



Predictive testing for cognitive functioning in female carriers of the fragile X syndrome using hair root analysis

R Willemsen, A Smits, L-A Severijnen, M Jansen, A Jacobs, E De Bruyn and B Oostra

J. Med. Genet. 2003;40;377-379
doi:10.1136/jmg.40.5.377

Updated information and services can be found at:
<http://jmg.bmj.com/cgi/content/full/40/5/377>

These include:

References

This article cites 22 articles, 3 of which can be accessed free at:
<http://jmg.bmj.com/cgi/content/full/40/5/377#BIBL>

Rapid responses

You can respond to this article at:
<http://jmg.bmj.com/cgi/eletter-submit/40/5/377>

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Topic collections

Articles on similar topics can be found in the following collections

[Genetics](#) (3972 articles)

Notes

To order reprints of this article go to:
<http://journals.bmj.com/cgi/reprintform>

To subscribe to *Journal of Medical Genetics* go to:
<http://journals.bmj.com/subscriptions/>

LETTER TO JMG

Predictive testing for cognitive functioning in female carriers of the fragile X syndrome using hair root analysis

R Willemsen, A Smits, L-A Severijnen, M Jansen, A Jacobs, E De Bruyn, B Oostra

J Med Genet 2003;**40**:377-379

The fragile X syndrome, the most common form of hereditary cognitive impairment, with a frequency of 1:4000 males and 1:6000 females, is caused by expansion of a trinucleotide repeat (CGG) within the *FMR1* gene.¹ In the normal population, the length varies from five to 50 repeats.² Subjects with the fragile X syndrome have more than 200 CGG repeats (full mutation, FM) and, as a consequence, the *FMR1* promoter region, including the CGG repeat, is hypermethylated.^{3,4} As methylation results in a lack of *FMR1* gene transcription, no *FMR1* protein (FMRP) is produced. The absence of FMRP in the brain (neurones) is responsible for the cognitive impairment in the fragile X syndrome.^{5,6} In addition, some subjects have intermediate sized alleles of between 50 and 200 CGG repeats (premutations; PM). The PM alleles are unmethylated with normal FMRP biosynthesis, but are unstable during transmission to the next generation.

Male fragile X patients are characterised by mild to severe learning difficulties. Macro-orchidism and facial abnormalities, including a long face with large, prominent ears and behavioural features such as hyperactivity, poor eye contact, and hand flapping, may be part of the fragile X phenotype in males. Generally, there is wide variation in the degree of clinical involvement in female FM carriers. Approximately 60% of females carriers have mild to moderate mental impairment, while the remaining 40% have normal intellectual capacity. The molecular basis for the phenotypic variability in both males and females is believed to be linked to the variable number of neurones in the brain that express FMRP. Mosaic males who are affected show a variable combination of PM and FM alleles, but the usual predominance of FM.⁷ In female carriers of FM, variation in the X chromosome inactivation ratio may account for the clinical variability in intellectual capacity. Some studies reported a correlation between the activation ratio and cognitive functioning using DNA isolated from peripheral blood,⁸⁻¹³ whereas another study failed to find such a correlation.¹⁴

Recently, a new diagnostic test has been described to identify fragile X patients on the basis of an absence of FMRP in their hair roots.¹⁵ The FMRP test on hair roots has high diagnostic power in males. Most hair roots in affected males are devoid of FMRP. In cells from female FM carriers, one of the two X chromosomes is inactivated and will not produce FMRP. As human hair roots are of clonal origin, they are labelled either positive or totally negative for FMRP expression, depending on which X chromosome is active, the normal *FMR1* allele or the mutant *FMR1* allele, respectively. Furthermore it has been suggested that hair root testing might be of value for predicting mental functioning in female carriers of FM because, like brain tissue, hair roots originate from the ectoderm during embryonic development. Thus, the X inactivation pattern in hair roots might be indicative of the X inactivation pattern in the brain and in this way reflect the number of neurones that express FMRP. Recently, we have made such an observation in monozygotic twin sisters who carry FM. One sister is intellectually normal and shows normal FMRP expression in her hair roots, whereas her

