Screening for the fragile X syndrome among the mentally retarded: a clinical study

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Abstract

The fragile X syndrome is characterised by mental retardation with other features such as a long face with large, protruding ears, macro-orchidism, and eye gaze avoidance. This X linked disorder is caused by an expanded CGG repeat in the first exon of the fragile X mental retardation (FMR1) gene which is associated with shut down of transcription and absence of the fragile X mental retardation protein (FMRP). Molecular testing is used for detection of patients and carriers of the fragile X syndrome.

In a screening programme for the fragile X syndrome in the south west of The Netherlands, 896 males and 685 females with an unknown cause for their mental retardation were scored on seven fragile X features. All were tested by DNA analysis and 11 new cases were diagnosed. The seven item checklist allowed exclusion from further testing in 86% of the retarded males (95% CI 0.83-0.88) without missing either any of the newly diagnosed cases or, in retrospect, any of the 50 previously diagnosed cases known to our department.

These results showed that clinical preselection for DNA testing in mentally retarded males is feasible using a simple scoring list, which will increase the efficiency of further testing eightfold.

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The fragile X syndrome is an X linked mental retardation syndrome, characterised by behavioural and physical features such as a long face with large, protruding ears, macro-orchidism, and eye gaze avoidance.[1–3] The fragile X syndrome was one of the first of a "novel" class of disorders caused by a trinucleotide repeat expansion. Affected subjects have expanded CGG repeats (>200) in the first exon of the fragile X mental retardation (FMR1) gene (the full mutation).[4–6] This expansion is associated with hypermethylation of the repeat and its flanking region resulting in silencing of transcription and absence of the FMR1 protein (FMRP).[7–9] In the normal population, the CGG repeat varies from six to 54 units.[10] The premutation with repeats in the range of 43 to 200 units occurs in phenotypically normal carriers (male and female) of the fragile X syndrome.[10]

The methodology for detection of the full and the premutation enabled accurate fragile X screening programmes. In recent screening studies in the United Kingdom, Australia, and The Netherlands, the prevalence of the fragile X syndrome was estimated as 1/4000 to 1/6000 for males.[11–13]

Since 1992, a fragile X screening programme has been conducted in the south west of The Netherlands among mentally retarded subjects in institutions giving residential care and special schools. A prevalence of 1/6000 for males was established. Over 50% of the cases in The Netherlands remain undetected until now, if we compare the estimated prevalence with the actual total number of cases diagnosed in the Dutch clinical genetic services.[11]

Here, we report on clinical selection criteria in 896 mentally retarded males to identify mentally retarded males at increased risk for the fragile X syndrome.

Patients and methods

Between 1992 and 1996, a screening programme for the fragile X syndrome was conducted in five institutions giving residential care (1878 subjects), 16 special schools (1488 subjects), one sheltered workshop (142 subjects), and two sheltered homes (49 subjects) for the mentally retarded in the south west of The Netherlands.[11] Persons with an unknown cause of their mental handicap were included for a physical examination and DNA analysis, after systematic information procedures for parents and guardians.[11] The study was approved by the Medical Ethical Committee of the Erasmus University and University Hospital Dijkzigt, Rotterdam and the respective institutional Ethical Review Committees.

Fifty fragile X males previously diagnosed by DNA analysis in our department were included for a physical examination. For 45 males the observer was not blinded for the diagnosis.

PHYSICAL EXAMINATION

Each subject was scored by one observer (BBAdV) for seven fragile X features: family history of intellectual handicap, elongated face, large/prominent ears, hyperextensible finger joints, soft/smooth skin, macro-orchidism, and personality. Four items were derived from the checklist developed by Laing *et al*[14] and three were added. Each item may score 0, 1, or 2 depending on the presence of the feature (table 1).

