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13

Fragile X Syndrome

More than 125 X-linked mental retardation (XLMR) syndromes have been identified, and of these, fragile X syndrome is the most common (43,59). The syndrome is the prototype of an ever growing list of disorders with dynamic mutations that result from instability of triplet repeats. The mutant gene in fragile X syndrome is *FMR1* (OMIM 309550)[a] and the repeated triplet is CGG (68). Several excellent reviews are available, the one by Nussbaum and Ledbetter (44) being particularly exhaustive, and many international fragile X workshops have been held (21).

PREVALENCE

Prevalence estimates are summarized in Table 13–1. Fragile X syndrome is not as prevalent as initially estimated. The discrepancy can be explained by the use of a molecular diagnosis, which is more accurate than the cytogenetic test previously employed.

Prevalence estimates of healthy female carriers are also summarized in Table 13–1. The higher estimate in the study of Falik-Zaccai et al. (16) is partially attributable to the current lack of an accurate definition of a premutation (meiotically unstable allele). Further studies are needed to establish whether this unexpectedly high prevalence of premutation carriers is unique to the specific populations studied or whether it applies to other populations, which is probable (54). Evidence that expansion to full mutation on transmission from a premutated mother is more likely to occur in male fetuses than in female fetuses (34) and may explain a relative lack of premutated males in the general population (50). Large population studies on an unselected series of newborns would be useful in settling the true prevalence of affected (fully mutated) males, normal transmitting (premutated) males, and fully mutated and premutated female carriers. Caution must be exercised in planning such studies to avoid untoward effects on the screened subjects (42).

[a]On-Line Mendelian Inheritance in Man number.

GENETICS

Many genetic and molecular papers have appeared (1,3,6,11,28,31,36,49,56,57,61,62). This section considers (a) gene structure and protein isoforms and (b) the origin and effects of full mutations.

Gene Structure and Protein Isoforms

The *FMR1* gene maps to Xq27.3 and has 17 exons spanning 38 kb of genomic DNA. The polymorphic CGG repeat is located in the 5′ untranslated region of exon 1 and is included in all *FMR1* transcripts (3). *FMR1* was shown to be ubiquitously transcribed during murine and human embryogenesis with the highest levels of differentiated neurons in the hippocampus and basal ganglia (1); in adult mice, it is expressed only in neurons and spermatogonia. The 4.4 kb full-length mRNA encodes a protein with a max-

Table 13–1 Prevalence Estimates[a] of Fragile X Syndrome and Healthy Female Carriers

Estimates	Reference
FRAGILE X SYNDROME	
67/100,000	Webb et al. (69)
25/100,000[b]	Morton et al. (40)
17/100,000[b]	de Vries et al. (14)
HEALTHY FEMALE CARRIERS	
386/100,000	Rousseau et al. (51)
407/100,000[c]	Ryynänen et al. (52)
1176/100,000[c]	Falik-Zaccai et al. (16)

[a]Estimates have been converted to standard epidemiologic form. With a standardized denominator, numerators can be compared more effectively.

[b]More realistic estimates by the use of molecular diagnosis, which is more accurate than the cytogenetic test previously employed.

[c]Difference probably reflects current lack of an accurate definition of a premutation.

imum size of 632 amino acids and a molecular weight of 70–80 kDa (15). Although 20 different transcripts can be produced by alternative splicing (3), only 4 or 5 of them and their corresponding protein products are actually detected in various tissues. Isoform 7 (ISO7), which lacks only the 21 amino acids of exon 12, makes almost all the FMR1 protein and corresponds to the highest band on Western blotting with an approximate molecular weight of 80 kDa (57).

Two KH domains (KH1 and KH2) and one RGG box, common to several RNA-binding proteins, have been identified in exons 8, 10, and 15, respectively (56). It has been shown that FMR1 can bind synthetic RNAs in vitro, and the importance of KH domains was underscored by the description of a severely retarded fragile X patient with a point mutation change in a highly conserved KH2 isoleucine to asparagine (Ile304Asn) (11), which impaired the RNA binding activity of FMR1. It has also been shown that ISO7 is localized in the cytoplasm (15). In contrast, minor isoforms are lacking exon 14 and, with a different C-terminus (ISO6 or ISO12), are confined to the nucleus (57). Although no specific nuclear localization signal (NLS) is present in the first half of FMR1, studies with deletion constructs indicate that the N-terminus of the protein (exons 1–5) can direct the protein to the nucleus; sequences in exon 14 are essential for its subsequent export to the cytoplasm (57).

