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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: fragile X mental retardation syndrome, fragile X-associated tremor/ataxia syndrome and fragile X-associated primary ovarian insufficiency

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

The term fragile X-associated disorders (FXD) refers to a family of conditions all caused by changes in fragile X mental retardation 1 gene (*FMR1*).

Fragile X mental retardation syndrome, fragile X syndrome (FXS), Martin–Bell syndrome

Males affected with FXS present with mild-to-severe mental retardation. Dysmorphic features often include large prominent ears, an elongated face, a prominent forehead, macrocephaly and a high arched palate, which is occasionally accompanied by a cleft palate, These dysmorphic features are generally more striking after early childhood. Macroorchidism, while not specific for FXS, is the most consistent finding, present in ~90% of boys by the age of 14. Behavioral disturbances including hyperactivity, hyperarousal, anxiety and aggressive outbursts are common. FXS represents the most common monogenic disorder responsible for autism and autism spectrum disorders. Approximately 30% of boys with FXS meet the criteria for autism.[1–3] This subgroup of boys presents with the same behavioral and social profile observed in children with idiopathic autism.[3] Strong gaze avoidance, even when the individual is seeking interaction, represents one of the hallmarks of FXS. In addition, tactile defensiveness and tantrum behaviour when subjected to excessive auditory or visual stimuli suggest a sensory processing disorder.

FXS is an X-linked disorder and females usually present with a milder phenotype. Females affected with FXS generally have IQs in the borderline to low normal range (mean IQ: 82). Most females present with learning disabilities, half meeting the criteria for intellectual and developmental disabilities[4] and approximately a quarter being mentally retarded (IQ < 70).[5]

Affected females have fewer behavioral problems than males, with shyness and social anxiety being the most commonly seen. Residual FMRP (protein produced by FMR1) levels in females are related to the X activation ratio (AR). Women may produce close to normal levels of FMRP when the normal X chromosome is preferentially activated (high AR), or much lower levels when the normal X chromosome is preferentially inactivated.

Fragile X-associated tremor/ataxia syndrome (FXTAS)

FXTAS is a late onset neurodegenerative disorder found among some male and female carriers of the premutation (see section 1.5 for a definition of the premutation). FXTAS is defined by clinical, neuro radiological, molecular and neuropathological criteria. Affected individuals primarily present with cerebellar ataxia and intention tremor. Less distinctive symptoms are cognitive decline or impairment, peripheral neuropathy, parkinsonism and urinary and bowel incontinence. MRI findings include increased signals in the middle cerebellar peduncle and the deep white matter of the cerebellum.

FXTAS is not fully penetrant in older male carriers of the premutation, with many individuals remaining asymptomatic.[6–8]

Fragile X-associated primary ovarian insufficiency (FXPOI)

FXPOI is characterized by a large spectrum of ovarian dysfunction phenotypes: an elevated follicle-stimulating hormone level, erratic menstrual function and an onset of menopause before 40 years of age.

1.2 OMIM# of the disease

300624 (FXS), 300623 (FXTAS).

1.3 Name of the analyzed genes or DNA/chromosome segments

FMR1, located in Xq27.3.

1.4 OMIM# of the gene(s): 309550

FMR1 has 17 exons spanning 39 kb of genomic DNA, and encodes the FMRP.

Mutations in FMR1 can lead to a deficiency of FMRP, responsible for FXS, or to overexpression and toxicity of FMR1 mRNA, responsible for FXTAS and FXPOI.

1.5 Mutational spectrum

FMR1 has a polymorphic (CGG) repeat in its 5′ untranslated region,[9–11] which is the major target of mutation of the gene.

Mutations affecting the (CGG) repeat are 'dynamic' and change the stability of the repeat in both somatic and germ cells on their mitotic proliferation, thereby favouring expansion of the repeat over generations (retractions are rare). The instability of the (CGG) repeat is

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responsible for the unusual and complex pattern of inheritance of the disease.

Four forms of the polymorphic CGG repeat have been defined. Two forms have been associated with phenotypic changes (full mutation and premutation) and three of the four forms are unstable on transmission (full mutation, premutation and intermediate or gray zone alleles) and should be considered during genetic counseling.

