BEHAVIORAL PHENOTYPES OF NEURODEVELOPMENTAL DISORDERS: PORTALS INTO THE DEVELOPING BRAIN

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HISTORICAL BACKGROUND

Increasing evidence indicates that specific neurodevelopmental disorders may be associated with particular patterns of behavior. A description of behavior was included by Langdon Down in the first published description of a specific mental retardation syndrome, Down syndrome (1). In his description, Down observed: "They have considerable powers of imitation, even bordering on being mimics. Their humorousness and a lively sense of the ridiculous often colors their mimicry." Later, he added: "Several patients who have been under my care have been wont to convert their pillow cases into surplices (vestments) and to imitate, in tone and gesture, the clergymen or chaplain which they have recently heard." He also commented on personality traits, saying: "Another feature is their great obstinacy—they can only be guided by consummate tact." Although these stereotypes were not confirmed in subsequent studies (2,3), the prospect of linking behavior and genetics was introduced in this first description of a neurogenetic disorder. Subsequent early clinical descriptions, such as that of tuberous sclerosis complex by Critchley and Earl (4), identified peculiar, and severe, behavioral problems in children and adults with that condition. Yet despite the early recognition of syndrome-specific behavioral and psychiatric features, neurogenetic disorders were not empirically investigated for behavioral deficits until 1990s, when new conceptual and methodologic procedures were introduced (5).

Two main reasons may explain this lack of interest after the early reports by Down and others. First, there was a general negative reaction against eugenics and claims for genetic bases of personality (6). This negative reaction estab-

lished a climate in which it was not considered appropriate for academic investigators to emphasize the genetics of behavior. Second, there has been a major emphasis on learning theory and its applications to the field of mental retardation, in which most genetic disorders are found. Tremendous strides have been made in the education of even the most severely mentally retarded persons. Advances in academic and social adaptive education, in conjunction with motor treatment, have placed greatest emphasis on how severely and multiply handicapped people could attain greater degrees of independence and social integration. With the emphasis on normalization, research into severe disorders in learning tended to be deemphasized. Moreover, the occurrence of associated psychiatric and behavioral problems was interpreted more in terms of learning theory rather than in being unlearned behaviors associated with behavioral phenotypes. The focus has been on addressing the potential of the individual person and the developmental possibilities. Yet this focus could not continue to ignore reports from families and clinical observations of characteristic patterns of behavior and stereotypes.

With the establishment of active and refined learningbased approaches and a better understanding of the interpretation of genetic findings, reappraisal and revision of attitudes toward research with behavioral phenotypes have begun. O'Brien suggested three reasons for this shift (7). First, research findings have been reliably reported with various syndromes. Second, there are continued reports from family members as large family organizations have developed in the United States and other countries that describe characteristic behavioral patterns and interpersonal responses. In meetings, parent groups frequently report similar behavior problems and difficulties in management across syndromes. The interest in parent groups in improving the life of their children has led to additional hypotheses and more refined observations on behavioral characteristics. Third, new techniques in genetics provide new insights into

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the extent and mechanisms of the human genome as the basis of behavior. Advances in other aspects of neuroscience, including neurophysiology and neuroanatomy, provide additional means of designating brain mechanisms that may be involved. With the establishment of these new methods of evaluation and the identification of rating scales to measure behavioral phenotypes, there is now an increased focus on behavioral phenotypes in developmental neuropsychiatry. Finally, comprehensive study of children with different developmental disabilities may increase our appreciation of the relative contribution of genetic variables in the pathogenesis of specific affective and behavioral disorders.

Nyhan introduced the term behavioral phenotype to describe outwardly observable behavior so characteristic of children with genetic disorders that its presence suggests the underlying genetic condition (8). In speaking of compulsive self-injury in Lesch-Nyhan disease (LND), a disorder that he initially described, Nyhan noted: "We feel that these children have a pattern of unusual behavior that is unique to them. Stereotypical patterns of behavior occurring in syndromic fashion in sizable numbers of individuals provide the possibility that there is a concrete explanation that is discoverable. In these children, there are so many anatomical abnormalities, from changes in hair and bones to dermatoglyphics, that it is a reasonable hypothesis that their behaviors are determined by an abnormal neuroanatomy that would be discoverable, possibly neurophysiologically, ultimately anatomically . . . these children all seem self-programmed. These stereotypical patterns of unusual behavior could reflect the presence of structural deficits in the central nervous system" (8).

Such observations have led to greater emphasis on assessment of behavior, and the recognition of behavioral phenotypes in some disorders has led to closer scrutiny of known neurodevelopmental conditions. Initially, the focus was on documenting the patterns of behavior because the study of brain and behavior requires the identification of welldefined syndromes for investigation. Now that developments in the neurosciences provide a means to understand the biological bases of such behavioral patterns, the focus has shifted to understanding the neurobiological mechanisms underlying characteristic behavioral patterns, including cognitive processes and social interactions. Such patterns are reported in numerous syndromes arising from genetic or chromosomal abnormalities. Thus, molecular analysis of the underlying genetic disorder has been initiated in several syndromes with the hope of revealing the biological basis of the behavioral phenotype. However, because of the rarity of many of these syndromes and the complexity of their genetic basis, establishing the validity of the association between syndrome and behavioral phenotype is difficult. Nevertheless, Flint pointed out that evidence from animal studies with relevance to human behavioral phenotypes shows that the pathway from genotype to phenotype may be accessible after careful delineation of each of the features of the behavioral phenotypes (9,10). However, in regard to the study of cognition, he suggested that we require a greater integration of different levels of understanding of cognition to exploit the genetic discoveries, "a rapprochement between molecular and systems neuroscience" (10).

Much of the research in behavioral genetics uses a "topdown" approach to the qualitative analysis of complex traits such as novelty seeking, memory, personality traits, and intelligence (11). Linkage or association strategies are used to examine naturally occurring alleles of candidate genes in a "wild-type population." These alleles are usually functional polymorphisms rather than mutations and, if they are quantitative trait loci, may be associated with individual differences in the trait in question. However, Tully suggested that such genes may have minor effects on the phenotype of the individual because alleles with a more striking effect could reduced fitness and would be selected against in evolution (12). Specifically, chromosomal deletions that may have cognitive and behavioral consequences may be associated with monosomy (13). The loss of one copy of genes that are dose sensitive may be significant in brain development. Such genes may play a fundamental role in development of the functional organization of the brain, but they may not be as important for individual differences in the general population. Moreover, partial variants of disorders such as LND that result in a range of enzyme levels may allow study of dose response to enzyme deficits.

