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Molecular mechanisms of developmental disorders

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Abstract

One of the central tenets of developmental psychopathology is the belief that we can learn more about normal functioning through the study of psychopathology and arrive at a better understanding of pathological conditions through investigations of normal behavior. Advances in knowledge from one area will inform us regarding mechanisms at work in the other. A similar perspective is the driving force behind recent scientific advances in our understanding of certain developmental disorders. In this paper, molecular findings for four developmental disorders are reviewed: Prader–Willi syndrome, fragile X syndrome, Williams syndrome, and lissencephaly. These disorders were chosen for discussion because putative genes for each of them have been isolated. The ways in which mutations within these genes disrupt normal cognitive and behavioral functioning are discussed. Although considerable progress has been achieved in understanding the genetic mechanisms for these illnesses, much more research is needed to identify the environmental and genetic factors that interact to contribute to the expression of the more complex behavioral disorders.

Developmental psychopathology has recently emerged as a distinct discipline in the behavioral sciences (Cicchetti & Cohen, 1995). At its core is the belief that the understanding of normal functioning of an organism is amplified through the analysis of pathological conditions. This perspective is not a new one; but it is one that has its roots in the writings of many theorists from such divergent fields as psychoanalysis, the neurosciences, and experimental psychology. Although these disciplines are often viewed as fundamentally separate, at least one common methodological approach exists among them. This is the belief that the study of human psychopathology can provide insight into normal functioning and, conversely, the study of normal behavior can inform us regarding abnormal functioning (Cicchetti, 1993).

A similar approach is required when using techniques from the basic sciences in the study of human psychopathology. Recent advances in recombinant DNA technologies have allowed investigators to apply molecular approaches to this field. Thus, there has been a shift from earlier efforts to establish *whether* genetic factors play an etiologic role in psychiatric disorders to more recent investigations into exactly *how* these factors cause disabilities. It is now clear that mutations in specific genes contribute to the etiology of various disorders. However, these molecular studies can only be successful in clarifying basic mechanisms when one compares and contrasts the normal functioning of a protein with its mutated counterpart.

Although these initial steps may take years of work as researchers borrow techniques from the fields of biochemistry, cell biology, and molecular biology to elucidate the structure and function of a protein, they have al-

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ready been successfully applied to a number of childhood disorders. In this paper we review recent advances in the application of molecular methods to four developmental disorders. Several cautionary remarks are in order at the outset to avoid a reductionistic reading of this review. We are very aware of the long-standing debate as to whether nature or nurture is central in the etiology of psychiatric disorders. In recent years the pendulum has swung from one extreme to the other and back again. Thus, in writing a review paper on molecular biology, one runs the risk of being misconstrued as advocating an overly mechanistic approach to the study of developmental disorders.

The fact is that neither genetics nor environmental theories have led to a fundamental understanding of the etiologies for the vast majority of psychiatric disorders. If we have learned anything from recent studies, it is that a delicate interplay exists between nature and nurture (Rende & Plomin, 1995; Rutter, 1991). It is only through the study of these interactions that we can hope to unravel how psychiatric disorders arise over the life span and disrupt the normal developmental trajectory.

In this paper we focus on Prader–Willi syndrome (PWS), fragile X syndrome, Williams syndrome, and lissencephaly. Although these disorders are relatively uncommon in the general pediatric population, they have been the subject of considerable research interest. All of these disorders are developmental disorders in the sense that a mutation has occurred in a gene whose protein product is necessary for the normal development, maturation, and functioning of the central nervous system (CNS). Studying these disorders reveals how these genes and their respective proteins are essential to normal development, which would not be easily appreciated in the absence of the pathological condition (Cicchetti & Cohen, 1995). It may be argued that a discussion of these disorders is biased, because the mutations being described act like sledge hammers in the profound effects they may have on the maturation of the brain. They are, however, among the first childhood developmental disorders for which genes have been identified and, as such, provide us with a glimpse of how these factors may work. Genes that contribute to the etiology of other disorders, such as attention deficit disorder or schizophrenia, have not yet been isolated and obviously cannot be discussed. However, it is possible that the insights gleaned from a discussion of these four disorders may be applied to other childhood disorders and at the very least will indicate the experimental approaches that are necessary. We will first briefly review methodologies that have been used to investigate the contribution of genetic factors to psychiatric disorders. We then describe laboratory findings for each of the four developmental disorders and the complex mechanisms by which the cloned genes and their protein products are thought to work.

Methods in Genetic Psychiatry

A variety of methodologies have been used to assess the contribution of genetic factors to the etiology of psychiatric disorders (for recent reviews see Lombroso et al., 1994; Rutter, 1994; Rutter et al., 1990a, 1990b). The results of some of these studies are summarized in Table 1.

Historically, *family studies* and *pedigree analyses* were among the first to be performed. These studies allowed investigators to determine the frequency of a disease phenotype within an extended family and to compare this with the prevalence in the general population. These investigations by themselves do not demonstrate that genetic factors are at work but rather that some factor, be it genetic or environmental, increases the risk for a specific disorder. Once it has been established that the prevalence of a particular disorder is higher in affected families than in the general population, it is possible to use the family data to test specific genetic models of inheritance using segregation analysis. It should be pointed out that modeling also will not prove that a particular illness is genetic, but it will help to rule out various modes of inheritance and suggest others that are more likely.

Twin studies are among the most important methodologies used to establish the heritabil-

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Table 1. *Chromosomal localization and gene abnormalities in select neuropsychiatric disorders*

Genetic Disorder	Chromosomal Location	Genetic Defect (Gene/Protein Name)
Autosomal-dominant		
Amyotrophic lateral sclerosis	21q21	Point mutations (SO dismutase)
Familial Alzheimer disease	1q31	Point mutations (PS-2)
	14q24	Point mutations (PS-1)
	21q21	Point mutations (APP)
Huntington disease	4p16	Triplet repeat (Huntingtin)
Myotonic dystrophy	19q13	Triplet repeat (DM-1)
Spinocerebellar ataxia	6p22	Triplet repeat (SCA1 or ataxin-1)
Von Recklinghausen	17q11	Deletions, insertions, translocation, and point mutations (neurofibromin)
Autosomal-recessive		
Angelman syndrome	15q11	Deletions (unknown)
Ataxia-telengiectasia	11q22 and 14q32	Translocations (ATM)
Friedreich ataxia	9q13–21	Unknown
Miller–Dieker syndrome	17p13	Deletions (lissencephaly-1)
Prader–Willi syndrome	15q11–13	Deletions (protein N)
Retinoblastoma	13q14	Deletions, point mutations (RB)
Waardenburg syndrome	2q35	Point mutation, deletion (Pax3)
Williams syndrome	7q11	Deletions (elastin, LIM-K)
Rubenstein–Taybi syndrome	16p13	Deletions, point mutation (CREB binding protein)
X-linked recessive		
Becker dystrophy	Xp21	Small deletion (dystrophin)
Duchene dystrophy	Xp21	Large deletion (dystrophin)
Fragile X syndrome	Xq27	Triplet repeat, point mutation (FMR-1)
Lesch–Nyhan syndrome	Xq27	Point mutation (HPRT deficiency)
Spinobulbar muscular atrophy	Xq21	Triplet repeat (androgen receptor)

ity of genetic disorders. These studies compare the occurrence of an illness in monozygotic and dizygotic sets of twins. For a condition in which there is a large genetic component, monozygotic twins should have a high rate of concordance because these twins share 100% of their genomes. In contrast, dizygotic twins share 50% of their genes, and their diagnostic concordance rate should be similar to that found among nontwin siblings. By comparing concordance rates between monozygotic and dizygotic twin pairs, investigators can assess the relative contribution of genetic and environmental factors in disease pathogenesis on a population level. A consistent finding from these studies is the lack of complete concordance among monozygotic twins for many disorders that are known to be inherited. These results suggest that although genetic factors may provide an underlying diathesis or vulnerability for a disorder, environmental factors play a critical role in the ultimate expression of symptoms.

Once genetic factors have been clearly established as etiologically important, *linkage analysis* is often used to pinpoint the chromosomal location of the mutated gene. With this technique, investigators compare the inheritance pattern of a disease phenotype with the inheritance pattern of specific DNA markers whose precise location in the genome is already known. During gamete formation a DNA marker located in the same chromosomal region as the disease gene will "cosegregate" with the illness more frequently than predicted by chance alone. Moreover, the closer the genetic marker lies to the disease locus, the more often it will travel with the disease allele and not be separated during meiotic "crossing over" events. By comparing the inheritance of markers and diseases, investigators have been able to identify the

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chromosomal loci for genes implicated in many disorders. One of the first examples of the successful application of these techniques was the localization of the gene responsible for Huntington disease to the short arm of chromosome 4 (Gussella et al., 1983). In some instances, direct observation of stained chromosomes under a microscope (karyotyping) may reveal large-scale mutations such as deletions, obviously facilitating chromosomal localization. In fact, all of the disorders discussed in this paper were initially characterized by karyotyping, making the process of localizing and cloning candidate genes that much easier.

Linkage analysis has worked well for those diseases that demonstrate classic Mendelian inheritance, such as dominant, recessive, or X-linked disorders. Indeed, only a small false positive rate has occurred over the years while identifying genes for these illnesses. However, because few if any psychiatric conditions are inherited as Mendelian disorders, traditional linkage approaches have not been successful. Many of the published reports of high linkage scores, and thus potential disease loci, have generated initial excitement and then invariable disappointment as the findings have either not been replicated or disappeared when additional family members were characterized.

