Genetics of Childhood Disorders: XIV.
A Gene for Rett Syndrome: News Flash

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When important advances in the neurosciences relating to childhood disorders occur, the scheduled series of columns will be interrupted. Recently, the gene that causes Rett syndrome was identified. The significance and relevance of this finding to our work as clinicians will be discussed in this column.

Rett syndrome is a neurodevelopmental disorder within the autism spectrum. The prevalence is estimated at 1 in 10,000. Because it is a sporadic illness, initially it was difficult to establish its genetic basis. Females are almost exclusively affected. These children develop normally for the first year or so of life. Milestones are reached on time, and no abnormalities are apparent. Then a rapid regression occurs, including loss of purposeful hand movements. Other clinical findings include growth retardation, loss of speech, microcephaly, ataxia, and often a severe interruption of normal cognitive progression. Affected children then enter a period of relative stability that lasts for decades after the initial regression.

The simplest explanation for the genetic findings is that the responsible gene lies on the X chromosome. If the mutated gene is present in males, they die as fetuses, as no additional X chromosome is available to produce functional protein. This explains the absence of the disorder among males. In females, the second X chromosome carries a complementary copy of the gene that provides some degree of protection. However, an insufficient amount of the protein is available and symptoms develop after the first few months of life.

The first task was to determine the chromosomal location for the responsible gene. Sufficient numbers of affected sisters had been characterized to allow investigators to focus their attention on an area of the X chromosome (Xq28). The search then began to identify the genes present in the region. A number of candidate genes were carefully examined for mutations, and these genes were systematically excluded. A recently isolated gene was found to be mutated in several individuals with the disorder. The gene encodes the methyl CpG-binding protein 2 (MeCP2). Mutations in 2 critical domains of the protein were discovered to be present in several affected probands.

What is the normal function of this protein? In higher organisms, such as vertebrates, a large number of genes are expressed in a tissue-specific manner. In fact, more than one third of genes are expressed only within the brain. Many of these genes are needed during critical periods of CNS development, and their expression must then be turned off. Other genes are required only after birth, and turning these genes on at the appropriate time is critical to normal and proper development. In addition, some genes are expressed only if they lie on either the maternal or paternal chromosome, a phenomenon called genomic imprinting (see recent columns). Disruption of this normal genetic mechanism is responsible for several human disorders, including Prader-Willi and Angelman syndromes.

A mechanism has evolved to regulate which genes are expressed and which must be repressed in both genomic imprinting and normal development. The stable silencing of a large fraction of genes allows cells to transcribe only those genes that are essential for a particular cell type. One of the first steps in this process involves the modification of chromosomal DNA by the addition of methyl groups to the cytosine nucleotide. Furthermore, the methylation that occurs is particularly enriched within stretches of DNA containing numerous cytosine and guanine pairs, so-called CpG islands. Such CpG islands are present throughout the genome, but they are most numerous within the promoters of genes, which are the regions of a gene known to regulate its expression pattern.

Initially, it was speculated that DNA methylation alone was sufficient to prevent the initiation of transcription. This turns out not to be the case. Several additional modifications are required to interfere with the binding of the transcriptional machinery to the promoter region of genes. Specific methyl-binding proteins must first recognize the methylated regions. The chromatin structure is then modified so that the promoter is no longer accessible to transcription factors or other enzymes required to initiate transcription. This regulatory mechanism allows the amounts of specific proteins to be finely tuned over the life of the organism.

The relationship between DNA methylation, chromatin structure, and gene expression has been recognized for some time. The protein that was recently implicated in Rett syndrome, MeCP2, links 2 steps in gene regulation: DNA methylation and histone deacetylation. MeCP2 has at least 2 functional domains that carry out these dual functions. At one end, a domain of the protein recognizes methylated CpG islands and allows the protein to bind tightly to these sequences. The second functional domain is then activated. This region is responsible for the recruitment of another group of proteins, termed the histone deacetylase complex, to the segment of DNA that must be repressed. The protein complex...
chemically modifies the structure of certain histones that are themselves highly enriched within the nuclei of all cells. Histones have long been known to associate with DNA and are required for compaction of DNA into packaged chromatin. Once histones are modified by deacetylation, they compact the DNA even further, making the underlying genes less accessible to transcriptional activators.

The discovery of mutations in a key player in this process suggests that patients with Rett syndrome suffer from inappropriate transcriptional activity. This hypothesis, while intriguing, requires more experimental findings. A number of laboratories are actively working to test it. However, it is reasonable to conclude that mutations in MeCP2 lead either directly or indirectly to inappropriate amounts of transcription of downstream genes that MeCP2 would normally silence.

The recent findings raise a number of questions. Why aren’t symptoms noted before 1 year of age? A similar delay occurs in a number of neurodegenerative disorders, including Huntington’s...
One possible explanation for the time lags has been that mutations of a number of different genes results in the production of toxic compounds, such as free radicals. Mutations in proteins needed to inactivate these compounds result in an inability to detoxify them, and they accumulate over time, eventually damaging neuronal structure and function.

It is possible that a similar mechanism is occurring in the brains of Rett syndrome patients, and neuroanatomical findings might help to clarify this issue. Unfortunately, only a single report exists in the literature of the neuropathological findings in Rett syndrome. Abnormalities were found in the architecture of cortical pyramidal neurons in layer II and III, which had a smaller number of dendrites than is typically seen. Moreover, the normally occurring arborization of these neurons was absent, with a corresponding decrease in the number of dendritic spines.