This chapter uses a developmental perspective to provide a definition and characterization of behavioral phenotypes in neurodevelopmental disorders, and it discusses etiologic factors, methods to understand underlying mechanisms, and natural history. It addresses the question: What do behavioral phenotypes that occur in specific neurogenetic disorders teach us, and how may they provide a portal to understand the developing brain? This question is considered by reviewing studies of neurogenetic disorders with behavioral phenotypes: (a) LND, an X-linked disorder, that results from the absence of an enzyme, hypoxanthine-guanine phosphoribosyltransferase (HPRT), that is involved in purine metabolism; (b) Prader-Willi syndrome (PWS) and Angelman syndrome (AS), in which the parental origin of the genes involved (uniparental disomy or UPD) is an important factor in the cause; (c) fragile X syndrome, a disorder caused by unstable trinucleotide repeat expansion that results in the absence of a gene that encodes an RNA-binding protein thought to play a role in translational regulation of selective messenger RNA transcripts; and (d) Williams syndrome (WMS), a contiguous gene disorder with an unusual cognitive phenotype in which language is preserved but the patient has severe visual spatial disabilities. Each of these neurogenetic disorders provides a portal to understand neurodevelopment. Other nongenetic disorders that are environmentally induced, such as fetal alcohol syndrome, are not discussed but also offer keys to understanding the developing brain (23).

DEFINITION AND CHARACTERIZATION

The study of behavioral phenotypes emphasizes the discovery, among individuals with known chromosomal, genetic, or neurodevelopmental disorders, of those mental and behavioral features causally related to the underlying condition. Examples are the characteristic self-mutilation of fingers and lips in LND, the hyperphagia and compulsive behaviors in PWS, gaze aversion in fragile X syndrome, and the superficial sociability, hyperlalia, and language disorder in WMS. When present, the behavior suggests the syndrome. As Nyhan suggested, these are "syndromes of behavior" (14). Still, despite their behavioral presentations, not all individuals with the disorder show the classic behavioral features, but the probability is greater that they will. The essential issue is that the behavior suggests the diagnosis.

Efforts to define what is meant by a behavioral phenotype are continuing. Harris proposed that behavioral phenotypes are stereotypic patterns of behavior that are reliably identified in groups of individuals with known neurodevelopmental disorders and are "not learned" (15,16). They may be the consequence of neurodevelopmental abnormalities that are potentially discoverable. This approach to definition is a phenonic approach that takes as its starting point observations of the behavior itself rather than beginning with a discrete and genetically identifiable condition, such as Down syndrome. Using the phenomic approach, the behavioral phenotype of Rett syndrome (17), with its characteristic hand and hand-to-mouth stereotypies, identified it as disorder with a behavior phenotype many years before the genetic origin was recognized. Moreover, the phenomic approach does not discount acquired disorders, such as fetal alcohol syndrome, as having behavioral phenotypes. The impact of alcohol on cellular signaling is now well known, with its consequences of cell death, abnormal midline brain development, behavioral problems, and learning disabilities (16,23).

Such considerations led Flint and Yule to propose the following definition that includes the characteristic types of behaviors: "The behavioral phenotype is a characteristic pattern of motor, cognitive, linguistic, and social abnormalities that is consistently associated with a biological disorder" (18). This does not mean that the behavior is present in all instances but that the probability of its occurrence is increased. In the future, more may be learned about brain mechanisms by comparing persons with behavioral involvement with others who have the same syndrome but without the behavioral features.

Although some investigators have sought to limit the study of behavioral phenotypes to known genetic disorders (11), knowledge of the genetic disorder is only the first step. Links from gene to behavior are complicated in that one gene may lead to the encoding of many, perhaps ten or more, different proteins; the number of genes and type of mutation determine complexity. For example, in LND, the

disorder of purine metabolism clearly leads to the overproduction of uric acid and renal stones, but the pathway to the movement disorder and self-injury is not direct and may be mediated through effects on the arborization of dopamine neurons (19). Moreover, there are variants of LND with different degrees of enzyme deficit, ranging up to 20%, that have clinical effects.

Thus, several caveats are necessary as we consider pathways from genes to behavior (11): (a) the behavioral descriptions, like other physical features of neurodevelopmental disorders, have increased probability of occurring and do not occur in all cases; they are not be fully expressed in all affected individuals; (b) the genetic background of the individual may affect the phenotypic expression; (c) the possibility exists that environmental factors may modify expression; (d) the behavioral presentation may be modified by the extent of mental retardation associated with the disorder; and (e) variability occurs in mouse models in which there may be species-specific factors so mutant mouse models do not replicate the behavioral features. One must consider the genetic background, strain differences, and the differences in the rodent physiology. In LND, the HPRTdeficient mouse has a uricase enzyme that breaks down uric acid. Therefore, it is not a model for the hyperuricemic metabolic disorder, but it still may be a useful model to study dopamine deficiency in the brain. Thus, aspects of the disorder may be modeled in transgenic mice or in other

In some animal models, links to specific pathophysiology have been established. The canine model of narcolepsy (20) is an interesting example of an approach to a human clinical disorder. Mutations for canine, autosomal recessive, narcolepsy were identified by linkage analysis in canine backcrosses, and homology was demonstrated between human chromosome 6 and canine chromosome 12. Canine narcolepsy is caused by a disruption of a G-protein–coupled receptor, the hypocretin (orexin) receptor 2 gene (Hcrt2) in three canine breeds. However, human narcolepsy is not associated with frequent hypocretin gene mutation (20). Nonetheless, most humans with narcolepsy have undetectable hypocretin 1 levels in cerebrospinal fluid.

PSYCHOPATHOLOGY AND BEHAVIORAL PHENOTYPES

Numerous neurogenetic disorders are associated with nonspecific behaviors that may be found in several syndromes. These include attention problems, hyperactivity, impulsivity, self-injury, aggression, autistic-like behavior, and preservative behaviors. Such presentations indicate vulnerability of the developing brain and perturbation of brain systems resulting in these clinical conditions. However, because these behaviors occur across many syndromes, they lack specificity and do not qualify as specific behavioral phenotypes. Still, these behavioral features should be included in the description of the disorders. For example, the relationship between aggression and antisocial behavior has been suggested in monoamine oxidase A (MAOA) deficiency. Brunner et al. described an association between abnormal behavior and MAOA deficiency in several males from a single large Dutch kindred (21). The affected males differed from unaffected males in that they tested in the borderline range of mental retardation and demonstrated increased impulsive behavior, that is, aggressive behavior, abnormal sexual behavior, and arson. Yet a specific psychiatric diagnosis was not made in four affected males who were examined by psychiatrists. Because MAOA deficiency leads to increased 5-hydroxytryptamine (5-HT) levels, the aggressive behavior in these persons may be an exception to studies linking low 5-HT with impulsive aggression. Brunner et al. suggested that even if a possible association between MAOA deficiency and abnormal behavior is confirmed in other kindreds, "the data do not support the hypothesis that MAOA constitutes an 'aggression gene'." These investigators noted that genes are essentially simple and code for proteins, whereas behavior is complex; thus, a direct causal relationship between a single gene and a specific behavior is highly unlikely. In MAOA deficiency, complexity is shown by the variability in the behavioral phenotype and by the highly complex consequences of MAOA deficiency on neurotransmitter function. Thus, the full pathway from gene to complex behavior must be considered; the concept of a gene that directly encodes behavior is simplistic (21). Still, a great deal may be learned by considering such pathways in neurogenetic syndromes.