One suggested reason for the failure lies with the heterogeneous nature of these disorders, whereby different genes are independently causing similar phenotypes. If mutations within different genes are responsible for a single disorder, then linkage analyses across multiple families cannot possibly reach significance. There is a growing sense, however, that the situation may be more complicated. The search for genes for the more complex mental and behavioral disorders, such as bipolar disorder and schizophrenia, has not been successful, even when large multigenerational pedigrees from relatively isolated populations have been used. These results have led investigators to conclude that single genes may not be responsible for many of these disorders. Rather, environmental factors in combination with multiple separate genes are thought to each contribute small effects to the expression of these disorders. Traditional linkage analysis is virtually impossible to perform for these multifactorial disorders, given the practical size limits of sample populations.

New techniques have been generated to provide the greater statistical power that is needed to detect the relatively small effects of multiple factors. These association studies employ candidate genes that are hypothesized to contribute to a particular trait or disorder. *Sib pair studies* are one paradigm often used to determine the frequency of specific alleles among affected pairs of siblings. If the same allele is shared among a large number of affected siblings, then the allele is likely to be, or is close to, the disease gene. Insulin-dependent diabetes mellitus (IDDM) provides a good example of the successful application of sib pair analysis to a complex, multifactorial disorder. IDDM is characterized by autoimmune destruction of the insulin-secreting beta cells of the pancreas and subsequent disruption of glucose metabolism. It tends to cluster in families and have a modest (36%) concordance rate in monozygotic twins. Genetic and environmental factors both contribute to IDDM susceptibility. It was only after association studies were conducted that several candidate genes were discovered, each of which makes a relatively small contribution to the expression of the disorder (Field et al., 1996; Rowe et al., 1995).

These techniques have also been applied recently to attentional disorders, and much suspicion has been directed at some naturally occurring variants of a receptor for the neurotransmitter dopamine. The receptor, known as DRD4, is normally found in multiple forms in the general population (Lichter et al., 1993). The basis for these polymorphisms is the repetition of a 48-nucleotide sequence at a particular site in the DNA coding region. Because every three nucleotides code for an amino acid, the receptor varies in the number of times a 16 amino acid sequence is repeated. The repeat sequences occur within an intracellular segment of the molecule that "informs" a cell when dopamine has been bound by the DRD4 receptor at the cell surface.

Within the general population, the most common variation is a receptor with four re-

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peated units, and a less frequent variation contains seven repeated units. Two recently published studies found a significant association of the seven-repeat variant with individuals who scored high for a personality trait that is often associated with hyperactive and impulsive behavior (Benjamin et al., 1996; Ebstein et al., 1996). Because these studies were conducted in adult cohorts, they are currently being replicated among well-characterized populations of children with attentional disorders.

Once the gene(s) implicated in a disorder have been identified, the focus shifts from genetics to cell biology in order to determine the normal function of the mutated protein. As in gene research, a number of different techniques have been developed to examine a protein's function within cells. One of these is the use of *animal models* in which evidence from other species is used to explore the potential role of the protein in humans. Animal models of disease have a number of distinct advantages. For example, animal physiological systems are often less complex and/or more easily manipulated than those in humans. This is especially true in neuropsychiatric studies in which the roles of volition, motivation, and self-awareness often confound human studies of neuropsychiatric function.

One methodology that is often used to explore the function of an unknown protein is the generation of "knockout mice." In this technique, mice are bred to lack the protein of interest by removing the gene that codes for the protein. This is accomplished by introducing a mutation into the normal gene and placing this mutated gene into embryonic stem cells. These cells are then placed into the uterus of a mouse, where some develop into heterozygote litter mates with one normal and one inactive copy of the gene. Cross-breeding such mice produces offspring with two inactivated copies of the gene. By creating and then studying such knockout animals, investigators are often able to obtain new information on the function of the protein of interest. In this way, researchers may determine the normal function of a protein by studying a mutated form.

Over the past decade knockout mice have produced a wealth of knowledge concerning the roles played by a variety of transcription factors and growth factors in the normal development of the CNS. Some of these studies have demonstrated that gene expression plays a far more important role in the establishment of behavior patterns than was previously suspected. One recent knockout provided startling results and attests to the importance of these genes in regulating even seemingly complex behavior. The removal of the transcription factor fosB produced mutant mice that were profoundly deficient in their ability to nurture their offspring, the majority of whom died from neglect within days (Brown et al., 1996). The only detectable differences between these mutant mothers and normal, fully nurturant mothers was the absence of fosB expression in the hypothalamus, a region known to be critical for proper nurturing behavior. The authors speculate that fosB expression within the hypothalamus induces the expression of other genes that are critical in strengthening the neuronal circuits that are necessary for normal nurturing behavior. A candidate gene whose expression might be regulated by fosB is the receptor for oxytocin, a hormone that is known to be involved in the normal induction of nurturing behavior.

Of course, these experiments were conducted in mice and nurturing in the human is considerably more complex than in the rodent. Many environmental and genetic factors contribute to nurturing behaviors. What these experiments suggest, however, is that the mutation of a single gene has such a detrimental effect on specific neuronal circuits that it creates the observed behaviors. Interestingly, fosB is a highly conserved gene across the animal kingdom and has been found in most species examined. Such conservation suggests that the protein serves a critical function.

It should be pointed out that not all knockout experiments have been helpful in providing functional information. The inactivation of a number of master control genes, including transcription factors and homeobox genes, might be predicted to have a major impact on CNS maturation; instead, the CNS appeared normal or had only minor abnormalities after the knockout of several of these genes. This led to the idea that important regulatory genes

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may have a certain degree of redundancy, wherein closely related genes may take over their functions. Indeed, there is some support for this hypothesis in the finding that "double" knockouts of two closely related homeobox genes expressed within the basal ganglia resulted in significant brain abnormalities, while knockout of only one produced very few findings (Anderson et al., 1995).

PWS

Within humans, the 22 autosomal pairs and two sex chromosomes contain an estimated 100,000 genes, each of which encodes the blueprint for at least one protein or enzyme. Approximately one-third of these genes encode proteins specifically expressed within the brain. Furthermore, many of these genes are expressed for brief, critical periods of neurological development during which they regulate the expression of additional genes necessary for normal brain maturation. This process of expressing between 30,000 and 40,000 brain-specific genes, each at its precise developmental moment, requires a highly orchestrated sequence of events and many levels of regulation.

For all genes, the basic flow of biologic information is from DNA to RNA to protein (Figure 1). The first step, DNA transcription, often requires the binding of transcription factors to specific chromosomal sites immediately adjacent to the gene being activated. These factors facilitate the initiation of transcription by allowing RNA-forming enzymes called polymerases to gain access to the DNA and to make an exact copy of one of the two DNA strands, termed the preliminary RNA transcript (preRNA). The preRNA message must be processed to produce a mature RNA molecule. During this editing step, a number of accessory proteins, termed splicing factors, bind to the preRNA molecule, remove noncoding regions, and splice together the segments that will code for the protein.

The mature RNA is rapidly exported from the nucleus to the ribosomes, which constitute the protein production machinery in the cytoplasm of a cell. It is here that messages are actually translated into proteins. Following translation, newly synthesized proteins are often modified in order to become fully functional. As we shall see below, disruptions in any one of these steps from DNA to RNA to functional protein have been associated with clinical syndromes.

PWS is a rare congenital disorder that is characterized by a constellation of symptoms including mild mental retardation and behavioral difficulties. The syndrome is perhaps best known for the food-related problems that first become evident in childhood. As early as 2 years of age, children with PWS develop food-seeking and overeating behavior (Cassidy, 1992). Preoccupation with food is a lifelong difficulty, and affected individuals almost invariably suffer from obesity. In addition to the unremitting food-seeking difficulties, children and adolescents with PWS display a number of behavioral difficulties including temper tantrums, stubbornness, and aggression (Dykens & Cassidy, 1995). Furthermore, obsessive–compulsive symptoms, outside of the food-related domain, have recently been described. These include ordering and arranging and concerns with symmetry and exactness (Dykens et al., in press).

Fifteen years ago researchers determined that the majority of cases of PWS were associated with a deletion on the paternally derived chromosome 15 (Ledbetter et al., 1981). PWS is an example of a disorder caused by imprinting, in which regions of one parental chromosome are "turned off" while genes from the other parental copy are expressed. Normally only the paternal genes are expressed in this region of chromosome 15, while the same genes on the maternal copy are silent. Thus, if a deletion is present on the paternal chromosome, the maternal genes are unable to compensate and the result is a complete absence of the proteins normally encoded by this region.

