The fragile X syndrome

Bert B A de Vries, Dicky J J Halley, Ben A Oostra, Martinus F Niermeijer

Abstract
The fragile X syndrome is characterised by mental retardation, behavioural features, and physical features, such as a long face with large protruding ears and macro-orchidism. In 1991, after identification of the fragile X mental retardation (FMR1) gene, the cytogenetic marker (a fragile site at Xq27.3) became replaced by molecular diagnosis. The fragile X syndrome was one of the first examples of a "novel" class of disorders caused by a trinucleotide repeat expansion. In the normal population, the CGG repeat varies from six to 54 units. Affected subjects have expanded CGG repeats (>200) in the first exon of the FMR1 gene (the full mutation). Phenotypically normal carriers of the fragile X syndrome have a repeat in the 43 to 200 range (the premutation).

The cloning of the FMR1 gene led to the characterisation of its protein product FMRP, encouraged further clinical studies, and opened up the possibility of more accurate family studies and fragile X screening programmes.

Keywords: fragile X syndrome; FMR1 gene; mental retardation

History
In 1943, Martin and Bell described sex linked mental retardation without dysmorphic features in a family in which both affected males and females were observed. Thirty-six years later, Lubs reported a marker X chromosome (later to be known as the fragile X chromosome) as an inconsistent finding in cytogenetic studies in leucocytes of some mentally retarded males. The folic acid and thymidine depleted cell culture medium was identified as the essential factor for the induction of this heritable fragile site at Xq27.1 During the seventies, the combination of X linked mental retardation and macro-orchidism was recognised.4,6 Later, other clinical characteristics were established, for example, a long face with large, protruding ears, and behavioural features, including avoidance of eye contact, hyperactivity, hand flapping, and perseverative speech.1,8

The fragile X syndrome was diagnosed at that time by cytogenetic detection of the fragile site at Xq27.3. It had some special features for an X linked disorder. Approximately 30% of the obligate carrier females were mildly or moderately retarded and a large portion (50%) had no cytogenetic fragile X expression. Also, mentally normal grandfathers linking two family branches with the fragile X syndrome were observed. These "normal transmitting males" without any clinical or cytogenetic features could apparently transmit the fragile X syndrome.

The gene involved in the fragile X syndrome, the Fragile X Mental Retardation (FMR1) gene, was identified in 1991.9-11 The gene defect was the first expansion of a trinucleotide repeat to be discovered and a whole class of disorders is now known to be associated with this type of mutation.12

Clinical studies
The original "Martin-Bell family" was restudied in 198113 and the typical cytogenetic and clinical features were found. The "Martin-Bell phenotype" was proposed as an eponym for the phenotype of affected males. Additional clinical features include high arched palate (52%), strabismus (36%), hyperextensible metacarpophalangeal joints (67%), hand calluses (29%), double jointed thumbs (53%), single palmar creases (25%), pes planus (71%), and heart murmur or click (18%) (figs 1 and 2, table 1).8,9,10 The facial features are often less noticeable, particularly in affected females and children (fig 3). The macro-orchidism often develops during or after puberty and is frequently absent in young patients.7,8 Seizures are observed in approximately 20% of young affected males, with a lower prevalence in adults.8,13,14

Fragile X infants often have relative macrocephaly persisting into adult life.15 However, the adult height of affected males is below the norm.16,17 A few patients present with either overweight or general persisting overgrowth which can be confused with either Prader-Willi syndrome or Sotos syndrome.19,20 Associations with other syndromes are reported; examples are the Robin sequence (micrognathia, glossophtosis, and cleft soft palate),21 the FG syndrome (congenital hypotonia, macrocephaly, distinctive face, and imperforate anus),21,22 and the DiGeorge anomaly (defects of thymus, parathyroids, and great vessels).23 However, there is no definite evidence for a causal relationship between the FMR1 gene.
mutation and these phenotypes. Several observations of the co-occurrence of the fragile X syndrome and sex chromosomal abnormalities like 47,XXX or 45,XO/46,XX\(^{26-34}\) suggest an increased rate of non-disjunction related to the fragile X chromosome.

