats (methylated) (Verkerk, 1991). In a literature review the detection rate of FXS was reported in the range of 0.77%–8.51%, depending on the study groups and the method of diagnosis (Yim *et al.,* 2008).

The aim of this study was to estimate the prevalence of FXS in Latvia and characterise the *FMR1* CGG-repeat structure in Latvian patients exhibiting mental retardation.

A group of 352 unrelated patients with mental retardation (MR) referred from clinical geneticists was screened by PCR (Chong *et al*., 1994) for the normal allele. In a sample of 245 chromosomes the CGG repeat number was determined by Applied Biosystems protocol on ABI Prism 310. For the normal allele prevalence of 29, 30 and 31 CGG repeats was found (Fig. 1). Five affected patients were identified (detection rate 2.56%). Final diagnosis of FXS was confirmed by Southern blotting. After active cascade testing in five FXS families five female permutation carriers, three females with full mutation, three affected males with full mutation, and one affected mosaic male were found.

DNA sequencing was used to identify AGG insert structure for samples with intermediate alleles. Stability of transmission to the next generation was found by AGG interspersion pattern analysis for 12 intermediate alleles (Table 1). Several authors have proposed a category of intermediate or 'grey zone' alleles (35 ± 54 repeats), which represent the overlap between alleles at the high end of the normal range in the general population and the lower limit of premutation alleles in fragile X families (Fu *et al*., 1991; Eichler *et al*., 1994). The basis of this designation is the instability of some small premutation alleles during transmission from one generation to the next. The *FMR1* CGG repeat usually includes 1 ± 3 interspersed AGG trinucleotides every 9 ± 10 CGG repeat, which is thought to stabilise the repeat by preventing slipped-strand mispairing during DNA replication (Eichler *et al*., 1994). All 12 analysed samples in our study

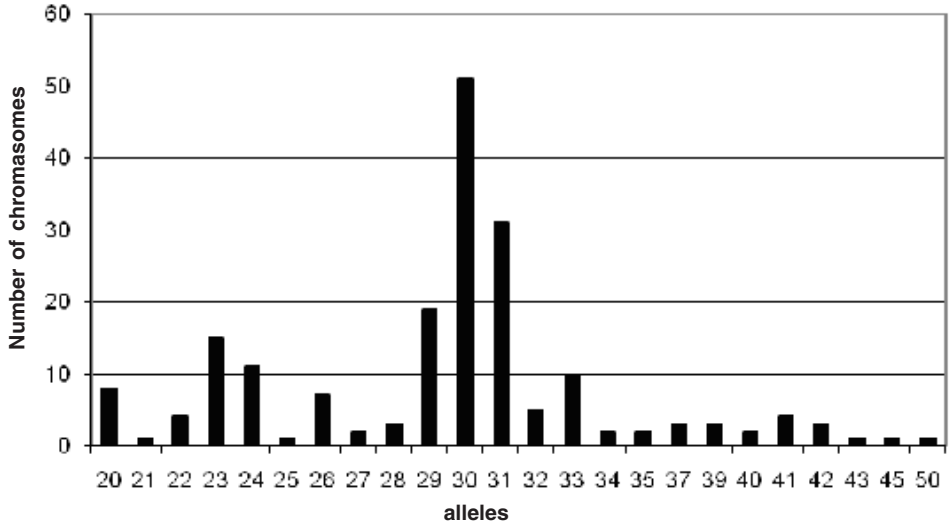

Fig. 1. Distribution of CGG repeats for normal alleles.

Table 1

CGG REPEAT STRUCTURE OF INTERMEDIATE ALLELES

Number of CGG repeats	**Structure of AGG inserts**
50	**9+9+29**
40	**9+9+20**
40	**9+9+20**
41	**10+9+9+9**
41	**9+9+21**
42	**9+9+22**
39	**9+9+9+9**
42	**10+9+20**
43	**10+9+22**
45	**9+9+25**
39	**10+9+98**
39	**9+28**

have shown stable CGG repeat structure with one to three interspersed AGG trinucleotides.

In future work the STR-based haplotype structure will be determined in Latvian FXS patients and their families. The relationship between genotype and phenotype for confirmed FXS cases will be studied. Statistical processing of study results to estimate the prevalence of FXS in Latvia is in progress.

The detection rate found in our survey among MR patients is similar to the detection rate found in literature. Taking into account the number of confirmed FXS cases we suggest that FXS is still clinically unrecognized in paediatrician practice. Our study is a first survey on FXS in Latvia. Estimation of FXS prevalence would provide a practical contribution in peadiatrics, children neurology and children neurology practice as well.

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FRAGILĀS X HROMOSOMAS SINDROMS LATVIJAS PACIENTIEM AR GARĪGO ATPALICĪBU

Vidēji 10%–12% gadījumu garīgās atpalicības iemesls ir fragilās X hromosomas sindroms (FXS – MIM 300624), viens no biežākajiem iemesliem kavētai garīgai attīstībai pēc 21 hromosomas trisomijas. Pilnas mutācijas sastopamības biežums vidēji ir 1 no 4 000 līdz 1 no 6 000 vīriešiem un 1 no 8 000 līdz 1 no 10 000 sievietēm. Pētījuma mērķis bija noteikt FXS sastopamības biežumu un raksturot *FMR1* gēna CGG atkārtojumu struktūru pacientiem ar kavētu garīgo attīstību Latvijā. 352 neradniecīgiem pacientiem ar garīgo atpalicību tika veikta normālās alēles sijājošā diagnostika ar polimerāzes ķēdes reakciju. 245 hromosomām veikta precīza CGG atkārtojumu skaita noteikšana, genotipējot pēc *Applied Biosystems* protokola. Hromosomām ar normālu CGG atkārtojumu skaitu tika konstatētas prevalējošās alēles ar 29, 30 un 31 CGG atkārtojumu. Diagnosticēti pieci pacienti ar pilnu mutāciju. 12 „pelēkās zonas" alēļu pārmantošanas stabilitāte nākamajām paaudzēm tika noteikta, analizējot AGG iestarpinājumu struktūru. Pētījumā konstatētās mutācijas frekvences būtiski neatšķiras no literatūras avotos minētajiem rādītājiem. Ņemot vērā diagnosticēto FXS pacientu skaitu, uzskatām, ka fragilās X hromosomas sindroms ir klīniski neatpazīts pediatrijā.