Syndrome of the month

The fragile X syndrome

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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.

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History

In 1943, Martin and Bell¹ described sex linked mental retardation without dysmorphic features in a family in which both affected males and females were observed. Thirty-six years later, Lubs² 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 Xq27.³ 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.⁷⁻⁸

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 ($\pm 50\%$) 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, 14} 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, 15, 16}

Fragile X infants often have relative macrocephaly persisting into adult life.¹⁷ However, the adult height of affected males is below the norm.^{17, 18} A few patients present with either overweight or general persisting overgrowth which can be confused with either Prader-Willi syndrome or Sotos syndrome.¹⁹⁻²⁴ Associations with other syndromes are reported; examples are the Robin sequence (micrognathia, glossoptosis, and cleft soft palate),²⁵ the FG syndrome (congenital hypotonia, macrocephaly, distinctive face, and imperforate anus),^{26, 27} and the DiGeorge anomaly (defects of thymus, parathyroids, and great vessels).²⁸ However, there is no definite evidence for a causal relationship between the FMR1 gene

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Table 1 Clinical features and medical problems of fragile X males*

	%
<i>Clinical features†</i>	
Long face	70
Prominent ears	70
High arched palate	52
Hyperextensible finger joints	67
Double jointed thumbs	53
Single palmar creases	25
Hand calluses	29
Flat feet	71
Heart murmur or click	18
Macro-orchidism	70
<i>Medical problems‡</i>	
Vomiting	31
Failure to thrive in infancy	15
Strabismus	36
Myopia or hyperopia	22
Hernia	15
Joint dislocation	3
Orthopaedic problems	21
Otitis media	85
Sinusitis	23
Seizures	22
Mitral valve prolapse	35
Apnoea	10
Autism	20
ADHD	80
Motor tics	19

*Majority of items scored in 100–280 patients.

‡Adapted from Merenstein *et al.*¹⁴

†Adapted from Hagerman.⁸

mutation and these phenotypes. Several observations of the co-occurrence of the fragile X syndrome and sex chromosomal abnormalities like 47,XXY or 45,XO/46,XX^{29–34} 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.^{35–39} 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.⁸ 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,^{43–45} a recent study indicated that the premutation does not seem to be a major risk for early menopause.⁴⁶

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.^{16–49–50} Similar abnormal dendritic spines were observed in fragile X knockout mice.⁵¹ Interestingly, in normal mouse brain, high FMRP expression is observed in the synaptoneuroosomes 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 abnormalities⁵³ 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.^{5–49–55–56}

The FMR1 gene

The identification of the FMR1 gene^{9–11} 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 exons⁵⁷ and an untranslated polymorphic CGG repeat in the first exon.⁵⁸ In the normal population, this CGG repeat varies from six to 54 units⁵⁸ (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,

Figure 1 Three affected males with characteristic facial features of the fragile X syndrome. Note the long, narrow face with large, everted ears and the poor eye contact.

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.

two types of mutations within the CGG repeat can be distinguished: the premutation (size 43 to 200 units) apparently without clinical effect and the full mutation (size >200 units) which, if fully methylated, is associated with mental retardation in all male and in 50–70% of female patients. At the protein level, FMR1 is absent from cells in cases with a fully methylated mutation only.

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) carriers⁵⁸ (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).^{58–60}

The CGG repeat is generally interspersed with two AGG triplets^{61–66} and their position at the 3' end influences repeat instability, with an instability threshold of 34–38 uninterrupted CGG repeats.^{61–65} 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.^{61–62–64} However, such premutation alleles can also be stably inherited through many generations.^{67–68} 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.^{69–71}

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 pre-conceptional enlargement.^{72–73} This seems to conflict with the finding of only premutations in the sperm of four males with a full mutation in somatic cells.⁷⁴ The latter observation might be explained by regression of a full mutation to a premutation in a limited number of cell

Figure 3 Two affected males and one female with less obvious facial features.

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.⁷⁵⁻⁷⁸ The latter causes the mental retardation. All males and a majority of the females with a hypermethylated full mutation are mentally retarded.⁷⁹⁻⁸³

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.⁸⁴⁻⁸⁵

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.⁸⁰⁻⁸⁶ Also, males with somatic mosaicism for a full mutation and a (partial) deletion of the FMR1 gene have been reported.⁸⁷⁻⁹¹

(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.⁹²⁻⁹⁹

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.¹⁰⁰⁻¹¹²

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.¹¹³ The missense mutation resided in one of the important domains of the FMR1 protein.¹¹⁴ 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.¹¹⁵ 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.¹¹⁶ 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

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

*For additional references see text.

(≈0.6 Mb distal to FRAXA) and FRAXF (1-2 Mb distal to FRAXA).^{117 119}

DNA ANALYSIS

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 *Hind*III, *Bgl*II, and *Pst*I, each with their specific fragment sizes.¹²⁰ 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.⁵⁸ 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.¹²¹⁻¹²⁵ 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.¹²⁵ 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.^{76 123} Methylation status is therefore unreliable in an early (<12 weeks of pregnancy) chorionic villus sample because it may differ from the fetal tis-

sue. 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.^{113 115 116} It is conceivable that other rare mutations in the FMR1 gene will be detected in the future.

FMRP ANTIBODY TEST

Recently, an FMR1 protein antibody test was developed for detecting the presence or absence of FMRP in lymphocytes.¹²⁶ 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 FMRP.¹²⁶ The proportion of cells with FMRP production allows a distinction between males with the fragile X syndrome (<30% staining cells) and normal subjects (>40% staining cells).¹²⁷ 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.¹²⁷ 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 FMR1 gene and its CGG related mutations in the first exon of the FMR1 gene that contains the CGG repeat (grey) (see text for further details).