Another question that remains unanswered relates to predominance of neurological symptoms. MeCP2 is expressed in a number of tissues in the body as well as in the brain, and yet disruptions of these other organ systems are not a significant part of the clinical findings. It is possible that the brain is particularly sensitive to disruption in the activity of MeCP2, although this will need to be determined experimentally. Finally, if mutations in MeCP2 lead to inappropriate expression of downstream genes, what are these target genes? It is possible that mutations will be found in these downstream genes that might result in the appearance of other developmental disorders.

Several additional points should be made. MeCP2 is one member of a larger family of proteins. Two other members of this family have been shown to bind to DNA and prevent transcription of downstream genes. These family members are as their target genes are now additional candidate genes that, when mutated, may cause similar disruptions to normal brain development and functioning. Moreover, as was discussed above, several enzymatic steps are required before genes can be repressed. Proteins that participate in this process include those present in the histone deacetylase complex as well as MeCP2 and probably a number of other yet-to-be-identified proteins. It is likely that mutations in some of these genes will have an effect on gene expression through inappropriate regulation of transcription.

Six different types of mutations were found in the 7 children who had mutations present in their MeCP2 gene. Two of these children were half-sisters and carried the same mutation. Several mutations are missense mutations that substitute one amino acid for another. These missense mutations are found in the methyl-binding domain and are thought to disrupt its ability to recognize and interact with methyl groups. The remaining mutations are found within the second functional region, the domain that recruits the deacetylation complex. Mutations in this domain also result in disruption of transcriptional repression. One of these mutations is caused by the insertion of a single nucleotide that leads to a shift in the downstream nucleotide sequence. This results in a completely different amino acid sequence in the portion of the protein beyond the mutation. The final mutation that was found results in a novel stop codon in the DNA sequence and causes the production of a shortened or truncated protein. Both of these mutations lead to severely impaired or nonfunctional protein being synthesized.

Separate mutations in a single gene are termed allelic heterogeneity. In the majority of disease-causing mutations studied so far, when different regions of a single gene are mutated, they lead to the same clinical phenotype among affected individuals. Occasionally, however, different mutations within the same gene will produce different clinical presentations. For example, mutations to the receptor for an important family of growth factors, fibroblast growth factors, lead to several clinical syndromes depending on where the mutation occurs.

It is important to note that mutations within the MeCP2 gene were found in only 7 of the 21 Rett syndrome patients studied. Several possibilities might explain these findings. Only the exonic DNA sequences that encode the MeCP2 protein were sequenced. Neither the intervening DNA segments, called introns, nor the promoter or additional regulatory regions were sequenced. It is likely that mutations in these regulatory areas will be found in some of the remaining patients with Rett syndrome in whom the coding sequence of the MeCP2 gene appears normal. If these additional mutations are found, they would be further examples of allelic heterogeneity in which mutations within a single gene disrupt its ability to produce sufficient amounts of functional protein.

It is also possible that mutations will be found in genes other than MeCP2, and yet the clinical symptoms of Rett syndrome will again be seen. Mutations that are present in separate genes within a single enzymatic pathway often lead to very similar clinical symptoms among affected individuals. These types of mutations are examples of genetic heterogeneity. In the case of Rett syndrome, this might be caused by mutations present in some of the other proteins required for the proper methylation of DNA sequences, or the deacetylation of histones. It will be interesting to determine whether disruptions in the functional activity of these proteins are responsible for some of the other cases of Rett syndrome not caused by mutations in the MeCP2 gene. A number of laboratories are actively working on this question.

WEB SITES OF INTEREST
http://www.rtsysyndrome.org/
http://www.rettsyndrome.org/main/announcement.htm
ADDITIONAL READINGS


Erratum

In the Letter to the Editor by Stein et al., "Evaluation of Adults for ADHD" (1999;38:940–941), the statistics for the control group were incorrect. The second sentence in the final paragraph of the letter should read, "Since Attention Problems scores were the single best factor which distinguished the ADHD group (mean = 15.6, SD = 6.3) from the controls (mean = 10.4, SD = 4.5), we recommend use of the Attention Problems factor for screening purposes."

Legal Issues Concerning Electronic Health Information: Privacy, Quality, and Liability. James G. Hodge, Jr, JD, LLM, Lawrence O. Gostin, JD, Peter D. Jacobsen, JD, MPH

Personally identifiable health information about individuals and general medical information is increasingly available in electronic form in health databases and through online networks. The proliferation of electronic data within the modern health information infrastructure presents significant benefits for medical providers and patients, including enhanced patient autonomy, improved clinical treatment, advances in health research and public health surveillance, and modern security techniques. However, it also presents new legal challenges in 3 interconnected areas: privacy of identifiable health information, reliability and quality of health data, and tort-based liability. Protecting health information privacy (by giving individuals control over health data without severely restricting warranted communal uses) directly improves the quality and reliability of health data (by encouraging individual uses of health services and communal uses of data), which diminishes tort-based liabilities (by reducing instances of medical malpractice or privacy invasions through improvements in the delivery of health care services resulting in part from better quality and reliability of clinical and research data). Following an analysis of the interconnectivity of these 3 areas and discussing existing and proposed health information privacy laws, recommendations for legal reform concerning health information privacy are presented. These include (1) recognizing identifiable health information as highly sensitive, (2) providing privacy safeguards based on fair information practices, (3) empowering patients with information and rights to consent to disclosure (4) limiting disclosures of health data absent consent, (5) incorporating industry-wide security protections, (6) establishing a national data protection authority, and (7) providing a national minimal level of privacy protections. *JAMA* 1999;282:1466–1471. Copyright 1999, American Medical Association.