Journal of Intellectual Disabilities

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Journal of Intellectual Disabilities 2009; 13; 239
DOI: 10.1177/1744629509348429

The online version of this article can be found at:
http://jid.sagepub.com/cgi/content/abstract/13/3/239

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ARTICLE

Evaluation of clinical checklists for fragile X syndrome screening in Brazilian intellectually disabled males

Proposal for a new screening tool

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Journal of Intellectual Disabilities

VOL 13(3) 239–248
ISSN 1744-6295(348429)
DOI: 10.1177/1744629509348429

Abstract Patients with fragile X syndrome present a variable phenotype, which contributes to the underdiagnosing of this condition. The use of clinical checklists in individuals with intellectual disability can help in selecting patients to be given priority in the molecular investigation of the fragile X mutation in the *FMR1* gene. Some features included in checklists are better predictors than others, but they can vary among different populations and with patient age. In the present study, we evaluated 20 features listed in four clinical checklists from the literature, using a sample of 192 Brazilian male patients presenting with intellectual disability (30 positive and 162 negative for fragile X mutation). After statistical analysis, 12 out of the 20 items analyzed showed significant differences in their distributions between the two groups. These features were grouped in a new checklist that can help clinicians in their referral for fragile X testing in patients with developmental delay.

Keywords fragile X checklist, fragile X syndrome, *FMR1* gene, X-linked intellectual disability

Introduction

Intellectual disability (ID) is one of the most common human disorders, affecting around 3 percent of the world population. In Brazil, according to the demographic census conducted in 2000, at least 2.8 million persons (1.6% of the population) had intellectual disability, which is certainly an underestimate, as the expected prevalence in developing countries is three times higher (Faria, 2006).

The most frequent cause of inherited ID is the fragile X syndrome (FXS, OMIN 300624), whose prevalence is 1:4000 to 1:6000 in males and 1:7000 to 1:10,000 in females (Mandel and Biancalana, 2004), accounting for 10–12 percent of all families with intellectual disabilities.

Fragile X syndrome is an X-linked disorder, almost exclusively caused by an expansion of a CGG repeat in the 5' untranslated region of the *FMR1* (fragile X mental retardation) gene. In the normal population, the CGG is polymorphic and ranges from 5 to 55 CGG repeats. In fragile X patients, however, the CGG is found to be expanded beyond 200 repeats, resulting in gene function loss (Fu et al., 1991; Oostra and Willemsen, 2009).

The phenotype of FXS is quite variable. The main and most consistent clinical feature is intellectual disability, whereas physical and behavioral features may vary with age and gender (Hagerman et al., 1991). Thus, the clinical diagnosis of FXS is difficult, requiring molecular laboratory testing. Since intellectual disability is a common disorder, providing fragile X molecular analysis to all such patients is costly and in many cases unnecessary. Several studies have attempted to characterize the behavioral

and physical phenotype of the FXS (Hagerman et al., 1996; Lachiewicz et al., 2000), and fragile X checklists have been prepared to provide clinical identification of males more likely to be affected by this syndrome (Arvio et al., 1997; Butler et al., 1991; de Vries et al., 1999; Hagerman et al., 1991; Johnson, 2008; Laing et al., 1991; Limprasert et al., 2000; Maes et al., 2000). The use of checklists to select patients with a high probability of being affected by FXS may reduce the number of individuals to be submitted to molecular evaluation by 60 to 80 percent (Mandel and Biancalana, 2004), greatly improving the cost-effectiveness of fragile X testing.

Our aim was to determine good clinical predictors of FXS among Brazilian patients with intellectual disability, thereby helping health professionals to improve their referrals for fragile X testing. We evaluated each item of the checklists published by Butler et al. (1991), Hagerman et al. (1991), Laing et al. (1991) and Giangreco et al. (1996), and propose a new checklist.

Material and methods

Subjects

This study included 192 male patients aged 2 to 31 years (mean 11.3 ± 5.16 years), presenting intellectual disability associated or not with other features, attending the Hospital São Paulo, Brazil, as outpatients, referred for fragile X testing. All patients had normal karyotypes. Patients with known dysmorphic and metabolic syndromes associated with intellectual disability were excluded. The institutional Research Ethics Committee approved this study, and informed consent was obtained from the patients' parents. Prepubertal (<12 years old) ($n = 126$) and postpubertal (>12 years old) ($n = 65$) patients were analyzed separately for some of the phenotypic characteristics.