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

	Persons studied			Persons with grey zone allele			Persons with premutation	Persons with full mutation			Characteristics of population studied
	M	F	M+F	M	F	M+F (CGG range)	M+F	M	F	M+F	
England Webb <i>et al</i> (1986), Turner <i>et al</i> (1996)	219	—	219	—	—	—	—	6	—	6 (2.7%)	Mentally retarded children in schools/institutions
Australia 472 — 472 Turner <i>et al</i> (1986), Turner <i>et al</i> (1996)	472	—	472	—	—	—	—	10	—	10 (2.1%)	Mentally retarded children in special schools
England 180 74 254 Jacobs <i>et al</i> (1992)	180	74	254	—	—	11 (41–49)	0	4	0	4 (1.6%)	Children with special educational needs
United States 299 140 439 Hagerman <i>et al</i> (1994)	299	140	439	—	—	—	1 (M)	1	3	4 (0.9%)	Retarded/learning disabled children
England 103 51 154 Slaney <i>et al</i> (1995)	103	51	154	—	—	—	0	4	0	4 (2.5%)	School children with learning difficulties
England 1013 — 1013 Murray <i>et al</i> (1996)	1013	—	1013	35	—	35 (41–60)	1 (M)	5	—	5 (0.5%)	Boys with learning difficulties
United States 888 391 1279 Meadows <i>et al</i> (1996)	888	391	1279	3	7	10 (50–60)	0	1	1	2 (0.2%)	Children in special needs education programmes
The Netherlands 870 661 1531 De Vries <i>et al</i> (1997)	870	661	1531	10	9	19 (43–60)	0	9	2	11 (0.7%)	Mentally retarded children/adults in institutions, special schools
Subtotal†	3353	1317	4670			75 (1.8%)	2 (0.04%)	24 (0.7%)	6 (0.5%)	30 (0.6%)	

*For references see text.

†Without (reassessed) studies of Webb *et al* (1986) and Turner *et al* (1986).

pregnancies using chorionic villi at 12.5 weeks of gestation¹²⁸ and uncultured amniotic fluid cells.¹²⁹ 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.^{130–131} Studies using DNA analysis indicate a prevalence for males of 1/4425 to 1/6045.^{38–132–133} 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.^{38–133–138}

In the general population, a premutation carrier frequency among females of $\pm 1/250$ has been observed.^{139–140} 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).¹⁴¹

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.^{142–149} 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.^{150–153} DNA testing is much more precise, especially for subsequent testing for (pre)mutation carriers in the family.^{38–132–136–138}

These programmes are welcomed by parents/guardians and well accepted by the staff of schools and institutes.^{38–154} An underdiagnosis of the fragile X syndrome of more than 50% even in well developed countries is found.³⁸ The relatively low “yield” of new cases

identified ($\pm 1\%$ of the tested patients have the fragile X syndrome) may be significantly increased by preselection based on physical features.^{35–36–38–39–155} 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.^{77–156} 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.^{157–158} Alternative splicing in the FMR1 gene occurs in various human tissues, leading to 24 distinct transcripts.^{156–159–160} Whether those transcripts lead to functional proteins is unclear. The localisation of FMRP is predominantly cytoplasmic.^{77–158}

In normal human brain, FMRP is found in nearly all neurones studied, but is absent or reduced in non-neuronal cells.^{158–161} 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.¹⁶¹ This is concordant with the presence of a premutation allele in the sperm of patients.⁷⁴

High FMRP expression was also found in other tissues not obviously involved in the fragile X phenotype,^{77–158–162–163} 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.¹⁵⁸

The FMRP has RNA binding capacity owing to characteristic sequence motifs in the protein: an RGG box and ribonucleoprotein particle (RNP) K homology domains (designated KH domains).¹⁶⁴⁻¹⁶⁷ A patient with a severe phenotype and a missense mutation in one of these KH domains had quantitatively normal FMRP production in lymphoblastoid cells.¹¹³⁻¹¹⁴

FMRP binds its own FMR1 transcript and, with unknown selectivity, 4% of fetal brain transcripts.¹⁶⁵ 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.¹⁶⁸⁻¹⁷² 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.¹⁷³ Eberhart *et al*¹⁷³ 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.¹⁷⁴⁻¹⁷⁵ The knockout mice brain and testes lacked pathological abnormalities, except for enlargement of the testes.¹⁷⁴

Mental retardation

Fragile X males show some variability in intellectual disability: in most prepubertal boys it is moderate (IQ $\approx$ 35-55), and in adults moderate to severe (IQ $\approx$ 20-40).⁸⁷⁻¹⁷⁶ This apparent decline in IQ probably reflects a slowing of cognitive development, stabilising after puberty.¹⁷⁶⁻¹⁸⁰

In affected males, a methylated, full mutation will lead to mental impairment whatever the repeat size of the mutation.⁷⁹⁻⁸⁷ 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.¹⁴⁻⁹³⁻¹⁸¹⁻¹⁸² However, others found no mean IQ differences.⁸⁰⁻⁸⁷ 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.⁹⁰

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.⁷⁹⁻⁹²⁻⁹⁵⁻⁹⁷⁻⁹⁹

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).⁷⁹⁻⁸¹⁻⁸³⁻¹⁸³ 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.⁸³⁻¹⁸³⁻¹⁸⁵ 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).¹⁸⁶ 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.¹⁸⁷ 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.¹⁸⁸ 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).¹⁸⁹ Approximately 30-50% of cases need ophthalmological help for strabismus, myopia, or hyperopia.¹⁹⁰

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.¹⁸⁶ 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.¹⁹¹⁻¹⁹²

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"¹⁹³⁻¹⁹⁴ 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.^{87 90} 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 retarded 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 parents 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 $\pm 50\%$ 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 conventional support for 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.

