

FRAGILE-X SYNDROME: GENETIC ASPECTS AND STOMATOLOGIC EVALUATIONS

F. MUZZI¹, F. SANTINI^{1,2}, G. ROMANINI¹, F.N. BARTULI^{1,2}, C. ARCURI^{1,2}

¹ "S. Giovanni Calibita" AFaR - Fatebenefratelli Hospital - Isola Tiberina, Rome, Italy

² University of Rome "Tor Vergata", Rome, Italy

SUMMARY

Fragile-X syndrome: genetic aspects and stomatologic evaluations.

Aim of the work. The fragile-X syndrome is the most common cause of inherited mental retardation and it is associated with the FMR1 gene on X chromosome. The origin of anatomic anomalies of maxillo-facial complex is still discussed in literature. The authors describe the syndrome and report a clinical case.

Methods. Genetical and clinical aspects and the incidence of caries, periodontal disease and occlusal abrasion are reviewed. Occlusal conditions, particularly openbite and crossbite, are considered.

Results. The incidence of fragile-X syndrome is 1: 2000 in males and 1:4000 in females, despite this the syndrome is diagnosed with a lot of difficulties yet, because of extreme variability of the phenomenological aspects. Patients often show severe mental retardation, linked to a peculiar profile of cognitive, behavioural, and emotional dysfunction and to distinctive anatomic features, which become more evident after puberty. Concerning oral characteristics, it doesn't seem to be a significant association between the syndrome and the incidence of caries or periodontal diseases, while an ogival shaped palate is peculiar.

Conclusions. Literature review suggests that when male patients with severe mental retardation without well-known cause are visited, the hypothesis of X-fragile syndrome should be considered. Even though the diagnostic hypothesis may arise from the observation of typical somatic features, the diagnosis can be confirmed only by genetical tests.

Key words: fragile-X syndrome, Martin-Bell syndrome, FMRP protein.

RIASSUNTO

La sindrome dell'X-fragile: aspetti genetici e considerazioni stomatologiche.

Obiettivi. La sindrome dell'X-fragile è ritenuta la forma più comune di ritardo mentale ereditario ed è associata al gene FMR1 sul cromosoma X. La natura delle anomalie del distretto orofacciale è ancora oggetto di studio. Gli autori descrivono la sindrome e riportano un caso clinico esplicativo.

Metodi: Viene effettuata una revisione degli aspetti genetici e clinici e dell'incidenza di patologie cariose, parodontali e del grado di abrasione dentale; vengono, inoltre, revisionati i dati relativi alle caratteristiche occlusali, in particolar modo *openbite* e *crossbite*.

Risultati. La Sindrome ha un'incidenza di 1:2000 nei maschi e di 1:4000 nelle femmine, nonostante ciò è ancora frequentemente sottodiagnosticata, a causa dell'estrema variabilità del quadro fenomenologico. I soggetti affetti presentano ritardo mentale severo, correlato ad alterazioni psico-comportamentali e somatiche caratteristiche delle sindromi più evidenti dopo la pubertà. A livello orale non sembra esserci un'associazione significativa con problematiche cariose o parodontali, mentre è peculiare il palato ogivale.

Conclusioni. Dalla revisione della letteratura si evince che ogni qualvolta si presentino all'osservazione pazienti maschi con ritardo mentale ad eziologia ignota, sarebbe opportuno ipotizzare una diagnosi di Sindrome dell'X-fragile. Quest'ultima può nascere dall'osservazione di alcune caratteristiche somatiche tipiche, anche se la diagnosi può essere confermata solo da test genetici.

Parole chiave: sindrome X-fragile, sindrome di Martin-Bell, proteina FMRP.

The Martin-Bell's syndrome, known also as fragile-X syndrome, is the most common cause of inherited mental retardation and it is transmitted as dominant linked of the X chromosome (1); it determines in males a particular pathologic phenotype associated with mental retardation. The gene responsible of the fragile-X syndrome is the FMR1 gene of the X-chromosome, and the syndrome is named due to the presence of a fragile site on the distal part of the long arm of X-chromosome (Xq27.3) also known as FRAXA (Fragile site A on X chromosome) (2).