Key points

- The objective of this study was to determine whether there is a correlation between FMRP expression in hair roots and cognitive functioning in female FM carriers.
- The expression of FMRP in hair roots was studied using an FMRP specific antibody test; the percentage of hair follicles that expressed FMRP was determined. In addition, the participants completed the Raven's Progressive Matrices (RPM) to measure their basic cognitive functioning.
- Female FM carriers had significantly lower median centile RPM score than their control relatives (n=12). No significant correlation was found between the centile scores of the female FM carriers and their control relatives on the RPM. In the female FM carrier group (n=34), a highly significant relationship was found between the centile score on the RPM and the percentage of hair roots that expressed FMRP.
- Cognitive functioning in the female FM carriers was much more strongly determined by the absence of FMRP than by genetic background. FMRP expression testing of hair roots has great potential as a prognostic indicator of cognitive functioning in female FM carriers.

affected sister shows a reduced percentage of hair roots that express FMRP.¹⁶ To investigate the molecular basis of the spectrum of cognitive functioning in female FM carriers and to evaluate the predictive power of the FMRP assay on hair roots, we compared FMRP expression in hair roots and basic cognitive functioning between female FM carriers and unaffected female control relatives.

METHODS

Participants

In this study we included women whose CGG repeat size had already been tested by DNA analysis. In total, 34 female carriers of a FM allele (>200 CGG repeats) and 12 female control relatives carrying either two normal alleles (sister, 5-50 CGG repeats, n=10) or one premutation allele (mother, 50-200 CGG repeats, n=2) were tested. After giving written informed consent, the women were visited at home to obtain hairs from the scalp and to measure cognitive functioning. The age of the participants varied from 16-67 years.

FMRP test on hair roots

At least 25 hair roots from different areas of the scalp were collected in an envelope and sent to the laboratory by regular mail on the same day. The FMRP assay was performed the next day as described previously.¹⁵ Briefly, hairs with a visible bulb were trimmed, fixed, and permeabilised. They were then incubated with monospecific antibodies against FMRP overnight at 4°C. Subsequently, indirect alkaline phosphatase

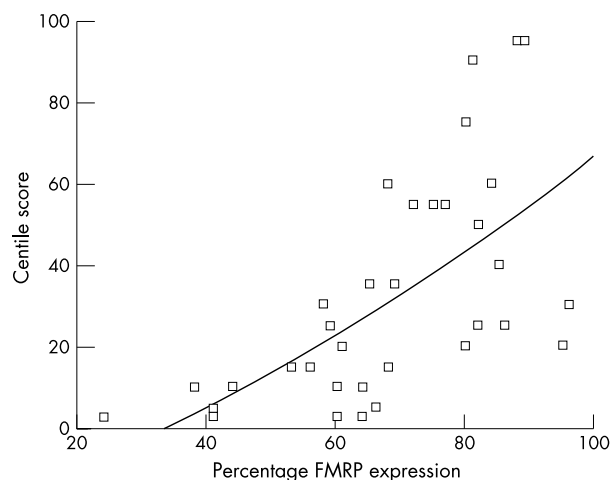


Figure 1 Diagram of the correlation between the Raven Progressive Matrices centile scores and the percentage of hair roots that expressed FMRP in the female FM carriers (n=34).

immunostaining was performed and the number of FMRP labelled hair roots was scored with a stereo zoom microscope and expressed as a percentage of the total number of hair roots examined. All hair roots were tested in a blind fashion. For detailed technical information see our website: www.eur.nl/fgg/ch1/fragx/

Cognitive testing

All the participants individually completed the Standard Raven's Progressive Matrices. The RPM was constructed by Raven¹⁷ in 1954 and ever since has been widely applied in both clinical practice and research. The RPM measures the eductive ability component of general intelligence (g) as defined in Spearman's theory of cognitive ability. Raw scores are converted into centile scores and into deviation IQs, although the latter may be less adequate.¹⁷ In the present study, raw scores were converted into centile norm scores using Burke's norms (Raven, 1998, No 2495).

