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Health problems in females carriers of premutation in the FMR1 gene

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Summary

Premutation in the FMR1 gene occur in the general population with an estimated prevalence 1 in 130–260 females and 1 in 250–810 males. Carriers of premutation are at risk of development of spectrum of neurological, psychiatric and immunological disorders in adulthood. Fragile X-associated disease caused by dynamic mutation (expansion of CGG repeats) can be divided into three disorders: FXS – Fragile X syndrome, FXPOI – Fragile X-associated primary ovarian insufficiency, FXTAS – Fragile X-associated tremor/ataxia syndrome, which can be present in few generations of one family. Immuno-mediated disorders are more common in premutation carriers as compared to control group, especially hypothyroidism and fibromyalgia. Although FMR1-associated conditions are not curable, timely diagnosis through genetic testing is important as it can lead to implementation of treatment strategies and behavioral interventions considered to improve symptoms. Knowledge of expanded allele status for females helps them to make more informed reproductive decisions.

Key words: depression, FMR1 gene, premutation

Introduction

Fragile X syndrome (FRAXA, FXS) (OMIM # 300624), Martin–Bell syndrome, Escalante syndrome, is the most common cause of inherited intellectual disability [1] with an estimated incidence of 1 in 4,000–6,000 males and 1 in 5,000–8,000 females. Premutation occur in the general population with an estimated prevalence 1 in 130–260 females and 1 in 250–810 males. FXS is inherited as X-linked dominant disease [2].

Fragile X syndrome shows unusual form of inheritance. In affected families are nonpenetrant males carriers who do not have symptoms of disease, but whose daughters – also without the symptoms of the disease – are at high risk of having children with FXS. Described inheritance pattern was documented by epidemiological investiga-

tions by Sherman et al. [3]. The risk of having affected child increases in successive generations only when mother transmits the gene (the form of anticipation). In 1991 the FMR1 gene causing FXS was cloned [4, 5]. The FMR1 gene is located in Xq27.3, has 17 exons, its mRNA has 4,000 base pairs. Exons 12, 14, 15 and 17 may be alternatively spliced forming different mRNA and different isoforms of FMRP. The structure of the FMR1 gene revealed that fragile X site contains CGG trinucleotide sequence in its 5' untranslated region. Repetitive CGG sequence is variable (polymorphic) in general population and contains 6–54 repeats, usually 29–30.

Above-mentioned specific mechanism of dynamic mutation with unusual pattern of inheritance is the cause of the disease [4–6]. Dynamic mutation is caused by expansion of CGG trinucleotide repeats in promotor region of the FMR1 gene. The affected persons are carriers of so-called full mutation with 200 or more CGG repeats, cytosines and the neighboring CpG island are methylated, causing the lack of transcription and product of the FMR1 gene. Full mutation causes fragile X syndrome, neurodevelopmental disease which is the most common cause of familial form of intellectual disability. Normal range of CGG repeats in the FMR1 gene is 6–54 (usually 29–30) and it involves intermediate range (35–44 CGG repeats) and so-called grey zone (45–54 CGG repeats). Gene with a normal range of CGG repeats is transmitted in a stable way, the number of CGG repeats does not change during meiosis. Intermediate values may be less stable, number of CGG repeats during meiosis may expand to full mutation in children. In the case of premutation the number of CGG repeats is 55–200 and is unstable during transmission from mother to child [6–8]. More than 99% of affected persons are the carriers of so-called full mutation with 200 or more CGG repeats, cytosines and neighboring CpG island are methylated causing lack of transcription of the gene and lack of gene protein product [9–11].

The diagnosis of FXS is very important not only for affected child but also for patient's siblings, parents and other family members, involves many generations. Fragile X-associated disease caused by dynamic mutation (expansion of CGG repeats) can be divided into three disorders: FXS, FXPOI, FXTAS. These diseases can be present in few generations of one family [12].

Premutation is the cause of two disorders with incomplete penetrance. One of them is Fragile X-associated primary ovarian insufficiency (FXPOI), characterized by broad spectrum of abnormal ovarian function with premature menopause (before the age of 40). Second one is Fragile X-associated tremor/ataxia syndrome (FXTAS), late onset neurodegenerative disorder more often males affecting than females [12, 13].