Scales for facilitating diagnosis based on physical and behavioural features have been developed and found to be effective.\(^{5-59}\) Recognition of behavioural features also assists in the diagnosis. In the younger child, hand flapping, hand biting, tactile defensiveness, poor eye contact, hyperactivity, and perseverative speech are suggestive of the diagnosis.\(^5\) The “autistic-like” behaviour of many fragile X males initiated the debate about whether autism is associated with the fragile X syndrome.\(^40-42\)

Although in female premutation carriers an increased incidence of premature menopause has been reported,\(^43-47\) a recent study indicated that the premutation does not seem to be a major risk for early menopause.\(^48\)

Although several neuroimaging and a few neuropathological studies have been done on the central nervous system in fragile X patients, knowledge on specific structural and cellular defects is still limited. Reduced size of the posterior cerebellar vermis on MRI in male patients and increased size of the caudate nucleus, thalamus, and hippocampus have been reported.\(^47-48\) These findings have neither been confirmed nor refuted by others.

Neuropathological studies of three fragile X males’ brains showed immature, thin, and long dendritic spines in different regions, with a reduction in mean synaptic contact but with preservation of neuronal density in the neocortex.\(^49-50\) Similar abnormal dendritic spines were observed in fragile X knockout mice.\(^51\) Interestingly, in normal mouse brain, high FMRI expression is observed in the synaptonematosomes which could be related to the high transcriptional activity necessary for function of neuronal spines.\(^52\) However, abnormally long, thin dendritic spines are also seen in cases with chromosomal abnormalities\(^53\) or unspecified mental retardation.\(^54\) Apparently, these are non-specific neuropathological changes, as is the neuronal heterotopia observed in two affected males and linked to their epilepsy. In the testes, macro-orchidism seems to be associated with increased tubular length and interstitial oedema.\(^49-55\)

The FMR1 gene

The identification of the FMR1 gene\(^5-11\) principally elucidated the special characteristics of the transmission of the defect in fragile X families rather than explaining the mental retardation itself. The FMR1 gene has a size of 38 kb with 17 exons\(^57\) and an untranslatable polymorphic CGG repeat in the first exon.\(^58\) In the normal population, this CGG repeat varies from six to 54 units\(^56\) (fig 4) with an average of 30 units. The mutations in the FMR1 gene can be divided into a major and a minor class (table 2). The major class of FMR1 gene mutations affects the CGG repeat, whereas mutations in the coding region are infrequently observed.

**CGG REPEAT EXPANSIONS**

On the basis of the size of the CGG repeat and its effect on protein and phenotypic expression,
The fragile X syndrome

affected offspring having

60

larger

premutation

depends

The intergenerational (in)stability

maladies in

premutation

missing parent and

The mutations in the CGG repeat are dynamic. They may change from generation to generation as well as within a single person during early embryogenesis. Stable alleles with a CGG repeat <55 units are defined as normal, and unstable alleles ranging from 43 to 200 repeat units are regarded as premutation alleles.

The premutation and its intergenerational instability

The premutation with repeats in the range of 43 to 200 units causes no phenotypic abnormalities in (male and female) carriers (fig 4). The intergenerational (in)stability of the premutation is dependent on the sex of the transmitting parent and on the size of the repeat. Only women with a premutation have a risk of having affected offspring with a full mutation. This risk of expansion into a full mutation depends on the size of their premutation; it is less than 20% for smaller premutation alleles (<70 CGG repeats), but more than 80% for larger premutation alleles (>80 CGG repeats).

The CGG repeat is generally interspersed with two AGG triplets and their position at the 3' end influences repeat instability, with an instability threshold of 34-38 uninterrupted CGG repeats. The potential predictive value of the size of the uninterrupted CGG repeat for risk prediction in the offspring of female premutation carriers has not been adequately evaluated until now.

In the 43 to 55 CGG repeat range, there is an overlap between normal and premutated alleles, often referred to as the "grey zone". Only the observation of intrafamilial instability identifies these "intermediate" alleles as premutation alleles. However, such premutation alleles can also be stably inherited through many generations. Alteration in one generation from alleles of <43 to >43 repeats is unknown, suggesting that such changes are very gradual. However, regressions from premutation to normal size (<43 repeats) have been observed within one generation.