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- Martin JP, Bell J. A pedigree of mental defect showing sex-linkage. *J Neurol Psych* 1943;6:154-7.
- Lubs H. A marker X chromosome. *Am J Hum Genet* 1969; 21:231-44.
- Sutherland GR. Heritable fragile sites on human chromosomes. I. Factors affecting expression in lymphocyte culture. *Am J Hum Genet* 1979;31:125-35.
- Escalante JA, Grunspun H, Frota-Pessoa O. Severe sex-linked mental retardation. *J Genet Hum* 1971;19:137-40.
- Turner G, Eastman C, Casey J, McLeay A, Procopis P, Turner B. X-linked mental retardation associated with macro-orchidism. *J Med Genet* 1975;12:367-71.
- Cantu JM, Scaglia HE, Medina M, et al. Inherited congenital normofunctional testicular hyperplasia and mental deficiency. *Hum Genet* 1976;33:23-33.
- Fryns JP. X-linked mental retardation and the fragile X syndrome: a clinical approach. In: Davies KE, ed. *The fragile X syndrome*. Oxford: Oxford University Press, 1989.
- Hagerman RJ. Physical and behavioural phenotype. In: Hagerman RJ, Cronister A, eds. *Fragile-X syndrome: diagnosis, treatment and research*. Baltimore: The Johns Hopkins University Press, 1996.
- Verkerk AJ, Pieretti M, Sutcliffe JS, et al. 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.
- Yu S, Pritchard M, Kremer E, et al. Fragile X genotype characterized by an unstable region of DNA. *Science* 1991; 252:1179-81.
- Oberle I, Rousseau F, Heitz D, et al. Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science* 1991;252:1097-102.
- Wells RD, Warren ST. *Genetic instabilities and hereditary neurological diseases*. New York: Academic Press, 1998.
- Richards BW, Sylvester PE, Brooker C. Fragile X-linked mental retardation: the Martin-Bell syndrome. *J Ment Defic Res* 1981;25:253-6.
- Merenstein SA, Sobesky WE, Taylor AK, Riddle JE, Tran HX, Hagerman RJ. Molecular-clinical correlations in males with an expanded FMR1 mutation. *Am J Med Genet* 1996;64:388-94.
- Partington MW. The fragile X syndrome II: preliminary data on growth and development in males. *Am J Med Genet* 1984;17:175-94.
- Wisniewski KE, Segan SM, Miezieski CM, Sersen EA, Rudelli RD. The fra(X) syndrome: neurological, electrophysiological, and neuropathological abnormalities. *Am J Med Genet* 1991;38:476-80.
- Butler MG, Brunswick A, Miller LK, Hagerman RJ. Standards for selected anthropometric measurements in males with the fragile X syndrome. *Pediatrics* 1992;89: 1059-62.
- Loesch DZ, Lafranchi M, Scott D. Anthropometry in Martin-Bell syndrome. *Am J Med Genet* 1988;30:149-64.
- Fryns JP, Haspelslagh M, Dereymaeker AM, Volcke P, Van den Berghe H. A peculiar subphenotype in the fra(X) syndrome: extreme obesity-short stature-stubby hands and feet-diffuse hyperpigmentation. Further evidence of disturbed hypothalamic function in the fra(X) syndrome? *Clin Genet* 1987;32:388-92.
- De Vries BB, Fryns JP, Butler MG, et al. Clinical and molecular studies in fragile X patients with a Prader-Willi-like phenotype. *J Med Genet* 1993;30:761-6.
- Schrander-Stumpel C, Gerver WJ, Meyer H, Engelen J, Mulder H, Fryns JP. Prader-Willi-like phenotype in fragile X syndrome. *Clin Genet* 1994;45:175-80.
- De Vries BB, Niermeijer MF. The Prader-Willi-like phenotype in fragile X patients: a designation facilitating clinical (and molecular) differential diagnosis. *J Med Genet* 1994;31:820.
- Beemer FA, Veenema H, de Pater JM. Cerebral gigantism (Sotos syndrome) in two patients with fra(X) chromosomes. *Am J Med Genet* 1986;23:221-6.
- De Vries BB, Robinson H, Stolte-Dijkstra I, et al. General overgrowth in the fragile X syndrome: variability in the phenotypic expression of the FMR1 gene mutation. *J Med Genet* 1995;32:764-9.
- Lachiewicz AM, Hoegerman SF, Holmgren G, Holmberg E, Arinbjarnarson K. Association of the Robin sequence with the fragile X syndrome. *Am J Med Genet* 1991;41:275-8.
- Loesch DZ, Hay DA, Sheffield LJ. Fragile X family with unusual digital and facial abnormalities, cleft lip and palate, and epilepsy. *Am J Med Genet* 1992;44:543-50.
- Piussan C, Mathieu M, Berquin P, Fryns JP. Fragile X mutation and FG syndrome-like phenotype. *Am J Med Genet* 1996;64:395-8.
- Macpherson JN, Curtis G, Crolla JA, et al. Unusual (CGG)n expansion and recombination in a family with fragile X and DiGeorge syndrome. *J Med Genet* 1995;32: 236-9.
- Kupke KG, Soreng AL, Muller U. Origin of the supernumerary X chromosome in a patient with fragile X and Klinefelter syndrome. *Am J Med Genet* 1991;38:440-4.
- Voelckel MA, Pellissier MC, Piquet C, et al. Fragile X syndrome in an extended family with special reference to an affected male with Klinefelter syndrome. *Am J Med Genet* 1991;38:374-7.
- Tejada MI, Mornet E, Tizzano E, Molina M, Baiget M, Boue A. Identification by molecular diagnosis of mosaic Turner's syndrome in an obligate carrier female for fragile X syndrome. *J Med Genet* 1994;31:76-8.
- Shapiro LR, Simensen RJ, Wilmut PL, et al. Asymmetry of methylation with FMR-1 full mutation in two 45,X/46,XX mosaic females associated with normal intellect. *Am J Med Genet* 1994;51:507-8.
- Fryns JP, Van den Berghe H. The concurrence of Klinefelter syndrome and fragile X syndrome. *Am J Med Genet* 1988;30:109-13.
- Brondum-Nielsen K. Sex chromosome aneuploidy in fragile X carriers. *Am J Med Genet* 1986;23:537-44.
- Laing S, Partington M, Robinson H, Turner G. Clinical screening score for the fragile X (Martin-Bell) syndrome. *Am J Med Genet* 1991;38:256-9.
- Hagerman RJ, Amiri K, Cronister A. Fragile X checklist. *Am J Med Genet* 1991;38:283-7.
- Butler MG, Mangrum T, Gupta R, Singh DN. A 15-item checklist for screening mentally retarded males for the fragile X syndrome. *Clin Genet* 1991;39:347-54.
- De Vries BB, van den Ouweland AM, Mohkamsing S, et al. Screening and diagnosis for the fragile X syndrome among the mentally retarded: an epidemiological and psychologi-