Origin and Effects of Full Mutations

In more than 95% of cases, fragile X syndrome is caused by a single type of mutation—expansion and hypermethylation of a potentially unstable CGG trinucleotide repeat in the 5′ UTR of the *FMR1* gene (full mutation). This causes transcriptional silencing of the gene and absence of the FMR1 protein. A full mutation appears to be generated by a lengthy multistep process requiring sequential action by a different mechanism (6). To date, no direct conversion of a wild-type to a fully mutated allele has been observed in fragile X families in which mothers of affected individuals were found to be carriers of an already expanded CGG triplet.

Different alleles at the CGG repeat are generally included in one of three classes depending on their total length: wild-type (6–50 repeats), premutation (50–200 repeats), and full mutation (200–1000 hypermethylated repeats). However, it has been shown that the boundaries between these classes are not absolute and that the initial instability depends not only on the total length but also on the repeat configuration. Detailed analysis of over 400 wild-type alleles has shown that the CGG repeat stretch is commonly interrupted by AGG triplets, mainly two, at intervals of 9–10 CGG units, which apparently have a stabilizing effect by preventing replication slippage.

The secondary structures formed by the full mutation and/or the increased number of CpG dinucleotide targets presumably favor the hypermethylation of the *FMR1* gene harboring a full mutation (28).

The question of when and in which cells the pre-to-full expansion takes place is still debated. It was initially proposed that expansion to full mutation was postzygotic because sperm cells obtained from male patients were shown to harbor only premutations (49). However, recent evidence demonstrates the presence of full mutations in the oogonia and spermatogonia of female fetuses and male fetuses, respectively (36), supporting the hypothesis of a selection process that favors premutations in the male germline.

CLINICAL PHENOTYPE

General Features and Variability

The phenotype is variable. In newborns, the birthweight may be elevated, relative macrocephaly may be found, and the anterior fontanelle may be large. Typical adult males have tall stature, large testes, relative macrocephaly, prominent forehead, hypoteloric sunken eyes, long narrow face, midface hypoplasia, prominent mandible, and large ears (Figs. 13–1 to 13–3). The phenotype tends to change with age. In infants and adolescents, the phenotype is milder and, often, macrocephaly, increased height, and hypotonia are the only findings. Hypotonia is virtually a constant feature and is usually accompanied by joint laxity. Mitral valve prolapse is frequently observed in hemizygotes after 18 years of age. On occasion, a small child may have the same phenotype as that found in typical adults.[b] A subgroup of patients have been noted to have short stature and obesity. Infrequently, hemizygotes have been noted to appear entirely normal. About one-third of female carriers with the full mutation have a behavioral phenotype that includes mild mental retardation, shyness, poor eye contact, and learning disabilities. Some, particularly the more retarded ones, have the typical long face, mandibular prognathism, and large everted ears (10,19,20,22,30,33,39,58,59a).

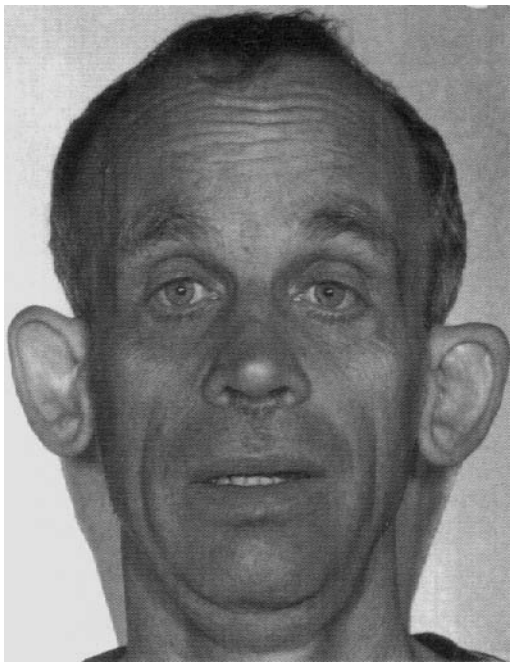

Figure 13–2 Fragile X syndrome. Narrow face and prominent ears. From McDermott et al. (39).