Full mutation (M)

Expansion of the (CGG) trinucleotide repeat, exceeding 200 triplets, with subsequent aberrant methylation of virtually all CG dinucleotides in the repeat and the adjacent regions including the gene promoter,[12] transcriptional silencing resulting in the absence, or highly diminished levels of FMR1 mRNA and protein. (The methylation is aberrant in that it is triggered by abnormal structures of the expanded CGG repeat sequence and is independent of the methylation of FMR1 that normally occurs with X inactivation in somatic tissues of any normal female embryo.)

Premutation (P)

Expansion of the (CGG) repeat to 55–200 triplets without aberrant methylation.

Premutation alleles are more or less unstable mitotically, dependent on their lengths. Females carrying a premutation have a risk of expansion to a full mutation on transmission to their offspring. This risk is strongly dependent on the size of the maternal premutation, and is >95% for maternal alleles with >100 CGG triplets.[13–15] This instability is thought to depend on the length of uninterrupted CGG tracts, with 'pure' CGG repeats being less stable than repeats with interspersed AGG sequences.[16] The smallest allele known to undergo transition to a full mutation in a single generation contained 56 consecutive CGG repeats uninterrupted by AGGs.[17]

Intermediate alleles (IA) and Normal alleles (N)

The smallest described normal allele has 6 repeats, with 29–30 repeats being the most common allele sizes.

Intermediate alleles are alleles at the boundaries of normal alleles (likely to be stable[18]/no genetic counselling) and premutation alleles (likely to show instability and with the possibility of transition to a full mutation in one generation upon maternal transmission/genetic counselling). Genetic counselling is recommended in cases of allele sizes in the intermediate range since these alleles may show instability, with the possibility of larger alleles in family relatives. It is known that alleles in the 45–54 CGG repeat range can show some instability on transmission[18] with no reported risk of transition to the full mutation (this instability is thought to depend on the number of consecutive CGG repeats uninterrupted by AGGs[16]). An allele with 52 CGG has been reported to expand to the full mutation in two generations through a 56 CGG repeat in a family.[17] In a second family, a grandmother of two boys presenting with a full mutation was the carrier of a 45 CGG repeat allele, whereas her two daughters were carrier of 80 and 90 CGG repeats, respectively.[19] Another allele containing 44 CGG is thought to have expanded to a full mutation in two generations thought a 61 CGG repeat allele in a third family, although in this case, the possibility of mosaicism associated with this 44 CGG allele was not excluded.[20] Risks associated with a 45–54 CGG allele are difficult to determine when it is found in the general population. Guidelines published by different associations have established different lower bound limits of the IA range: 45 CGG repeats American College of Medical Genetics (ACMG[21]) or 50 CGG repeats (Clinical Molecular Genetics Society (CMGS): http://www.cmgs.org/BPGs/pdfs%20current%20bpgs/Fragile%20X.pdf and EMQN: http://www.emqn.org/emqn/digitalAssets/0/233_EMQN_guidelines_FRAX_2006.pdf).

Mosaicism of full and premutation alleles (MoMP) is not uncommon, occurring in ~12–41% of all patients with FXS. In rare cases, the mutation is even associated with a low percentage of normal cells (MoMN or MoMPN genotype). Some individuals with full mutation show methylation mosaicism (methylation mosaic: MoMe) in their lymphocytes.

Fragile X syndrome

For ~99% of reported cases, FXS is a result of the full mutation preventing transcription and translation of the gene into FMRP.[22]

Other rare loss of function (LOF) mutations, such as point mutations[23–25] or deletions, have also been reported to cause FXS. Various deletions involving all or a segment of FMR1 have also been found associated with abnormal expansion (reviewed in Coffee *et al*[26]).

FXTAS and FXPOI

The premutation does not cause mental retardation (ie, FXS), but is associated with a gain of function toxicity at the mRNA level, increasing an individual's risk for FXTAS and FXPOI.