- cal survey. Collaborative Fragile X Study Group. *Am J Hum Genet* 1997;61:660-7.
- 39 Giangreco CA, Steele MW, Aston CE, Cummins JH, Wenger SL. A simplified six-item checklist for screening for fragile X syndrome in the pediatric population. *J Pediatr* 1996;129:611-14.
 - 40 Fisch GS. What is associated with the fragile X syndrome? *Am J Med Genet* 1993;48:112-21.
 - 41 Cohen IL, Sudhalter V, Pfadt A, Jenkins EC, Brown WT, Vietze PM. Why are autism and the fragile-X syndrome associated? Conceptual and methodological issues. *Am J Hum Genet* 1991;48:195-202.
 - 42 Reiss AL, Freund L. Behavioral phenotype of fragile X syndrome: DSM-III-R autistic behavior in male children. *Am J Med Genet* 1992;43:35-46.
 - 43 Schwartz CE, Dean J, Howard-Peebles PN, et al. Obstetrical and gynecological complications in fragile X carriers: a multicenter study. *Am J Med Genet* 1994;51:400-2.
 - 44 Turner G, Robinson H, Wake S, Martin N. Dizygous twinning and premature menopause in fragile X syndrome. *Lancet* 1994;344:1500.
 - 45 Partington MW, York Moore D, Turner GM. Confirmation of early menopause in fragile X carriers. *Am J Med Genet* 1996;64:370-2.
 - 46 Kenneson A, Cramer DW, Warren ST. Fragile X premutations are not a major cause of early menopause. *Am J Hum Genet* 1997;61:1362-9.
 - 47 Reiss AL, Freund L, Tseng JE, Joshi PK. Neuroanatomy in fragile X females: the posterior fossa. *Am J Hum Genet* 1991;49:279-88.
 - 48 Reiss AL, Abrams MT, Greenlaw R, Freund L, Denckla MB. Neurodevelopmental effects of the FMR-1 full mutation in humans. *Nat Med* 1995;1:159-67.
 - 49 Rudelli RD, Brown WT, Wisniewski K, et al. Adult fragile X syndrome. Clinico-neuropathologic findings. *Acta Neuropathol (Berl)* 1985;67:289-95.
 - 50 Hinton VJ, Brown WT, Wisniewski K, Rudelli RD. Analysis of neocortex in three males with the fragile X syndrome. *Am J Med Genet* 1991;41:289-94.
 - 51 Comery TA, Harris JB, Willems PJ, et al. Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proc Natl Acad Sci USA* 1997;94:5401-4.
 - 52 Weiler IJ, Irwin SA, Klintsova AY, et al. Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. *Proc Natl Acad Sci USA* 1997;94:5395-400.
 - 53 Marin-Padilla M. Structural organization of the cerebral cortex (motor area) in human chromosomal aberrations. A Golgi study. I. D (13-15) trisomy, Patau syndrome. *Brain Res* 1974;66:375-91.
 - 54 Purpura DP. Dendritic spine "dysgenesis" and mental retardation. *Science* 1974;186:1126-8.
 - 55 Nistal M, Martinez-Garcia F, Regadera J, Cobo P, Paniagua R. Macro-orchidism: light and electron microscopic study of four cases. *Hum Pathol* 1992;23:1011-18.
 - 56 Johannisson R, Rehder H, Wendt V, Schwinger E. Spermatogenesis in two patients with the fragile X syndrome. I. Histology: light and electron microscopy. *Hum Genet* 1987;76:141-7.
 - 57 Eichler EE, Richards S, Gibbs RA, Nelson DL. Fine structure of the human FMR1 gene. *Hum Mol Genet* 1993;2:1147-53.
 - 58 Fu YH, Kuhl DP, Pizzuti A, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell* 1991;67:1047-58.
 - 59 Heitz D, Devys D, Imbert G, Kretz C, Mandel JL. Inheritance of the fragile X syndrome: size of the fragile X premutation is a major determinant of the transition to full mutation. *J Med Genet* 1992;29:794-801.
 - 60 Turner AM, Robinson H, Wake S, Laing SJ, Leigh D, Turner G. Counselling risk figures for fragile X carrier females of varying band sizes for use in predicting the likelihood of retardation in their offspring. *Am J Med Genet* 1994;51:458-62.
 - 61 Eichler EE, Holden JJ, Popovich BW, et al. Length of uninterrupted CGG repeats determines instability in the FMR1 gene. *Nat Genet* 1994;8:88-94.
 - 62 Hirst MC, Grewal PK, Davies KE. Precursor arrays for triplet repeat expansion at the fragile X locus. *Hum Mol Genet* 1994;3:1553-60.
 - 63 Kunst CB, Warren ST. Cryptic and polar variation of the fragile X repeat could result in predisposing normal alleles. *Cell* 1994;77:853-61.
 - 64 Hirst MC. FMR1 triplet arrays: paying the price for perfection. *J Med Genet* 1995;32:761-3.
 - 65 Zhong N, Yang W, Dobkin C, Brown WT. Fragile X gene instability: anchoring AGGs and linked microsatellites. *Am J Hum Genet* 1995;57:351-61.
 - 66 Zhong N, Ju W, Pietrofesa J, Wang D, Dobkin C, Brown WT. Fragile X "gray zone" alleles: AGG patterns, expansion risks, and associated haplotypes. *Am J Med Genet* 1996;64:261-5.
 - 67 Drugge U, Holmgren G, Blomquist HK, Dahl N, Gustavson KH, Malmgren H. Study of individuals possibly affected with the fragile X syndrome in a large Swedish family in the 18th to 20th centuries. *Am J Med Genet* 1992;43:353-4.
 - 68 Smits AP, Dreesen JC, Post JG, et al. The fragile X syndrome: no evidence for any recent mutations. *J Med Genet* 1993;30:94-6.
 - 69 Vits L, De Boule K, Reyniers E, et al. Apparent regression of the CGG repeat in FMR1 to an allele of normal size. *Hum Genet* 1994;94:523-6.
 - 70 Brown WT, Houck GE, Ding X, et al. Reverse mutations in the fragile X syndrome. *Am J Med Genet* 1996;64:287-92.