Checklist evaluation

Characteristics included in four fragile X checklists were considered in this study. The checklist presented by Hagerman et al. (1991) comprises 13 items: intellectual disability, large ears, large testes, hyperactivity, family history of intellectual disability or autism, shorter attention span, tactile defensiveness, hyperextensible finger joints, perseverative speech, hand flapping, hand biting, poor eye contact and single palmar crease (Sydney line).

The complete checklist of Butler et al. (1991) consists of the same 13 items listed by Hagerman et al. (1991) plus plantar crease and pale blue eyes. Laing et al. (1991) proposed a clinical score based on five items: family history of intellectual handicap, face length, ear configuration, personality

(lack of eye contact followed by friendliness and verbosity with echolalic speech patterns) and body habitus (a slim physique with tall stature, rounded shoulders, hyperextensible finger joints and lack of body hair; or an obese physique with feminine distribution of body fat and stria). Giangreco et al. (1996) proposed a simplified checklist for pediatric populations, based on the presence of six items: intellectual disability, family history, elongated face, large or prominent ears, attention deficit hyperactivity disorder, and autistic-like behavior. The measurements of the ears and face were considered according to age percentiles. Testes measurement was done by the use of an orchidometer and correlated with the expected percentile for the age.

In this study, the checklists were applied to all patients by the same clinical geneticist, in order to prevent discrepancies in scoring.

Molecular analysis

Genomic DNA was obtained from peripheral blood leukocytes by a salting-out method. DNA samples were screened for full mutation in the *FMR1* gene by PCR, according to the technique described by Haddad et al. (1996). Southern blot analysis (Mingroni-Netto et al., 1996) was performed to validate the PCR positive results. Patients with and without the full mutation were classified as fragile X positive and fragile X negative, respectively.

Statistical analysis

The aim of this study was to identify predictive variables for FXS testing in Brazilian males with intellectual disabilities. A chi-square test and logistic regression analyses were used to determine statistical differences in checklist items between patients with and without FXS. Table 1 shows the *p*-value for each feature in patients referred for FXS testing. A value of $p < 0.05$ indicates that the feature is significantly different between fragile X negative and positive patients. For the characteristics that were statistically different between fragile X positive and negative patients, a ROC curve was done for cutoff value determination for the new checklist proposed.

Furthermore, a chi-square test was performed in order to identify differences among pre- and postpubertal populations.

Results

Of the 192 patients studied, 162 were fragile X negative and the other 30 were fragile X positive (full mutation of the *FMR1* gene). Premutation was not found in any patient. The geneticist who performed the clinical evaluation was blind to the molecular results. The distribution of their clinical features according to the checklist items is shown in Table 1. Intellectual

Table 1 **Frequencies of checklist items[a] in 192 patients with intellectual disability, with and without fragile X syndrome**

Item[a]	*Fragile X negative (n = 162)*		*Fragile X positive (n = 30)*		*Chi-square value*	*p*
	Present	*Absent*	*Present*	*Absent*		
Hand flapping	91	71	28	2	14.833	0.0001
Tactile defensiveness	52	110	20	10	12.905	0.0003
Plantar crease	100	62	28	2	11.378	0.0007
Large testes	58	104	20	10	9.997	0.0016
Family history of intellectual disability	68	94	22	8	9.995	0.0016
Hyperextensible finger joints	69	93	22	8	9.594	0.0020
Family history of intellectual disability or autism	71	91	22	8	8.824	0.0030
Large ears	111	51	27	3	5.778	0.0162
Elongated face	127	35	29	1	5.547	0.0185
Hand biting (habit of)	57	105	17	13	4.931	0.0264
Poor eye contact	110	52	26	4	4.314	0.0378
Single palmar crease (Sydney line)	29	133	8	22	1.250	0.2635
Large and prominent ears	115	47	27	3	4.751	0.2930
Pale blue eyes	2	160	1	29	0.725	0.3945
Hyperactivity	151	11	29	1	0.516	0.4725
Body habitus[b]	151	11	29	1	0.516	0.4725
Personality[c]	154	8	28	2	0.153	0.6955
Shorter attention span	155	7	29	1	0.062	0.8036
Perseverative speech	129	33	24	6	0.002	0.9631

