Genetics of Childhood Disorders: XI. Fragile X Syndrome

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Fragile X syndrome is a common form of mental retardation with an estimated incidence of 1 per 2,000 to 4,000 in the general population. The physical manifestations associated with the syndrome include macroorchidism, large ears, a prominent jaw, moderate to severe mental retardation, and autistic-like behavior. However, physical features are not always reliable indicators of the presence of the condition, particularly in prepubertal children and females. For years, investigators had noted that the phenotype of fragile X syndrome cosegregated with an unusual disruption of the X chromosome. Karyotyping of cells grown in folate-depleted cell culture media revealed that many patients had a "fragile" site on one of their X chromosomes that appeared as a constriction on the distal long arm.

This genetic syndrome is of particular interest to clinicians and scientists who want to understand brain development and function in children. Individuals with fragile X syndrome manifest neurodevelopmental abnormalities that include varying levels of cognitive dysfunction, particularly in the domains of executive, visual-spatial, and visual motor abilities, and frequently display behavioral symptoms of autism, attention-deficit/hyperactivity disorder, and social anxiety. Morphological variations of brain structure have been observed in this population and include abnormalities in the cerebellar vermis, caudate, hippocampus, and lateral ventricles.

In 1991, Verkerk and colleagues identified a single gene that was associated with symptoms of the disorder. The gene, known as fragile X mental retardation gene 1 (*FMR-1*), exhibited a novel form of mutation that had not been previously described. It was determined that a sequence of 3 nucleotides (CGG) was repeated many times in patients. This region of the *FMR-1* gene

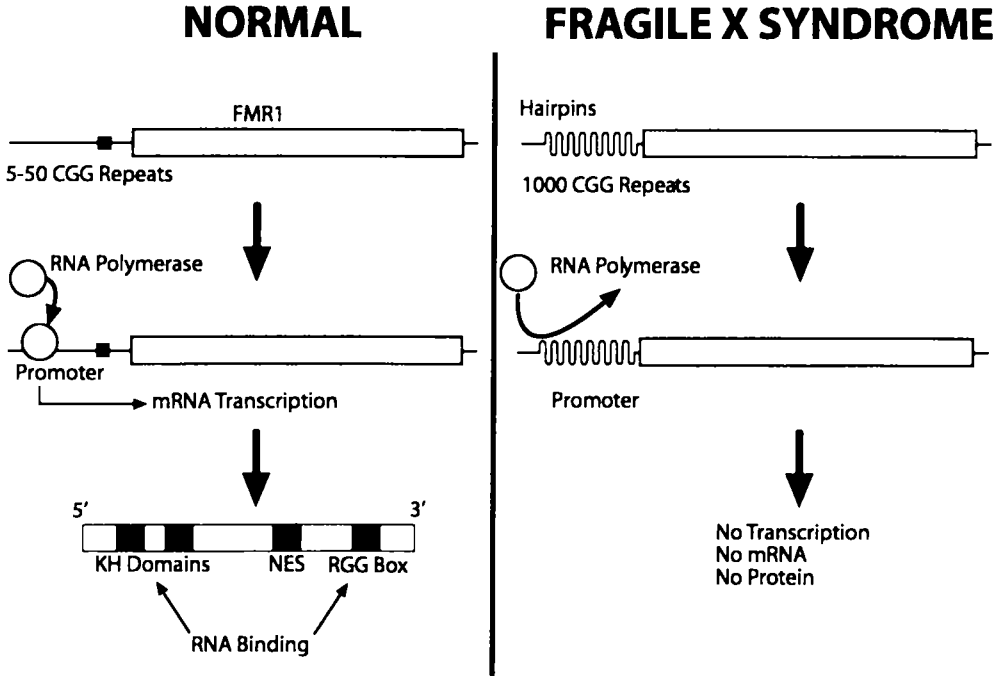

Fig. 1 The illustration demonstrates a leading hypothesis to explain the mechanism of the fragile X full mutation. Both a normal and mutated *FMR-1* gene are shown. The mutated version on the right illustrates the consequences of an expanded CGG repeat on DNA structure and transcription. The protein structure is shown on the bottom, and the functionally important amino acid domains that are known are indicated. The presence of a nuclear export signal (the NES domain) together with the presence of 2 RNA binding domains (the KH domain and RGG box) suggest that a normal function of the FMR1 protein is to bind to mature messenger RNA molecules and transport them out of the nucleus where they are synthesized and into the cytosol where they may be translated into proteins.

is highly variable in the general population, meaning that its length is found to vary considerably from one person to the next. In normal individuals, the repeated sequence ranges in size from 5 to approximately 50 repeats. In premutated but unaffected patients, however, this sequence was found to enlarge to up to 200 repeats, and in affected patients repetitions of more than a thousand nucleotides can be seen. The stability of the region depends directly on the length of the CGG region and probably also on the presence of single interspersed AGG islets anchoring the CGG region. The enlargement of this triplet repeat across generations is responsible for the increasing severity of the disorder that is often seen over several generations.

Although the CGG region is transcribed into RNA, it precedes the nucleotide sequence that will be translated into protein (Fig. 1). Thus, the expansion is not exerting its effects by introducing novel and destabilizing amino acids into the protein structure. This is in contrast to several disorders caused by triplet repeat expansions such as Huntington disease in which the expansion occurs within the coding sequence and leads to the abnormal inclusion of a large series of glutamine residues within the protein itself (see last month's column). This large tract of glutamines is thought to disrupt the normal functioning of the huntingtin protein.