FXTAS

A preliminary study by Jacquemont *et al*[27] demonstrated an age-related penetrance of tremor and ataxia of 17, 38, 47 and 75% for male carriers of the premutation, aged 50–59, 60–69, 70–79 and 80 years or older, respectively. Allele distributions in patients with FXTAS show that 80% of the expansions are >70 CGG repeats. It is unknown whether there is a strict lower limit for the size of the CGG repeat required for carriers to develop FXTAS; however, the motor and cognitive symptoms are correlated to the size of the allele. The penetrance of FXTAS may be very high in carriers of very large alleles (>90 CGG).[28]

FXPOI

In all, 21% of female carriers of the premutation present with premature ovarian failure (ie, menopause before the age of 40), compared with only 1% in the general population.[29] Data from several studies show that 11 out of 81 (13.6%) of pedigrees with familial premature ovarian failure and 7 out of 301 (2.3%) of women with sporadic premature ovarian failure had the premutation.[30–32] The probability of having FXPOI increases with increasing repeat size in the low premutation repeat range, but thereafter the risk of FXPOI becomes stable or even decreases for women with repeat sizes over 100.[33–35]

1.6 Analytical methods

Many analytical methods are used for genetic testing of fragile X-associated disorders (FXS, FXTAS and FXPOI), each with their own strengths and weaknesses. The performance and interpretation of genetic tests are discussed in several guidelines: EMQN (http://www.emqn.org/emqn/digitalAssets/0/233_EMQN_guidelines_FRAX_2006.pdf), CMGS (http://cmgsweb.shared.hosting.zen.co.uk/BPGs/pdfs%20current%20bpgs/Fragile%20X.pdf) or ACMG (http://www.acmg.net/Pages/ACMG_Activities/stds-2002/fx.htm).

Diagnostic laboratory methods include Southern blotting (DNA specifically cleaved with restriction endonucleases) and/or direct amplification of the CGG repeat with flanking primers.

Southern blotting allows for the identification of all expansions, as well as the determination of the methylation status.[36] Accurate sizing of the CGG repeats requires the use of PCR, and is a crucial step in order to establish risk for individuals, notably carrier females at risk of having affected children, as well as to distinguish intermediate alleles from premutations.

The basic PCR methods are adapted from Fu *et al*[10] and are widely used as a pre-screening test. Standard PCR amplification may not reliably amplify large premutation alleles, particularly in carrier females. In males in whom no normal allele is visible, in females in whom only one normal allele is distinguishable (homoallelism), or when an allele is in the intermediate or premutation range, Southern blot analysis (or another test allowing the detection of the whole range of expansions) should be undertaken. One downside of this strategy is the rare occurrence of mosaic individuals who carry a full mutation and an unexpected amplifiable normal size fragment because of cellular mosaicism (MoMN) or abnormal karyotype (XXX, XXY or XXYY).

xPCR tests specifically optimized to detect large expansions and/or methylation status have been described.[37] Such tests use a methylation-sensitive restriction enzyme or bisulphite treatment of DNA before amplification.[38–41] The interpretation of a methyl-sensitive PCR technique can prove difficult in a female with a full mutation due to the presence of the methylated inactive normal X chromosome. Furthermore, the methyl-sensitive PCR techniques are not suitable for early prenatal diagnosis, as the tests do not directly detect fully expanded alleles but are based on DNA methylation, which is not completed in chorionic villi samples of full mutation fetuses. Some PCR tests based on triplet primed–PCR strategy have been described, which should distinguish between normal homozygous females and females with a normal allele and an expansion.[42–45]

In males, rare mutations such as deletions encompassing the CGG repeat and the promoter can be detected using Southern blot or PCR.

Detection of other deletions as well as a search for point mutations in FMR1 coding sequence are not usually offered by routine laboratories.

Alternative immunocytochemical tests have been described, but are not widely used in the diagnostic setting.[46]

1.7 Analytical validation

Results may be misinterpreted because of the specific pitfalls of each method and the technical limitation of each protocol. The limitations of each test should be clearly stated in the interpretation section of the molecular diagnosis report for all cases, regardless of positive or negative screening. Two independent methods, for example, PCR plus Southern, should be used for the testing of individuals in fragile X families.