 - 71 Van den Ouweland AMW, Deelen WH, Kunst CB, et al. Loss of mutation at the FMR1 locus through multiple exchanges between maternal X chromosomes. *Hum Mol Genet* 1994;3:1823-7.
 - 72 Malter HE, Iber JC, Willemsen R, et al. Characterization of the full fragile X syndrome mutation in fetal gametes. *Nat Genet* 1997;15:165-9.
 - 73 Moutou C, Vincent MC, Biancalana V, Mandel JL. Transition from premutation to full mutation in fragile X syndrome is likely to be prezygotic. *Hum Mol Genet* 1997;6:971-9.
 - 74 Reyniers E, Vits L, De Boule K, et al. The full mutation in the FMR-1 gene of male fragile X patients is absent in their sperm. *Nat Genet* 1993;4:143-6.
 - 75 Pieretti M, Zhang FP, Fu YH, et al. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell* 1991;66:817-22.
 - 76 Sutcliffe JS, Nelson DL, Zhang F, et al. DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum Mol Genet* 1992;1:397-400.
 - 77 Verheij C, Bakker CE, de Graaff E, et al. Characterization and localization of the FMR-1 gene product associated with fragile X syndrome. *Nature* 1993;363:722-4.
 - 78 Hansen RS, Gartler SM, Scott CR, Chen SH, Laird CD. Methylation analysis of CGG sites in the CpG island of the human FMR1 gene. *Hum Mol Genet* 1992;1:571-8.
 - 79 Rousseau F, Heitz D, Biancalana V, et al. Direct diagnosis by DNA analysis of the fragile X syndrome of mental retardation. *N Engl J Med* 1991;325:1673-81.
 - 80 Rousseau F, Heitz D, Tarleton J, et al. A multicenter study on genotype-phenotype correlations in the fragile X syndrome, using direct diagnosis with probe StB12.3: the first 2,253 cases. *Am J Hum Genet* 1994;55:225-37.
 - 81 Taylor AK, Safanda JF, Fall MZ, et al. Molecular predictors of cognitive involvement in female carriers of fragile X syndrome. *JAMA* 1994;271:507-14.
 - 82 Smits A, Smeets D, Hamel B, Dreesen J, de Haan A, van Oost B. Prediction of mental status in carriers of the fragile X mutation using CGG repeat length. *Am J Med Genet* 1994;51:497-500.
 - 83 De Vries BB, Wiegers AM, Smits AP, et al. Mental status of females with an FMR1 gene full mutation. *Am J Hum Genet* 1996;58:1025-32.
 - 84 Devys D, Biancalana V, Rousseau F, Boue J, Mandel JL, Oberle I. Analysis of full fragile X mutations in fetal tissues and monozygotic twins indicate that abnormal methylation and somatic heterogeneity are established early in development. *Am J Med Genet* 1992;43:208-16.
 - 85 Wohrle D, Hennig I, Vogel W, Steinbach P. Mitotic stability of fragile X mutations in differentiated cells indicates early post-conceptual trinucleotide repeat expansion (see comments). *Nat Genet* 1993;4:140-2.
 - 86 Nolin SL, Glicksman A, Houck G Jr., Brown WT, Dobkin CS. Mosaicism in fragile X affected males. *Am J Med Genet* 1994;51:509-12.
 - 87 De Vries BB, Wiegers AM, de Graaff E, et al. Mental status and fragile X expression in relation to FMR-1 gene mutation. *Eur J Hum Genet* 1993;1:72-9.
 - 88 De Graaff E, Rouillard P, Willems PJ, Smits AP, Rousseau F, Oostra BA. Hotspot for deletions in the CGG repeat region of FMR1 in fragile X patients. *Hum Mol Genet* 1995;4:45-9.
 - 89 Mannermaa A, Pulkkinen L, Kajanoja E, Ryyanen M, Saarikoski S. Deletion in the FMR1 gene in a fragile-X male. *Am J Med Genet* 1996;64:293-5.
 - 90 De Graaff E, De Vries BBA, Willemsen R, et al. The fragile X phenotype in a mosaic male with a deletion showing expression of the FMR1 protein in 28% of the cells. *Am J Med Genet* 1996;64:302-8.
 - 91 Schmucker B, Ballhausen WG, Pfeiffer RA. Mosaicism of a microdeletion of 486 bp involving the CGG repeat of the FMR1 gene due to misalignment of GTT tandem repeats at chi-like elements flanking both breakpoints and a full mutation. *Hum Genet* 1996;98:547-52.
 - 92 McConkie-Rosell A, Lachiewicz AM, Spiridigliozzi GA, et al. Evidence that methylation of the FMR-1 locus is responsible for variable phenotypic expression of the fragile X syndrome. *Am J Hum Genet* 1993;53:800-9.
 - 93 Hagerman RJ, Hull CE, Safanda JF, et al. High functioning fragile X males: demonstration of an unmethylated fully expanded FMR-1 mutation associated with protein expression. *Am J Med Genet* 1994;51:298-308.
 - 94 Loesch DZ, Huggins R, Hay DA, Gedeon AK, Mulley JC, Sutherland GR. Genotype-phenotype relationships in fragile X syndrome: a family study. *Am J Hum Genet* 1993;53:1064-73.
 - 95 Rousseau F, Robb LJ, Rouillard P, Der Kaloustian VM. No mental retardation in a man with 40% abnormal methylation at the FMR-1 locus and transmission of sperm cell mutations as premutations. *Hum Mol Genet* 1994;3:927-30.
 - 96 Feng Y, Zhang F, Lokey LK, et al. Translational suppression by trinucleotide repeat expansion at FMR1. *Science* 1995;268:731-4.
 - 97 Smeets HJ, Smits AP, Verheij CE, et al. Normal phenotype in two brothers with a full FMR1 mutation. *Hum Mol Genet* 1995;4:2103-8.
 - 98 Wang Z, Taylor AK, Bridge JA. FMR1 fully expanded mutation with minimal methylation in a high functioning fragile X male. *J Med Genet* 1996;33:376-8.
 - 99 De Vries BB, Jansen CA, Duits AA, et al. Variable FMR1 gene methylation of large expansions leads to variable phe-