How, then, does the expanded triplet repeat produce its effects within the *FMR-1* gene? When more than 200 CGG repeats are present, there is a high likelihood that the promoter region of the *FMR-1* gene will be hypermethylated. Methylation is a chemical modification that occurs in certain DNA regions and, in particular, to DNA enriched in CGG triplets. When hypermethylation occurs, the enzyme necessary for transcription is unable to bind to the promoter region and initiate transcription. The end result is that no messenger RNA is produced, a condition referred to as "transcriptional silencing" of a gene.

What is the consequence of large stretches of CGG repeats? In vitro structural studies have shown that nucleotide regions enriched in C and G nucleotides tend to bend into a hairpin shape. This abnormal conformation becomes increasingly stable as the length of the uninterrupted CGG region grows. The increase in stability with length is also thought to explain why an expansion of the triplet repeat is favored. In addition, this explains why AGG islands could have a stabilizing effect on the CGG region by interrupting the CGG sequence and shortening uninterrupted sequences and preventing them from forming the hairpins. The hairpin structure is also known to be a signal that leads to an increase in methylation that eventually blocks *FMR-1* transcription.

Exactly how and when the premutated allele expands into its full mutational form is still a matter of debate. Interestingly, it occurs only when passed through the female germ line. There is now evidence suggesting that the expansion event occurs in a postzygotic stage early in embryogenesis. This would explain the common mosaic status of many individuals with fragile X syndrome. Mosaic status refers to the presence of different repeat lengths in cells originating from various parts of the body. This makes it possible for a male with the fragile X full mutation to have only premutated alleles in his sperm or for monozygotic twins to have CGG expansions of different sizes.

The *FMR-1* gene is expressed at its highest levels in the brain and testes, but it has been found in many other adult and fetal tissues. Its protein product (FMRP) is mainly localized in the cytoplasm of cells, particularly in Purkinje cells of the cerebellum and neurons of the hippocampus and basal forebrain.

Current research efforts are devoted to understanding the normal function of the protein. For example, the amino acid sequence that is found in FMRP was compared with the amino acid sequence of other known proteins. Several domains are present that are highly homologous to domains on other proteins of known function (Fig.). The similarities between various domains of proteins suggested a similarity in their function as well. Three amino acid domains (2 KH domains and an RGG box) have been located on FMRP. Both the KH domains and RGG box are known to be important functional domains in proteins that bind to RNA. FMRP was then shown to be able to bind to RNA transcripts, as well as with the ribosomal subunit involved in the translation of messages into proteins. Another domain, termed the nuclear export signal, is found on FMRP and is a signal that leads to the export from the nucleus into the cytoplasm of proteins that contain it. Thus, FMRP may play a role in the transport of messages between the nucleus and cytoplasm. In this manner, FMRP would participate in the translational machinery that converts messenger RNA into protein.

Recently, 2 additional genes have been identified that are very similar to *FMR-1*. The protein products of these genes, named FXR1P and FXR2P, are highly homologous with FMRP at the amino acid level. Both of these proteins have the KH domains and RGG box present in FMRP. FMRP has the ability to interact with FXR1P and FXR2P, and these protein-protein interactions may be important for the function of this new family of RNA-binding proteins. However, different distributions of these proteins have been found in various tissues, and they could signify different functions for each of these proteins. Furthermore, the fact that the absence of FMRP in fragile X males leads to mental retardation even in the presence of normal FXR1P and FXR2P suggests at least partial independence of function.

It is clear that the absence of FMRP is responsible for abnormal brain development within the cerebellar vermis, caudate, and hippocampus. What remains to be determined is exactly how the absence of this gene leads to the observed variations in brain morphology. The recent creation of an experimental knockout mouse for *FMR-1* gene may shed some light on the underlying mechanisms and help explain how mutations of these proteins result in abnormal brain development.

WEB SITES OF INTEREST

http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?309550
http://www.fraxa.org/
http://www-cap.stanford.edu/research

ADDITIONAL READINGS

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Prenatal Exposure to Cigarette Smoking Is Associated With a Decrease in Arousal in Infants. Patricia Franco, MD, José Groswasser, MD, Sergio Hassid, MD, Jean Pierre Lanquart, MD, Sonia Scaillet, MD, André Kahn, MD, PhD

Objective: Sudden infant death syndrome has been related to both exposure to prenatal cigarette smoke and impaired arousability from sleep. We evaluated whether healthy infants born to mothers who smoked during pregnancy had higher auditory arousal thresholds than those born to mothers who did not smoke and whether the effects of smoking occurred before birth. *Study Design:* Twenty-six newborns were studied with polygraphic recordings for 1 night: 13 were born to mothers who did not smoke, and 13 were born to mothers who smoked (>9 cigarettes per day). Other infants with a median postnatal age of 12 weeks were also studied, 21 born to nonsmoking mothers and 21 born to smoking mothers. White noise of increasing intensity was administered during rapid eye movement sleep to evaluate arousal and awakening thresholds. *Results:* More intense auditory stimuli were needed to induce arousals in newborns ($P = .002$) and infants ($P = .044$) of smokers than in infants of nonsmokers. Behavioral awakening occurred significantly less frequently in the newborns of smokers ($P = .002$) than of nonsmokers. *Conclusions:* Newborns and infants born to smoking mothers had higher arousal thresholds to auditory challenges than those born to nonsmoking mothers. The impact of exposure to cigarette smoke occurred before birth. J Pediatr 1999;135:34–38. Reproduced with permission from Mosby-Year Book, Inc.