PREVALENCE

With increasing attention to neurogenetic disorders, the number of identifiable behavioral phenotypes is increasing. Careful observations of behavior are necessary when considering intervention for neurogenetic disorders. Although standardized rating scales and personality profiles have been developed to measure behavioral phenotypes (22,23), profiles pertinent to the specific disorder are needed. Besides behavioral phenotypes, isolated special abilities that occur in genetically based syndromes require assessment. These include special abilities in calculation and in music (24). These special abilities may potentially be related to the proposed modular organization of the central nervous system.

BEHAVIORAL PHENOTYPES OF SPECIFIC NEURODEVELOPMENTAL DISORDERS

The sections that follow discuss four syndromes in which behavioral phenotypes have been identified: LND, PWS/ AS, fragile X syndrome, and WMS. Characteristic behaviors are highlighted, findings on origin are discussed, and potential neurochemical and neuroanatomic abnormalities are reviewed. Behavioral and pharmacologic therapies have had limited success in many of these conditions, so better characterization of the individual condition is essential to establish treatment. Neuroanatomic studies, brain imaging studies, and continuing investigations of neurotransmitter systems, endocrine rhythms, and sleep studies may provide information that will be helpful in the future in treatment.

Lesch-Nyhan Disease

LND is a rare (1:380,000) sex-linked recessive disease caused by an inborn error of purine nucleotide metabolism. It is caused by an almost complete deficiency of the enzyme HPRT, which is involved in the purine salvage (purine base recycling) pathway (25). Self-injury is the major behavioral manifestation; this behavior was sufficiently characteristic that Nyhan introduced the term "behavioral phenotype" as a descriptor (8). LND is of psychosocial and psychiatric importance because of the lifelong suffering experienced by the involved child and his family, the uniqueness of the behavioral phenotype, and the resources needed for lifelong patient supervision. Moreover, an understanding of the neurobiological basis of this disease may contribute to a better understanding of brain mechanisms involved in self-injurious and compulsive behaviors.

Genetic and Metabolic Aspects

The HPRT-encoding gene is located on the X chromosome in the q26-q27 region and is made up of nine exons and eight introns totaling 57 kilobases (kb). The *HPRT* gene is transcribed to produce a mRNA of 1.6 kb that contains a protein-encoding region of 654 nucleotides. More than 270 mutations throughout the coding regions have been identified (79). Techniques that provide information on the three-dimensional structure of the HPRT protein make it possible to correlate structure and function of the enzyme (26). Eads et al. reported the effects of single amino acid substitutions on the stability and activity of HPRT (26).

The gene involved in LND is on the X chromosome, so the disorder occurs almost entirely in males; occurrence in females is extremely rare. The metabolic abnormality is the result of an abnormal gene product—a deficiency in the enzyme HPRT. This enzyme is normally present in each cell in the body and is highest in the brain, especially in the basal ganglia. Its absence prevents the normal metabolism of hypoxanthine and results in excessive uric acid production and manifestations of gout without specific drug treatment (i.e., allopurinol). The full disease requires the virtual absence of the enzyme. Other syndromes with partial HPRT deficiency are associated with gout without the neurologic and behavioral symptoms. Page and Nyhan reported that HPRT levels are related to the extent of motor symptoms,

the presence or absence of self-injury, and possibly the level of cognitive function (27). Hypoxanthine accumulates in the cerebral spinal fluid, but uric acid does not because it is not produced in the brain and does not cross the blood-brain barrier.

Behavioral Phenotype

Self-injurious behavior usually is expressed as self-biting; however, other patterns of self-injurious behavior may emerge with time. It is not uncommon for self-injury to progress to deliberate self-harm (19,28). Characteristically, the fingers, mouth, and buccal mucosa are mutilated. The biting pattern is often asymmetric, so the patient may mutilate the left or right side of the body and may become anxious if he perceives that this side of the body is threatened. Other associated maladaptive behaviors include head or limb banging, eye poking, pulling of fingernails, and psychogenic vomiting (28).

Self-mutilation in LND is conceptualized as a compulsive behavior that the child tries to control but generally is unable to resist. With increasing age, the affected child becomes more adept at finding ways to control his self-injury. He may enlist the help of others to protect him against these impulses or may learn self-restraint.

A language pattern that consists of repeated ambivalent statements with anxiety and coprolalia (vulgar speech) is characteristic. Moreover, the patient may be compulsively aggressive and may inflict injury on others through pinching, grabbing, or using verbal forms of aggression. Frequently, he will apologize for this behavior immediately afterward and will say that the behavior was out of his control.

Etiologic Factors

The cause of the neurologic and behavioral symptoms is not clearly established; however, abnormalities in dopamine function have been demonstrated in three autopsied cases (29). The behavior is not caused by either hyperuricemia or by excess hypoxanthine because LND partial variants whose HPRT levels are greater than 2 do have hyperuricemia but they do not self-injure. Moreover, infants treated for hyperuricemia from birth whose uric acid level is normalized still develop self-injury despite having normal levels of uric acid.

Wong and Harris et al. used positron emission tomography to investigate how dopamine dysfunction contributes to the self-injurious behavior (30). These authors documented reductions in dopamine transporter density of 68% in putamen and 42% in caudate in six patients with classic LNS and self-injurious behavior. To clarify the relationship between presynaptic dopamine transporter binding in the striatum and self-injurious behavior further, Harris, Jinnah and Wong (30a) studied seven patients with Lesch–Nyhan variants (HPRT levels 1.8% to 20.0%) and two patients with HPRT levels less than 1.5%, all nine without self-injurious behavior (age range, 12 to 37 years). The extent of motor

findings was documented on quantitated neurologic examination. Two patients with HPRT levels less than 1.5% and two patients with HPRT levels of 1.8% and 2.5% with severe movement disorder were not different in WIN 35,428 dopamine transporter binding in positron emission tomography imaging than the previously described classic patients with LND who did injure themselves. The study of variant cases with motor symptoms but with no self-injurious behavior suggests that reductions in dopamine receptor density are not a sufficient explanation of the self-injury. However, these authors found that HPRT level and the extent of motor deficit were correlated with dopamine transporter binding in caudate and putamen in the nine cases. Dopamine transporter binding was significantly correlated with HPRT levels in whole cells. Moreover, when the movement disorder was rated on the Fahn-Marsden dystonia rating scale, putamen dopamine transporter density was significantly correlated with symptom severity. These findings suggest that dopamine reduction is linked to the extent of the movement disorder, but it may not be a sufficient explanation for self-injurious behavior, and other neurotransmitters need to be examined. Moreover, these variant subjects with levels from 2% to 20% showed cognitive deficit profiles similar to those of classic LND (31).