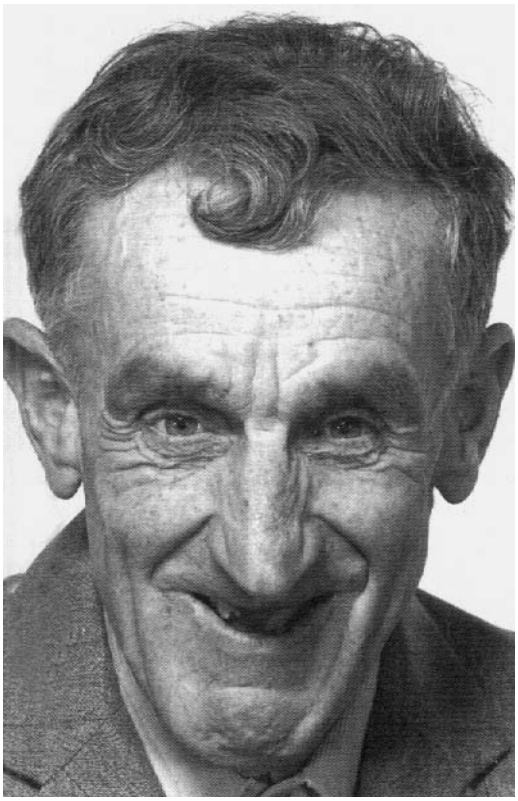

Figure 13–1 Fragile X syndrome. Long face, prominent forehead, sunken eyes, broad based nose, prominent chin, and prominent ears. From McDermott et al. (39).

Typical Craniofacial Appearance

In typical adult males, an increased head circumference and dolichocephaly are found together with a high quadrangular forehead, prominent supraorbital ridges, and a long narrow face. Puffiness under the eyes occurs in

[b]Parvari et al. (45) reported an 8.5 Mb deletion, including the *FMR1* gene, in a 4-year-old boy with a facial appearance consistent with fragile X syndrome and a height and weight above the 97th centile.

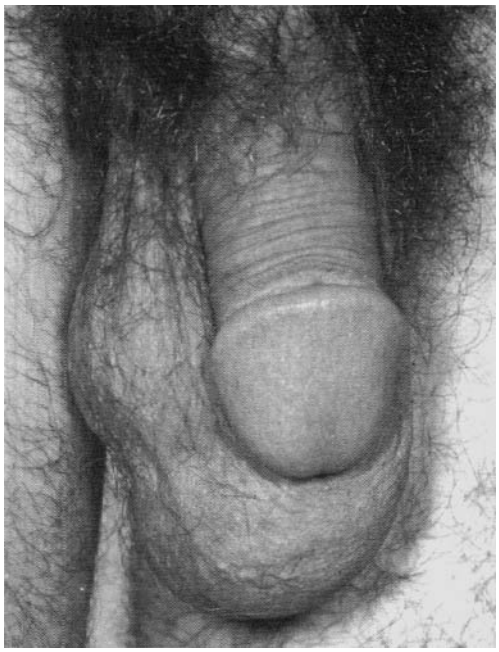

Figure 13–3 Fragile X syndrome. Macroorchidism. From McDermott et al. (39).

some cases. The palpebral fissure length is increased and the inter-inner canthal distance is decreased. Strabismus and refractive errors may be present. The nose often appears broadly based and midface hypoplasia is found (Figs. 13–1 and 13–2). The chin becomes long and prominent during adolescence. The ears are large, outstanding, and may be somewhat soft. The helices may be simple and the lobules may be absent. Otitis media is common and the palate is highly arched (20,39).