In the majority of remaining cases of PWS, the genetic mechanism is a maternal duplication of the entire chromosome 15 (Nicholls et al., 1989). In these cases, there is no paternally derived chromosome 15 at all. The imprinting rule still applies, however, and nei-

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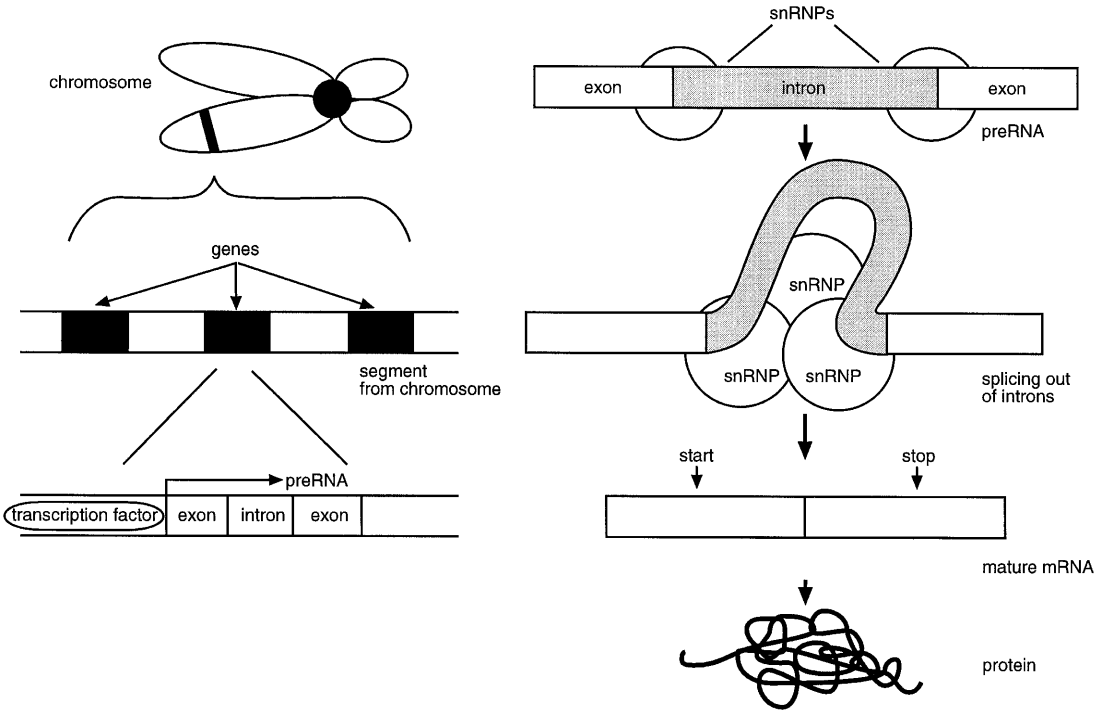


Figure 1. Biological flow of information. In humans, approximately 100,000 genes are present on the 22 pairs of autosomal and 2 sex chromosomes, one of which is shown here. The binding of transcription factors to regulatory elements immediately upstream of the transcription start site results in the production of a preRNA molecule. These preRNA molecules are then processed by the removal of intervening sequences (introns) and the splicing together of exons. The family of snRNP molecules is required for this step, and one of these (SNRP-N) has been implicated in the etiology of Prader–Willi syndrome. The mature RNA molecule is transported from the nucleus to the protein synthesis apparatus in the cytoplasm where translation begins at a translation start codon and is terminated at a stop codon.

ther maternal chromosome expresses transcripts. The effect of this mutation is the absence of any functional protein.

In 1992 two groups reported that the loss of a single gene from this region was strongly associated with PWS (Leff et al., 1992; Ozcelik et al., 1992). An earlier group had isolated this gene and determined that its protein product was a member of the family of splicing proteins required for the proper editing of mature RNA messages (McAllister et al., 1988). These proteins are collectively termed snRNPs (pronounced "snurps"), an acronym for small nuclear ribonucleoproteins. The novel member was found to be highly expressed in the nervous system and was thus called SNRP-N or protein N (McAllister et al., 1988; Schmauss et al., 1992).

The finding of a splicing protein that was highly expressed in the brain was initially met with considerable excitement. Although many transcripts and proteins are preferentially expressed within the CNS, as mentioned above, related transcripts and proteins may be found within other tissues. The process by which a single gene gives rise to multiple related messages is termed alternative splicing. Through this mechanism, several proteins may be produced from a single gene, and each protein may contain slightly different amino acid se-

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quences that have presumably been modified for proper functioning in the tissue in which it is expressed.

Investigators have long wondered how these alternatively spliced proteins were produced. The discovery of tissue-specific splicing factors such as protein N led to the hypothesis that brain-specific snRNPs were necessary for the production of brain-specific messages. If protein N were mutated and unable to function properly, a variety of brain-specific proteins might be absent or improperly edited. This was suggested as the mechanism by which a diverse array of behavioral and neurophysiological symptoms are produced in PWS. SNRP-N thus seemed a good candidate gene for the disorder: the gene mapped to the chromosome region deleted in PWS patients, its RNA transcript was enriched within the CNS, and its protein possessed a seemingly brain-specific function.

Further evidence implicating SNRP-N in the pathogenesis of PWS has accumulated. For example, knockout of SNRP-N in mice causes some of the abnormal eating behaviors seen in PWS (Cattanach et al., 1992). Because the hypothalamus is the brain region that controls many aspects of metabolism, growth, and behavior, it was suggested that protein N might regulate the expression of specific messages in the hypothalamus (Leff et al., 1992).

However, many of the features of PWS cannot be explained by hypothalamic dysfunction. In addition, SNRP-N is expressed at equal levels throughout the brain and is not particularly enriched within the hypothalamus. Therefore, some investigators have suggested that, in addition to SNRP-N, other genes are lost within the deleted region and their collective loss produces the PW phenotype (Glenn et al., 1996). When several adjacent genes are deleted and their absence contributes to the observed phenotype of a disease, the disorder is termed a contiguous gene syndrome. We will return to this concept in our discussions of other developmental disorders.

The debate over the extent of the role of SNRP-N in the pathogenesis of PWS has continued. A recent case report questions whether SNRP-N has any role in this disorder. Schulze and colleagues (1996) described a 29-year-old man with some of the classic symptoms of PWS. Cytogenetic studies revealed that his chromosome 15 was damaged by a translocation, a mutation in which a fragment at the end of one chromosome breaks off and changes places with a fragment at the end of another chromosome. As might be expected, translocations can disrupt the activity of genes when the breakpoints occur in coding or regulatory sequences. Careful genetic mapping studies revealed that the SNRP-N gene was unaffected in this case by the translocation and remained active on chromosome 15. Moreover, protein N was found at normal levels in the patient's tissues (Schulze et al., 1996). The coexistence of PWS symptoms and normal levels of the SNRP-N gene product challenges the prevailing understanding of the genetics of this disorder. However, because this was a single case report, it will be important to replicate these findings in additional subjects.

Fragile X Syndrome

Children with fragile X syndrome are born with mild to severe mental retardation, as well as facial, testicular, and connective tissue abnormalities (Baumgardner et al., 1995; Martin & Bell, 1943). Although investigators have recently become interested in attempting to identify specific linguistic, cognitive, or behavioral deficits consistently affected in these patients, no distinctive patterns have yet been found. Abnormal speech patterns are present in the majority of cases and include echolalia and high pitched jocular speech, as well as dysfluency, dyspraxia, and poor articulation. A number of papers describe the frequent occurrence of gaze aversion, stereotypies, hyperactivity, and attentional difficulties, as well as aggressive and self-injurious behaviors (Bregman et al., 1987). Female carriers are found to do poorly on the arithmetic, block design, and digit span subtests of the Weschler scales, suggesting deficits in numerical and visuospatial abilities (Kemper et al., 1986).

The disorder is the second most common form of mental retardation associated with a

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chromosomal abnormality after Down syndrome, and it is more common in males than in females. Estimates suggest that the disorder accounts for up to 20% of all boys with IQ levels between 30 and 55 (Turner et al., 1980). 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 the presence of folic acid revealed that many patients had a "fragile" site on one of their X chromosomes that appeared as a constriction on the distal long arm (Lubs, 1969).

It was also noted that the syndrome has a distinct pattern of inheritance that has been termed anticipation. Anticipation refers to the tendency of certain genetic illnesses to become more severe over successive generations, and in fragile X syndrome this phenomenon is called the Sherman paradox (Sherman et al., 1985). Thus, a patient's grandmother might have mild developmental difficulties and his mother might have more significant cognitive problems, while the patient himself has severe retardation.

The molecular abnormalities that underlie anticipation have now been clarified (Nelson, 1995). In 1991 Verkerk and colleagues (1991) identified a single gene that was associated with the 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 three nucleotides (CGG) was repeated many times in these patients. This region of the *FMR-1* gene is highly polymorphic 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 (Fu et al., 1991; Snow et al., 1993). In mildly affected patients, however, this sequence was found to enlarge to up to 200 repeats, and in severely affected patients repetitions of up to several thousand nucleotides are seen. The enlargement of this triplet repeat across generations is responsible for the increasing severity of the disorder over time, thus explaining the Sherman paradox (Fu et al., 1991).

The triplet repeat occurs within the first exon of the transcript, but it precedes the nucleotide sequence that will be translated into protein (Figure 2). Thus, the expansion is not exerting its effects by introducing novel and destabilizing amino acids into the FMR-1 protein structure. How, then, does the repeat exert its effects? Two hypotheses have been offered. The first is that the enlargement interferes with the stability of the FMR-1 RNA transcripts. Instead of being normally processed, transcripts are rapidly degraded. As a result few mature messages ever leave the nucleus. The second hypothesis proposes that the region containing the triplet nucleotide expansion is chemically modified and therefore is not transcribed (Bell et al., 1991; Knight et al., 1993). As a result, there would be less expression of FMR-1 messages. The result of either of these mechanisms would be a decrease in functional FMR-1 protein.