Future investigation will need to take into account the existence of a variety of mutations in the *HPRT* gene structure. Why partial HPRT deficiency does not lead to neurologic and behavioral symptoms remains unclear; perhaps neurotrophic factors are active with minute amounts of the enzyme. It is advisable to study combined drug and behavioral treatment. An emphasis on parental training is of particular importance for drug compliance and generalization of treatment effects. As in other inborn errors, continuous family support is essential. Harris provides a description of a comprehensive treatment program for LND (19).

Prader-Willi Syndrome

PWS is a neurodevelopmental disorder characterized by obesity, short stature, cryptorchidism, mental retardation, hyperphagia, learning disability, short stature, hypogonadism, hypotonia, small hands and feet, and dysmorphic facies. Patients have an increased prevalence of daytime sleepiness, scoliosis, and other orthopedic abnormalities. Because of the obesity, heart failure and diabetes may occur as complications. Although it is a rare disorder (1 in 10,000 to 15,000), its behavioral phenotype has assumed prominence in genetics because of its relationship with AS, which has a different behavioral phenotype, although both disorders involve genomic imprinting of the same region of chromosome 15.

Genetics

PWS may result from both chromosomal deletion and maternal UPD. In UPD, two copies of the maternal chromo-

some are inherited with no paternal contribution (32). Without the presence of the chromosome donated by the father, the normal imprinting of the two maternally donated chromosomes leads to absence of gene expression in this interval. This results in a functional abnormality that is essentially equivalent to the structural abnormality found in a deletion in the 15q11-q13 region that is associated with the disorder. Moreover, in about 5% of cases, abnormalities in the mechanism of imprinting may occur when the imprinting control center itself has a mutation.

Several genes are included in the most commonly deleted region in PWS. Some are paternally imprinted, and others are maternally imprinted (33). Among these, ZNF 127, NDN, SNURF-SMRPN, IPW are paternally imprinted. Another gene, UBE3A (E6-AP ubiquitin lipase), is maternally imprinted. Others genes in this region that are expressed from both maternal and paternal chromosomes include three y-aminobutyric acid (GABA) receptor subunits (GABRB3, GABRA5, GABRG3) (33). Because similar phenotypes result from deletions and from imprinting in PWS, it is less likely that nonimprinted genes play a role in PWS or AS. Among these genes, a specific gene for PWS has not been established, so several of these genes may contribute to the phenotype. For example, the SMRPN gene is involved in protein slicing and is expressed throughout the brain; however, it is not thought that the PWS is the direct outcome of this deficit. The NCD (necdin) gene does lead to failure to thrive in certain mouse strains, so it may be a factor; however, those mice that survive do develop into apparently normal adults. Thus, the disorder is most likely linked to the loss of more than one gene in this region. Conversely, the mutation of a single gene, *UBE3A*, has been found in cases of AS (34).

Behavioral Phenotype

The extent of cognitive impairment is variable in PWS. Some patients test in the normal range of intelligence, but most test in the mild to moderate range of mental retardation. Others may test in the severe range of mental retardation. The behavioral phenotype includes unusual food-related behavior (compulsive food seeking, hoarding, gorging), skin picking, irritability, anger, low frustration tolerance, and stubbornness. Standardized methods of assessment have substantiated increased rates of depression, anxiety, and compulsive behavior. Up to 50% of children and adults with PWS demonstrate behavioral disorders.

Compulsive eating is the most disabling of these behavioral manifestations and leads to obesity and the complications of severe obesity, such as respiratory impairment and diabetes. The hyperphagia, which has been consistently found, has received the most systematic behavioral evaluation. When not carefully supervised, patients may steal food and, in some instances, eat unpalatable food, although this can be avoided with appropriate supervision. Holm and

Pipes evaluated food-related behavior in the PWS (36). They found that behavioral problems were most commonly related to food and included food stealing, foraging for food, gorging, and indiscriminate eating with little food selectivity. No special circumstances that resulted in food stealing or gorging were identified.

Besides the food-related compulsions, emotional lability with temper tantrums, stubbornness, negativism, skin picking and scratching, and non-food-related obsessions have been examined. A questionnaire survey involving 369 cases identified compulsive and impulsive aggressive behavior (37). These authors used the Overt Aggression Scale, the Yale-Brown Obsessive-Compulsive Disorder Scale, a clinical global rating, and DSM-III-R criteria to diagnose selfstimulation and self-injury, compulsive behavior, and obsessive behaviors. These investigators found that skin picking was the most common form of self-injury, observed in 19.6% of this sample. Other types of self-injury with lower frequency were nose picking, nail biting, lip biting, and hair pulling. The second behavioral problem area was compulsive behavior; food hoarding was the most severe manifestation and occurred in 17.7%. Other compulsive behaviors included counting, symmetric arrangements of objects, checking, and hand washing, but they were less common. Obsessive thinking was far less characteristic, with only 1.4% rated in the severe range on an item dealing with concerns about contamination. State et al. reviewed the evidence in regard to compulsive behaviors in PWS and the relationship with obsessive compulsive disorder (38). Behavioral problems identified in the preschool years persist throughout the school years and continue into adolescence and adulthood.

Etiologic Factors

Investigators have proposed that the genetic abnormality in PWS leads to hypothalamic dysfunction that results in aspects of the clinical phenotype, such as dysregulation of feeding, delay in sexual development, sleep disorder, and abnormality of thermoregulation. In support of hypothalamic dysfunction, Swaab et al., in a postmortem study, found reduction in oxytocin cells in certain regions of the hypothalamus (35). However, other brain regions and neuropeptides may be involved in PWS. Because the loci of GABA subunits is in the area around the 15q11-13 region, GABA has been measured in PWS, and abnormalities have been reported in plasma levels in some patients.

To clarify the mechanism leading to the behavioral phenotype further, differences between deletion and maternal UPD causes have been assessed (39). Similar studies have been completed in AS (40). Differences in intellectual functioning in PWS with a paternal 15q11-q13 deletion versus maternal UPD of chromosome 15 were evaluated using measures of intelligence and academic achievement in 38 patients with PWS (24 with deletion and 14 with UPD).