Recent studies report an incidence of the fragile-X syndrome of 1:2000 in males and 1:4000 in female (3). The syndrome is associated to cognitive and behavioural deficits and distinctive features, more evident after puberty, can be recorded (4). Not all affected subjects, though, present the typical phenotype, behavioural and linguistic characteristics, therefore the fragile-X syndrome is usually diagnosed not in an early age and through genetic evaluation. The aim of the present work is to give dentists a more detailed description of behavioural aspects and facial features of the syndrome, in order to guarantee to patients and their parents an early diagnosis, an adequate medical and psycho-social assistance together with therapeutical and preventional programs that are priority in these subjects.

GENETICAL ASPECTS

The gene FMR1, whose mutation is responsible for fragile-X syndrome was identified in 1991. The FMR1 gene is located on chromosome X in the region Xq27.3. In correspondence of the untranslated 5'UTR of the gene, there is a region (corresponding to the first exon) containing a CGG repeat (triplet) as part of an island CPG, extending upstream from the site of initiation of transcription. Normally, this sequence has an average of 30 triplets, with a variability in the population between 5 and 50. In individuals affected by fragile-X syndrome, the number exceeds 200 triplets and can reach up to 2000 and beyond, showing a considerable instability. The mutation of FMR1, accounting for more than 95% of cases of fragile-X syndrome, consists of amplification of triplets located in the untranslated portion of the first exon of the gene (5). The triplets' amplification is followed by a cytosine methylation and

chromatin compaction, with a block of transcription which follows the inactivation of gene (5). Therefore, the FMR1 gene, while keeping intact its coding sequence, is "switched-off" and is no longer able to direct the synthesis of its proteic product (6). The fragile-X syndrome arises from a lack of the protein encoded by the FMR1 gene, called FMRP (7). The few cases of illness associated with deletions or single point mutations confirmed that the lack of FMRP can generate the X-fragile syndrome (8). The full mutation described above is preceded by a pre-mutation, where the number of CGG triplets varies from about 50 to 200, but, in this case, there isn't any methylation reactions, so that, even if the number of repetitions is inherited with instability, premutated genes keep their normal function (1). Subjects holding premutations, both male and female, do not therefore present mental retardation, but have an unstable gene, predisposed to further amplification. This second step occurs in female meiosis, the stage in which the premutated gene is transmitted from a carrier mother to her descendants (1). The FMRP protein is particularly concentrated in cells of the central nervous system, where it performs regulatory functions on the activity of other genes, presumably responsible for the synthesis of neurotransmitters (1). During child growth, the mRNA level gradually decreases and shows a more heterogeneous distribution, in particular in the ectoderm tissues, such as brain, nerve, hair follicles, sensory cells and adrenal (9). This distribution is in accordance with the one observed on embryonal tissues of a few weeks human fetus. In adults the expression of mRNA for FMR1 remains high in the brain and testicles, which are the tissues that appeared to be altered in patients affected by the syndrome; high levels of expression are also found in ovary, thymus, spleen and oesophagus. Lower levels are found in kidneys, liver, colon, uterus, thyroid, lungs, while the transcript is completely absent in heart and aorta (9). The tissutal distribution of the FMRP protein is quite similar to that of mRNA in FMR1; the protein is particularly abundant in brain, especially in the hippocampus, an important region of the brain for learning and memory, and in the cerebellum, where part of the information responsible for the coordination of movements is processed. FMRP is also present in testicles, especially in sper-

matogonia (10), in ovary, in oesophageal epithelium, in thymus, in eye and spleen; the expression is moderate in colon, uterus, thyroid and liver, and is completely absent in muscles (11). This pattern of expression suggested that FMRP, although expressed in many tissues, could play a key role in neuroepithelial tissues and especially in brain and gonads. At sub-cellular level, FMRP is found more in the cytoplasm, but it was also found into the nucleus (12) and into the nucleolus (13). In neurons it is present mainly around the nucleus, in the dendrites and in the proximal part of axons. It is unclear the sub-cellular localization of the transcript for FMR1: from experiments of RT-PCR performed on preparations of synaptosomes, it is clear that the mRNA of FMR1 is present in synaptic terminals (14).