1.8 Estimated frequency of the disease (incidence at birth ('birth prevalence') or population prevalence)

Fragile X syndrome

The prevalence of FXS in males is estimated 1/4000. The prevalence of the full mutation in females should be 1/4000 as well, but not all full mutation females present with cognitive and/or behavioral symptoms. The data are based on population screening from cohorts of children with special education needs. This generally underestimates the prevalence, as many individuals with IQs in the borderline range are not tested. Other approaches have estimated the prevalence of the full mutation in the general population around 1/2500.[47]

FXTAS

There are no population-based studies on the prevalence of FXTAS. This has been estimated based on (i) the prevalence of the premutation in the general population, (ii) the penetrance of FXTAS among premutation carriers, (iii) the relationship between the premutation allele size and the penetrance of neurological signs in FXTAS. Given an estimated prevalence of the premutation of 1/800 in males and an estimated lifetime penetrance of FXTAS of 40%, the prevalence of FXTAS would be 1/2000 males. Clinical manifestation of FXTAS are essentially associated to alleles >60 CGGs, which represent $\sim 50\%$ of all premutation alleles in the general population. Taken this into consideration, the prevalence of FXTAS drops to 1/4000.[48] This estimate is subject to the uncertainty of both, the overall prevalence of premutation alleles in the general population and the penetrance of FXTAS for smaller premutation sizes.

1.9 If applicable, prevalence in the ethnic group of investigated persons

Some populations may have a higher prevalence of FXS because of the founder effect, with the founding group having more unstable alleles in the intermediate or premutation range.[49–51] Epidemiological studies are necessary to better estimate the prevalence of the premutation and full mutation in different ethnic groups.

1.10 Diagnostic setting

Guidelines for genetic counseling and testing protocols for FXS and fragile X-associated disorders have been established by various multidisciplinary groups and are regularly updated.[52–55] Information on the disorders are available online at http://www.nfxf.org.

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comments:

A. (Differential) diagnostics

Fragile X syndrome

Considering that

- FXS is the most common form of inherited mental retardation
- Clinical features are neither constant nor specific,
- Behavioral changes and dysmorphic features are not always present,
- Dysmorphic features can become more apparent with age,
- Phenotypic characteristics can be mild or absent in females, ie, they can often only have mild or moderate learning disabilities,
- Testing for the most common mutations are easily performed in routine molecular diagnostic laboratories,

The search for an abnormal expansion in FMR1 should be part of the routine screening in males and females who present with developmental delay, mental retardation or borderline intellectual abilities, autism spectrum disorder characteristics, and/or behavioral or dysmorphics features typical of FXS.

When applying the above guidelines at the national level in France[56] and Greece,[57] and other European countries probably as well, approximately 2–3% of individuals tested are positive for an abnormal CGG repeat expansion.

FXTAS

- Clinicians should test for FMR1 premutation if any of the following criteria apply.[8]
- Onset of cerebellar ataxia of unknown cause in an individual over 50 years.
- Onset of intention tremor of unknown cause in an individual over 50 years with concurrent parkinsonism or cognitive decline.
- Previous diagnosis of multiple system atrophy or a cerebellar subtype MCP sign on T2/FLAIR MRI images in a patient with signs consistent with FXTAS.
- Individual with signs consistent with FXTAS if he/she could be a carrier based on his/her position in the pedigree in case of

- Positive family history of a FMR1 premutation or mutation,
- Family history of mental retardation,
- Family or patient history of primary ovarian insufficiency,

For unexplained cerebellar gait ataxia with an onset >50 years of age, the positive diagnostic yield for the premutation is 1–4%.[48] For patients with probable multiple system atrophy (cerebellar subtype), the positive yield is 2–3%.[48,58]

FXPOI

FXPOI should be on the differential diagnostic list of a female with primary ovarian insufficiency,[35] regardless of her family history. A premutation has been identified in up to 13% of women with familial premature ovarian failure and in approximately 2–3% of women with sporadic premature ovarian failure. FMR1 premutation screening should be recommended to all women with primary ovarian insufficiency, an elevated follicle-stimulating hormone level before the age of 40 years without an otherwise known cause, particularly if increased FSH is accompanied by infertility.

Families with one or more individuals who were tested positive for either FXPOI or FXTAS should benefit from appropriate genetic counseling regarding the risk of transmission of FXS.