FMRP

Protein product of FMR1 gene, FMRP, belongs to a group of proteins selectively binding RNA. FMRP consists of 632 amino acids and has five domains, including two KH domains (hnRNP K-protein homology) in central part and RGG box domain

containing arginine and glycine residues (Arg-Gly-Gly) in carboxyl end. FMPR is expressed in many tissues, the highest levels are in brain and testes and this correlates with clinical symptoms of FXS. In brain, FMRP is expressed in differentiated neurons, mainly in hippocampus and granular layer of the cerebellum [12, 13]. Expression of FMRP can be detected in synapses but not in axons.

FMRP plays an important role in regulation/repression of translation of other cellular transcripts. This process is called repression of translation. Lack of FMRP causes weak control of the translation of cellular mRNA and excessive protein expression [9, 10]. Mutations in the genes encoding the following proteins: neuroligins, neurexin 1, PTEN, PSD95, MAPK1, SHANK3, have been described in patients diagnosed with autism spectrum disorder. Molecular overlapping with FXS and autism spectrum disorders is based on dysregulation of the genes associated with autism when FMRP is absent [14].

Molecular bases of disorders associated with premutation

In premutation carriers FMR1 mRNA transcripts are significantly increased (2–8x) [15]. Pathophysiological bases of disorders associated with premutation seem to be related to RNA toxic effect, FMR1 mRNA toxic-gain-of-function mechanism. Different mRNA–protein interactions lead to aberrant protein expression and sequestration of cytoplasmic proteins thus reducing their capacity to carry out their role [16]. Sellier et al. [17] showed that RNA-binding protein DGCR8 binds to expansions of CGG repeats of pathogenic length and it results in partial sequestration of DGCR8 and its partner DROSHA within CGG-RNA aggregates. Consequently, miRNA regulation is disturbed resulting in decreased levels of mature miRNA in neurons. Overexpression of DGCR8 protects neurons from death induced by expanded CGG repeats. Although sequestration of the proteins related to CGG repeats seems to be dominant mechanism, several alternative models explaining RNA toxicity have been proposed. The RNA containing CGG repeats triggers conformational transition in one or more proteins that harbor prion-like domains, similarly to the formation of amyloid deposits in Alzheimer's disease. Aberrant polypeptide products may aggregate and become neurotoxic [15].

Clinical symptoms in females with FXS

Females carriers of full mutation having two X chromosomes, one of which is inactivated, show much milder symptoms of the disease. Half of them may show mild form of intellectual disability or learning problems as well as emotional problems, depression, anxiety. Phenotypic features are subtle with slightly elongated face and enlarged ears [18].

Clinical symptoms in females carriers of premutation

Carriers of premutation are at risk of development of spectrum of problems from mild cognitive and behavioral problems in childhood to neurological, psychiatric and immunological disorders in adulthood.

Before getting to know the molecular basis of the fragile X syndrome, FMR1 gene (in 1991), it was thought that the females carriers of premutation do not show clinical features of the disease but more often give birth to children with genetic disease – fragile X syndrome. Short before description of FMR1 gene, Cronister et al. [19] observed significantly increased incidence of premature ovarian failure (menopause before 40) in females carriers of premutation (up to 20 %) as compared to general population (1%). Early estrogen deficiency leads to low bone density, earlier onset of osteoporosis and bone fractures, impaired endothelial function, earlier onset of coronary heart disease and increased cardiovascular mortality in females carriers of FMR1 gene premutation [20].

Additional clinical symptoms among premutation carriers were identified in 1990s, they involved psychiatric problems such as depression and anxiety disorder [21–25]. Psychiatric problems were initially thought to be associated with the stress of raising children with fragile X syndrome. Results of more extensive controlled studies have established that depression occur in about 40% of premutation carriers [23]. Now we know that carriers are intrinsically more vulnerable to depression and anxiety disorder because these problems can occur before having children affected by fragile X syndrome. This intrinsic vulnerability is also sensitive to the stress of raising a child with FXS. Risk for emotional problems appears to be dependent on stressful life events. Emotional functioning in the females premutation carriers should be interpreted in light of the additional impact of FMR1 neighboring genes, hormonal levels, marital satisfaction, and differences in severity of child behavioral problems associated with FXS [26]. Recent studies suggest that the premutation may affect the hippocampus and may be related to psychiatric symptomatology. MRNA toxicity in vulnerable hippocampal regions may contribute to memory problems and psychiatric symptoms in permutation carriers [26].

Au et al. [27] observed migraines in 54.2% of females carriers of premutation when compared to control group (25.3%). Headaches were more common among mothers of children with FXS (26.9%) than matched controls (13.6%) [28]. Headaches were not significantly increased in carriers from the general population, so this may imply a relationship between migraines and stress in family with FXS [20].