[a] Butler et al. (1991), Hagerman et al. (1991), Laing et al. (1991) and Giangreco et al. (1996).
[b] Slim physique with tall stature, rounded shoulders, hyperextensible finger joints and lack of body hair, or feminine distribution of body fat, striae, soft skin and lack of body hair.
[c] Initial shyness and lack of eye contact followed by friendliness and verbosity with echolalic speech patterns.

disability, present in all four checklists, was excluded from the statistical analysis because it was the inclusion criterion of patients in this study.

The features that showed a p value < 0.01 between fragile X positive and negative patients were hand flapping, tactile defensiveness, plantar crease, large testes, family history of intellectual disability, hyperextensible finger joints, familial history of intellectual disability or autism. Additional features which showed a difference at < 0.05 included: large ears, elongated face, hand biting, and poor eye contact.

No significant differences were found between fragile X positive and negative patients regarding hyperactivity, shorter attention span, perseverative speech, complete palmar crease, pale blue eyes, body habitus and personality.

Based on these findings we propose a new checklist with the 10 most significant characteristics. The determination of the cutoff point was determined by a ROC curve (Figure 1).

Table 2 shows the number of individuals selected for laboratory testing among our 192 patients, based on different scores, and the rate of detection of FXS patients.

In order to investigate interactions between the evaluated features, logistic regression analysis was performed. It demonstrated that the best predictors among the features related to fragile X syndrome were: family history of intellectual disability, large testes, tactile defensiveness, and hand flapping.

When patients were subdivided into groups according to age, large ears were more prevalent in patients older than 12 years old ($p = 0.0187$), while hand biting and poor eye contact were more frequent in patients up to 12 years old ($p = 0.0236$ and $p = 0.0087$, respectively).

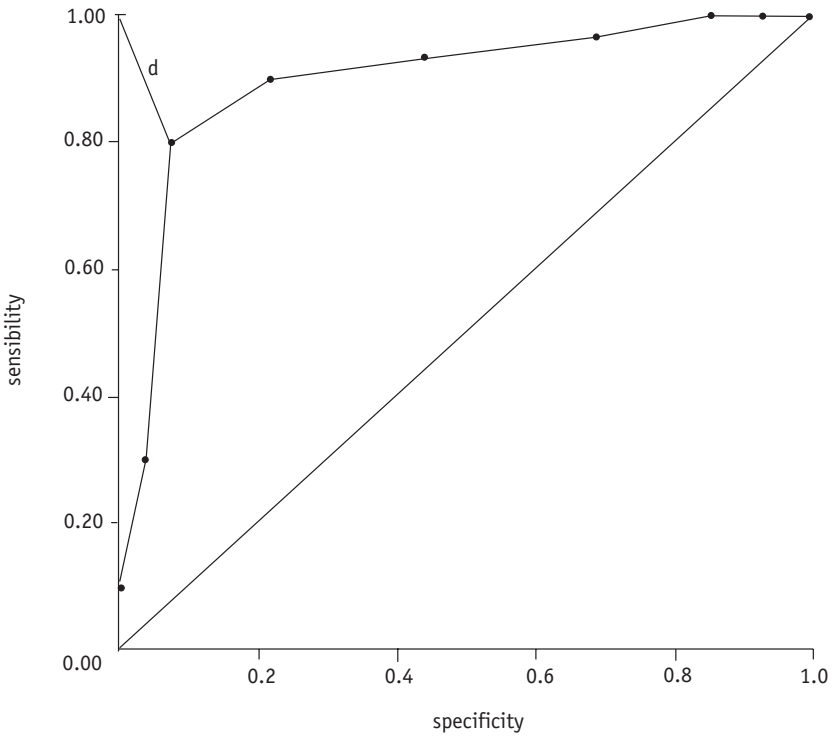

Figure 1 **ROC curve for cutoff determination according to the number of features considered to be predictive of FXS**

Table 2 **Number of individuals selected from a sample of 192 intellectually disabled males for FXS molecular testing by using different checklist scores, and the estimated number of patients detected**

Cutoff score	*Individuals selected for testing*		*FXS patients detected*	
9	31	16.1%	10	33.3%
8	46	23.9%	25	83.3%
7	82	42.7%	27	90.0%
6	116	60.4%	29	96.7%
4	172	89.6%	30	100.0%

Discussion

To increase the efficiency of screening programs for fragile X syndrome, a pre-selection of clinical features will be required.