B. Predictive testing

For FXS

Current guidelines state that genetic testing of children is recommended only if a clear benefit for the child can be demonstrated. The test is not generally recommended for asymptomatic children but the topic is controversial and some clinics perform testing in children with no or little symptoms. McConkie-Rosell *et al*[59] studied how parental approaches to communicating information about genetic disorders to children may determine how the children manage stress as well as their adjustment and adaptation to that information.

For FXTAS

There are no current guidelines on the presymptomatic testing.

In families, genetic counseling for the FXS should remain the priority when considering whether an asymptomatic individual should be tested for the premutation (eg, testing the grandparents of an affected child to identify which side of the family is at risk for further involvement).

Only a subgroup of carriers develops symptoms that significantly impact activities of daily living (one-third of individuals affected with FXTAS, or $\approx 10\%$ of carriers in the 60–69 age group[60]).

For FXPOI

The possibility of early menopause leading to reduced fertility should be included in the genetic counseling of women identified with a premutation.

C. Risk assessment in relatives

Families with a diagnosis of a fragile X-associated disorder (FXS, FXTAS or FXPOI) should be referred for genetic counseling. Counseling and diagnostic testing may be offered to relatives at risk of being carriers, and can help determine the risk for females of having children with FXS. The premutation is also a risk factor for the development of FXPOI in females and FXTAS in males and females.

D. Prenatal

Prenatal diagnostics should be offered to women with a fragile X allele containing 55 or more CGG repeats.[61] Options include freshly dissected chorionic villi sampling or amniotic fluid cells (a cell culture allowing a larger amount of DNA being required for Southern blot analysis). A prenatal measurement of the CGG number can be accurately and reliably obtained with either sample, with the size of the expansion being the most important piece of information. Prenatal diagnostics performed on chorionic villi allow for the definite determination of the fetal status, and can be performed earlier in the pregnancy than amniocentesis. In rare cases in which a large premutation cannot be distinguished from a small full mutation based on the repeat size estimation, an amniocentesis is necessary to determine the methylation status of the fetus. The prenatal testing can not be perfomed on chorionic villi DNA by using a methylation-sensitive method, because methylation of the full mutation allele is not always present during the 8–10th week of pregnancy.[62] In case a premutation is detected on chorionic villi DNA, a second test may be carried out on DNA from cultured amniotic fluid cells, in order to exclude a risk of expansion size discrepancy between the two tissues (with a full mutation being present in amniotic cells). Nevertheless, such a discrepancy has never been reported.

Prenatal diagnosis should include a CGG repeat PCR study of the parents and the fetus. Indirect familial genotyping using microsatellite markers should also be applied when possible. For both, chorionic villi sampling and amniocentesis, a reliable prenatal diagnostic requires careful exclusion of maternal DNA contamination.

A female fetus with a full mutation has a 50% risk of being affected with cognitive deficits ($IQ<70$ in 25% of the cases), which is generally milder than that observed in males with a full mutation. No other tests are currently available to predict the future clinical status of a female fetus with a full mutation.

Prenatal testing is not indicated for a male with the premutation, as all of his daughters are expected to carry a premutation.

A male with the full mutation is also expected to transmit alleles of premutation size to his daughters but it is documented on a restricted number of observation: rare reported cases of daughters of affected males show that they carry a premutation and a study showing that the sperm of four males with the full mutation had premutation size repeats.[63] There has been one report of a male with a mosaicism transmitting a full mutation to his daughter.[64] There is, however, some controversy on the level of methylation of the large expansion found in the lymphocytes of his daughter.[65] Prenatal testing should be proposed for a female fetus of a full mutation father as a cautionary measure.

2. TEST CHARACTERISTICS

The analytical validity of a genetic test is determined by its ability to accurately and reliably determine the genotype of interest. The clinical validity is a measurement of the accuracy (such as clinical sensitivity and specificity as well as predictive value) of a test to identify and/or predict a clinical condition.[66]

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity

The analytical sensitivity (ie, the proportion of positive tests when the genotype is truely present) depends on the size of the expansion, the gender of the patient and the analytical method used. The analytical sensitivity should be indicated in the laboratory report.

