Imagine a ten-year-old boy with ninety pounds of raw, compressed energy. He has a particular attachment to his Playmobil trucks, rarely sharing his prized possessions with even his older brother. His long face, pronounced forehead, and goofy ears make him unique but it is his personality that is more striking. At the age of ten, he should be zooming around on his bike with his neighborhood pals or reading childhood classics like *James and the Giant Peach* with his fifth grade classmates. However, he lacks the balance to ride a two-wheeler and he can’t read, or speak, very well. He avoids eye contact with his parents and teachers and most of the time, prefers to be alone.

Our imaginary ten-year-old boy typifies the cognitive, behavioral, and motor characteristics of a child with Fragile X Syndrome (FXS). FXS is a genetic disorder resulting from a defective gene on the X chromosome, one of the pair of chromosomes that determines sex. Individuals with the disorder tend to have elongated faces, large protruding ears, and seizure disorder; delay in speech and language development, hand-flapping, impulsivity, and poor eye contact; and difficulty in managing transitions, emotional upsets, and relationships. FXS sometimes presents as an autism spectrum disorder, causing autism in 30% of FXS-affected children, which represents about 5% of all autism cases.

**FRAGILE X SYNDROME: AN OVERVIEW**

FXS arises from a mutation in the fragile X mental retardation 1 gene (FMR1) in the DNA of the X chromosome. This mutation triggers various regulatory mechanisms that eventually result in the silencing or shutting down of the FMR1 gene. Since the gene is turned off, the body can no longer produce fragile x mental retardation protein (FMRP), which is the protein programmed by the FMR1 gene. FMRP has an important function in tissue of regulating the production of other proteins, especially those associated with the proper development of brain cells or neurons, by binding to mRNA in cells. A failure to produce normal quantities of this protein results in FXS.

Today, one in 4000 boys and one in 6,000 girls have FXS, making this genetic defect the most common inherited cause of mental retardation. Although FXS is often considered a pediatric developmental disorder, the effects of FXS may persist into adulthood. Adults with FXS may concurrently suffer from fragile X-associated tremor ataxia syndrome, fibromyalgia, hypothyroidism, neuropathy, and psychiatric problems including anxiety and depression. Additionally, epilepsy is highly correlated with FXS. Similar to other spectrum disorders, the severity of disability in individuals with FXS greatly vary. Often times, females have milder presentation of the FXS, likely because of a second normal
X chromosome. In contrast, males not only more frequently present the clinical symptoms but also do so to a greater degree.

**The Pioneers**

J. Purdon Martin and Julia Bell were the first to discover and describe FXS in 1943. The pair proposed sex-linked inheritance as the explanation for the severe mental retardation present in eleven males in a single family over two generations. The high incidence of mental retardation among the boys, but little to none in girls, suggested that the inheritance pattern of this mental retardation is X-linked, or associated with the X chromosome. X-linked traits occur more frequently in males, who only have one X chromosome, than in females, who have two.

Twenty six years later, the syndrome got its name, “Fragile X,” when Herbert Lubs examined the X chromosome in four mentally retarded boys and two of their mentally normal female relatives. Lubs found a constriction near the end of the long arm of the X chromosome, which made the chromosome appear to be broken or “fragile.”

In the late 1970s, interest in FXS picked up as more and more families came forward with similar mental retardation and chromosome abnormalities. With the development of a chemical diagnostic test for chromosomal abnormality, scientists were able to study the pattern of FXS within families in order to better understand its transmission. What they found however was hard to explain.

**The Paradox**

FXS inheritance patterns deviated from the norm of X-linked traits. In the mid-eighties, Stephanie Sherman and colleagues studied the inheritance pattern of individuals with FXS and observed that the effects of FXS worsened with the next generation. To illustrate, the daughter of an unaffected carrier male was more likely to have affected children than her grandmother (the mother of the unaffected male carrier) was. This pattern was coined as the “Sherman Paradox.” To explain this pattern, Sherman and colleagues proposed that a two-step process is required for the eventual expression of the full disorder. Even though a generation will not express the disorder, those individuals will carry the FXS mutation (premutation) and transmit a more severe version (full mutation) of the defect to their offspring, who will then exhibit the FXS traits. Sherman was right on.

A few years later, Dr. Stephen T. Warren of Emory University led an international team of scientists in the discovery of the FMR1 gene in 1991. Researchers identified the gene associated with the “fragile” site on the X chromosome and a fragment that contains a long repeat of a trinucleotide sequence of DNA bases, CGG. They found that FXS phenotype was associated with an abnormal expansion of a piece of the FMR1 gene. In the general population, a normal individual has anywhere between five and fifty copies of the CGG repeat in the non-coding region of the gene. When repeats number between fifty and two hundred copies, the expansion is considered to be a premutation and is unstable. The premutation form can expand to full mutation (more than two hundred copies of the CGG repeat) by transmission of the premutation from mother to child. In general, the larger the permutation, the
greater the risk that the child will carry the full mutation. Sherman paradox resolved.