Table 1 Clinical scoring for fragile X features

	Score
Family history of mental retardation*	
Affected sib, affected maternal uncle, aunt, nephew, niece, or first cousin	2
For any other affected relative (compatible with X linked inheritance)	1
Face*	
Long jaw and high, wide forehead	2
Only one of these findings	1
Ears*	
Large (by measurement) and protruding from side of head	2
Large only	1
Joints	
Hyperextension of metacarpophalangeal joint digit V (>90°) and I	2
Only hyperextension of metacarpophalangeal joint digit V	1
Skin	
Soft and velvety on the palms with redundancy of skin on the dorsum of hand	2
Only soft and velvety on palms	1
Testes†	
Both testes >30 ml	2
One testis >30 ml	1
Personality*	
Initial shyness and lack of eye contact followed by friendliness and verbosity with echolalic speech	2
Some of these characteristics	1
Total	

*Items adapted from Laing *et al*.[14]
†Using orchidometer.

Additionally, the height, head circumference, and dysmorphic features unrelated to the fragile X syndrome were recorded.

DNA ANALYSIS

The CGG repeat status of the FMR1 gene was tested as described previously.[11]

STATISTICAL ANALYSIS

The data were analysed with version 6.1.3 of SPSS for Windows and the software Confidence Interval Analysis (CIA) written by Gardner and Altman. The data are presented as percentages with 95% confidence intervals. These confidence intervals concern the effects of sampling variation on the precision of the estimated statistics; the smaller the interval, the higher the precision.

The magnitude of the discrepancy of the percentage of fragile X patients presenting with a certain feature compared to the reference, that is, the non-fragile X patients, was represented by the discrimination, d. This measure implies the percentage of non-fragile X patients with a certain feature (m_b) subtracted from the fragile X patients with that feature (m_a) divided by the standard deviation of all patients (σ): $(m_a - m_b)/\sigma$. A d value >0.80 was considered to represent a large effect.[15]

Results

Among the 896 males and 685 females without a diagnosis who underwent a brief physical examination and the DNA test, 11 new patients were diagnosed (nine males and two females) as reported previously.[11]

PHYSICAL EXAMINATION

Table 2 shows the results of the physical examination in 887 males without a full mutation grouped according to age (⩽16 years and >16 years) compared to the nine newly identified fragile X males.

Fig 1 shows the total scores for the seven clinical features in 826 males compared to the nine newly diagnosed fragile X cases and the 50 previously diagnosed fragile X cases. In 61 males, one or more of the features (mainly family history, table 2) could not be scored and these cases were excluded from further analysis for which the total score was required. Using a score of ⩾5 as cut off for molecular testing, one could have excluded 716 of 835 males (specificity 0.87, 95% CI 0.84-0.89) from further molecular testing without missing a fragile X diagnosis either prospectively (sensitivity 1.00, 95% CI 0.66-1.00) or retrospectively (sensitivity 1.00, 95% CI 0.93-1.00).

Twenty nine males without a diagnosis in whom the presence of a full mutation in the FMR1 gene had been ruled out before the start of the programme were not available for physical examination. Theoretically, the exclusion rate might have been different when these males were included. However, if all 29 males had several fragile X features, the exclusion rate would still be 83% for the seven item checklist.

In table 3, the data for 683 females without a full mutation are shown. Because of the small number of newly diagnosed fragile X females, further calculations on predictiveness of features are not presented for females.

Table 2 Fragile X features in mentally retarded males without the fragile X syndrome compared to fragile X males