Genotype search for the full mutation

Southern blot (or another technique capable of detecting the full mutation): Almost a 99% sensitivity in detecting the full mutation, missing only the rare individuals with heterogeneity of expansions among different tissues.

Genotype search for the premutation

Southern blot (or another technique capable of detecting a premutation): Almost a 98% sensitivity in detecting a premutation, missing only the rare individuals with heterogeneity of mutations among different tissues. A 99% sensitivity is achieved when an additional method capable of distinguishing the premutation from intermediate alleles is used (ie, the combining of Southern blot and PCR across the CGG repeat).

Comment

A comparison study on the DNA in blood cells and skin fibroblasts has shown a striking difference in the relative amounts of premutation and full mutation alleles in the tissues of two out of four mosaic fragile X males (MoMP).[67] Some extremely rare cases of tissue mosaicism have been described[68,69] that could lead to a false-negative test independent of the technical approach for blood analysis when the full mutation is not present in this tissue.

Even though PCR methodology is widely used in the screening of the mentally retarded probands, this approach is subject to specific pitfalls:

- Some individuals are mosaics for an abnormal expansion and an apparently normal allele (MoMN or MoMPN): In such cases, a CGG PCR test will be negative even though a mutation is present because a normal signal will be obtained[70,71] (V Biancalana personal data: 1 MoMN/100 M and MoMP).
- A 49 bp tandem duplication adjacent to the triplet repeat in FMR1 has been described in the Finish population[72] that affects annealing of the primers commonly used in the molecular analysis of the CGG repeat by PCR. One concern is that a female with a full mutation and a variant allele may be genotyped as normal as a result of the two PCR products generated by the variant.

Genotype search for deletion with loss of the promoter

A large deletion will be detected in males by absence of a specific signal on PCR and Southern blotting, and in females as well when the normal X chromosome is not randomly inactivated. But these mutations are only found in about 1% of FXS patients.

Genotype search for point mutation

Sequencing (males) ~100%. But there is hardly any clinical phenotype and/or family history with a fair chance to detect another mutation in a patient when a full mutation and a deletion of the promoter region were excluded.

2.2 Analytical specificity
(proportion of negative tests when the genotype is not present)

Depends on the analytical method.

Genotype search for full mutation

Southern blot: It is 99% specific in males and females. Some point mutations affecting a restriction site of the enzymes used have been reported. These polymorphisms could mimic a full mutation in FMR1.[73,74]

Genotype search for premutation

Southern blot (or another technique capable of detecting the premutation): It is 99% specific in detecting the premutation. False-positive findings can occur in an individual who has an intermediate allele in the upper high CGG repeat range. The specificity is almost 100% when combining Southern blot and CGG repeat PCR techniques.

Comments

A PCR test showing no amplificate in a male and thereby suggesting the presence of an expansion should always be confirmed with another method, that is, by Southern blotting. PCR artefacts or deletions encompassing the primer(s) sites could mimic an expansion and may lead to a false-positive result.[75]

Genotype search for deletion with loss of promoter

Southern blot: Almost 99% specific. Rare polymorphisms of the restriction site of an enzyme have been reported. These polymorphisms may mimic a deletion in FMR1, leading to a false-positive result.[76–79]

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)

Fragile X syndrome

Full mutation: ~99% (Southern)
Deletion with loss of promoter: ~1% (Southern)
Other LOF mutations: <1% (all methods)
The yield of fragile testing by southern blot in males with mental retardation is ≈2%.[56]

FXTAS

Premutation: ~99% (Southern and PCR)
The yield of the premutation testing in individuals with late onset cerebellar ataxia is ≈1–3%.[48]

FXPOI

Premutation: ~99% (Southern and PCR)
The yield for premutation testing is ≈2% and 10% in sporadic and familial premature ovarian failure.[35]

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)

Full mutation: ~100%
Deletion with loss of promoter: ~100%
Premutation: ~100%.

2.5 Positive clinical predictive value
(lifetime risk of developing the disease if the test is positive)

Fragile X syndrome

Full mutation (>200 CGGs), with aberrant promoter methylation: It is 100% for males. Females have two X chromosomes and the clinical expression is likely to be correlated to the normal process of X inactivation, in particular in the brain. Approximately 50% of full mutation carrier females present cognitive deficits, 25% present mental retardation with an IQ<70.