The timing of any transition from pre- to full mutation is not fully understood. The presence of full mutations in the ovary of female fetuses, without evidence of a premutation, and simulation studies support the hypothesis of preconceptional enlargement. This seems to conflict with the finding of only premutations in the sperm of four males with a full mutation in somatic cells. The latter observation might be explained by regression of a full mutation to a premutation in a limited number of cell

Figure 2 Some clinical features in fragile X subjects. (A) High arched palate, (B) dental crowding, (C) hyperextensible MP joints, (D) hand calluses, and (E) pes planus.

The latter observation might be explained by regression of a full mutation to a premutation in a limited number of cell
lineages. In the germline, we have then to assume a selection for those cells with a premutation.

The full mutation

Subjects with the fragile X syndrome have CGG repeats above 200 units, the "full mutation" (fig 4). The expansion into full mutation is usually accompanied by hypermethylation of the repeat and its flanking regions, resulting in a shut down of transcription and the absence of the FMR1 protein. The latter causes the mental retardation. All males and a majority of the females with a hypermethylated full mutation are mentally retarded.

In somatic cells of fragile X patients, the mitotic instability of the repeat of the full mutation causes longer and shorter expansions, so all fragile X patients are somatic mosaics. The instability occurs during early embryogenesis and results in this somatic heterogeneity.

In fragile X patients, two special subclasses of mosaicism can be distinguished on the basis of size and methylation pattern.

(1) "Size mosaics" are those patients with both a full and a premutation, which is observed in 20-40% of the male patients. Also, males with somatic mosaicism for a full mutation and a (partial) deletion of the FMR1 gene have been reported.

(2) "Methylation mosaics" are subjects with variations between cells in the methylation status of a full mutation. The proportion of leucocytes with an unmethylated full mutation may vary from low (≤10%) to 100%. A few intellectually normal males with a high proportion (>60%) of leucocytes with an unmethylated full mutation have been reported.

OTHER MUTATIONS

Mutations other than CGG repeat expansions are most often large deletions (partial or complete) of the FMR1 gene, with or without surrounding regions. Several unrelated cases have been reported.

The first intragenic mutation in the FMR1 gene, a de novo point mutation (an Ile304Asn substitution) in exon 11, was reported in a profoundly retarded male with fragile X features. The missense mutation resided in one of the important domains of the FMR1 protein. Intragenic mutations may result in an absence of FMRP and thereby cause the fragile X syndrome. One single de novo base pair deletion in exon 5 and an inherited two base pair substitution in exon 2 resulting in a loss of protein production have been described. A C to T point mutation in the 14th nucleotide of intron 10 of the FMR1 gene causing a deletion of exon 10 was reported in three unrelated fragile X patients. All five male patients showed the mental retardation and physical features characteristic of the fragile X syndrome.

Molecular diagnosis

Before the cloning of the FMR1 gene, cytogenetic detection of the fragile site at Xq27.3 (FRAXA) was the only method for (prenatal) diagnosis and carrier detection. However, this method was inadequate for carrier detection and other fragile sites localised near FRAXA caused diagnostic confusion, like FRAXE.

Table 2 Mutations in the FMR1 gene and their effect

<table>
<thead>
<tr>
<th>Mutation</th>
<th>No of cases</th>
<th>FMRP</th>
<th>MR</th>
<th>Physical features</th>
<th>References *</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGG repeat expansion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premutation (43-200 CGGs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full mutation (&gt;200 CGGs)</td>
<td>Innumerable</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>58, 79, 198</td>
</tr>
<tr>
<td>&gt;60% methylated</td>
<td>Innumerable</td>
<td>Reduced/absent</td>
<td>+</td>
<td>+</td>
<td>75-77, 79</td>
</tr>
<tr>
<td>+ premutation</td>
<td>Innumerable</td>
<td>Reduced</td>
<td>+</td>
<td>+</td>
<td>80, 86, 87, 93, 157, 158</td>
</tr>
<tr>
<td>+ deletion</td>
<td>8</td>
<td>Absent</td>
<td>+</td>
<td>+</td>
<td>87-91</td>
</tr>
<tr>
<td>&lt;40% methylated</td>
<td>16</td>
<td>&gt;60%</td>
<td>-</td>
<td>Absent or minor</td>
<td>92-99</td>
</tr>
<tr>
<td>Other mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 base pair change (exon 2)</td>
<td>1</td>
<td>Absent</td>
<td>+</td>
<td>+</td>
<td>115</td>
</tr>
<tr>
<td>1 base pair deletion (exon 5)</td>
<td>1</td>
<td>Absent</td>
<td>+</td>
<td>+</td>
<td>115</td>
</tr>
<tr>
<td>1 base pair change (exon 11)</td>
<td>1</td>
<td>Present</td>
<td>+</td>
<td>+ (less typical)</td>
<td>113</td>
</tr>
<tr>
<td>1 base pair change (intron 10)</td>
<td>3</td>
<td>Truncated</td>
<td>+</td>
<td>+</td>
<td>116</td>
</tr>
<tr>
<td>Partial or complete deletion of FMR1 gene</td>
<td>14</td>
<td>Absent</td>
<td>+</td>
<td>(some atypical)</td>
<td>100-112</td>
</tr>
</tbody>
</table>