Regarding the function of the protein, the most accredited hypothesis is that within the neurons it is not only responsible for the transport of specific mRNA from nucleus to cytoplasm, but also to their synaptic localization and subsequent regulation of local translation. In particular, lately, it has been shown the role of FMRP as translational repressor of some messengers at synapsis.

It was observed that FMRP is predominantly a cytoplasmic protein (10) that co-localizes with ribosomes (11), modulating the translation of mRNA. It has been demonstrated that FMR1 is a repressor for *in vivo* translation and that, although FMRP is predominantly localized in the cytoplasm, it can also operate as shuttle between the nucleus and the cytoplasm, playing a role in exporting specific mRNAs from the nucleus to cytoplasm.

Finally, considering its association with polyribosomes and its localization in the synapse, FMRP might be a key-molecule in local protein synthesis: thus it was thought that the translation of its own mRNA is also regulated at the synapse.

Normal genes, premutations and complete mutations are easily distinguishable through DNA molecular analysis, which have now become routine and are the necessary precondition for genetic advice (1). Most diagnostic centers offer screening based on the discovery of increased CGG triplet in affected patients. This is based both on the Southern Blot technique of leukocyte DNA digested with specific endonucleases, and on the direct amplification of the CGG

triplet, using primers. The direct amplification (using the PCR method) is more rapid, but is unable to detect complete mutations because the extensions of more than 100-200 triplets are difficult to amplify. Southern Blot technique is slow and costly, but it can discover all the amplification of CGG triplets and the mutilation of the FMR1 promoter, if appropriate endonucleases are used.

Recently immunocytochemistry alternative tests, based on direct detection of FMRP using a monoclonal antibody and indirect visualization of antigen-antibody complex with the activation of alkaline phosphatase, have been described. Another test, however, detects the presence of FMRP in blood lymphocytes, using a conventional optical microscope. Finally, another test, detects the presence of FMRP in hair roots. None of these tests, however, was commercially introduced on a large scale.

Each patient with a X-fragile syndrome diagnosis identifies a family in which there may be other subjects at risk of transmitting the same condition to their children and grandchildren. Therefore it is necessary to offer the family the tools to recognize of carriers and to give them a genetic advice that includes the calculation of the risk of recurrence of the syndrome and eventually the possibility of prenatal diagnosis. The commitment to prevent, however, should not get to neglect patients' treatment, which must necessarily be based on knowledge of their needs. The mental retardation is usually moderate in degree and is associated with a rather complex personality which should be dealt with the rehabilitation treatment, waiting for genetic progress that may make effective treatments available. In this sense, a possible way is the reactivation of the FMR1 gene, assuming that the removal of the transcriptional block caused by DNA hyper-methylation and histones' deacetylation leads to a recovery of the function of gene (1). This way presents different difficulties because of the high toxicity of the methylating and acetylating substances necessary to the process of gene reactivation.

Perhaps the peptides of nucleic acids (PNAs), small molecules that invade specific sequences in the DNA, may offer better hope for the future. It is assumed that the link between the PNA molecules and the methylated DNA could result in the de-methylation and

the de-acetylation that lead to the reactivation of the FMR1 gene. However even after the development of appropriate PNAs, many problems will remain unsolved, not least, the individual system to address the PNA to the brain.

The current method of treatment for the fragile-X syndrome is palliative and includes specific therapies based on the cognitive and behavioural characteristics of each patient, in order to help people suffering from fragile-X to achieve their maximum potential, even in relation to symptom-specific therapies of correlated diseases (15). The current medical therapy is, therefore, a motorial and psychological rehabilitation started in early age. A good psychological assistance from specialist teachers can significantly improve the potential of the child and help him to live with others harmoniously.