Full mutation with incomplete aberrant promoter methylation or without aberrant promoter methylation: Owing to the lack of precision in measuring the levels of mosaic, a predictive value can not be estimated.

Comment

As males have a single X chromosome, almost all males with a full mutation fully methylated will develop FXS. The extremely rare exceptions likely involve particular forms of the mutation with somatic mosaicism.

The mitotic instability of the repeat of the full mutation in somatic cells in early embryogenesis before the methylation of expanded CGG repeat leading to their stabilization[80–82] causes somatics mosaicism in most individuals.[83] As the expansion size of a methylated full mutation does not have an influence on the severity of the clinical phenotype,[36,84] somatic mosaicism is of no consequence for the clinical expression when the expansions are in the full mutation range. There are, however, two special subclasses of mosaicism based on size and methylation status.

'Methylation mosaics' (MoMe) are rare individuals who have a partially unmethylated full mutation expansion in leucocytes. The proportion of cells with an unmethylated full mutation may vary from 5 to 100%. In some cases, the mental impairment may be less severe than that seen in individuals with a full methylated mutation. The absence of mental retardation has been reported in cases with little or no methylation ('high-functioning' fragile X males) but mild intellectual deficits may remain present likely because of the reduced FMRP levels related to a decrease in translation of FMRP.[36,85–94]

'Size mosaic' (MoMP) are individuals with a mixture of premutation and full mutation alleles, sometimes associated with a deleted allele. They have a risk of mental retardation similar to that of full mutation carriers,[84] although they may occasionally be more 'high functioning'.[95,96] Blood cells and skin differences in fragile X mosaics has been reported.[67] The mosaicisms in the brain and skin, being both ectodermal in origin, may be similar to one another, but different from blood which has a mesodermal origin. Thus, the ratio of full mutation to premutation in skin fibroblast may be a better indicator of the risk of mental impairment than the ratio found in blood cells.

FXTAS

Full mutation with aberrant promoter methylation: 0%

Full mutation without aberrant promoter methylation: unknown.

Premutation: Published penetrance figures have not taken premutation sizes into account:

Male carriers: It is 17, 38, 47 and 75% for men aged 50–59, 60–69, 70–79, and over 80 years, respectively.

Premutation <70 CGG repeats: The penetrance of FXTAS is much lower.[58]

There are no published studies on penetrance figures for premutation female.

FXPOI

Full mutation with aberrant promoter methylation: 0%

Premutation: It is 21–23%

2.6 Negative clinical predictive value (probability of not developing the disease if the test is negative)

Almost 100%.

3. CLINICAL UTILITY

Clinical utility refers to the ability of genetics test results, either positive or negative, to provide information that is of value in the clinical setting.[66]

3.1 (Differential) diagnosis: the tested person is clinically affected

(To be answered if in 1.10 'A' was marked.)

3.1.1 Can a diagnosis be made other than with a genetic test?

No (continue with 3.1.4).

3.1.2 Describe the burden of alternative diagnostic methods on the patient

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

3.1.4 Will disease management be influenced by the result of a genetic test?

Yes.

Fragile X syndrome

Therapy, management**:** Currently, there is no pharmaceutical treatment for the cognitive deficits in FXS, and although various drugs have been used to treat the associated behavioral problems, there is a paucity of controlled studies that formally measure the effectiveness of such therapies.[97] Atypical antipsychotics, stimulants and SSRIs are prescribed depending on the problematic target symptoms.

More and more is known, however, on the physiopathology of FXS[98] and this body of research strongly suggests that mGluR5 (metabotropic glutamate receptor type 5) antagonists may be an effective treatment for FXS.[97] Lithium, which reduces excess activity in the translational activation pathway regulated by group I mGluRs (mGluR5 and mGluR1) was assessed in 15 young males (6–23 years of age) with FXS by Berry-Kravis *et al*.[99] They observed significant improvement in behavioral functioning, adaptive behavior, and verbal memory. An open-label, single-dose trial of fenobam, a mGluR5 antagonist, was recently conducted[100] and improvement in prepulse inhibition was observed. A double-blinded phase 2 trial was completed in Europe evaluating the effects of AFQ056, a new specific mGluR5 antagonist in 30 adult males with FXS aged 18 to 35 years. In this trial, Jacquemont *et al*[101] reported significant improvement in behavioral functioning in patients with a fully methylated FMR1 promoter. Large scale phase 3 trials are being conducted in 2011 by the same groups.