*For additional references see text.
The fragile X syndrome

There is a fragile X syndrome

The fragile X syndrome

Figure CGG repeat

precaution of the full mutation

methylating in chorionic

in about because it

sample enzyme even

is found for delicate

with stabilisation

PCR size repeat

for detection of

methylated full mutations

island in sensitive restriction

methylation fragment

The actual diagnostic standard is the size of the

CGG repeat amplification in patients and carriers. Two major methods (Southern blots and PCR) are used. For Southern blotting different restriction enzymes may be used, such as HindIII, BglII, and PstI, each with their specific fragment sizes. A double digest, including a methylation sensitive restriction enzyme, enables detection of the methylation of the CpG island in the CGG region. This is also valuable for identification of males with incompletely methylated full mutations (“methylation mosaics”). The double digest may also be used in distinguishing between an unmethylated large premutation and a small methylated full mutation.

An alternative method for determining CGG repeat size is the polymerase chain reaction or PCR analysis. However, CGG repeats of more than =150 units are generally difficult to amplify and will often not give a detectable product after the PCR.

Most laboratories use both methods (restriction enzyme and PCR analysis) for postnatal and prenatal diagnosis. When a premutation is found in a chorionic villus sample, confirmation in amniotic fluid or a cord blood sample might be considered to ascertain the stability of the premutation during fetal life. In the (near) future, when sufficient information about stability becomes available, this precaution may become unnecessary. Absence of methylation of the fully expanded mutation in chorionic villi at 11 weeks of gestational age has been observed in a fetus with hypermethylation of the full mutation in fetal tissue. Methylation status is therefore unreliable in an early (<12 weeks of pregnancy) chorionic villus sample because it may differ from the fetal tissue. Nevertheless, testing of methylation status may be useful in amniotic cells.

The methods described above can identify repeat amplifications in the CGG repeat and large deletions in the FMR1 gene. Subtle mutations outside the CGG repeat will not be detected. At present, only a limited number of mutations in the coding region of the FMR1 gene have been found using sequencing techniques. It is conceivable that other rare mutations in the FMR1 gene will be detected in the future.

FMRF antibody test

Recently, an FMRF protein antibody test was developed for detecting the presence or absence of FMRF in lymphocytes. This rapid detection method allows the diagnosis of fragile X syndrome from a blood smear; cells from fragile X males with a methylated full mutation produce no FMRF. The proportion of cells with FMRF production allows a distinction between males with the fragile X syndrome (<30% staining cells) and normal subjects (>40% staining cells). The test does not detect dysfunctional proteins, neither does it differentiate between normal and premutation alleles.

As a result of lyonisation, females with a full mutation may have staining of up to 80% of the lymphocytes, overlapping with 40% of the normal females who have staining in less than 80% of their lymphocytes. The test is therefore not suitable for detecting females with a full mutation or a premutation.

The main application for this rapid and cheap test is screening for the fragile X syndrome among mentally retarded males or male neonates. DNA studies are needed to confirm the diagnosis and to ascertain carrier status in their relatives.

Recently, the antibody test was successfully used in the prenatal diagnosis of at risk male

Figure 4 Part of the FMRF gene and its CGG related mutations in the first exon of the FMRF gene that contains the CGG repeat (grey) (see text for further details).
pregnancies using chorionic villi at 12.5 weeks of gestation and uncultured amniotic fluid cells. Further tests are needed to establish the reliability of this technique for prenatal diagnosis because of methylation differences between villi and fetal tissue at week 11.