Statistical analysis

Using a paired samples correlation analysis, we examined the relation between the normalised RPM centile scores of 12 pairs of FM carrier participants and their unaffected control relatives. A regression analysis resulting in a Pearson correlation coefficient was completed to examine the correlation between the percentage of hair roots that expressed FMRP and the RPM centile score as a measure of cognitive functioning for a group of 34 FM carriers.

RESULTS

The contribution of genetic background and environmental factors to cognitive functioning in female carriers of the FM was investigated by studying FMRP expression in hair roots and cognitive functioning of female FM carriers and a group female control relatives. All the members of the female control relatives group scored normal percentages of FMRP expression in their hair roots (75-100%). The median centile RPM scores in the female FM carriers and in their control relatives were 31 and 70, respectively. The means of the normalised centile RPM scores in the female FM carriers and in their control relatives were 0.6446 and 0.6774, respectively. Female FM carriers scored significantly lower than their control relatives; however, the paired sample analysis did not show any significant correlation between the two groups ($r=0.35$, $p>0.2$). This finding prompted us to continue our study only on the group of female FM carriers, because the centile RPM scores of the normal control relatives did not make any significant

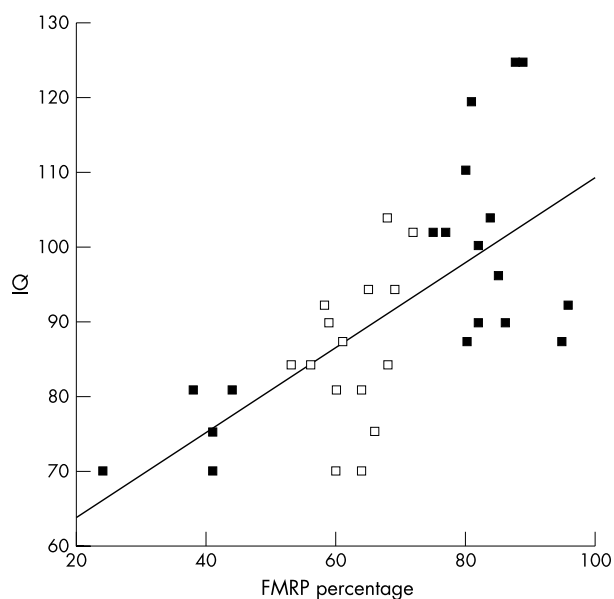


Figure 2 Diagram of the correlation between the IQ scores (converted from RPM raw scores) and the percentage of hair roots that expressed FMRP in the female carriers (n=34).

contribution to the final outcome of cognitive functioning. Further analysis was performed on 22 additional female FM carriers, bringing the total of female FM carriers to 34. Regression analysis of the FMRP expression on the RPM centile scores showed a significant F statistic ($F(1,33) = 19.973$, $p=0.00$). Fig 1 shows a plot of the correlation between the percentage of hair roots that expressed FMRP and the RPM centile scores of the female FM carriers. The analysis showed a highly significant relationship between the two parameters in this group ($r=0.614$, $p<0.01$). In addition, fig 2 shows a plot of the correlation between the percentage of hair roots that expressed FMRP and the IQ scores of the female FM carriers ($Rsq= 0.4356$).

DISCUSSION

Our study found evidence that female FM carriers with normal intelligence had a normal percentage of hair roots that expressed FMRP (75-100%), whereas FM carriers with mental impairment had a reduced percentage of hair roots that expressed FMRP. This observation confirms the notion that although human intelligence is influenced by many genetic factors, a single mutation in the *FMR1* gene significantly reduces cognitive functioning in affected female FM carriers. Our comparison between female FM carriers and control relatives shows that it is not necessary to obtain data on cognitive functioning from control relatives to assess the intellectual outcome of female FM carriers using the FMRP test on hair roots.