There are almost no empirical studies on the effectiveness of behavioral treatments among patients with FXS.[102] The behavioral phenotype in FXS has, however, been extensively studied and a detailed review and recommendations for behavioral interventions in individuals with FXS were provided by Hills-Epstein and Sobesky.[103] Patients with FXS seem to benefit from non-pharmacological interventions, such as speech, occupational and sensory integration therapies. Other guidelines for the health supervision of FXS children are available and include advice for both physical and behavioral components of the syndrome.[52]

FXTAS

No controlled trials have been carried out in individuals with FXTAS, but a significant amount of empirical information has been gathered through clinical practice regarding various treatment modalities.[104,105]

3.2 Predictive setting: the tested person is clinically unaffected but has an increased risk based on family history

(To be answered if in 1.10 'B' was marked.)

3.2.1 Will the result of a genetic test influence the individual's lifestyle or prevention strategies

If the test result is positive (please describe)

Identification of a female fragile X carrier allows women to make informed reproductive decisions, which take into account the risk of primary ovarian insufficiency and the risk of having a FXS affected child.

Early family planning may enable conception in a female premutation carrier likely to suffer from primary ovarian insufficiency.

A woman with a premutation or a full mutation may decrease her risk of having a child affected with FXS by taking advantage of prenatal diagnostics, donor eggs, adoption and so on. Preimplantation genetic diagnosis (PGD) is possible but particular technical difficulties exist for FXS. Ovarian dysfunction in premutation carriers reduces the chances of a successful pregnancy using PGD due to a low yield of available eggs.[106]

If the test result is negative (please describe)
Determining that a female patient is not a carrier can relieve the anxiety related to genetic risk and allow for confident family planning.

3.2.2 What lifestyle and prevention strategies does an at-risk individual have if genetic testing is not performed (please describe)?
No special options; prevention is not possible.

3.3 Genetic risk assessment for the family members of an affected individual
(To be answered if in 1.10 'C' was marked.)

3.3.1 Does the result of a genetic test resolve the genetic situation of the family?
Once an individual has been shown to be affected by any one of the fragile X-associated disorders, cascade counseling and testing may be offered to relatives at risk of being a carrier, taking into account the unusual pattern of mutation inheritance.

3.3.2 Can genetic testing of a patient save genetic testing of family members?
Given the X-linked transmission, the presence of a premutation in a father automatically determines the status of the children: his sons will be non-carriers and daughters will be premutation carriers. Genetic testing in the sons is not necessary.

Given the exclusive maternal transmission of a full mutation, genetic testing of the father of a patient affected with fragile X is not necessary.

3.3.3 Does a positive genetic test result in a patient allow to predict the genetic status of a family member?
Yes, a positive test in a female (full mutation) allows to identify her mother as a carrier. A positive test in a male allows to identify his mother and his daughter as carriers and his sons as non carriers.

3.4 Prenatal diagnosis
(To be answered if in 1.10 'D' was marked.)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?
All females carriers of a premutation or a full mutation can be offered a prenatal diagnosis.

Male carriers of a full mutation can be offered a prenatal diagnosis as a cautionary measure in case of a female fetus (see 1.10.D).

4. IF APPLICABLE, FURTHER CONSEQUENCES OF GENETIC TESTING

Molecular confirmation of the diagnosis will limit unnecessary further etiological investigations, which can often be invasive and unpleasant. Although there is no cure for fragile X, the diagnosis helps guiding the appropriate physical, cognitive and behavioral management of the affected individual.

Many parents feel guilty, and may be relieved after a genetic diagnosis is obtained. Parents also find encouragement and support in dealing with daily anxieties and difficulties by becoming members of clubs and associations that welcome affected families.

A molecular diagnosis enables a female carrier to make informed reproductive decisions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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