**Prevalence and screening**

Several genetic epidemiological studies of the fragile X syndrome have been done using various techniques (cytogenetics, DNA) and clinical selection criteria, for example, level of mental retardation, macro-orchidism, or autism. Studies using DNA analysis indicate a prevalence for males of 1/4425 to 1/6045. The combined results of studies from England, the United States, Australia, and The Netherlands (table 3), irrespective of methodological differences, showed, in a total of 4670 mentally retarded subjects, 30 (0.6%) newly diagnosed fragile X patients.

In the general population, a premutation carrier frequency among females of ±1/250 has been observed. This reflects the proportional excess of relatively stable premutation sized alleles over full mutation alleles. This high frequency of premutations might partially be explained by a founder effect in the population studied (French-Canadian).

The identification of the gene paved the way for screening programmes; however, it is still an open question if large scale testing, for example, among the mentally retarded or young adult females, is desirable. Testing mentally retarded subjects leads to diagnosis and improved support for newly diagnosed fragile X patients. Such programmes have been in place since cytogenetic testing was introduced. DNA testing is much more precise, especially for subsequent testing for (pre)mutation carriers in the family.

These programmes are welcomed by parents/guardians and well accepted by the staff of schools and institutes. An underdiagnosis of the fragile X syndrome of more than 50% even in well developed countries is found. The relatively low "yield" of new cases identified (±1% of the tested patients have the fragile X syndrome) may be significantly increased by preselection based on physical features. However, the yield of identified and informed (pre)mutation carriers surpasses the number of identified affected males. Precise identification of the various allele classes may have major implications for screening programmes in the general population. For accurate counselling of females identified with a premutation sized allele, more knowledge of the allele's stability is desirable.

**FMRP**

The translation of the FMR1 gene starts distal to the CGG repeat and results in a 70-80 kDa protein. In lymphoblastoid cells the normal and premutated FMR1 alleles are transcribed equally, resulting in FMR1 transcripts with a half life of 12 hours, and identical levels of FMR1 protein expression. Alternative splicing in the FMR1 gene occurs in various human tissues, leading to 24 distinct transcripts. Whether those transcripts lead to functional proteins is unclear. The localisation of FMRP is predominantly cytoplasmic.

In normal human brain, FMRP is found in nearly all neurones studied, but is absent or reduced in non-neuronal cells. In normal human testes, both the primordial germ cells (prenatally) and spermatogonia (postnatally) show high FMRP expression, with low expression in the Sertoli and Leydig cells. Remarkably, in fragile X patients a proportion of the primordial germ cells and all early spermatogonia show FMRP expression. This is concordant with the presence of a premutation allele in the sperm of patients.

High FMRP expression was also found in other tissues not obviously involved in the fragile X phenotype, for example, dividing mesodermal cells during wound healing and in cardiac hyperplasia, whereas in other circumstances dermis and skeletal and cardiac muscle were negative for FMRP.