Central Nervous System and Performance

Central nervous system findings and performance have been discussed by many authors (9,10,19,20,22,30,33,41,46–48,53,59a,63,67,75). IQs in hemizygotes have been reported to range from 20–70. IQs in females with the full mutation range from 50–107. Delayed speech is very common. Retardation seems to increase with age. Those with higher IQs exhibit dysfluencies and stuttering and, frequently, a characteristic intonation. With lower IQs, less verbal ability is found together with repetitive phrases. Females who carry the full mutation may have learning disabilities. Even psychosis has been noted in some cases (9,19,20,22,53,63,75).

Neuropsychiatric evaluation shows delayed milestones, hypotonia, increased deep tendon reflexes, emotional instability, and automutilation, particularly handbiting. An adverse response to touch on the skin is found. Aggressive behavior occurs in about 50%. Seizures may be found during infancy; a characteristic EEG pattern has been reported as trains of medium-high voltage spikes discharging from the temporal region during sleep. A cognitive profile has been reported to consist of attention deficit hyperactivity disorder, oppositional defiant disorder, enuresis, and encopresis. Fragile X boys show greater variability in total sleep time than normal and difficulty in sleep maintenance compared with controls. In some cases, autism is found, characterized by avoidance of eye contact, hand flapping, or other stereotypic movements, perseverative speech, and echolalia (4,19,20,22,23,30,41,67).

MRI of the brain shows volume conservation of tissue, diminished white-to-gray matter ratio, relatively enlarged caudate nucleus and hippocampus, and increased cerebrospinal fluid, particularly in the lateral ventricles. In males, a relative decrease in the size of the superior temporal gyrus and cerebellar vermis occurs with a relative increase in the size of the fourth ventricle. Likewise, in young females, a relative decrease in the size of the cerebellar vermis is found together with an increase in the size of the fourth ventricle. Neuropathologic studies have demonstrated more long dendritic spines and fewer shorter ones than normal with significant immature morphology in both temporal and visual cortical areas (29,46–48).

Connective Tissue Findings

Joint laxity involves particularly the thumbs but also the fingers, metacarpophalangeal joints, knees, and ankles. The skin feels velvety and soft over the dorsa of the hands. Single palmar creases are found in about one-fourth of the cases. Flat feet are common. Mitral valve prolapse, encountered in approximately 80% of hemizygotes after 18 years of age, is accompanied by aortic dilatation in about 15% of the cases (27).

Genitourinary System

Macro-orchidism, either unilateral or bilateral, is easier to detect in postpubertal males (Fig. 13–3). The testes tend to be softer than normal and the scrotum may be hyperpigmented. There is increased seminiferous tubule length and interstitial edema. The penis is often enlarged (9,14,20,32).

Early menopause and an increased rate of twinning, both reported in premutation carriers, are indications of ovarian failure, which has been found in about 20% of carriers. The data supporting increased twinning conflict and need to be resolved (19,33,55).

DIAGNOSIS

Molecular diagnosis of the CGG amplification is available since the cloning of the *FMR1* gene in 1991; it relies on Southern blotting and hybridization of specific probes, while PCR is employed to detect repeat lengths in the premutation range. The cytogenetic test in low-folate media is obsolete and has been abandoned. A rapid method based on antibody detection of the FMR1 protein in the cells of blood smears is useful for screening affected males (73,74) and has been adapted for uncultured amniotic cells (72) and hair roots (65,71,74).

Prenatal diagnosis depends on the availability of sufficient DNA to perform Southern blotting after double digestion including a methylation-sensitive enzyme (usually EagI or BssHII), while the sex of the fetus can be determined by standard karyotype or Y-specific PCR analysis. False positives, due to suboptimal amplification, and false negatives, due to the possible presence of reverted alleles in the wild-type range, can occur when performing PCR alone on a sample from a male fetus. Furthermore, only direct DNA analysis after digestion with methylation-sensitive enzymes can demonstrate the methylation status of the *FMR1* CpG island, especially in the presence of a full mutation (58). Given that in extra-embryonic tissues, such as chorionic villi, a full mutation may remain largely undermethylated until 10–11 weeks of gestation (5,35,60), chorionic villus sampling might not reveal the hypermethylation already present in the embryonic tissues and may need confirmation by amniocentesis.