Preliminary information concerning the function of the FMR-1 gene product is beginning to emerge. Hinds et al. (1993) reported that the FMR-1 gene product is localized to the brain and the testes. This pattern of expression is consistent with the cognitive difficulties and testicular abnormalities seen in the disorder. However, during early embryonic development, the protein is found to be expressed in all tissues. This more ubiquitous pattern of expression suggests that FMR-1 may play a role in the development of many tissues.

The FMR-1 protein contains two regions with a particular sequence of amino acids. These domains of approximately 50 amino acids are termed KH domains, and proteins that contain these domains bind tightly to RNA molecules (Siomi et al., 1993, 1994). As mentioned earlier, RNA molecules must be modified into mature messages prior to their leaving the nucleus for translation into proteins. RNA modification does not occur by itself: it requires the participation of a large group of proteins. The ability of these accessory proteins to bind tightly to RNA molecules is an important functional attribute. Thus, when investigators find a KH domain on a protein, they immediately suspect that the protein may be involved either in the processing of RNA transcripts or in the transla-

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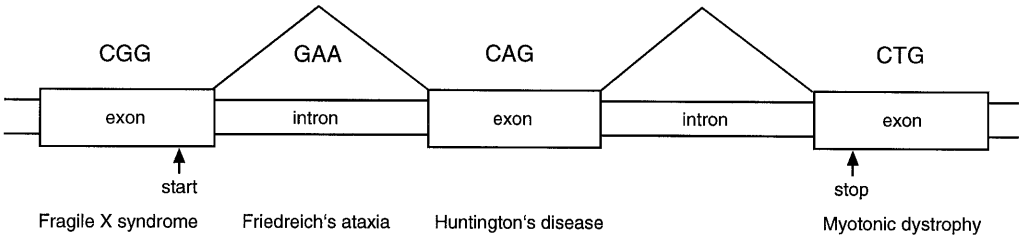


Figure 2. The location and types of triplet repeat for four neuropsychiatric disorders. In this factitious gene, three exons (larger boxes) are separated by two introns (smaller boxes). Introns are normally removed to produce mature messenger RNA. The triplet repeats of fragile X syndrome (CGG) lie within the untranslated region immediately upstream of the translation initiation site (start). In Fredriech's ataxia, the triplet repeat (GAA) lies in an intron where it is thought to interfere with the correct splicing of the message. The triplet repeat found in Huntingtons' disease (CAG) lies within the coding region itself, and leads to the production of a protein containing a long polyglutamine tract. In myotonic dystrophy, the triplet repeat (CTG) is found after the protein termination site (stop) in the downstream untranslated region, and is thought to interfere with the production of mature messages.

tion of that transcript into functional protein. Obviously, a mutation in this kind of RNA binding protein might have a serious effect on the cells' ability to produce mature messages or translate those messages into protein.

The importance of the RNA-binding domain for the function of the FMR-1 protein is underscored by a recent case study of a fragile X patient. The investigators did not find the expected triplet repeat mutation, but instead they found a point mutation that led to the change of a single highly conserved amino acid normally present within KH domains (Musco et al., 1996; Siomi et al., 1994). The authors concluded that the KH domain is critical to the proper function of FMR-1 and that the mutation within this domain interfered with the protein's ability to bind to RNA molecules. This is an example of how different kinds of mutations within the same gene may produce similar clinical pictures. In the majority of cases, fragile X syndrome is caused by the enlarged triplet repeat discussed earlier; however, any mutation that disrupts the functional activity of the FMR-1 protein will lead to the disorder.

A recent report extended these findings by examining the intracellular location of the FMR-1 protein (Khandijan et al., 1996). The fragile X protein was consistently associated with ribosomes. Ribosomes are single strands of RNA to which a number of accessory proteins are bound. The conclusions that emerged from the intracellular localization studies were that the FMR-1 protein binds to the RNA strands of ribosomes through its KH domains. These authors speculated that the fragile X protein might be particularly important in tissues that produce a large volume of proteins for export, such as neurotransmitter-producing neurons and androgen-producing testicular cells.

Although its precise function in protein synthesis has yet to be elucidated, considerable progress has occurred since the identification of the fragile X gene 5 years ago. Future research will in all likelihood concentrate on determining exactly how the FMR-1 protein participates in the translation of mature RNA messages into functional proteins.

It was originally thought that the enlargement of the *FMR-1* gene through triplet repeats was an anomaly found only in fragile X syndrome. However, at least seven other disorders have been identified in which the mutated gene contains a triplet repeat region (Nelson & Warren, 1993; Warren, 1996). Interestingly, all of the illnesses that contain this mutational form are neuropsychiatric disorders and are characterized by the inheritance pattern of anticipation (Figure 2). It is not yet clear why triplet repeats have been found only in neuropsychiatric diseases, and this remains an area of active research.

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Williams Syndrome

Williams syndrome is a developmental disorder characterized by distinct facial features, a variable degree of mental retardation, cardiovascular disease (usually supravalvular aortic stenosis or SVAS), and, most distinctively, relatively normal verbal abilities in the presence of significant visuospatial deficits (Morris et al., 1988). The disorder, first described by Williams in 1961, has a rather low prevalence of 1 per 20,000 live births; nevertheless, it has remained a topic of considerable interest because of the unusual cognitive profile. Deficits in affected individuals have been described as a difficulty in visualizing a complete picture, because they are only able to see its parts and not integrate the parts into a whole pattern (Ewart et al., 1993). By contrast, their strengths frequently lie in their verbal skills. Indeed, some elements of speech may be enhanced, such as the quality of vocabulary, auditory memory, and social use of language. Many patients sing or play musical instruments with considerable expertise. Because of patients' engaging personalities, language skills, and loquaciousness, mental retardation is often underestimated (Gosch & Pankau, 1994, 1996). The most frequently reported behavioral problems relate to poor concentration, attention-seeking behavior, and motor restlessness. Most patients achieve a high degree of autonomy, and the majority of adult Williams syndrome individuals live at home with their parents and attend a day care center (Plissart et al., 1994).

The presence of cognitive strengths in some areas with deficits in others raised the possibility that the gene(s) responsible for Williams syndrome was critical for the development of specific cognitive abilities. This is in contrast to other forms of mental retardation in which there is a more uniform depression across many cognitive skills, suggesting that the environmental or genetic factors at work are affecting multiple areas of the CNS. As such, Williams syndrome has the potential to be particularly informative, because it raises the possibility of identifying a factor important for the development of specific cognitive skills. This should not be interpreted as meaning that other environmental and genetic factors are not involved. However, Williams syndrome is an example of how the careful assessment and study of a pathological condition may teach us something about the normal development of specific psychological skills.

As the search for the gene associated with Williams syndrome began, a number of investigators noted that the cardiovascular symptoms of these patients cosegregated with a gene termed elastin. Elastin was previously shown to be a major component of skin, blood vessels, and lung tissues, and the elastin gene mapped to chromosome 7. Nearly all Williams syndrome patients have a deletion of a large segment of this region of chromosome 7 (Lowery et al., 1995; Nickerson et al., 1995). Such a deletion would result in half the normal production of elastin, and this deficiency was proposed as the defect underlying the vascular abnormalities and the characteristic facies seen in Williams syndrome (Lowery et al., 1995).

Very quickly, however, investigators suspected that a deletion of the elastin gene alone should not result in the cognitive deficits that are found in patients. Elastin was not detectable in fetal or adult nervous tissue (Frangiskakis et al., 1996), and it was hard to understand how a protein not found in the CNS could contribute to the cognitive abnormalities. Moreover, there are a number of families that exhibit a hereditary form of SVAS without any other Williams traits, suggesting that the cardiovascular problems could be inherited separately from other aspects of the phenotype (Frangiskakis et al., 1996). Indeed, these families with only vascular problems were soon found to have mutations in the elastin gene. Investigators began to suspect that the disparate symptoms observed in Williams syndrome were the result of mutations not only of the elastin gene, but also of additional contiguous genes on chromosome 7.

Investigators next sought to identify those genes that are adjacent to elastin. Tassabehji and colleagues (1996) were the first to report the deletion of a second gene in 20 out of 20 Williams syndrome patients. When they examined the sequence of this gene, it was

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found to be nearly identical to a previously characterized gene, *LIM-kinase*. Earlier work had established that *LIM-kinase* codes for a protein involved in signaling pathways and is found in high concentrations in the brain (Mizuno et al., 1994; Proschel et al., 1995). Thus, researchers began to suspect that mutations in the *LIM-kinase* gene might be responsible for the cognitive deficits seen in Williams syndrome.

Additional support for this hypothesis was provided when researchers analyzed families with the characteristic vascular symptoms and cognitive abilities but who had few of the typical facial features and no mental retardation (Frangiskakis et al., 1996). The cardiovascular and cognitive deficits cosegregated with a relatively small deletion on chromosome 7. The researchers reasoned that if they could sequence the entire nucleotide sequence normally present in this region, they would be able to identify any genes that were present. Only two genes were found: the elastin gene and the *LIM-kinase* gene. Based on these data, the investigators argued that mutation of the *LIM-kinase* gene was responsible for the observed impairments in visuospatial cognition (Frangiskakis et al., 1996). If the findings are replicated, this paper is significant because it represents the first linkage of a single gene product to a specific cognitive deficit.