---

**Table 3 Overview of DNA screening programmes among institutes and schools for the mentally retarded**

<table>
<thead>
<tr>
<th>Persons studied</th>
<th>Persons with grey zone allele</th>
<th>Persons with premutation</th>
<th>Persons with full mutation</th>
<th>Characteristics of population studied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M+F</td>
<td>M+F</td>
<td>M+F</td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>219 — 219</td>
<td>— —</td>
<td>— —</td>
<td>(0.8%)</td>
</tr>
<tr>
<td>Webb et al</td>
<td>140 — 140</td>
<td>— (0.1%)</td>
<td>— (0.2%)</td>
<td>Mental retardation in schools/institutions</td>
</tr>
<tr>
<td>Turner et al</td>
<td>180 — 180</td>
<td>— —</td>
<td>— —</td>
<td>Mental retardation in special schools</td>
</tr>
<tr>
<td>Jacobs et al</td>
<td>110 — 110</td>
<td>— —</td>
<td>— —</td>
<td>Children with special educational needs</td>
</tr>
<tr>
<td>United States</td>
<td>439 — 439</td>
<td>— —</td>
<td>— —</td>
<td>Boys with learning difficulties</td>
</tr>
<tr>
<td>Hagerman et al</td>
<td>0 — 0</td>
<td>— —</td>
<td>— —</td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>154 — 154</td>
<td>— —</td>
<td>— —</td>
<td></td>
</tr>
<tr>
<td>Slaney et al</td>
<td>35 — 35</td>
<td>— —</td>
<td>— —</td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>51 — 51</td>
<td>— —</td>
<td>— —</td>
<td></td>
</tr>
<tr>
<td>Murray et al</td>
<td>1013 — 1013</td>
<td>35 (41-60)</td>
<td>1 (M) (0.5%)</td>
<td>Mental retardation/learning disabled children</td>
</tr>
<tr>
<td>United States</td>
<td>3591 — 3591</td>
<td>0 — 0</td>
<td>0 — 0</td>
<td>Children in special needs education programmes</td>
</tr>
<tr>
<td>Meadows et al</td>
<td>2729 — 2729</td>
<td>10 (50-60)</td>
<td>0 — 0</td>
<td>(0.2%)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>1531 — 1531</td>
<td>6 (43-60)</td>
<td>0 — 0</td>
<td>Mental retardation/adults in institutions, special schools</td>
</tr>
<tr>
<td>De Vries et al</td>
<td>1370 — 1370</td>
<td>75 (1.8%)</td>
<td>2 (0.04%)</td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>3353 — 3353</td>
<td>0 — 0</td>
<td>6 (0.5%)</td>
<td></td>
</tr>
</tbody>
</table>

*For references see text.
The FMRP has RNA binding capacity owing to characteristic sequence motifs in the protein: an RGG box and ribonucleoprotein particle (RNP) KH homology domains (designated KH domains). Recently, a more specific binding of FMRP was found via RNA with the 60S ribosomal subunit, suggesting a function of FMRP in ribosomal function and in translation of certain proteins. A nuclear localisation signal encoded by the 3' part of the gene, as well as a nuclear export signal encoded by exon 14, were seen. Eberhart et al postulated an interaction of FMRP with mRNA(s) in the nucleus, followed by export to the cytoplasm as part of a mRNP particle where it associates with ribosomes. A role of the FMRP in the translation machinery could explain the high expression in (some) actively dividing pre- and postnatal tissues.

Effects of the absence of FMRP were studied in knockout mice. These mice showed relevant learning deficits, hyperactivity, and macroorchidism as observed in fragile X patients. The knockout mice brain and testes lacked pathological abnormalities, except for enlargement of the testes.

**Mental retardation**

Fragile X males show some variability in intellectual disability: in most prepubertal boys it is moderate (IQ=35-55), and in adults moderate to severe (IQ=20-40). This apparent decline in IQ probably reflects a slowing of cognitive development, stabilising after puberty.

In affected males, a methylated, full mutation will lead to mental impairment whatever the repeat size of the mutation. It has been suggested that males with a “size mosaic” DNA pattern (premutation together with the full mutation with an associated residual FMR1 protein production) might have better mental functioning than fragile X males with a full mutation only. However, others found no mean IQ differences. Apparently, the proportion of brain neurones producing FMRP in “size mosaic” males is insufficient for normal cognitive functioning. This is also shown by the presence of mental retardation in a male mosaic for a partial deletion of the CGG repeat and a full mutation, who had FMRP production in 28% of his lymphocytes.

An indication of the proportion of cells expressing FMRP needed for normal mental functioning is shown in mentally normal males with incompletely methylated full mutations in more than 60% of their leucocytes.

An insufficient proportion of FMRP producing cells also explains cognitive impairment (IQ=85) in females who have a full mutation; 52-82% of the women with a full mutation were shown to have mental impairment (IQ<85). In these heterozygotes, the proportion of abnormal FMR1 alleles on the active X chromosome (which are unable to produce FMRP) has an influence on cognitive development. Apparently, there is no large scale transmission/passage of corrective molecules from FMRP producing cells to non-FMRP producing neurones.

**Management**

Mental retardation and behavioural problems dominate the clinical presentation. The mental retardation is not amenable to intervention. However, careful medical follow up and sometimes intervention are required as the physical and behavioural problems of fragile X patients are related to their stage of development (table 1). Seizures observed in approximately 20% of males and 5% of females necessitate timely diagnosis and treatment. During infancy, connective tissue abnormalities, such as congenital hip dislocations and inguinal hernia, may be present. In later life the connective tissue dysplasia may lead to scoliosis, flat feet, and mitral valve prolapse. The mitral valve prolapse requires evaluation by a cardiologist and a recommendation for antibiotic prophylaxis before surgical or dental procedures.