In the past, molecular studies on the *FMR1* gene in female FM carriers to establish a relation between genotype-phenotype have focused on the size of the CGG repeat, the ratio of active normal X chromosomes to normal inactive X chromosomes (AR ratio), and FMRP expression in lymphocytes on the one hand and on IQ values on the other. The results of these studies were not unequivocal,^{8-14 18-22} which in part can be explained by methodological issues and the choice of biopsy tissue (blood). Genotype-phenotype correlations in leucocytes do not necessarily reflect the situation in brain tissue, because the rapid turnover of peripheral leucocytes may lead to high variation in clones that either carry the *FMR1* mutation on the active X chromosome or do not. Earlier studies using the FMRP test on lymphocytes did show a weak statistical correlation; however, the significance was not high

enough to use this method as a reliable predictive test.²⁰⁻²² We addressed these issues by using cells (hair roots) which during embryonic development originate from the same germ layer as brain tissue and were able to show that in female FM carriers there is a highly significant correlation between cognitive functioning and the percentage of hair roots that express FMRP. This genotype-phenotype correlation is preliminary in nature but still illustrates the potential power of the FMRP assay on hair roots as a predictive test for cognitive functioning in female FM carriers. Further sophistication of FMRP expression in hair roots as a predictive test for cognitive functioning should include larger numbers of hair samples to establish whether the reliability of the test can be increased. Research is in progress to increase the number of female FM carriers to 100.

This study focused primarily on cognitive functioning in female FM carriers and the relation with FMRP expression in hair follicles. Although the spectrum of involvement in male carriers (who are always affected) varies from mildly to severely retarded, predictive testing is in most cases not informative. In contrast, cognitive functioning in female FM carriers can vary from unaffected to moderately retarded, which makes a test for predicting the absence or presence of mental impairment very useful. The availability of a test to establish the genotype-phenotype relationship in female FM carriers would enable parents to choose educational intervention at an early stage, including adequate support and child adapted educational programmes (special schools) as has been suggested for less severely retarded fragile X males.²²

We conclude that cognitive functioning in female FM carriers is more strongly determined by the absence of FMRP than by genetic background or environmental factors. In addition, we conclude that the FMRP test on hair roots might be a strong prognostic indicator of cognitive functioning in female FM carriers and has great potential as a predictive test.

ACKNOWLEDGEMENTS

The authors wish to thank Drs L Sandkuyl, J Kremer-Nas, S van 't Padje, Y De Diego Otero, and J Abma for their contributions. This work was supported by the Dutch Brain Foundation grant No 7F99.(2).19 (RW).

Authors' affiliations

R Willemsen, L-A Severijnen, B Oostra, Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands
A Smits, A Jacobs, Department of Human Genetics, University Hospital, Nijmegen, The Netherlands
M Jansen, E De Bruyn, Institute of Family and Child Care Studies, University of Nijmegen, The Netherlands

Correspondence to: Dr R Willemsen, Department of Clinical Genetics, Erasmus MC, PO Box 1738, 3000 DR Rotterdam, The Netherlands; r.willemsen@erasmusmc.nl