To understand the possible function of *LIM-kinase*, it is necessary to review how signals are transmitted within cells. *LIM-kinase* is a member of a ubiquitous class of signaling enzymes known as protein kinases. Protein kinases transfer highly charged phosphate groups to substrate proteins (Figure 3). This process, termed protein phosphorylation, is essential for the normal regulation of protein function in cells. The addition of large, negatively charged phosphate groups to proteins causes an alteration in their shape; and because the activity level of a protein is highly dependent on its 3-dimensional structure, phosphorylation leads to dramatic changes in enzymatic activity.

Many growth factors and hormones exert their biological effects by inducing phosphorylation of proteins. The phosphorylation of these substrates produces a wide array of "downstream" intracellular effects including transcriptional control of genes, activation of metabolic enzymes, opening of ion channels, and proliferation and migration of cells (Hyman & Nestler, 1996). The series of events described here—an extracellular signal binding to its receptor on the plasma membrane, protein kinases being activated on the inside of the cell, and downstream effector molecules becoming phosphorylated—is one of the most common regulatory pathways encountered within cells.

It is not surprising then that the activity of many protein kinases must be closely regulated in order to control the rate of phosphorylation of substrate proteins. Loss of this tight regulation may have disastrous effects on the cellular life cycle. For example, many forms of cancer are caused by mutations of specific protein kinases. The mutated kinases are no longer regulated and proceed to phosphorylate their substrates constitutively and at inappropriate points in the life cycle, resulting in the abnormal proliferation of cells. To prevent this from occurring normally, many kinases have a companion regulatory domain that is distinct from the catalytic domain. When regulatory proteins bind to these adjunct domains, the enzymatic activity of the protein kinase is modulated, sometimes being activated and sometimes repressed. It is this regulation of activity that prevents the abnormal proliferation of cells.

LIM-kinase, the candidate gene for Williams syndrome, contains a catalytic domain and a nearby regulatory domain. The identities of the proteins that bind to the regulatory domain and of the substrate proteins that are phosphorylated by *LIM-kinase* remain unknown (Sanchez–Garcia & Rabbits, 1994). Research in developmental neurobiology, however, suggests that brain-enriched protein kinases play a major role in the growth and maturation of the CNS (Abeliovich et al., 1993; Grant et al., 1992). The recent findings on Williams syndrome have led to the proposal that *LIM-kinase*, its regulatory proteins, and substrates will be found enriched within regions of the brain that contribute to visuospatial abilities, such as the posterior parietal lobes (Frangiskakis et al., 1996).

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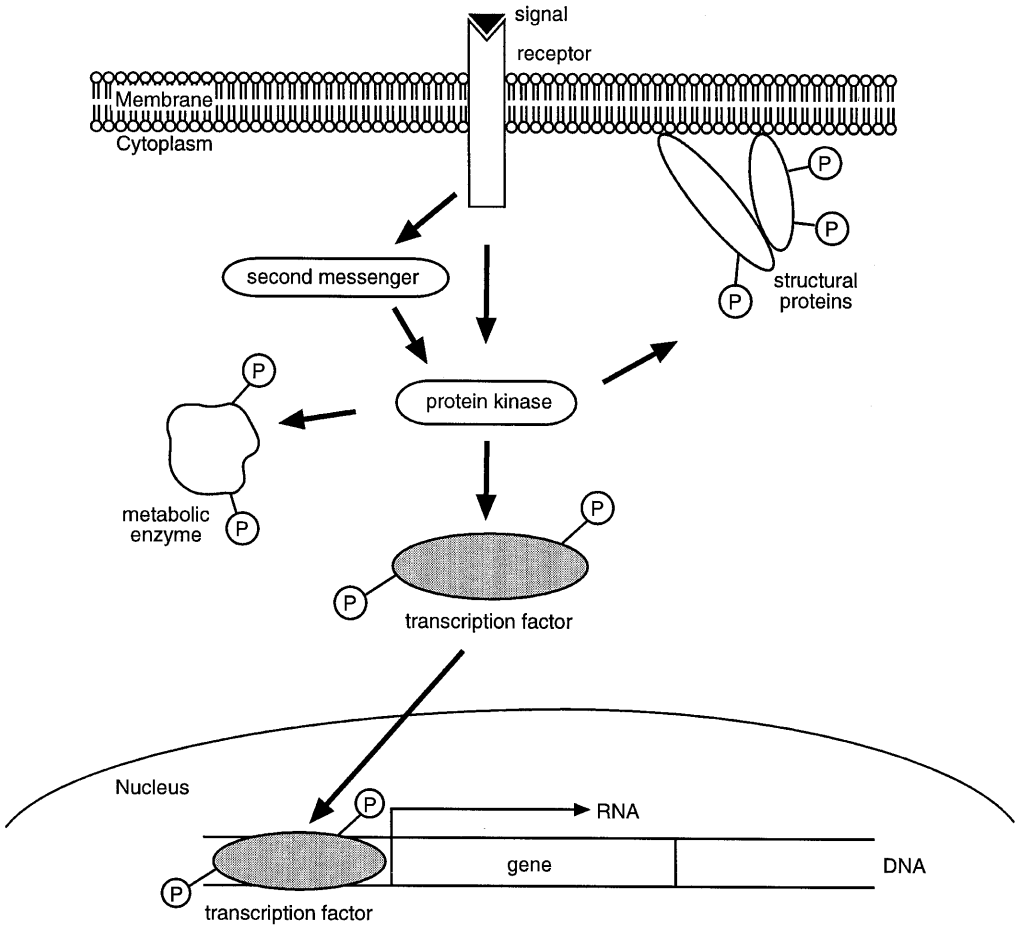


Figure 3. Signal transduction pathways. Many biological events within cells are initiated by growth factors, hormones, and other extracellular factors. The first step in this process involves the binding of a ligand (signal) to specific receptors on the surface of the cell, and a conformational change in the intracellular domain of the receptor. The net result is the phosphorylation of substrate molecules, either through a kinase domain on the receptor itself or through activation of a protein kinase through second messenger pathways. The phosphorylation of three classes of proteins is depicted, although many other types of proteins are regulated by phosphorylation. This posttranslational modification of proteins modulates their activity, either through the activation, or inactivation, of their enzymatic activity.

There are a number of reasons to remain cautious about these preliminary results. In some of the families studied, the deleted region did not always produce the Williams phenotype (Frangiskakis et al., 1996). Although incomplete penetrance of an abnormal genotype is not unusual in behavioral disorders, this finding raises the possibility that the LIM-kinase deletion is unrelated or only tangentially related to the pattern of cognitive deficits. In addition, another group of investigators recently isolated a new gene that appears to lie within the Williams syndrome region, raising the possibility that additional genes participate in the expression of the phenotype (Peoples et al., 1996). Although it remains an exciting hypothesis, replication is necessary and additional research must be done to determine the exact role of LIM-kinase in the etiology of Williams syndrome.

Lissencephaly

Lissencephaly, from the Greek words meaning "smooth brain," is the name given to a

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number of disorders of brain formation in which there is an absence of gyri, or ridges, on the surface of the brain. There is also a loss of the normal neuronal migration pattern within the inner layers of the cerebral cortex, the brain region responsible for higher mental processing. Although several distinct lissencephaly syndromes exist (Dobyns & Truwit, 1995), only the two most common forms are reviewed here: isolated lissencephaly (ILS) and Miller–Dieker syndrome (MDS). Patients diagnosed with ILS have a smooth cerebral surface. In addition, while the normal cerebral cortex is divided into six distinct cellular layers, the brains of ILS patients have only four. The ILS pattern resembles what is found in more immature fetal brains (Dobyns et al., 1993). As might be expected, these malformations produce profound mental retardation. Patients with MDS have the same neuroanatomic features. In addition, however, they often have a characteristic facies and a variety of congenital malformations of the limbs not found in patients with ILS (Dobyns et al., 1993).

In 1993 a gene on chromosome 17 was isolated and was found to contain large deletions in two unrelated MDS patients (Reiner et al., 1993). The gene was named LIS-1, and its expression was detected in the CNS. Because of the combination of neurological and nonneurological symptoms, Dobyns and colleagues (1993) suspected that MDS might be another example of a contiguous gene syndrome, whereby the deletion affected LIS-1 as well as other nearby genes on chromosome 17. By contrast, ILS might be caused by subtle, less easily detectable deletions within LIS-1 only, leaving neighboring genes intact. When these investigators used high-resolution techniques to examine the chromosomes of ILS patients, 40% were found to have subtle deletions in LIS-1. Thus, LIS-1 appeared to be an important factor in the pathogenesis of both major forms of lissencephaly, while MDS appeared to result from the deletion of additional contiguous genes.

As a first step toward understanding the function of LIS-1, investigators compared the amino acid sequence of LIS-1 with other known proteins. Hattori and colleagues (1994) reported a 99% identity between LIS-1 and a subunit of the bovine protein PAF-acetylhydrolase or PAFAH. An even higher degree of similarity was detected between mouse LIS-1 and human LIS-1, which differed by only 1 out of 410 amino acids (Peterfy et al., 1994). Both sets of authors argue that a protein so highly conserved across mammalian species must play an essential role in cellular processes.