Some children fail to thrive because of gastro-oesophageal reflux, tactile defensiveness, or difficulties in sucking. The latter of these problems requires attention from a specialised speech therapist or physiotherapist, while the gastro-oesophageal reflux can be treated by dietary advice or medication or both. Surgery is rarely needed. The frequent otitis media and sinusitis in approximately 50% of affected children require adequate intervention (antibiotics or polyethylene tubes or both). Approximately 30-50% of cases need ophthalmological help for strabismus, myopia, or hyperopia.

Behavioural problems include attention deficit and hyperactivity at a young age. Although fragile X patients are generally friendly, some may show aggressive behaviour in adulthood. Influencing these behavioural problems is difficult, although behavioural therapy and avoidance of overwhelming stimuli may alleviate some of the symptoms. In some countries, pharmacological intervention for the behavioural problems is common. However, adequately controlled studies on their effectiveness in the fragile X syndrome are scarce.

The need for special education and training, especially in the younger child, is of primary importance. Speech therapists and physiotherapists can help with language and motor development.

**Conclusions**

Since the original observation of the fragile X syndrome as a familial sex linked mental retardation syndrome in 1943, its clinical and molecular diagnosis has greatly improved. The biological basis for the unique phenomena of normal transmitting males and the “Sherman paradox” became evident after the cloning of the FMR1 gene in 1991, as well as the nature of the CGG repeat at the 5' end of the FMR1 gene and its intergenerational instability. The precise mechanism of the repeat expansion is...
still unknown.195 196 The FMR1 protein shows widespread expression in nearly all tissues studied, with both nuclear and cytoplasmic localisation. The protein seems to shuttle between nucleus and cytoplasm and it can bind to ribosomes via mRNA. Why the major clinical symptoms are restricted to the brain and testis remains unresolved.

The intellectual disability is the most consistent clinical symptom in males with the methylated full mutation. FMR1 protein expression in less than 30% of lymphocytes is associated with mental retardation and other clinical symptoms.85 86 Size mosaicism (combined presence of premutation and full mutation) does not seem to mitigate mental retardation while it is likely that at least 50% (of brain) cells expressing FMRP will be required to function at a normal IQ level.

The identification of the gene defect enabled screening programmes to be implemented, but the practicability and desirability of screening the mentally retarded or young adult females is still under debate.142 149 197 Testing mentally disabled subjects leads to diagnosis and improved support for newly diagnosed fragile X patients and allows identification of all premutation carriers in the proband's family.132 136 138 Subsequently, a fragile X diagnosis assists the patients in coping with problems and eventually accepting their mentally handicapped child. The diagnosis in a child will generally result in parents looking for treatment. Some parents ask for pharmacotherapy to suppress the behavioural problems. However, its value is still a subject of debate. Most parents hope for a cure, either through protein replacement or gene therapy or both. However, before studying such future therapy strategies, one should consider that protein production probably needs to be restored in at least 50% of the cells (based on studies in blood) to achieve normal cognitive function. Before this long term aim, efforts should be concentrated on improving the diagnostic rate and subsequently the presentation and contact of fragile X syndrome patients. In the meantime, (basic) research can concentrate on increasing our understanding of the actual mechanism(s) of the neuronal dysfunction underlying the mental retardation.

We are grateful to Professor H Gailudder and Professor P J Wil- lems for their comments on the manuscript, and to the Rotter- dam Foundation of Clinical Genetics for its financial support. We would like to thank Dr A M W van den Ouweland, Dr R Willemijn and Dr A T Hoogenwege who contributed to the fragile X studies in our centre.

37 De Vries BB, van den Ouweland AM, Mokhammam S, et al. Screening and diagnosis of the fragile X syndrome in the mentally retarded: an epidemiological and psychologi-


The fragile X syndrome.

B B de Vries, D J Halley, B A Oostra and M F Niermeijer

doi: 10.1136/jmg.35.7.579

Updated information and services can be found at:
http://jmg.bmj.com/content/35/7/579

*These include:*

**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 online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/