The patients with UPD had significantly higher verbal IQ scores than those with deletion (p < .01). The magnitude of the difference in verbal IQ was 9.1 points (69.9 versus 60.8 for UPD and deletion PWS patients, respectively). Only 17% of subjects with the 15q11-q13 deletion had a verbal IQ greater than or equal to 70, whereas 50% of those with UPD had a verbal IQ greater than or equal to 70. Performance IQ scores did not differ between the two PWS genetic subtype groups. This report documents the difference between verbal and performance IQ score patterns among patients with PWS of the deletion versus the UPD subtype. Comprehensive treatment of behavioral problems in PWS is described by Holm et al. (41).

Angelman Syndrome

In contrast to PWS, investigators have shown that one gene in the deleted region can lead to AS (34). AS is a neurologic disorder with a heterogeneous genetic origin. It most frequently results from a de novo interstitial deletion in the 15q11-q13 region, but it is also caused by paternal UPD or an imprinting mutation. The remaining 20% to 30% of patients with AS exhibit biparental inheritance and a normal pattern of allelic methylation in the 15q11-q13 region. In this biparental inheritance group, mutations in the *UBE3A* gene have been shown to be a cause of AS. Moncla et al. described the phenotypic expression in 14 patients with AS involving eight UBE3A mutations (34). These were made up of 11 familial cases from five families and three sporadic cases. Some subtle differences from the typical phenotype of AS were noted. Consistent features were psychomotor delay, a happy disposition, a hyperexcitable personality, EEG abnormalities, and mental retardation with severe speech impairment. The other main features of AS—ataxia, epilepsy, and microcephaly—were either milder or absent in various combinations among these cases. Moreover, myoclonus of cortical origin was commonly observed with severe myoclonic seizures. Most of these patients were overweight. This study showed that ataxia, myoclonus, EEG abnormalities, speech impairment, characteristic behavioral phenotype, and abnormal head circumference are attributable to a deficiency in the maternally inherited *UBE3A* allele. Finally, analysis of mutation transmission showed an unexpectedly high rate of somatic mosaicism in normal carriers. These clinical findings have important consequences for genetic counseling in AS.

Fragile X Syndrome

The fragile X syndrome is characterized by mental retardation, behavioral characteristics, and the physical findings of a long face with large, protruding ears and macroorchidism (42). Fragile X syndrome is the most common known cause of inherited mental retardation, and it may also result in learning disabilities and social deficits in those who do not

test in the mentally retarded range. After the identification of the fragile X mental retardation (FMR1) gene, the cytogenetic marker (a fragile site at Xq27.3) was replaced by molecular diagnosis. Recognition of this gene has broadened our understanding of the spectrum of the fragile X syndrome.

Genetics

Fragile X syndrome is caused by massive expansion of CGG triplet repeats located in the 5'-untranslated region of the FMR1. The cloning of the FMR1 gene led to the characterization of its protein product FMRP. The full mutation is associated with a process of methylation; the addition of methyl groups along the "backbone of the DNA helix" (42). In patients with fragile X syndrome, the expanded CGG triplet repeats are hypermethylated, and the expression of the FMR1 gene is repressed, which leads to the absence of FMR1 protein (FMRP) and subsequent mental retardation. The encoded protein is a ribosome-associated, RNA-binding protein thought to play a role in translational regulation of selective messenger RNA transcripts. FMRP is an RNAbinding protein that shuttles between the nucleus and cytoplasm. This protein has been implicated in protein translation because it is found associated with polyribosomes and the rough endoplasmic reticulum (43). A similar mechanism is proposed for FMR2, which encodes a large protein of 1,311 amino acids and is a member of a gene family encoding proline-serine-rich proteins that have properties of nuclear transcription factors (44).

The fragile X syndrome was one of the first examples of a "novel" class of disorders caused by a trinucleotide repeat expansion in the X chromosome. In the genetically normal population, the CGG repeat varies from six to 54 units. Affected subjects have expanded CGG repeats (more than 200) in the first exon of the *FMR1* gene (the full mutation). Phenotypically normal carriers of the fragile X syndrome have a repeat in the 43 to 200 range (the premutation). The process of methylation silences transcription so a fully methylated full mutation results in no FMR1 protein's being produced. The absence of FMR1 protein results in fragile X syndrome. Two additional disorders result in a fragile site at Xq27.3; there are FRAXE, which is usually associated with a milder form of mental retardation, and FRAXF, which is not consistently associated with mental retardation. These two mutations also have CGG repeat expansions and are distal to the FMR1 site. The transcriptional silencing of the FMR2 gene also has been implicated in FRAXE mental retardation. FRAXE individuals have been shown to exhibit learning deficits, including speech delay and reading and writing problems.

The frequency of the premutation and mutation may be variable in different populations because of founder effects (42). Thus, the prevalence in an English study was 1 in 2,200, and in an Australian study it was 1 in 4,000, but it

was higher in Finland, where it is proposed that the initial settlers included one or more fragile X carriers.

Behavioral Phenotype

There is a substantial degree of genetic and phenotypic heterogeneity in the physical, cognitive, and behavioral phenotype. The behavioral phenotype has been the subject of considerable study and includes mental retardation and learning disabilities, language impairment, hand flapping, gaze aversion, perseveration, and neuropsychiatric disturbance, principally attention-deficit/hyperactivity disorder and pervasive developmental disorder—like symptoms. These patients are more interested in social interactions than those with autistic disorder; the avoidance of social contact may be secondary to hyperarousal or increased sensitivity to stimuli associated with social situations. The behavioral phenotype may be more helpful than the physical phenotype in diagnosis because most prepubertal patients do not have macroorchidism or the characteristic long face.

Attentional difficulty and concentration problems are commonly associated, and hyperactivity may be a presenting symptom in nonretarded boys with fragile X syndrome. Self-injury, most commonly hand biting and scratching, may be elicited by excitement and by frustration. Female patients with fragile X syndrome may be unaffected, although abnormalities in social interaction, thought process, and affect regulation have been reported in carriers. Both schizotypal features and depression have also been found in carriers.

Most girls with the full mutation show shyness and social anxiety. In women with the full mutation, the social anxiety is associated with social awkwardness and schizotypal features. Anxiety disorders, avoidance disorder, and mood disorder symptoms are common (42).

Gaze Aversion

Gaze aversion is a striking feature of affected males with fragile X syndrome. There is consistency in gaze aversion over repeated trials in the same individual; nearly all male patients with fragile X syndrome who are more than 8 or 9 years old avert their gaze on greeting another person. Their unusual greeting is characterized by both head and gaze aversion along with an appropriate recognition of the social partner (45). This greeting response is qualitatively different from gaze aversion that is described in autistic patients. Those with Down syndrome and nonspecific mental retardation do not show this behavioral pattern on greeting. The idiosyncratic gaze behavior in fragile X syndrome may disrupt social interactions. Despite their apparent social anxiety and aversion to eye contact, male patients with fragile X syndrome are otherwise socially responsive and can be affectionate.