LIS-1 may contribute to the lissencephaly syndromes by altering the normal migration of neurons during development. One possible mechanism is suggested by the similarity of LIS-1 to PAFAH. This latter protein is known to contribute to the regulation of calcium levels within cells, and it is thought to modulate rapid changes in intracellular calcium concentration (Honda et al., 1991; Nakamura et al., 1991a; Yu & Feuerstein, 1994; Yue et al., 1992). A rapid rise in intracellular calcium levels has multiple biological effects. One of these is the alteration of the meshwork of supportive fibers within the cell, which is called the cytoskeleton. Alterations in the cytoskeleton would affect the maturation of neurons and ultimately their migration patterns (Komuro & Rakic, 1992; Rakic & Komuro, 1995). In theory, then, a mutation in LIS-1 would result in abnormal intracellular calcium levels and a disruption in normal brain architecture.

During fetal life, all of the neurons of the CNS are produced by cell divisions in a thin layer of the CNS, the ventricular zone. As CNS development proceeds, neurons migrate out from the ventricular zone into overlying regions, eventually forming the six distinct layers of the cerebral cortex. Some of the highest levels of LIS-1 in the CNS were detected in the ventricular zone, the cortical plate, and the developing cortex (Reiner et al., 1995), a finding consistent with the enzyme's putative role in neuronal differentiation or migration.

Although impressive strides have been made in the understanding of LIS-1 and its potential role in lissencephaly syndromes, additional research is necessary to fill in the details surrounding its function. It is unclear at the present time whether LIS-1 is necessary

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for the initiation of neuronal migration or for some other step in the process by which neurons find their ultimate destination and connectivity. Future research will attempt to address these issues by generating knockout mice that will be used to examine the resulting neuronal migration patterns.

Future Directions and Conclusions

Research in the past two decades has produced tremendous advances in recombinant DNA techniques. In this article we have reviewed the successful application of these methodologies to four developmental disorders of childhood. The findings not only confirm the complexity of normal development but also provide an outline of the steps used to identify and characterize genes contributing to neuropsychiatric disorders.

A key finding that has emerged from the recent investigations of these four disorders includes the demonstration that mutations of single genes can lead to complex behavioral symptoms. For example, in PWS and fragile X syndrome, multiple systems are affected, including the regulation of food intake, cognition, temperament, and behavior. The proteins believed to be responsible for these disorders produce their effects by interacting with other intracellular components. Thus, the splicing factor N in PWS is believed to edit multiple brain transcripts, while the FMR-1 protein is thought to be a necessary component for the translation of neuronal proteins. In addition, significant interference of maternal nurturing behavior occurs in mice after the knockout of the transcription factor *fosB*, a gene that regulates the expression of additional genes. Thus, a major conclusion from these studies is that disruption of normal development may result after the mutation of a single gene, especially if the affected protein is itself essential for the expression or processing of multiple downstream genes.

Several points should also be made regarding the molecular findings for lissencephaly and Williams syndrome. The process of wiring the nervous system during development is enormously complex. Billions of neurons must first establish their proper identities, migrate considerable distances to their final destination points, send out their own dendritic and axonal processes, and finally establish proper connections within specific brain regions. Disruptions of earlier stages of neurodevelopment, such as the establishment of neuronal identity or proper migration patterns, will generally lead to more global disturbances in CNS development and, consequently, have a greater effect on cognitive functioning. Thus, lissencephaly usually leads to profound retardation, because the early migration of cortical neurons to the gray matter of the cortex is disrupted. In Williams syndrome the disruption occurs later in neurodevelopment, presumably when neurons have already established their identities and have migrated to their final sites. This may in part explain the relative sparing of certain cognitive skills in this syndrome. The LIM-kinase protein that appears to be mutated is involved in signaling pathways within more mature neurons. It is intriguing to speculate that the visuospatial deficits that occur are the result of a disruption within highly specialized neurons of the parietal lobes required for the establishment and consolidation of this particular cognitive skill.

Our understanding of normal and abnormal brain development has been helped enormously over the years by the analyses of mice born with specific brain abnormalities. A number of these “accidents of nature” were discovered in various laboratories in mice displaying distinctive gaits or abnormal brain growth. These mice were kept alive in the hope that one day molecular techniques would improve to the point of being able to identify the specific mutations responsible for the brain pathology. Investigators are now characterizing many of the genes responsible for the observed CNS abnormalities. They have discovered several proteins that are critical for normal brain development in these mice. Moreover, all of the characterized proteins have also been discovered to be present in humans and are believed to be critical factors for human brain development as well. Once again, we see that studies of normal function and psychopathology are mutually informative.

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One example of the information gleaned from these animal models is a better appreciation of how the normal layering of the cerebral cortex occurs. Neurons that are migrating into the growing cortex require support from radial glia cells that help to guide them from deep within the brain where they are born to their final destination. A number of proteins have been recently identified that are secreted into the extracellular matrix to guide the migrating neurons. These factors have either attractive or repulsive properties toward the growing neurons and instruct them exactly when and where to stop their migration. Researchers have suggested that mutations of these factors will be found in a number of neuropsychiatric disorders in which subtle abnormalities of neuronal migration and architecture have occurred. It is to be expected that investigations of this kind will continue over the next several years.

Many aspects of the initial phases of neuronal development appear to be relatively "hard wired." Thus, the birth of neurons, their differentiation, and initial migration patterns are dictated to a large extent by the precise turning on and off of CNS genes. Of course, environmental factors such as intrauterine viral infections, drugs, or toxins are able to disrupt normal growth, although exactly how this occurs in most cases remains unclear. In contrast, in later phases of nervous system development when synaptic connections are being established between neurons, environmental factors play a much more critical role. It has been established that neuronal activity is a necessary component to the establishment or strengthening of synaptic connections. This has been most clearly elucidated within the visual system where environmental stimulation of the developing visual pathways is a critical factor leading to the proper organization of the visual cortex. Moreover, synaptic activity within the hippocampus is required for many aspects of memory consolidation and learning.

These discussions clearly show that genetic and environmental factors both contribute to the normal developmental process. The molecular studies reviewed here have addressed the genetic side of this equation. It will be equally important to investigate in greater detail the contribution of environmental factors and explain exactly how they affect and interact with our genetic blueprint. These studies will hopefully focus on how factors such as poor nutrition, stress, or lack of a stimulating environment lead to developmental difficulties. Or, conversely, what are the genetic factors, if any, that buffer some individuals, and lead to a relatively normal development despite overwhelming environmental disadvantages? Such studies will be more easily conducted initially in animal models where either genetic or environmental factors may be kept relatively constant.

In what directions will these research endeavors move over the next years? Certainly the details of the cell biology for the four disorders we have discussed will be clarified. Efforts will continue to determine exactly how protein N, FMR-1, LIM-K, and LIS-1 exert their biological effects; in particular it is likely that the various accessory proteins and substrates for these proteins will be identified. Researchers will also turn to other developmental disorders caused by mutations of single genes and attempt to pinpoint their genetic etiologies.

However, as discussed earlier, the vast majority of psychiatric disorders are probably not caused by single-gene mutations. Single-gene disorders were studied first for the simple reason that the responsible genes proved to be much easier to identify than the multiple environmental and genetic factors that contribute to the etiology of more complex behavioral disorders. For the latter, researchers will need to use recently developed association strategies that do not make assumptions regarding inheritance patterns or the number of genes that are involved in the expression of a particular disorder. Moreover, it is becoming clear that for many disorders, there is only a small contribution by each of these genes and environmental factors to the ultimate expression of symptoms. Teasing apart exactly how each of these factors make a particular individual more, or less, vulnerable to a specific syndrome will be the next challenge of basic research.

Once the molecular bases for disorders be-

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gan to be discovered, expectations were high that gene therapies might be initiated. If researchers were capable of removing normal genes when generating knockout mice, would it not be as easy to reverse the process and replace a defective gene with a normal copy? The early excitement for this work has been replaced by considerably more caution. Attempts to replace genes in humans are fraught with technical difficulties. For example, extensive effort has been expended for gene therapy with patients with cystic fibrosis. In this single-gene disorder that affects the ability to produce secretions within the lungs, it was expected that scientists would rapidly develop the technology to restore a normal copy of the cystic fibrosis gene to the epithelial cells within the lungs. To date, these efforts have been unsuccessful because efficient methods to deliver the normal gene to the appropriate tissue and in sufficient quantities are lacking.

The situation is many times more complex in developmental disorders in which affected genes may be expressed only during critical periods of brain development. Introducing a normal copy of a defective gene will first require knowledge that a mutation exists in the fetus. For the majority of neurodevelopmental and mental retardation syndromes, there is no prior family history and thus no way of knowing that there is a problem with the growing nervous system. In addition, methods have not yet been developed to introduce a normal copy of a gene early in embryonic development. These considerations raise a number of significant ethical questions that must be addressed before genetics-based treatments for mental retardation syndromes and other child psychiatric disorders are considered over the next decades.