GENERAL CLINICAL CHARACTERISTICS

The fragile-X syndrome is the most common form of inherited mental retardation, with an estimated prevalence of 1:4000 in males and 1:8000 in females (3). Boys are affected in more severely compared to girls, although they often present less relevant somatic features than individuals with other chromosomal diseases. For this reason, the syndrome often goes undiagnosed (11). Usually male subjects have severe mental retardation, delayed development of language, tendency to repeat words at random (ecolalia) and behavioural difficulties in interpersonal relationships, problems with attention, hyperactivity, irritability, frequent accesses of anger, shyness, anxiety, poor ability to cope with new and different situations and, sometimes, real close to the environment (16). Commonly these patients show an increased sensitivity to visual, sound and tactile (such as reflection of retreating when touched) stimulation, an exaggerated repetition of gestures, a tendency to sniff out and put objects in their mouths, poor provision to visual contact with other people, incontinence and a tendency to clap and bite hands. The affected females are generally less compromised in neuropsychiatric aspects compared to males and show a wider range of functional capabilities preserved.

Many fragile-X females with preserved mental capacities anyway show problems in learning, in planning and organizing things and speeches, loss of concentration, logical derailments, difficulties in arithmetic and in social skills. The difference in phenotype between males and females with the fragile-X syndrome is due to the fact that males have just one X chromosome, while females have two X chromosomes: therefore in females the FMRP protein production, necessary to a normal maturation of neurons and synapses in the brain, and whose synthesis is controlled by the gene affected by the fragile-X, is maintained at higher levels compared to males, due to the presence of the not affected X chromosome, resulting in a minor damage to the neurological development (3). In addition to psycho-behavioural problems, peculiar somatic defects are associated with the syndrome. These defects are more easily detectable after puberty (3). The most important somatic feature is the increase in testicular volume or macrorchidism (testicular volume > 30ml), which can be observed after the complete sexual development. Therefore, the macrorchidism associated with mental retardation must be suspicion of fragile-X syndrome. Other alterations in somatic characteristics, not always observable, are large and prominent ears, elongated and narrow facies, facial asymmetries, reduced interocular distance, prominent forehead, large cranial circumference, prominent thumbs, iperextensible joints and reduced height (11) (Figs. 1, 2). Muscular hypotonia and dysplasia of connective tissue, which can sometimes cause a prolapse of the mitral valve, are often present (1). Sometimes the intellectual deficits and somatic characteristics are associated with not particularly severe epileptic crisis (1). The main cause of such disorders and their relationship with partial or total lack of FMRP are still unknown.

ODONTOSTOMATOLOGICAL CLINICAL ASPECTS

Despite many attempts to classify the facial features in fragile-X subjects, conflicting opinions remain on the nature of the anomalies and their degree of severity. In 1983 Hagerman et al. identified in 15 out



Figura 1, 2

Extra-oral view of M (male, 16 years): The morphological analysis of the face underlines modestly prominent ears and a slight increase of longitudinal dimension and cranial circumference. The diagnosis of fragile-X syndrome arisen from the observation of hyperactivity from early years of age, seldom associated with anger crisis and correlated to behavioural difficulties and it has been confirmed through molecular genetic exams. Even though a poor visual contact and a moderate tactile tolerance could be observed, M. shows to be quite collaborative and lively. A sedative therapy for control of anxiety had been prescribed, in association with psychological and logopedic support in order to correct language dysfunction, which was characterized by the tendency to repeat words at random (ecolalia) and difficulty to directly answer to questions.

of 23 examined fragile-X patients a high and curved palate (17); one year after Meryash et al. described prominent palate crests in 12 out of 18 subjects suffering from the syndrome (18). In 1986 Shellart et al. performed a study comparing a group of 16 adolescent and adult fragile-X patients with a sample of healthy peers (19). The presence of caries, the incidence of soft and hard tissue diseases and the type of occlusion, including palatal size and form and level of occlusal abrasion, were examined. The presence of caries was evaluated using the system of damaged, lost or filled surfaces (DMFS system); the authors observe a rate inferior to the one expected and suggest that caries does not seem to be a problem more frequent in the fragile-X examined sample. Also oral soft and hard tissues didn't seem to be altered: although

some abnormalities were detected, they were not considered to be related to or characteristic of the syndrome (19). Perhaps it should be clear that just two areas on each patient were radiographically examined, thus, in our opinion, the statements on the influence of the syndrome on hard tissues should not be considered conclusive.