Speech and Language

Speech and language in fragile X syndrome is generally delayed, even thought the IQ may be in the normal range. Deficits in both receptive and expressive language include dysfluency, production of incomplete sentences, echolalia, palilalia (reiteration of the speaker's own words and phrases in a perseverative manner), verbal perseveration, and poor fluency in conversation. Compulsive utterances and shifts in speech pitch are common, and auditory processing and memory deficits are present.

Etiology

The FMR1 protein is expressed most abundantly in neurons and testes with the localization primarily in the cytoplasm. High concentrations of FRM1 mRNA have been found at the synapse in rat brains, especially in areas involved in synaptogenesis in the hippocampus, cerebral cortex, and cerebellum (46). Hinton et al. found thin and immature dendritic branches with small synapses in neuroanatomic studies of the neocortex in three male patients with fragile X syndrome (47). The expression of the FMR2 protein also has been characterized. To characterize the expression of the FMR2 protein, polyclonal antibodies were raised against two regions of the human FMR2 protein and were used in immunofluorescence experiments on cryosections of mouse brain. The FMR2 protein is localized in neurons of the neocortex, Purkinje cells of the cerebellum, and the granule cell layer of the hippocampus. FMR2 staining is shown to co-localize with the nuclear stain 4,6-diamidino-2-phenylindole (DAPI) and confirms that FMR2 is a nuclear protein. The localization of FMR1 and FMR2 protein to the mammalian hippocampus and other brain structures involved with cognitive function is consistent with the learning deficits seen in patients with fragile X syndrome. Comprehensive treatment of fragile X syndrome is described by Hagerman and Cronister (48).

Williams (Williams-Beuren) Syndrome

WMS is a rare (1 in 25,000), genetically based neurodevelopmental disorder associated with a characteristic physical, linguistic, cognitive, and behavioral phenotype. This syndrome provides a unique opportunity to study personality development, linguistic functioning, and visuospatial development. The syndrome is characterized by congenital facial and cardiovascular anomalies (supravalvular aortic stenosis and peripheral pulmonary stenosis), failure to thrive, and mental retardation that may be accompanied by transient idiopathic infantile hypercalcemia (49). Adolescents with WMS have expressive language abilities that are better than expected for their mental age. Because of their hyperverbal speech, the investigation of WMS allows the study of the dissociability of components of language and other cognitive brain systems. In mentally retarded patients with WMS,

linguistic abilities may be selectively spared, unlike language learning disability occurring in normally intelligent children (50).

In WMS, a deletion of 1.5 Mb on one copy of chromosome 7 results in the specific physical, cognitive, and behavioral features. Molecular dissection of the WMS phenotype may lead to identification of genes important in human cognition and behavior.

Genetics

WMS is caused by a chromosomal deletion at 7q11.23. A contiguous gene deletion disorder, it results from hemizygous deletion of about 20 genes (51). This chromosomal region is highly repetitive, and the deletion arises from recombination between misaligned repeat sequences flanking the WMS region. The deletion breakpoints cluster within the repeats, so most patients with WMS have similar, although not identical, deletions of 1.5 Mb.

The first deleted gene identified in the critical region was that for elastin (ELN). Studies of patients having deletions or point mutations confined to this gene showed that hemizygosity for ELN causes supravalvular aortic stenosis but not the other typical features of WMS.

Several other genes have now been identified that are deleted in most patients with WMS. These include the following: *LIMK1*, which codes for a protein tyrosine kinase expressed in the developing brain; that for syntaxin 1A (STX1A), which encodes a component of the synaptic apparatus; *RFC2*, which codes for a subunit of the replication factor C complex involved in DNA replication; and *FZD3*, homologous to the *Drosophila* tissue-polarity gene, "frizzled" (51).

Cognitive Phenotype

Bellugi et al. proposed that "the cognitive hallmark of WMS is dissociation between language and face processing (relative strengths) and spatial cognition (profound impairment)" (50). The WMS phenotype demonstrates specific dissociations in the higher cognitive functions. These investigators proposed that general cognitive deficits are present but linguistic abilities are spared. They found extreme spatial cognitive deficits with intact face processing. Of special interest is the social phenotype in WMS: an overly friendly, engaging personality and excessive sociability with strangers (52). WMS subjects show an unusual positive response in their social judgments of unfamiliar persons.

Howlin et al. investigated cognitive, linguistic, and academic assessments in a representative sample of 62 adults with WMS (average age of the group was 26 years; mean full-scale IQ was 61) (53). Less difference was found in verbal and performance IQ and between receptive and expressive language skills in the adults than that found in children. Still, subtest scores documented an almost identi-

cal cognitive profile to that found in children. Reading, spelling, arithmetic, and social adaptation remained at a low level, with functioning around a 6- to 8-year age equivalent. The consistency in intellectual abilities in both child and adult studies of patients with WMS supports the notion of a syndrome specific pattern of cognitive, linguistic, and adaptive functioning.

The use of adult neuropsychological models to explain developmental disorders of genetic origin such as WMS has been challenged (54,55). It is assumed that uneven cognitive profiles found in childhood or adulthood in WMS characterize infant starting states and that modules underlying these abilities start out either intact or impaired. However, findings from two experiments with infants with WMS (selected for study based on claims of innate modularity) suggest a within-syndrome double dissociation: for numerosity judgments, WMS subjects do well in infancy but poorly in adulthood, whereas for language, WMS subjects show poor performance in infancy but do well in adulthood. The theoretic and clinical implications of these findings in WMS emphasize the importance of an developmental approach to neurogenetic disorders. Karmiloff-Smith et al. previously proposed that in WMS, language follows a different path to normal acquisition and may turn out to be more like second language learning (56).

Finally, Tager-Flusberg et al. tested the hypothesis that the WMS phenotype involves sparing abilities involved in the domain of understanding other minds (mentalizing or theory of mind) (57). They compared a group of mentally retarded adults with WMS to an age-, IQ-, and languagematched group of adults with PWS, and a group of agematched normal adults, on a task that tests mentalizing ability. The task involved identifying the correct labels to match photographs of complex mental state expression focused on the eye region of the face. The adults with WMS performed significantly better than the adults with PWS on this task, and about half the group performed in the same range as the normal adults. Such findings provide support for the proposal that mentalizing is a distinct cognitive domain. The authors proposed that this sparing of cognitive capacity could be "linked to the relative sparing of limbiccerebellar neural substrate in WMS, which is also connected to cortico-frontal regions that are known to be involved in understanding complex mental states."