It should be emphasized that the molecular findings discussed in this article do not diminish the importance of experiential and environmental factors in determining disease pathogenesis and clinical outcome. Indeed, the clinical course in a patient is the result of an interaction of genetic, cultural, familial, therapeutic, and environmental factors and adopting a viewpoint of genetic determinism would be incorrect. As we discussed earlier, basic research has determined that environmental factors affect the extent to which certain genes are expressed, although exactly how this occurs and the molecular bases for these interactions will be the focus for future investigations. We anticipate that the next several years will clarify some of the details of the delicate interplay between these factors and the complex process of development.

References

Abeliovich, A. C., Goda, Y., Silva, A., Stevens, C., & Tonegawa, S. (1993). Modified hippocampal long-term potentiation in PKC gamma-mutant mice. *Cell, 75*, 1253–1262.

Anderson, S., Qiu, M-S., Bulfone, A., Meneses, J., Pederson, R., & Rubenstein, J. (1995). Functional analysis of DLX-1 and DLX-2 using gene replacement. *Society for Neuroscience Abstract,* 32018.

Baumgardner, T., Reiss, A., Freund, L., & Abrams, M. (1995). Specification of the neurobehavioral phenotype in males with fragile X syndrome. *Journal of Pediatrics, 95*, 744–752.

Bell, M., Hirst, M., Nakahori, Y., MacKinnon, R., Roche, A., Flint, T., Jacobs, P., Tommerup, N., Tranebjerg, L., Froster–Iskenius, U., Kerr, B., Turner, G., Lindenbaum, R., Winter, R., Pembrey, M., Thibodeau, S., & Davies, K. (1991). Physical mapping across the fragile X: Hypermethylation and clinical expression of the fragile X syndrome. *Cell, 64,* 861–866.

Benjamin, J., Li, L., Patterson, C., Greenberg, B., Murphy, D., & Hamer, D. (1996). Population and familial association between the D4 dopamine receptor gene and measures of novelty seeking. *Nature Genetics, 12*, 81–84.

Bregman, J., Dykens, E., Watson, M., Ort, S., & Leckman, J. (1987). Fragile X syndrome: Variability of phenotypic expression. *Journal of the American Academy of Child and Adolescent Psychiatry, 4*, 463–471.

Brown, J., Ye, H., Bronson, R., Dikkes, P., & Greenberg, M. (1996). A defect in nurturing in mice lacking the immediate early gene fosB. *Cell, 86*, 297–309.

Cassidy, S. (Ed.). (1992). *Prader–Willi syndrome and other chromosome 15q deletion disorders*. Berlin: Springer–Verlag.

Cattanach, B., Barr, J., Evans, E., Burtenshaw, M., Beechey, C., Leff, S., Brannan, C., Copeland, N., Jenkins, N., & Jones, J. (1992). A candidate mouse model for Prader–Willi syndrome. *Nature Genetics, 2*, 270–274.

Cicchetti, D. (1993). Developmental psychopathology: Reactions, reflections, projections. *Developmental Review, 13*, 471–502.

Cicchetti, D., & Cohen, D. (1995). Perspectives on devel-

https://doi.org/10.1017/S0954579498001503 Published online by Cambridge University Press

opmental psychopatholgy. In D. Cicchetti & D. Cohen (Eds.), *Developmental Psychopathology* (Vol. 1, pp. 3–20). New York: Wiley.

Dobyns, W., Reiner, O., Carrozzo, R., & Ledbetter, D. (1993). Lissencephaly: A human brain malformation assocaited with deletion of the *LIS1* gene located at chromosome 17p13. *Journal of the American Medical Association, 270,* 2838–2842.

Dobyns, W., & Truwit, C. (1995). Lissencephaly and other malformations of cortical development: 1995 update. *Neuropediatrics, 26,* 132–147.

Dykens, E., & Cassidy, S. (1995). Correlates of maladaptive behavior in childen and adults with Prader–Willi syndrome. *American Journal of Medical Genetics (Neuropsychiatry), 60,* 546–549.

Dykens, E., Leckman, J., & Cassidy, S. (in press). Obsessions and compulsions in Prader–Willi syndrome. *Journal of Child Psychology and Psychiatry*.

Ebstein, R., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D., Bennett, E., Nemanov, L., Katz, M., & Belmaker, R. (1996). Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. *Nature Genetics, 12,* 78–80.

Ewart, A., Morris, C., Atkinson, D., Jin, W., Sternes, K., Spallone, P., Stock, A., Leppert, M., & Keating, M. (1993). Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. *Nature Genetics, 5,* 11–16.

Field, L., Tobias, R., Thomson, G., & Plon, S. (1996). Susceptibility to insulin-dependent diabetes mellitus maps to a locus (IDDM11) on human chromosome 14q24.3-q31. *Genomics, 33,* 1–8.

Frangiskakis, J., Ewart, A., Morris, C., Mervis, C., Betrand, J., Robinson, B., Klein, B., Ensing, G., Everett, L., Green, E., Proschel, C., Gutowski, N., Noble, M., Atkinson, D., Odelberg, S., & Keating, M. (1996). *LIM-kinase1* hemizygosity implicated in impaired visuospatial constructive cognition. *Cell, 86,* 59–69.

Fu, Y., Kuhl, D., Pizzuti, A., Pieretti, M., Sutcliffe, J., Richards, S., Verkerk, A., Holden, J., Fenwick, R., Jr., Warren, S., Oostra, B., Nelson, D., & Caskey, C. (1991). Variation of the CGG repeat at the fragile X site results in genetic instability: Resolution of the Sherman paradox. *Cell, 67,* 1047–1058.

Glenn, C., Saitoh, S., Jong, M., Filbrandt, M., Surti, U., Driscoll, D., & Nicholls, R. (1996). Gene structure, DNA methylation, and imprinted expression of the human SNRPN gene. *American Journal of Human Genetics, 58,* 335–346.

Gosch, A., & Pankau, R. (1994). Social–emotional and behavioral adjustment in children with Williams–Beuren syndrome. *American Journal of Medical Genetics, 53,* 335–339.

Gosch, A., & Pankau, R. (1996). Longitudinal study of the cognitive development in children with Williams–Beuren syndrome. *American Journal of Human Genetics, 61,* 26–29.

Grant, S., O'Dell, T. J., Karl, K., Stein, P., Soriano, P., & Kandel, E. (1992). Impaired long-term potentiation, spatial learning, and hippocampal development in *fyn* mutant mice. *Science, 258,* 1903–1910.

Gusella, J., Wexler, N., Conneally, P., Naylor, S., Anderson, M., Tanzi, R., Watkins, P., Ottina, K., Wallace, M., & Sakaguchi, A. (1983). A polymorphic DNA marker genetically linked to Huntington's disease. *Nature, 306,* 234–238.

Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., & Inoue, K. (1994). Miller–Dieker lissencephaly gene encodes a subunit of brain platelet-activationg factor. *Nature, 370,* 216–218.

Hinds, H., Ashley, C., Sutcliffe, J., Nelson, D., Warren, S., Housman, D., & Schalling, M. (1993). Tissue specific expression of FMR-1 provides evidence for a functional role in fragile X syndrome. *Nature Genetics, 3,* 36–43.

Honda, Z., Nakamura, M., Miki, I., Minami, M., Watanabe, T., Seyama, Y., Okado, H., Toh, H., Ito, K., Miyamoto, T., & Shimuzu, T. (1991). Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. *Nature, 349,* 342–346.

Hyman, S., & Nestler, E. (1996). Initiation and adaptation: A paradigm for understanding psychotropic drug action. *American Journal of Psychiatry, 153,* 151–162.

Kemper, M., Hagerman, R., Ahmad, R., & Mariner, R. (1986). Cognitive profiles and the spectrum of clinical manifestations in heterzygous fra(X) females. *American Journal of Medical Genetics, 23,* 139–156.

Khandijan, E., Corbin, F., Woerly, S., & Rousseau, F. (1996). The fragile X mental retardation protein is associated with ribosomes. *Nature Genetics, 12,* 91–93.

Knight, S., Flannery, A., Hirst, M., Campbell, L., Christodoulou, Z., Phelps, S., Pointon, J., Middleton–Price, H., Barnicoat, A., Pembrey, M., Holland, J., Oostra, B., Bobrow, M., & Davies, K. (1993). Trinucleotide repeat amplification and hypermethylation of a CpG island in *FRAXE* mental retardation. *Cell, 74,* 127–134.

Komuro, H., & Rakic, P. (1992). Selective role of N-type calcium channels in neuronal migration. *Science, 257,* 806–809.

Ledbetter, D., Riccardi, V., Airhart, S., Strobel, R., Keenan, B., & Crawford, J. (1981). Deletions of chromosome 15 as a cause of the Prader–Willi syndrome. *New England Journal of Medicine, 304,* 325–329.

Leff, S., Brannan, C., Reed, M., Ozcelik, T., Francke, U., Copeland, N., & Jenkins, N. (1992). Maternal imprinting of the mouse *Snrpn* gene and conserved linkage homology with the human Prader–Willi syndrome region. *Nature Genetics, 2,* 259–264.

Lichter, J., Barr, C., Kenned, J., Van Tol, H., Kidd, K., & Livak, K. (1993). A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. *Human Molecular Genetics, 2,* 767–773.

Lombroso, P., Pauls, D., & Leckman, J. (1994). Genetics mechanisms in childhood psychiatric disorders. *Journal of the American Academy of Child and Adolescent Psychiatry, 33,* 921–938.