Fragile X checklists have been shown to be effective in the selection of patients among intellectually disabled men for further laboratory testing. However, the predictive features may differ among ethnic groups. Therefore, establishing checklists for specific populations might increase the number of FXS-positive patients selected.

In a previous study of Brazilian patients, Christofolini et al. (2007) compared the checklists of Butler et al. (1991) and Laing et al. (1991) by using them for identifying fragile X patients in a cohort of 200 intellectually disabled males. Butler et al.'s (1991) checklist proved to be more efficient, although it includes features which are uncommon in the Brazilian population, such as pale blue eyes.

As shown in Table 1, a significant association ($p = 0.0015$) between fragile X syndrome and a positive familial history of ID was detected: out of 90 patients with familial intellectual disability, 22 (24.4%) were fragile X positive. This remarkable association with familial intellectual disability has been found in many studies that investigated FXS (Butler et al., 1991; Christofolini et al., 2007; de Vries et al., 1997; 1999; Hagerman et al., 1991).

Large testes have been reported as common in males with FXS. According to some authors, this feature is more evident after puberty, being difficult to screen in younger boys (Giangreco et al., 1996; Hagerman et al. 1991; Hjalgrim et al., 1998; Kirkilionis et al., 1988). Another trait that becomes more evident postpubertally is the long face (Hagerman, 1987; Thake et al., 1985). In our sample, we did not find significant differences between pre- and postpubertal patients for these features, although they tended to differ when comparing boys under 12 years who were fragile X positive and negative (large testes $p = 0.0514$, elongated face $p = 0.0594$).

Hagerman (1987) reported that hyperextensible finger joints, hyperactivity and handflapping were frequently observed in prepubertal individuals with FXS. We found a significantly higher frequency of hand flapping in both pre- and postpubertal FXS patients ($p = 0.0148$ and 0.0038, respectively) when compared to other mentally impaired patients.

Large ears were more frequently present in adult fragile X positive than in fragile X negative patients ($p = 0.0187$), whereas there was no difference between FXS positive and negative up to 12 years old ($p = 0.1309$). This finding can be explained by the fact that this feature becomes more evident with age.

In the study of Giangreco et al. (1996), behavior appeared to be very important as a predictor of FXS in prepubertal individuals. In our investigation, some behavioral features were more frequent in children than in adults, such as hand biting ($p = 0.0236$) and poor eye contact ($p = 0.0087$). Furthermore, in our sample, the frequency of hand biting and poor eye contact was significantly higher only in the prepubertal fragile X positive patients.

Based on these results, we propose a fragile X checklist for the Brazilian population comprising the following 10 features: family history of intellectual disability, elongated face, large ears, hyperextensible finger joints, large testes, plantar crease, hand biting, hand flapping, tactile defensiveness, and poor eye contact. We considered the attribution to be of value 1 if the characteristic is present and 0 if it is absent. The ROC curve showed a cutoff value of 8, with sensitivity 0.8 and specificity 0.93 (shown as 'sensibility'.

When we analyzed the scores obtained for patients with and without the *FMR1* gene mutation, based on the 10 features considered predictive for FXS, we observed that the fragile X negative patients were concentrated around the median scores and the fragile X positive patients were clustered at the higher scores.

Adopting score 8, 46 percent of the sample would have been tested, with a detection rate of 83.3 percent of fragile X positive patients. Using score 4, 172 patients (89.6%) of the total sample would have been tested, with a detection rate of 100 percent of fragile X positive patients.

In spite of the ethnical heterogeneity of the Brazilian population, we succeeded in selecting a relatively small number of predictive features for FXS.

Given the high frequency of intellectually disabled males in our population, implementing adequate checklist screening methods will increase the cost-effectiveness of FXS laboratory testing, without the risk of a significant level of underdiagnosis.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

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Date accepted 16/08/09