Linking Genes and Cognition

An approach to studying cognition is to carry out genetic and psychometric testing of patients who have small deletions within the WMS critical region. *LIMK1* and *STX1A* are good candidate genes to investigate cognitive or behavioral aspects of WMS. The gene for *LIMK1* was implicated as a cause of the visuospatial characteristics of WMS (58); however, other investigators were unable to substantiate this association in three further cases (59). The genes for *STX1A*

and FZD9 were proposed as involved, based on brain-specific gene expression in the developing (FZD9) or adult (STX1A) central nervous system. However, when these genes were underexpressed by 50%, as is expected in WMS, Korenberg et al. reported that deletion of these genes was not associated with significant effects on overall cognition (51). However, these authors did propose that genes responsible for mental retardation and other features of the disorder are "located in the region telometric to RFC2 through GTF21 at the telometric border of the deletion." Moreover, mild cognitive deficits reported in a subject deleted for elastin and LIMK1 genes (59) were consistent with findings in those with deletion of genes in the WMSTF through LIMK1 region having mild cognitive deficits. Thus, studies of patients with rare and atypical deletions may be informative in identifying candidate genes to understand the cognitive deficit.

Linking Anatomic and Behavioral Changes

WMS is associated with specific neuromorphologic and neurophysiologic findings. There is proportional sparing of frontal, limbic, and neocerebellar structures on magnetic resonance imaging (60). Abnormal functional organization of the neural systems that underlie language processing is revealed through studies using event-related potentials (61). Event-related potential studies suggest abnormal cerebral specialization for spared cognitive functions in WMS. The lack of uniformity in the cognitive, neuromorphologic, and neurophysiologic domains of WMS makes it a compelling model for elucidating the relationships among cognition, the brain, and, ultimately, the genes.

Another approach is to investigate anatomic changes in brain regions in WMS that may the result of gene deletions. In WMS, Galaburda and Bulligi found that the overall shape of the brain is not consistently abnormal (62), although in some cases abnormal brain shape is apparent. The most consistent anatomic finding is abnormal length of the central sulcus producing an unusual configuration of the dorsal central region. This includes the distal portion of the superior-parietal lobule and dorsal frontal gyrus. These regions may be linked to abnormal behavior in patients with WMS. Cytoarchitecture of WMS forebrain appears mostly normal, although subtle dysplastic changes are noted. Abnormal neuronal size of cortical neurons was suggested in one region and may be linked to increased subcortical connectivity. Elastin does not stain in the cerebellum, whereas Lim kinase does stain in cortical neurons.

Thus, in WMS, the link of neuroanatomy and behavior seems to fit a dorsal ventral dichotomy and not a frontal-caudal, left-right, or cortical subcortical dichotomy. Gallaburda and Bellugi proposed that the dorsal portions of the hemispheres, the frontal and parietal-occipital regions, may be involved (62). They noted that some language functions are preserved that are linked to ventral systems. Face recog-

nition, also a ventral function, is preserved despite severe visuospatial dysfunction, a dorsal function. Anatomic findings also suggest possible involvement of the visually linked lateral nucleus of the amygdala. Galaburda and Bellugi speculated that this could be related to the lack of appropriate fear in WMS of new and unfamiliar faces, perhaps also threatening ones. Moreover, because this region may receive auditory projections, WMS subjects may not be sensitive to threatening voice and speech. Further work is needed at architechtonic and histologic levels to confirm sparing of ventral regions. To understand the linking of genes with neuroanatomy, it is necessary to find more genes with brain developmental effects. Of particular interest in this regard is the proposal that the region deleted in WMS may be a hotspot in mammalian brain evolution (51). Hagerman outlined a comprehensive approach to treatment of WMS (63).

ANIMAL MODELS: SIMULATIVE OR SUBSTITUTIVE

Animal models may be used to elucidate critical brain mechanisms involved in disorders with behavioral phenotypes. Early animal models focused on the impact of traumatic events during the developmental period, as exemplified by the social isolation and chronic stress (learned helpless) models of depression (64). These animal models generally simulated rather than substituted for the disorder. Animal models have used pharmacologic challenges to study neurochemical mechanisms linked to aberrant behavior or have introduced transgenic mice as substitutive models of conditions with behavioral phenotypes. Examples of these models include pemoline models of stereotyped self-biting behavior in the rat (65), SNAP mutant mouse model of attentiondeficit/hyperactivity disorder (66), and transgenic and knockout mouse models for fragile X syndrome (67,68), MAOA deficiency (69), and LND (70–72). These models may contribute to the understanding of psychopathology. However, despite genetic replication of a disorder in the mouse, the behavior may not be replicated, so even these animal models often simulate aspects of the condition and are not fully substitutive.

Molecular genetic techniques combined with techniques to manipulate the developing mouse embryo make it feasible to produce such genetic animal models. Embryonic stem cells are isolated from a pregnant mouse with identifiable coat color that acts as a donor. The embryonic stem cells are grown in cell culture and then are genetically modified with the insertion of genetic material or through mutation of endogenous genes. Modified embryonic stem cells are microinjected into a blastula that is isolated from another mouse that ordinarily has a different coat color. The blastula is then reimplanted into a female host mouse and develops in utero. The inserted stem cells are incorporated into the

developing fetuses, and progeny that contain genetically altered cells are chimeras that can be identified by their mosaic coat colors. As adults, these chimeras, in which genetically modified cells have been involved in the establishment of the germ cell line, may then transmit the altered gene to their own offspring. It takes several generations to produce an affected animal, by using these embryonic stem cell techniques that depend on whether the needed phenotype can be produced in the heterozygous, homozygous, or hemizygous condition.

To illustrate these animal models, we may contrast animal models of LND based on the use of the neurotoxin 6-hydroxydopamine (6-OHDA), transgenic mouse models of LND, calcium channel blocker models of self-injury, and the mutant mouse model of fragile X syndrome.

Lesch-Nyhan Disease 6-OHDA (Rat Model)

After the demonstration of dopamine deficiency in striatum in autopsies of brain in three human patients with LND (35), Breese et al. administered 6-OHDA to neonatal and adult rats to test the effects of dopamine depletion in an animal model (73,74). These authors demonstrated that the age at which neural function is disrupted is an important factor in the type of motor and behavioral symptoms observed after a neural insult to basal ganglia structures. They documented a relationship between dopaminergic supersensitivity and self-injurious behavior. Rats treated with 6-OHDA in the neonatal period demonstrated self-biting with mutilation when they were challenged as adults with L-DOPA or a D1 dopamine agonist, but no such self-injurious behavior was found in the adult rats treated with 6-OHDA. Because of the self-biting, the neonatal 6-OHDA-treated rat was proposed as a model for LND, and dopamine deficiency was linked to self-injury. In these studies, rats that were not HPRT deficient were given injections of 6-OHDA at 5 days of age to denervate basal ganglia regions. These brain regions developed supersensitive dopamine receptors. Self-biting was documented in the lesioned animals when they were challenged as adults with a dopamine agonist; however, untreated adult rats did not show this behavior.