The presence of malocclusion was evaluated using Angle classification and identifying the presence of crossbite and openbite, either front or rear. No significant differences were noted between fragile-X subjects and healthy subjects according to Angle classification, but, when crossbite and openbite were used as evaluation criteria, a significant difference between the two groups arisen; fragile-X subjects in fact presented a more higher incidence of transverse



Figura 3, 4

Intra-oral view of M: the palate appears to be ogivally shaped and it is associated with Class II malocclusion and deep-bite, while there isn't any anterior or posterior cross-bite; gnathologic examination shows hyperlaxity of ligaments during mouth opening, together with pain crisis in auricular area, which could be related to temporomandibular disorders. None periodontal or teeth alterations could be observed.

and vertical alteration of the occlusion (19). It must be also considered that the openbite may be the result of habits, such as thumb sucking, rather than a craniofacial bone alteration. An high and arched shaped palate is, perhaps, considered a peculiarity of fragile-X syndrome (Figs. 3, 4).

The evaluation of occlusal abrasion showed different results from expectations, in fact there were significant differences between healthy and fragile-X individuals.

Other features observed but not quantified included a limitation in mouth opening and an excessive gagging (19).

Finally, it must be remembered that drug therapy used in fragile-X patients often has systemic impacts and facial effects. Many of the drugs used to treat the syndrome, in fact, have stomatologic side effects and interact with drugs used in dentistry.

About 50% of adults and a less percentage in childhood have a mitral valve prolapse, clinically associated with a heart murmur, which is an indication to the prescription of antibiotic prophylaxis before performing invasive dental procedures to avoid infective endocarditis.

The recurrent media otitis is common in children, such as the recurrent sinusitis in older patient. Sometimes it is difficult for these mentally compromised

subjects to distinguish the pain of dental origin from the one caused by otorhinolaryngoiatric diseases. Another frequent disturbance in the fragile-X patients is gastro-oesophageal reflux, with frequent vomiting, which exposes the teeth to gastric acids.

In about 20% of affected children, there are partial or generalized epileptic crisis, usually treated with carbamazepine, which is often suspended in late adolescence when access spontaneously regress (3).

It should be added that these patients may be at risk when undergoing general anesthesia because of their heart defects and their articular hiperestensibility that can cause problems in positioning patients during surgery (20).

Conclusions

The fragile-X syndrome is the most common inherited form of mental retardation. It is also the cause of other problems in development, such as specific dysfunction in learning and important behavioural difficulties.

Despite its relatively high diffusion (1:2000 in males and 1:4000 in females) (21), the syndrome is still relatively known and certainly underdiagnosed, with the consequence that many families at risk of

passing this condition do not receive an adequate genetic counselling.

Whenever a mental retardation with unknown aetiology is observed in a male subject, it should be considered that he may have a high probability of suffering from this syndrome, with all the related implications on general and oral health.

There have been many attempts to establish a protocol based on stomatognathic and cranio-facial features of fragile-X syndrome, but it was not possible to clearly describe a specific clinical picture. Probably the cause of these difficulties is related to the fact that research on neuropsychiatric disorders of genetic origin is in full development, and it is influenced by constant discoveries, both regarding basic research and specialist genetic and neurological research. Considering the risks of dental treatment in fragile-X patient, prevention of dental problems is a priority. The aim of the review was to give clinical and epidemiological data of dento-facial characteristics in order to provide a valid scientific support to dentists and guarantee an adequate medical and psycho-social care for patients suffering from fragile-X syndrome and their parents, achievable within a multidisciplinary prevention program.

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Correspondence to:

Dott.ssa Fabiana Muzzi
Via Federico Nansen, 100
00154 Rome, Italy
E-mail: rena_mu@yahoo.com
Tel: +39 347 4867313
Fax: +39 06 86214633