	Fragile X			*Non-fragile X**			
	Prospective % (95% CI) (n=9)	*Retrospective % (95% CI) (n=50)*	*Subtotal % (95% CI) (n=59)*	*⩽16 years % (95% CI) (n=330)*	*>16 years % (95% CI) (n=557)*	*Subtotal % (95% CI) (n=887)*	*d†*
Fragile X related features							
Family history of mental retardation	33 (8-70)	86 (73-94)	78 (73-94)	16 (12-20)	21 (18-24)	19 (16-22)	0.70
Elongated face	67 (30-93)	48 (34-63)	51 (38-64)	2 (1-4)	9 (7-12)	7 (5-8)	0.67
Large and prominent ears	33 (8-70)	26 (15-40)	27 (16-40)	9 (6-13)	13 (10-16)	11 (9-14)	0.23
Hyperextensible joints							
MC digit V	44 (14-79)	40 (26-55)	41 (28-54)	31 (26-36)	19 (16-22)	23 (21-26)	0.33
MC digit V and digit I	0 (0-34)	18 (9-31)	15 (7-27)	6 (4-9)	1 (1-3)	3 (2-4)	0.22
Soft/smooth skin	33 (8-70)	20 (10-34)	22 (12-35)	1 (0-3)	6 (4-8)	4 (3-6)	0.33
Macro-orchidism							
Moderate/one testicle	11 (0-48)	8 (2-19)	9 (3-19)	4 (2-6)	14 (11-17)	10 (8-12)	−0.02
Both testicles	67 (30-93)	58 (43-72)	59 (46-72)	2 (1-4)	8 (6-11)	6 (5-8)	0.85
Personality	56 (21-86)	64 (49-77)	63 (49-75)	2 (1-4)	5 (4-7)	4 (3-5)	0.96
Non-fragile X related features							
Microcephaly	0 (0-34)	0 (0-07)	0 (0-06)	7 (4-10)	8 (6-11)	8 (6-10)	
Non-fra(X) related dysmorphic features (⩾3 or microcephaly)‡	0 (0-34)	0 (0-07)	0 (0-06)	20 (15-24)	29 (25-33)	25 (23-28)	

*For some items (predominantly family history) some patients (<7%) could not be scored.
†Discrimination measure.
‡Facial and hands.

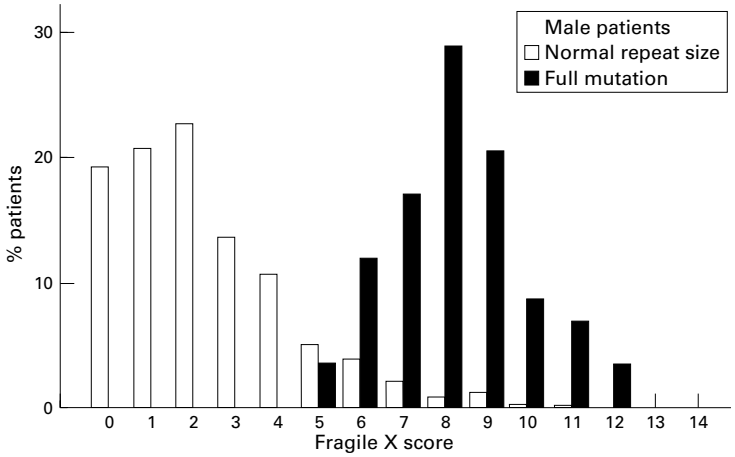

Figure 1 Clinical score for the fragile X males (n=59) versus the non-fragile X males (n=826).

*Table 3 Fragile X features in females for different age categories**

	≤16 years % (95% CI) (n=216)	*>16 years % (95% CI) (n=467)*	*Total % (95% CI) (n=683)*
Fragile X related features			
Family history of mental retardation	21 (15-26)	13 (10-16)	16 (13-18)
Elongated face	5 (2-8)	4 (3-7)	4 (3-7)
Large and prominent ears	2 (1-5)	3 (2-6)	3 (2-5)
Hyperextensible joints			
MC digit V	34 (27-40)	18 (14-21)	23 (20-26)
MC digit V and digit I	5 (2-8)	2 (1-4)	3 (2-5)
Soft/smooth skin	1 (0-3)	5 (4-8)	4 (3-6)
Personality	1 (0-3)	4 (2-6)	3 (2-5)
Non-fragile X related features			
Microcephaly	9 (6-14)	21 (18-25)	17 (15-20)
Non-fra(X) related dysmorphic features (≥3 or microcephaly)†	18 (13-23)	47 (43-52)	38 (34-42)

*For some items (predominantly family history) some patients (<5%) could not be scored.
†Facial and hands.