**FXS and the Brain**

Sherman and Warren laid down significant groundwork for future studies on FXS. Since the discovery of FMR1 gene and the advent of new technology, much has been learned about the syndrome, including its etiology and behavioral and cognitive manifestations, through studies at the cellular level, in animals, and in humans.

Mental retardation, delay in speech and language processing, and impaired motor control suggest developmental deficits in the brain of individuals with FXS. Brain imaging studies in children and adults with FXS have shown abnormal sizes, connectivity, and activity in several cortical regions involved in cognition. For example, the amygdala, hippocampus, and thalamus are significantly enlarged. These brain regions are responsible for social and emotional behavior, learning and memory, and sensory processing, respectively. Therefore, abnormalities in brain region may be correlated with FXS behavioral deficits. Functional imaging studies demonstrate that individuals with FXS cannot properly recruit appropriate brain regions that are normally employed by non-FXS individuals to perform certain cognitive tasks. A correlation between the FMR1 gene product, FMRP, and FXS was established when blood sera of affected individuals had significantly reduced levels of the protein, as compared to unaffected individuals.

Microscopic analyses have given scientists a magnified view of FXS at the cellular level. Neurons of affected individuals have increased levels of dendritic spines, which are sites on neurons that receive input from other neurons. The spines of FXS individuals tend to be abnormally long and thin, indicative of immaturity. Presently, these morphological changes are believed to underlie the cognitive deficits associated with FXS.

**A Search For a Model**

To better understand FXS, scientists have developed models in mice. Scientists mimic the FMR1 gene deficit by knocking out, or eliminating, the FMR1 gene. The FMR1-knockout mouse is the most widely studied preclinical model of FXS, as its behavior closely parallels the observed FXS condition in humans. For example, FMR1-knockout mice have increased anxiety, hyperactivity, and impaired memory. These animal models have thus revealed that mutations in the FMR1 are acting to reduce expression of the gene. However, one caveat to this approach is that the FMR1 gene is entirely absent, rather than silenced, in the knockout mouse. So, the process underlying the accumulation of these repeats cannot be studied.

In 2007, a team of Israeli researchers sought to address the inadequacy of the mouse model. The scientists used human embryonic stem cells to track the mechanism underlying the reduced production of FMRP. Researchers created a stem cell line from embryos of a female with a permutation of FXS. This cell line developed full mutation of FMR1 and was implanted into a mouse. A tumor of pluripotent cells, or cells that could form a variety of tissue types, developed and was isolated for further study. Before these cells differentiated, the researchers found that the FMR1 gene remained active and produced FMRP. However, as differentiation began and progressed, researchers observed epigenetic modifications of the DNA, which are changes unrelated to DNA mutations that affect the activity of a gene. In the
tumor cells’ nuclei, chemical molecules called methyl groups were added to the FMR1 gene, preventing its transcription into protein. Through this process called methylation, the gene is effectively silenced or shut down. Taken together with the characteristic abnormal morphology, the FMRP is important for normal function.

**The Developing Fragile X Brain**

Organization of the brain depends on the proper proliferation, differentiation, and migration of new neurons and glial cells. During normal development, FMR1 is widely expressed throughout the embryonic brain and levels of FMRP peak at the end of the first postnatal week in a normal mouse. Inactivation, then, of the FMR1 gene affects the early development of the nervous system. Studies in stem cell cultures have demonstrated that loss of FMRP does not affect the birth of new neurons. However, more and more cells express those markers characteristic of neurons in the absence of FMRP, which may be the cause of the hyperabundance of dendritic spines typical of FXS-affected individuals. Thus, current research suggests that FMRP may be important in normal differentiation and pruning of new neurons in the brain and the cognitive and behavioral impairments in FXS-affected individuals may be due to the disorganization of specific brain regions, as demonstrated in brain imaging. Furthermore, hyperabundance of dendrite spines may provide a potential explanation for the increased rates of seizures in FXS-affected individuals.

Normal levels and functioning of FMRP in brain is necessary for successful neuron-to-neuron communication. In cell cultures, FMRP is found in high concentrations in developing axons and growth cones. Growth cones of axons in FMR1-knockout mice have impaired dynamics and an excess of filopodia, or slender structures that help guide the developing axon. Errors in axonal development in the absence of FMRP provide a potential mechanism for the failure of the recruitment of certain brain regions and proper neuronal communication during cognitive tasks in individuals with FXS.

**Are we there yet?**

The ultimate goal is a cure. To date, scientists have used a variety of approaches to studying FXS in the hopes of one day finding that cure and reversing mental retardation in our friends and family affected by FXS. We still have gaps in our knowledge of FMRP and how tightly this protein controls the manifestations of FXS in individuals. But history has shown us that each discovery brings us closer to the treasure. X marks the spot? Not quite yet, but someday.

**Further readings**