- notype in three males from one fragile X family. *J Med Genet* 1996;33:1007-10.
- 100 Wohrle D, Kotzot D, Hirst MC, et al. A microdeletion of less than 250 kb, including the proximal part of the FMR-I gene and the fragile-X site, in a male with the clinical phenotype of fragile-X syndrome. *Am J Hum Genet* 1992;51:299-306.
 - 101 Gedeon AK, Baker E, Robinson H, et al. Fragile X syndrome without CCG amplification has an FMR1 deletion. *Nat Genet* 1992;1:341-4.
 - 102 Tarleton J, Richie R, Schwartz C, Rao K, Aylsworth AS, Lachiewicz A. An extensive de novo deletion removing FMR1 in a patient with mental retardation and the fragile X syndrome phenotype. *Hum Mol Genet* 1993;2:1973-4.
 - 103 Meijer H, de Graaff E, Merckx DM, et al. A deletion of 1.6 kb proximal to the CGG repeat of the FMR1 gene causes the clinical phenotype of the fragile X syndrome. *Hum Mol Genet* 1994;3:615-20.
 - 104 Albright SG, Lachiewicz AM, Tarleton JC, et al. Fragile X phenotype in a patient with a large de novo deletion in Xq27-q28. *Am J Med Genet* 1994;51:294-7.
 - 105 Trotter Y, Imbert G, Poustka A, Fryns JP, Mandel JL. Male with typical fragile X phenotype is deleted for part of the FMR1 gene and for about 100 kb of upstream region. *Am J Med Genet* 1994;51:454-7.
 - 106 Hirst M, Grewal P, Flannery A, et al. Two new cases of FMR1 deletion associated with mental impairment. *Am J Hum Genet* 1995;56:67-74.
 - 107 Quan F, Zonana J, Gunter K, Peterson KL, Magenis RE, Popovich BW. An atypical case of fragile X syndrome caused by a deletion that includes the FMR1 gene. *Am J Hum Genet* 1995;56:1042-51.
 - 108 Prior TW, Papp AC, Snyder PJ, Sedra MS, Guida M, Enrile BG. Germline mosaicism at the fragile X locus. *Am J Med Genet* 1995;55:384-6.
 - 109 Gu Y, Lugenbeel KA, Vockley JG, Grody WW, Nelson DL. A de novo deletion in FMR1 in a patient with developmental delay. *Hum Mol Genet* 1994;3:1705-6.
 - 110 Wolff DJ, Gustashaw KM, Zurcher V, et al. Deletions in Xq26.3-q27.3 including FMR1 result in a severe phenotype in a male and variable phenotypes in females depending upon the X inactivation pattern. *Hum Genet* 1997;100:256-62.
 - 111 Gronskov K, Hjalgrim H, Bjerager MO, Brondum-Nielsen K. Deletion of all CGG repeats plus flanking sequences in FMR1 does not abolish gene expression. *Am J Hum Genet* 1997;61:961-7.
 - 112 Hammond LS, Macias MM, Tarleton JC, Shashidhar Pai G. Fragile X syndrome and deletions in FMR1: new case and review of the literature. *Am J Med Genet* 1997;72:430-4.
 - 113 De Boule K, Verkerk AJ, Reyniers E, et al. A point mutation in the FMR-1 gene associated with fragile X mental retardation. *Nat Genet* 1993;3:31-5.
 - 114 Verheij C, de Graaff E, Bakker CE, et al. Characterization of FMR1 proteins isolated from different tissues. *Hum Mol Genet* 1995;4:895-901.
 - 115 Lugenbeel KA, Peier AM, Carson NL, Chudley AE, Nelson DL. Intragenic loss of function mutations demonstrate the primary role of FMR1 in fragile X syndrome. *Nat Genet* 1995;10:483-5.
 - 116 Wang YC, Lin ML, Lin SJ, Li YC, Li SY. Novel point mutation within intron 10 of FMR-1 gene causing fragile X syndrome. *Hum Mutat* 1997;10:393-9.
 - 117 Sutherland GR, Baker E. Characterisation of a new rare fragile site easily confused with the fragile X. *Hum Mol Genet* 1992;1:111-13.
 - 118 Flynn GA, Hirst MC, Knight SJ, et al. Identification of the FRAXE fragile site in two families ascertained for X linked mental retardation. *J Med Genet* 1993;30:97-100.
 - 119 Hirst MC, Barnicoat A, Flynn G, et al. The identification of a third fragile site, FRAXF, in Xq27-q28 distal to both FRAXA and FRAXE. *Hum Mol Genet* 1993;2:197-200.
 - 120 Oostra BA, Jacky PB, Brown WT, Rousseau F. Guidelines for the diagnosis of fragile X syndrome. National Fragile X Foundation. *J Med Genet* 1993;30:410-13.
 - 121 Hirst M, Knight S, Davies K, et al. Prenatal diagnosis of fragile X syndrome. *Lancet* 1991;338:956-7.
 - 122 Dobkin CS, Ding X-H, Jenkins EC, et al. Prenatal diagnosis of the fragile X syndrome. *Lancet* 1991;338:957-8.
 - 123 Sutherland GR, Gedeon A, Kornman L, et al. Prenatal diagnosis of fragile X syndrome by direct detection of the unstable DNA sequence. *N Engl J Med* 1991;325:1720-2.
 - 124 Maddalena A, Hicks BD, Spence WC, Levinson G, Howard-Peebles PN. Prenatal diagnosis in known fragile X carriers. *Am J Med Genet* 1994;51:490-6.
 - 125 Halley D, Van Den Ouweland A, Deelen W, Verma I, Oostra B. Strategy for reliable prenatal detection of normal male carriers of the fragile X syndrome. *Am J Med Genet* 1994;51:471-3.
 - 126 Willemsen R, Mohkamsing S, de Vries B, et al. Rapid antibody test for fragile X syndrome. *Lancet* 1995;345:1147-8.
 - 127 Willemsen R, Smits A, Mohkamsing S, et al. Rapid antibody test for diagnosing fragile X syndrome: a validation of the technique. *Hum Genet* 1997;99:308-11.
 - 128 Willemsen R, Oosterwijk JC, Los FJ, Galjaard H, Oostra BA. Prenatal diagnosis of the fragile X syndrome. *Lancet* 1996;348:967-8.
 - 129 Willemsen R, Los F, Mohkamsing S, et al. Rapid antibody test for prenatal diagnosis of fragile X syndrome on amniotic fluid cells: a new appraisal. *J Med Genet* 1997;34:250-1.