When comparing males and females (tables 2 and 3), both without an expanded CGG repeat within the FMR1 gene, there is a significantly larger proportion of females with microcephaly than males (17% and 8% respectively, 95% CI for differences 0.06-0.13). Also, females show significantly more frequent non-fragile X related dysmorphic features compared to males (38% and 25% respectively, 95% CI for differences 0.08-0.17) and, after removing all subjects with microcephaly, the latter difference is still present (30% of the females and 22% of the males, 95% CI for difference 0.03-0.13). This difference is mainly contributed by the adults, as neither the microcephaly nor the dysmorphic features are significantly different between males and females younger than 17 years (95% CI for differences −0.02-0.07 and −0.08 to 0.05 respectively).

Discussion

Since the recognition of the fragile X syndrome as a major cause of mental retardation, screening programmes have been in place.[16] These programmes became more accurate with the advent of DNA analysis of the FMR1 gene.[11 13 17 18] To increase the efficiency of screening programmes, a preselection of clinical features will be required. In the current study, among a large sample of mentally retarded subjects, a method of clinical preselection was studied. To improve insight into the significance of the clinical features for preselection, seven features (table 1) were used to screen the 1581 subjects with mental retardation of unknown aetiology, most of which have been used in different fragile X checklists before.[14 19 20] However, these checklists had been validated using chromosome analysis and, generally, scores were obtained by more than one observer. Giangreco *et al*[21] reported a six item checklist based on physical and DNA studies among 273 boys and 62 girls referred for DNA analysis for the fragile X syndrome. Based on their checklist, they could exclude 60% from further testing, albeit with a wide 95% confidence interval owing to the relative small sample size. Arvio *et al*[22] reported preselection of 44 retarded males from 344 retarded males, diagnosing six new cases in the selected group but without testing the majority of the unselected males.

As might be expected, our data show that the individual clinical symptoms were not specific for the fragile X syndrome. It is the combination of these features which considerably increases the likelihood of the fragile X diagnosis. The seven item score list allows significant reduction of 86% of the mentally retarded males who should be tested for the fragile X syndrome without missing a fragile X case prospectively (score <5 out of 14). In retrospect, the checklist identified all 50 previously diagnosed fragile X males who had been examined by the same observer (BBAdV). Most of these males were not examined blind. However, this did not seem to influence the scoring as the findings in the "retrospective" group do not significantly differ from the "prospective" group of fragile X patients for the different features, except for family history (table 2). It is unlikely that the latter was influenced by knowing the diagnosis.

As in most clinical dysmorphology studies, some of the clinical features might be differently scored in some patients by different observers. Four items (family history, ears, joints, and testes) are objective measurements whereas the other three features (face, skin, and personality) are to some degree subjective. By using one observer, interobserver variation has been avoided. The observer in the current study has extensive experience of the fragile X syndrome. Therefore, clinicians who are less familiar with the syndrome might achieve a lower exclusion rate than the one reported in this study. For these clinicians particularly, a checklist would be helpful to assess whether a mentally retarded person should be tested for the fragile X syndrome or not.

Females with mental retardation of unknown cause, but with a normal repeat in their FMR1 gene, show the various fragile X features equally as often as mentally retarded males with an unknown cause. However, these females less often have the large/prominent ears and the personality features compatible with the fragile X syndrome. Remarkably, the adult retarded females more frequently show microcephaly and other dysmorphic features

than the retarded males, for which we do not have an explanation.

In conclusion, preselection for DNA testing in mentally retarded males is feasible and will increase the efficiency of DNA analysis by exclusion of 86% of the retarded patients. Using the seven item checklist will make DNA analysis more cost effective and increase the diagnostic rate eightfold.

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