HPRT-Deficient Mouse

For LND, molecular techniques were used to produce two HPRT-deficient strains of mice. One strain was produced by retroviral interruption of the human *HPRT* gene in the embryonic stem cells (72). Another model was produced through the selection of embryonic stem cells for spontaneous mutations in the *HPRT* gene (70,71). In both instances, the mouse strains produced had nondetectable levels of HPRT. However, neither strain showed the spontaneous behavioral abnormalities or neurologic presentation

seen in patients with LND. Tests of both cognitive functions and motor functions were intact in these animals. Similar findings were documented in a double knockout that is HPRT/APRT deficient (75).

This HPRT-deficient transgenic mouse model of LND (70) may still contribute to our understanding of LND. Reductions in dopamine of 40% or more (76) have been documented (77) in these animals. Because of questions about strain differences, Jinnah et al. studied the caudate nucleus in five HPRT-deficient strains of mice and made comparisons to littermate controls (77). Reductions of dopamine and also of the dopamine transporter of 35% to 40% were found in these animals. These results indicate an abnormality in the dopamine system despite apparently normal spontaneous behavior.

The absence of behavioral changes in the HPRT-deficient mice was unexpected. Originally, it was thought that uricase, which is present in rodents but is not present in primates, may act in a protective manner to lessen behavioral manifestations because uric acid, which normally builds up in the blood in LND, and hypoxanthine, which accumulates in cerebrospinal fluid, would not do so in mice because of the presence of uricase. This explanation is consistent with the inability of treatment with allopurinol, a xanthine oxidase inhibitor that prevents the accumulation of uric acid, to improve the behavior disorder in patients with LND. However, the mice were still found to have reduced dopamine in brain (76). Thus, it is the consequence of the HPRT deficits on dopamine, and possibly other neurotransmitter systems, that leads to the behavior in humans. This mouse differs from the Breese rat model in that dopamine depletion is complete in the Breese rat model but only 40% to 50% reduced in the HPRT-deficient mouse. Dopamine depletion is substantially greater in human subjects (76) than in the mice; therefore, this difference may account for the differences in behavior.

Bay K 8644 Model of Self-Injurious Behavior

Another approach to study self-injury is the calcium channel blockers model proposed by Jinnah et al. (78). The L-type calcium channel agonist (+/-) Bay K 8644 causes motor abnormalities in adult mice. These authors showed that administration of this drug could also cause the self-injurious biting, particularly when it was given to young mice. Self-biting was provoked by injecting small quantities of (+/-) Bay K 8644 directly into the lateral ventricle of the brain, a finding indicating a central effect of the drug. Similar behaviors can be elicited by administration of another L-type calcium channel agonist, FPL 64176. The self-biting elicited by (+/-) Bay K 8644 can be inhibited by pretreating the mice with dihydropyridine L-type calcium channel antagonists such as nifedipine, nimodipine, or nitrendipine. Moreover, self-biting is not inhibited by nondihydropyri-

dine antagonists including diltiazem, flunarizine, or verapamil. The known actions of (+/-)Bay K 8644 as an Ltype calcium channel agonist, the reproduction of similar behavior with another L-type calcium channel agonist, and the protection from this behavior that results from certain L-type calcium channel antagonists implicate calcium channels, and their possible association with neurotransmitter deficits, in the mediation of the self-biting behavior (78).

Fragile X Mouse Model

The mutant mouse model of fragile X syndrome demonstrates another use of an animal model for a neurogenetic disorder. Transgenic fragile X knockout mice were developed to provide an animal model to study the physiologic function of the fragile X gene (*FMR1*) and to understand better the clinical phenotype caused by the absence of the fragile X protein.

The fragile X mouse model demonstrates macroor-chidism and cognitive, affective, and behavioral features similar to the human condition (79). In the Morris water maze test, the Fmr1 knockout mice learned to find the hidden platform nearly as well as the control animals, but they showed impaired performance after the position of the platform was modified. The fragile X knockout mouse exhibited subtle deficits in spatial learning but normal early-phase long-term potentiation.

Jin and Warren expanded these studies by examination of late-phase hippocampal long-term potentiation, the protein synthesis-dependent form of long-term potentiation, in the Fmrl knockout mice (43). Initially, they found that latephase long-term potentiation was normal and proposed that either absence of fragile X mental retardation protein has no influence on long-term potentiation or that any such influence is too subtle to be demonstrated by this technique. Moreover, when they examined spatial learning in this knockout mouse using the hippocampus-dependent Morris water maze, near-normal performance was observed. However, because the knockout mouse strain they used differed from that used in the earlier investigations that did show learning deficits, their studies were repeated using the same mouse knockout line that showed the deficit. Now significant, but subtle, increased swim latencies on the Morris maze test in reversal trials were found to be in agreement with the earlier studies. Thus, strain differences among mouse strains influence the behavior in the Fmrl knockout phenotype. Because the finding were subtle, these authors chose to investigate a paradigm less dependent on hippocampal function, one using the conditional fear paradigm. In this paradigm, the knockout animals showed significantly less freezing behavior than their wild-type littermate with two types of stimuli, contextual and conditional fear stimuli. These researchers concluded that that amygdala dysfunction may also be involved in fragile X syndrome.

These examples from LND and fragile X syndrome illus-

trate how animal models may contribute to our understanding of behavioral phenotypes: self-biting in LND and learning and fear responses in fragile X syndrome.

CONCLUSION

The study of behavioral phenotypes in neurodevelopmental disorders demonstrates the complexity in mapping pathways from genes to cognition and complex behavioral phenotypes. Behavioral phenotypes occur in disorders with mendelian inheritance (LND) and nonmendelian inheritance (PWS/AS, FRX). An investigation of these syndromes demonstrates that recognition of the involved gene is only the first step. Identification of the involved protein and of its expression in brain is critical. To clarify the mechanism, the use of animal models, neuroanatomic study, brain imaging techniques, systems neuroscience, and detailed descriptions of behavior are needed. The study of partial variants of the disorder (LND, WMS), comparison of deletion versus UPD (PWS, AS), and the study of atypical subjects who exhibit some but not all features of the disorder (WMS) are essential in understanding developmental pathways. Moreover, a neurodevelopmental model is essential because brain modularity of function cannot be assumed. Animal models must be carefully chosen because genetic background may influence the expression of the disorder. Such models may be important in simulating aspects of the disorder, but they may not substitute for the human condition. Flint proposed that success in the study of behavioral phenotypes requires a screen for regions of monosomy, the use of a sophisticated battery of neuropsychological and behavioral tests to describe the phenotype, a transcript map to identify quickly the genes that are likely to affected by the deletion, and a way to of deciding which genes are dosage sensitive (5). These are challenges that lie ahead as we continue to investigate behavioral phenotypes as portals to understanding the developing brain.

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638

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