 - 130 Webb T. The epidemiology of the fragile X syndrome. In: Davies KE, ed. *The fragile X syndrome*. Oxford: Oxford University Press, 1989.
 - 131 Sherman S. Epidemiology. In: Hagerman RJ, Cronister AC, eds. *Fragile X syndrome*. Baltimore: The Johns Hopkins University Press, 1996.
 - 132 Turner G, Webb T, Wake S, Robinson H. Prevalence of fragile X syndrome. *Am J Med Genet* 1996;64:196-7.
 - 133 Murray A, Youings S, Dennis N, et al. Population screening at the FRAXA and FRAXE loci: molecular analyses of boys with learning difficulties and their mothers. *Hum Mol Genet* 1996;5:727-35.
 - 134 Hagerman RJ, Wilson P, Staley LW, et al. Evaluation of school children at high risk for fragile X syndrome utilizing buccal cell FMR-1 testing. *Am J Med Genet* 1994;51:474-81.
 - 135 Jacobs PA, Bullman H, Macpherson J, et al. Population studies of the fragile X: a molecular approach. *J Med Genet* 1993;30:454-9.
 - 136 Slaney SF, Wilkie AO, Hirst MC, et al. DNA testing for fragile X syndrome in schools for learning difficulties. *Arch Dis Child* 1995;72:33-7.
 - 137 Van den Ouweland AM, de Vries BB, Bakker PL, et al. DNA diagnosis of the fragile X syndrome in a series of 236 mentally retarded subjects and evidence for a reversal of mutation in the FMR-1 gene. *Am J Med Genet* 1994;51:482-5.
 - 138 Meadows KL, Pettay D, Newman J, Hersey J, Ashley AE, Sherman SL. Survey of the fragile X syndrome and the fragile X E syndrome in a special education needs population. *Am J Med Genet* 1996;64:428-33.
 - 139 Reiss AL, Kazazian H Jr, Krebs CM, et al. Frequency and stability of the fragile X premutation. *Hum Mol Genet* 1994;3:393-8.
 - 140 Rousseau F, Rouillard P, Morel ML, Khandjian EW, Morgan K. Prevalence of carriers of premutation-size alleles of the FMR1 gene and implications for the population genetics of the fragile X syndrome. *Am J Hum Genet* 1995;57:1006-18.
 - 141 Sherman SL. The high prevalence of fragile X premutation carrier females: is this frequency unique to the French Canadian population? *Am J Hum Genet* 1995;57:991-3.
 - 142 Palomaki GE, Haddow JE. Is it time for population-based prenatal screening for fragile-X? *Lancet* 1993;341:373-4.
 - 143 Bonthron D, Strain L. Population screening for fragile-X syndrome. *Lancet* 1993;341:769-70.
 - 144 Bundey S, Norman E. Population screening for fragile-X syndrome. *Lancet* 1993;341:770.
 - 145 Howard-Peebles PN, Maddalena A, Black SH, Schulman JD. Population screening for fragile-X syndrome. *Lancet* 1993;341:770.
 - 146 Palomaki GE. Population based prenatal screening for the fragile X syndrome. *J Med Genet* 1994;1:65-72.
 - 147 Anonymous. Fragile X syndrome: diagnostic and carrier testing. Working group of the genetic screening subcommittee of the clinical practice committee. American College of Medical Genetics. *Am J Med Genet* 1994;53:380-1.
 - 148 Laxova R. Fragile X screening: what is the real issue? *Am J Med Genet* 1995;57:508-9.
 - 149 Craft N. Study supports screening for the fragile X syndrome. *BMJ* 1995;310:148.
 - 150 Gustavson KH, Blomquist HK, Holmgren G. Prevalence of the fragile-X syndrome in mentally retarded boys in a Swedish county. *Am J Med Genet* 1986;23:581-7.
 - 151 Kahkonen M, Alitalo T, Airaksinen E, et al. Prevalence of the fragile X syndrome in four birth cohorts of children of school age. *Hum Genet* 1987;77:85-7.
 - 152 Turner G, Robinson H, Laing S, Purvis-Smith S. Preventive screening for the fragile X syndrome. *N Engl J Med* 1986;315:607-9.
 - 153 Webb TP, Bundey S, Thake A, Todd J. The frequency of the fragile X chromosome among schoolchildren in Coventry. *J Med Genet* 1986;23:396-9.
 - 154 Turner G, Robinson H, Laing S, et al. Population screening for fragile X. *Lancet* 1992;339:1210-13.
 - 155 Arvio M, Peippo M, Simola KO. Applicability of a checklist for clinical screening of the fragile X syndrome. *Clin Genet* 1997;52:211-15.
 - 156 Ashley CT, Sutcliffe JS, Kunst CB, et al. Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. *Nat Genet* 1993;4:244-51.
 - 157 Feng Y, Lakkis L, Devys D, Warren ST. Quantitative comparison of FMR1 gene expression in normal and premutation alleles. *Am J Hum Genet* 1995;56:106-13.
 - 158 Devys D, Lutz Y, Rouyer N, Bellocq 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.
 - 159 Verkerk AJ, de Graaff E, De Boule K, et al. Alternative splicing in the fragile X gene FMR1. *Hum Mol Genet* 1993;2:399-404.
 - 160 Sittler A, Devys D, Weber C, Mandel JL. Alternative splicing of exon 14 determines nuclear or cytoplasmic localisation of fmr1 protein isoforms. *Hum Mol Genet* 1996;5:95-102.
 - 161 Tamanini F, Willemsen R, van Unen L, et al. Differential expression of FMR1, FXR1 and FXR2 proteins in human brain and testis. *Hum Mol Genet* 1997;6:1315-22.
 - 162 Abitbol M, Menini C, Delezoide AL, Rhyner T, Vekemans M, Mallet J. Nucleus basalis magnocellularis and hippocampus are the major sites of FMR-1 expression in the human fetal brain. *Nat Genet* 1993;4:147-53.