Shelton et al. [29] observed executive dysfunction amongst premutation carriers, specifically reduced attention, processing speed, response inhibition and working memory processes.

Premutation in FMR1 gene leads to aberrant regulation of the hypothalamic–pituitary–adrenal axis, causing increase in stress hormone, cortisol, levels as it was shown

in the mouse model of premutation. Aberrant secretion of cortisol and stress may lead to inflammation processes and activation of immunologic system [30].

Immune-mediated disorders are more common in premutation carriers as compared to control group, especially hypothyroidism and fibromyalgia.

Lozano et al. [31] showed that premutation carriers are at 2.7-fold–3.3-fold greater risk of thyroid problems than the controls. Untreated hypothyroidism may lead to cognitive impairments and psychological symptoms – anxiety and depression. Thyroid function should be tested as a matter of routine in females carriers of FMR1 premutation [32, 33].

Winarni et al. [34] analyzed health of 344 females carriers of premutation in FMR1 gene. Among them 44.77 % had at least one immune-mediated disease. The most common was autoimmune thyroid disease (24.4%), then fibromyalgia (10.2%), irritable bowel syndrome (9.9%), Raynaud's phenomenon (7.6%), rheumatoid arthritis (3.8%), Sjörgen syndrome (2.6%), systemic lupus erythematosus (2.03%), multiple sclerosis (1.74%).

Although FMR1-associated conditions are not curable, timely diagnosis through genetic testing is important as it can lead to implementation of treatment strategies and behavioral interventions considered to reduce symptoms. Knowledge of expanded allele status allows to make informed decisions about procreation and family planning [35].

Molecular analysis

Molecular analysis involves two complementary methods: PCR with primers flanking CGG repeats to establish number of the CGG repeats in 5' region of FMR1 gene and Southern blot of the genomic DNA to establish methylation status and size of the full mutation, which is usually a difficult PCR template. The use of two methods – Southern blot and PCR – allows to detect 99% of mutations in FMR1 gene. The remaining 1% includes missense mutations and complete or partial deletions of FMR1 gene [7].

Genetic counseling

Fragile X syndrome diagnosis may be very difficult because FXS has no distinctive physical characteristics(phenotype) to prompt diagnosis and psychomotor delay is relatively common in general population. Children with FXS typically present with developmental delay, behavioral disorders and learning difficulties. The disease is usually diagnosed at the age of 3, 18 months after the family first identifies concerns. Late diagnosis delays parents' understanding of their reproductive risk, which can be very important for reproductive choices. In an American study of families with FXS 55% of parents already had another child before the first child was diagnosed with FXS [36, 37].

The diagnosis of FXS can have far-reaching implications for extended family members. Genetic counseling should involve the family as soon as possible. The family members at risk of inheriting abnormal gene should be informed about possibility of diagnostic genetic tests. The spectrum of symptoms observed in FXS should be explained to them. They should be informed about increased risk of being affected or being a carrier in extended family members. It is important to inform them about possibility of testing siblings of proband, especially if they present learning difficulties or behavioral disorders, because these symptoms may be mild symptoms of FXS [7].

Transition from premutation to full mutation happens only if mother transmits abnormal allele. The probability of having child with full mutation depends on maternal premutation size. Female with premutation of 60 CGG repeats is at relatively low risk of having child with FXS, while in female having 90 or more CGG repeats the risk of having a child with full mutation is 50% [38]. A male carrier of premutation will transmit premutation to all his daughters, who will also be carriers of premutation and who will be burdened with a high risk of disease in their children. Males with FXS, full mutation in FMR1 gene in somatic cells will have premutation in sperm and will have daughters with premutation. Males with FXS rarely are able to have children. When a proband is a carrier of full mutation, his/her mother is a premutation carrier or a carrier of FMR1 gene fullmutation. The risk of having next child with FXS is 50% and is the same for each subsequent pregnancy. There is a possibility of prenatal testing of the fetus to detect which allele has fetus inherited [38].

Conclusions

There is a strong evidence to suggest that females with FMR1 premutation are at increased risk for multiple reproductive, immune, cognitive and psychiatric problems. Hypertension and hypothyroidism should be evaluated medically and, if present, treated, since the lack of treatment may aggravate CNS function. In the case of neurological problems including migraines, neuropathy, sleep apnea and psychoses, treatment should be initiated. Exercise, stress reduction techniques, avoidance of toxins, and healthy food should be recommended for premutation carriers [16, 20].

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