REFERENCES

- Kooy RF**, Willemsen R, Oostra BA. Fragile X syndrome at the turn of the century. *Mol Med Today* 2000;**6**:193-8.
- Fu YH**, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick R, Jr., Warren ST, Oostra BA, Nelson DL, Caskey CT. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell* 1991;**67**:1047-58.
- Verkerk AJ**, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP, Eussen BE, Van Ommen GJB, Blonden LAJ, Riggins GJ, Chastain JL, Kunst CB, Galjaard H, Caskey CT, Nelson DL, Oostra BA, Warren ST. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991;**65**:905-14.
- Oberlé I**, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boue J, Bertheas MF, Mandel JL. Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science* 1991;**252**:1097-102.
- Verheij C**, Bakker CE, de Graaff E, Keulemans J, Willemsen R, Verkerk AJ, Galjaard H, Reuser AJ, Hoogeveen AT, Oostra BA. Characterization and localization of the FMR-1 gene product associated with fragile X syndrome. *Nature* 1993;**363**:722-4.
- Devys D**, Lutz Y, Rouyer N, Bellocaq JP, Mandel JL. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. *Nat Genet* 1993;**4**:335-40.
- Nolin SL**, Glicksman A, Houck GE, Brown WT, Dobkin CS. Mosaicism in fragile X affected males. *Am J Med Genet* 1994;**51**:509-12.
- Rousseau F**, Heitz D, Oberlé I, Mandel JL. Selection in blood cells from female carriers of the fragile X syndrome: inverse correlation between age and proportion of active X chromosomes carrying the full mutation. *J Med Genet* 1991;**28**:830-6.
- Abrams MT**, Reiss AL, Freund LS, Baumgardner TL, Chase GA, Denckla MB. Molecular-neurobehavioral associations in females with the fragile X full mutation. *Am J Med Genet* 1994;**51**:317-27.
- Reiss AL**, Freund LS, Baumgardner TL, Abrams MT, Denckla MB. Contribution of the FMR1 gene mutation to human intellectual dysfunction. *Nat Genet* 1995;**11**:331-4.
- De Vries LBA**, Wiegers AM, Smits APT, Mohkamsing S, Duivenvoorden HJ, Fryns JP, Curfs LMG, Halley DJJ, Oostra BA, Van den Ouweland AM, Niermeijer MF. Mental status of females with a FMR1 gene full mutation. *Am J Hum Genet* 1996;**58**:1025-32.
- Sobesky WE**, Taylor AK, Pennington BF, Bennetto L, Porter D, Riddle J, Hagerman RJ. Molecular/clinical correlations in females with fragile X. *Am J Med Genet* 1996;**64**:340-5.
- Riddle JE**, Cheema A, Sobesky WE, Gardner SC, Taylor AK, Pennington BF, Hagerman RJ. Phenotypic involvement in females with the FMR1 gene mutation. *Am J Ment Retard* 1998;**102**:590-601.
- Taylor AK**, Safanda JF, Fall MZ, Quince C, Lang KA, Hull CE, Carpenter I, Staley LW, Hagerman RJ. Molecular predictors of cognitive involvement in female carriers of fragile X syndrome. *JAMA* 1994;**271**:507-14.
- Willemsen R**, Anar B, de Diego Otero Y, de Vries BB, Hilhorst-Hofstee Y, Smits A, van Looveren E, Willems PJ, Galjaard H, Oostra BA. Noninvasive test for fragile X syndrome, using hair root analysis. *Am J Hum Genet* 1999;**65**:98-103.
- Willemsen R**, Olmer R, De Diego Otero Y, Oostra BA. Twin sisters: monozygotic with the fragile X mutation, but with a different phenotype. *J Med Genet* 2000;**37**:603-4.
- Raven J**. The Raven's progressive matrices: change and stability over culture and time. *Cognit Psychol* 2000;**41**:1-48.
- Rousseau F**, Heitz D, Tarleton J, Macpherson J, Malmgren H, Dahl N, Barnicoat A, Mathew C, Mornet E, Tejada I, Maddalena A, Spiegel R, Schinzel A, Marcos J, Schorderet DF, Schaap T, Maccioni L, Russo S, Jacobs PA, Schwartz C, Mandel JL. A multicenter study on genotype-phenotype correlations in the fragile X syndrome, using direct diagnosis with probe StB 12.3: The first 2,253 cases. *Am J Hum Genet* 1994;**55**:225-37.
- Willemsen R**, Mohkamsing S, De Vries B, Devys D, Van den Ouweland A, Mandel JL, Galjaard H, Oostra B. Rapid antibody test for fragile X syndrome. *Lancet* 1995;**345**:1147-8.
- Willemsen R**, Smits A, Mohkamsing S, Vanbeerendonck H, Dehaan A, Devries B, Vandenuweland A, Sijstermans E, Galjaard H, Oostra BA. Rapid antibody test for diagnosing fragile X syndrome: a validation of the technique. *Hum Genet* 1997;**99**:308-11.
- Kaufmann WE**, Abrams MT, Chen W, Reiss AL. Genotype, molecular phenotype, and cognitive phenotype: correlations in fragile X syndrome. *Am J Med Genet* 1999;**83**:286-95.
- Tassone F**, Hagerman RJ, Ikle DN, Dyer PN, Lampe M, Willemsen R, Oostra BA, Taylor AK. FMRP expression as a potential prognostic indicator in fragile X syndrome. *Am J Med Genet* 1999;**84**:250-61.