- 163 Hinds HL, Ashley CT, Sutcliffe JS, *et al.* Tissue specific expression of FMR-1 provides evidence for a functional role in fragile X syndrome. *Nat Genet* 1993;3:36-43.
- 164 Siomi H, Siomi MC, Nussbaum RL, Dreyfuss G. The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. *Cell* 1993;74:291-8.
- 165 Ashley C, Jr., Wilkinson KD, Reines D, Warren ST. FMR1 protein: conserved RNP family domains and selective RNA binding. *Science* 1993;262:563-6.
- 166 Gibson TJ, Rice PM, Thompson JD, Heringa J. KH domains within the FMR1 sequence suggest that fragile X syndrome stems from a defect in RNA metabolism. *Trends Biochem Sci* 1993;18:331-3.
- 167 Siomi H, Choi M, Siomi MC, Nussbaum RL, Dreyfuss G. Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. *Cell* 1994;77:33-9.
- 168 Khandjian EW, Corbin F, Woerly S, Rousseau F. The fragile X mental retardation protein is associated with ribosomes. *Nat Genet* 1996;12:91-3.
- 169 Tamanini F, Meijer N, Verheij C, *et al.* FMRP is associated to the ribosomes via RNA. *Hum Mol Genet* 1996;5:809-13.
- 170 Siomi MC, Zhang Y, Siomi H, Dreyfuss G. Specific sequences in the fragile X syndrome protein FMR1 and the FXR proteins mediate their binding to 60S ribosomal subunits and the interactions among them. *Mol Cell Biol* 1996;16:3825-32.
- 171 Willemsen R, Bontekoe C, Tamanini F, Galjaard H, Hoo-geveen A, Oostra B. Association of FMRP with ribosomal precursor particles in the nucleolus. *Biochem Biophys Res Commun* 1996;225:27-33.
- 172 Corbin F, Bouillon M, Fortin A, Morin S, Rousseau F, Khandjian EW. The fragile X mental retardation protein is associated with poly(A)⁺ mRNA in actively translating polyribosomes. *Hum Mol Genet* 1997;6:1465-72.
- 173 Eberhart DE, Malter HE, Feng Y, Warren ST. The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. *Hum Mol Genet* 1996;5:1083-91.
- 174 Anonymous. Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium. *Cell* 1994;78:23-33.
- 175 Kooy RK, D'Hooge R, Reyniers E, *et al.* Transgenic mouse model for the fragile X syndrome. *Am J Med Genet* 1996;64:241-5.
- 176 Curfs LM, Wieggers AM, Fryns JP. Intelligence and the fra(X) syndrome: a review. *Genet Couns* 1991;2:55-62.
- 177 Lachiewicz AM, Gullion CM, Spiridigliozzi GA, Aylsworth AS. Declining IQs of young males with the fragile X syndrome. *Am J Ment Retard* 1987;92:272-8.
- 178 Fisch GS, Arinami T, Froster-Iskenius U, *et al.* Relationship between age and IQ among fragile X males: a multicenter study. *Am J Med Genet* 1991;38:481-7.
- 179 Hay DA. Does IQ decline with age in fragile-X? A methodological critique. *Am J Med Genet* 1994;51:358-63.
- 180 Fisch GS, Simensen R, Tarleton J, *et al.* Longitudinal study of cognitive abilities and adaptive behavior levels in fragile X males: a prospective multicenter analysis. *Am J Med Genet* 1996;64:356-61.
- 181 Staley LW, Hull CE, Mazzocco MM, *et al.* Molecular-clinical correlations in children and adults with fragile X syndrome. *Am J Dis Child* 1993;147:723-6.
- 182 Cohen IL, Nolin SL, Sudhalter V, Ding XH, Dobkin CS, Brown WT. Mosaicism for the FMR1 gene influences adaptive skill development in fragile X-affected males. *Am J Med Genet* 1996;64:365-9.
- 183 Rousseau F, Heitz D, Oberle 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.
- 184 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.
- 185 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.
- 186 Hagerman RJ. Medical follow-up and pharmacotherapy. In: Hagerman RJ, Cronister A, eds. *Fragile X syndrome: diagnosis, treatment, and research*. Baltimore: The Johns Hopkins University Press, 1996:283-331.
- 187 Loehr JP, Synhorst DP, Wolfe RR, Hagerman RJ. Aortic root dilatation and mitral valve prolapse in the fragile X syndrome. *Am J Med Genet* 1986;23:189-94.
- 188 Goldson E, Hagerman RJ. Fragile X syndrome and failure to thrive. *Am J Dis Child* 1993;147:605-7.
- 189 Hagerman RJ, Altschul-Stark D, McBogg P. Recurrent otitis media in the fragile X syndrome. *Am J Dis Child* 1987;141:184-7.
- 190 Boucher CA, King SK, Carey N, *et al.* A novel homeodomain-encoding gene is associated with a large CpG island interrupted by the myotonic dystrophy unstable (CTG)_n repeat. *Hum Mol Genet* 1995;4:1919-25.
- 191 Sobesky WE. The treatment of emotional and behavioral problems. In: Hagerman RJ, Cronister A, eds. *Fragile X syndrome: diagnosis, treatment, and research*. Baltimore: The Johns Hopkins University Press, 1996.
- 192 Scharfenaker S, O'Connor R, Stackhouse T, Braden M, Hickman L, Gray K. An integrated approach to intervention. In: Hagerman RJ, Cronister A, eds. *Fragile X syndrome: diagnosis, treatment, and research*. Baltimore: The Johns Hopkins University Press, 1996.
- 193 Sherman SL, Jacobs PA, Morton NE, *et al.* Further segregation analysis of the fragile X syndrome with special reference to transmitting males. *Hum Genet* 1985;69:289-99.
- 194 Opitz JM. On the gates of hell and a most unusual gene. *Am J Med Genet* 1986;23:1-10.
- 195 De Graaff E. The fragile X syndrome: complex behavior of a simple repeat. Thesis, Erasmus University Rotterdam, 1996.
- 196 Wells RD. Molecular basis of genetic instability of triplet repeats. *J Biol Chem* 1996;271:2875-8.
- 197 Murray J, Cuckle H, Taylor G, Hewison J. Screening for fragile X syndrome: information needs for health planners. *J Med Screen* 1997;4:60-94.