Lowery, M., Morris, C., Ewart, A., Brothman, L., Zhu, X., Leonard, C., Carey, J., Keating, M., & Brothman, A. (1995). Strong correlation of elastin deletions, detected by FISH, with Williams syndrome: Evaluation of 235 patients. *American Journal of Human Genetics, 57,* 49–53.

Lubs, H. (1969). A marker X chromosome. *American Journal of Human Genetics, 21,* 231–244.

Martin, J. P., & Bell, J. (1943). A pedigree of mental defect showing sex-linkage. *Journal of Neurology and Psychiatry, 6,* 154–157.

McAllister, G., Amara, S., & Lerner, M. (1988). Tissue-specific expression and cDNA cloning of small nuclear ribonucleoprotein-associated polypeptide N. *Proceedings of the National Academy of Science USA, 85,* 5296–5300.

Mizuno, K., Okano, I., Ohashi, K., Nunoue, K., Kuma,

https://doi.org/10.1017/S0954579498001503 Published online by Cambridge University Press

K., Miyata, T., & Nakamura, T. (1994). Identification of a human cDNA encoding a novel protein kinase with two repeats of the LIM/double zinc finger motif. *Oncogene, 9,* 1605–1612.

Morris, C., Demsey, S., Leonard, C., Dilts, C., & Blackburn, B. (1988). Natural history of Williams syndrome: Physical characteristics. *Journal of Pediatrics, 113,* 318–326.

Musco, G., Stier, G., Joseph, C., Morelli, M., Nilges, M., Gibson, T., & Pastore, A. (1996). Three dimensional structure and stability of the KH domain: Molecular insights into the fragile X syndrome. *Cell, 85,* 237–245.

Nakamura, T., Honda, Z., Izumi, T., Sakanaka, C., Mutoh, H., Minami, M., Bito, H., Seyama, Y., Matsumoto, T., & Noma, M. (1991). Molecular cloning and expression of platelet-activating factor receptor from human leucocytes. *Journal of Biological Chemistry, 266,* 20400–20405.

Nelson, D. (1995). The fragile X syndromes. *Seminars in Cell Biology, 6,* 5–11.

Nelson, D., & Warren, S. (1993). Trinucleotide repeat instability: When and where? *Nature Genetics, 4,* 107–108.

Nicholls, R., Knoll, J., Butler, M., Karam, S., & Lalande, M. (1989). Genetic imprinting suggested by maternal heterodisomy in nondeletion Prader–Willi syndrome. *Nature, 342,* 281–285.

Nickerson, E., Greenberg, F., Keating, M., McCaskill, C., & Shaffer, L. (1995). Deletions of the elastin gene at 7q.11.23 occur in approximately 90% of patients with Williams syndrome. *American Journal of Human Genetics, 56,* 1156–1161.

Ozcelik, T., Leff, S., Robinson, W., Donlon, T., Lalande, M., Sanjines, E., Shinzel, A., & Francke, U. (1992). Small nuclear ribonucleoprotein polypeptide N (SNRPN) and expressed gene in the Prader Willi syndrome critical region. *Nature Genetics, 2,* 265–269.

Peoples, R., Perez–Jurado, L., Wang, Y., Kaplan, P., & Francke, U. (1996). The gene for replication factor C subunit 2 (RFC2) is within the 7q11.23 Williams syndrome deletion. *American Journal of Human Genetics, 58,* 1370–1373.

Peterfy, M., Gyuris, T., Basu, R., & Takacs, L. (1994). Lissencephaly-1 is one of the most conserved proteins between mouse and human: A single amino-acid difference in 410 residues. *Gene, 150,* 415–416.

Plissart, L., Borghgraef, M., Volcke, P., Van den Berghe, H., & Fryns, J. (1994). Adults with Williams–Beuren syndrome: Evaluation of the medical, psychological and behavioral aspects. *Clinical Genetics, 46,* 161–167.

Proschel, C., Blouin, M., Gutowski, N., Ludwig, R., & Noble, M. (1995). Limk1 is predominantly expressed in neural tissues and phosphorylates serine, threonine and tyrosine residues in vitro. *Oncogene, 11,* 1271–1281.

Rakic, P., & Komuro, H. (1995). The role of receptor/channel activity in neuronal cell migration. *Journal of Neurobiology, 26,* 299–315.

Reiner, O., Albrecht, U., Gordon, M., Chianese, K., Wong, C., Gal–Gerber, O., Sapir, T., Siracusa, L., Buchberg, A., Caskey, C., & Eichele, G. (1995). Lissencephaly gene expression in the CNS suggests a role in neuronal migration. *Journal of Neuroscience, 15,* 3730–3738.

Reiner, O., Carrozzo, R., Shen, Y., Whenert, M., Faustinella, F., Dobyns, W., Caskey, C., & Ledbetter, D. (1993). Isolation of a Miller–Dieker lissencephaly gene containing G protein β-subunit-like repeats. *Nature, 364,* 717–721.

Rende, R., & Plomin, R. (1995). Nature, nurture, and the development of psychopathology. In D. Cicchetti & D. Cohen (Eds.), *Developmental Psychopathology* (Vol. 1, pp. 291–314). New York: Wiley.

Rowe, R., Wapelhorst, B., Bell, G., Risch, N., Spielman, R., & Concannon, P. (1995). Linkage and association between insulin-dependent diabetes mellitus (IDDM) susceptibility and markers near the glucokinase gene on chromosome 7. *Nature Genetics, 10,* 240–242.

Rutter, M. (1991). Nature, nurture, and psychopathology. *Development and Psycyopathology, 3,* 125–136.

Rutter, M. (1994). Psychiatric genetics: Research challenges and pathways forward. *American Journal of Medical Genetics, 54,* 185–198.

Rutter, M., Bolton, P., Harrington, R., Le Couteur, A., Macdonald, H., & Simonoff, E. (1990a). Genetic factors in child psychiatric disorders. I. A review of research strategies. *Journal of Child Psychology and Psychiatry, 31,* 3–37.

Rutter, M., Macdonald, H., Le Couteur, A., Harrington, R., Bolton, P., & Bailey, A. (1990b). Genetic factors in child psychiatric disorders. II. Empirical findings. *Journal of Child Psychology and Psychiatry, 31,* 39–83.

Sanchez–Garcia, I., & Rabbitts, T. (1994). The LIM domain: A new structural motif found in zinc-finger-like proteins. *Trends in Genetics, 9,* 315–320.

Schmauss, C., Brines, M., & Lerner M. (1992). The gene encoding the small nuclear ribonucleoprotein-associated protein N is expressed at high levels in neurons. *Journal of Biological Chemistry, 267,* 8521–8529.

Schulze, A., Hansen, C., Skakkebaek, N., Brondum–Nielsen, K., Ledbetter, D., & Tommerup, N. (1996). Exclusion of *SNRPN* as a major determinant of Prader–Willi syndrome by a translocation breakpoint. *Nature Genetics, 12,* 452–454.

Sherman, S., Jacobs, P., Morton, N., Froster–Iskenius, U., Howard–Peebles, P., Nilsen, K., Partington, M., Sutherland, G., Turner, G., & Watson, M. (1985). Further segregation analysis of the fragile X syndrome with special reference to transmitting males. *Human Genetics, 69,* 289–299.

Siomi, H., Siomi, M., Nussbaum, R., & Dreyfuss, G. (1993). The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. *Cell, 74,* 291–298.

Siomi, H., Choi, M., Siomi, M., Nussbaum, R., & Dreyfuss, G. (1994). Essential role for KH domains in RNA binding: Impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. *Cell, 77,* 33–39.

Snow, K., Doud, L., Hagerman, R., Pergolizzi, R., Erster, S., & Thibodeau, S. (1993). Analysis of CGG sequence at the FMR-1 locus in fragile X families and in the general population. *American Journal of Human Genetics, 53,* 1217–1228.

Tassabehji, M., Metcalfe, K., Fergusson, W., Carette, M., Dore, J., Donnai, D., Read, A., Proschel, C., Gutowski, N., Mao, X., & Sheer, D. (1996). LIM-kinase deleted in Williams syndrome. *Nature Genetics, 13,* 272–273.

Turner, G., Daniel, A., & Frost, M. (1980). X-linked mental retardation, macro-orchidism, and the Xq27 fragile site. *Journal of Pediatrics, 96,* 837–841.

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Warren, S. (1996). The expanding world of trinucleotide repeats. *Science, 271,* 1374–1375.

Williams, J., Barratt–Boyes B., & Lowe, J. (1961) Supravalvular aortic stenosis. *Circulation, 24,* 1311–1318.

Yue, T., & Feuerstein, G. (1994). Platelet-activating factor: A putative neuromodulator and mediator in the pathophysiology of brain injury. *Critical Review in Neurobiology, 8,* 11–24.

Yue, T., Stadel, J., Sarau, H., Friedman, E., Gu, J., Powers, D., Gleason, M., Feuerstein, G., & Wang, H. (1992). Platelet activating factor stimulates phosphoinositide turnover in neurohybrid NCB-20 cells: Involvement of pertussis toxin-sensitive guanine nucleotide-binding proteins and inhibition by protein kinase C. *Molecular Pharmacology, 41,* 281–289.

https://doi.org/10.1017/S0954579498001503 Published online by Cambridge University Press