DIFFERENTIAL DIAGNOSIS

A subgroup of fragile X patients have short stature and obesity with a superficial resemblance to Prader-Willi syndrome (12). Several patients initially thought to have Sotos syndrome were diagnosed as having fragile X syndrome. Pitfalls in clinical diagnosis justify the view that every mentally retarded patient should be tested for fragile X syndrome in the absence of another reasonable diagnosis. Difficulties in the diagnosis of fragile X syndrome in young children have been discussed by Stoll (59a).

GUIDELINES FOR HEALTH SUPERVISION

Useful guidelines for health supervision of fragile X children have been published by the American Academy of Pediatrics (2) and include advice for both physical and behavioral aspects of the syndrome. After confirming the diagnosis with molecular testing and appropriate parental counseling for recurrence risk for subsequent pregnancies, a series of medical examinations can be envisioned depending on the age of the child. Development during the first year of life can be normal, although hypotonia and irritability may be apparent. In early childhood, the following examinations are important: ophthalmologic examination (strabismus, myopia), echocardiogram if a murmur or click is present (mitral valve prolapse), and orthopedic examination (flat feet, scoliosis, and loose joints). Inguinal hernias should also be excluded. A history of seizures or startle episodes should be reviewed; performing an EEG may be appropriate, although antiepileptic medication after a single seizure is not advisable, given the self-limited course of epileptic manifestations in adolescence (41). Hyperactive behavior and severe attention deficit, which are a major concern during school age, can be treated pharmacologically (24). Torrioli et al. (64) reported the results of a preliminary clinical trial, suggesting that L-acetylcarnitine might be beneficial in ameliorating the

hyperactive behavior of affected children. However, socialization and school integration, possibly within a mainstream program with individual support, are important in helping to overcome these problems. Sports and regular physical activity are important to counteract hypotonic posture and to improve motor coordination. Speech, language, and occupational therapy should be goal oriented to help adolescents and young adults attain as much autonomy as possible. Support from family organizations is important, especially for parents and sibs.

Treatments aimed at recovering the function of the *FMR1* gene have been attempted with folic acid because of its action on cytogenetic expression of the fragile site. Although some reports indicated some beneficial effects on behavior (15), others have not confirmed these observations (18,70). Folate supplementation has no efficacy for the treatment of fragile X syndrome patients. Observations on intellectually normal (58) or minimally affected (25,37) males with an unmethylated full mutation confirm that the abnormally amplified CGG tract per se can still be transcribed and translated. Even if translation may not be completely efficient (17), lymphoblastoid cell lines containing only unmethylated full mutations of two such males have shown the presence of FMR1 protein in every cell, but at reduced levels (58). Given the observations of these exceptional individuals and knowing that the coding sequence of the mutated *FMR1* gene is intact, Chiurazzi et al. (7) tested the possibility of restoring its activity in vitro, employing a DNA demethylation protocol. These authors obtained in vitro reactivation of FMR1 expression after inducing DNA demethylation with 5-azadeoxycytidine in lymphoblastoid cell lines from patients.

Given that the effect of DNA hypermethylation in silencing the *FMR1* gene seems to be potentiated by deacetylation of histones, Chiurazzi et al. (8) performed another set of experiments with phenylbutyrate, an inhibitor of histone deacetylase, and showed that treatment of fragile X cell lines restores FMR1 transcription. Although the effect was weak, it appeared to be synergistic with that of 5-azadeoxycytidine. Phenylbutyrate is structurally similar to L-acetylcarnitine, used in vivo by Torrioli et al. (64) in the clinical trial discussed above.

McConkie-Rosell et al. (38) carried out a longitudinal study of women-at-risk for inheriting the fragile X mutation. Problems addressed were (a) how upsetting the women perceived their information to be, (b) how serious a problem they perceived fragile X syndrome to be, and (c) their feelings about the carrier testing process. Such information is useful for counseling purposes.

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