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Molecular Basis of Genetic Neuropsychiatric Disorders

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The genetic basis of several childhood neuropsychiatric disorders has been clarified over the last decade and provides a springboard for the development of intervention strategies [1,2]. Aberrant brain development during the fetal and childhood period has been implicated in several neurodevelopmental and psychiatric disorders. The chromosomal location has been mapped for numerous autosomal-dominant, autosomal-recessive, and X-linked disorders; in some cases, specific genes have been implicated in the pathophysiology of the disease [3,4]. The functional relevance of the genes in question is being studied. This article presents an overview of the molecular pathways implicated in neuropsychiatric disorders, with a discussion of some of the mutations that occur in signaling molecules critically involved in learning.

Fragile X syndrome

Our understanding of the molecular basis of fragile X mental retardation syndrome (FXS) has advanced tremendously over the past decade [5]. FXS is a recessive X-linked mental retardation disorder, and the phenotype associated with the syndrome cosegregates with a “fragile” site on the X-chromosome. A break point appears to be present on one of the X-chromosomes in cells grown in medium lacking folic acid [6]. Because of the mutation that is present, this chromosomal region does not stain normally in karyotypic analyses, and this chromosomal abnormality suggested to investigators that a gene involved in the disorder might lie near the disrupted site.

Certain unusual aspects were observed in the phenotypic expression of the disorder before the gene of interest was actually cloned. About 20% of men carrying the mutant version of the gene did not manifest any cognitive deficits. This was intriguing, because if the gene involved lies on the X-chromosome, why were these men with an apparent fragile site unaffected? These individuals are called “normal transmitting males” and pass the mutated copy of the gene to their daughters who also may show few symptoms; however, it was observed that their grandson carried a much higher risk for manifesting the full-blown syndrome. The progressive increase in the severity of a disorder over several generations is called “anticipation.”

The molecular basis of anticipation is now clear. A novel type of mutation in the 5' untranslated sequence of the *FMRI* gene, called a triplet repeat expansion, was identified as the cause for FXS [7]. The size of the expansion increases over generations, and, thus, the mutation and the disorder become more severe over time. Triplet repeats are made up of three consecutive nucleotides repeated several times as a tandem unit. The three nucleotides repeated in FXS are cytosine-guanine-guanine (CGG). In normal individuals, this triplet sequence is repeated between 6 and 50 times in the *FMRI* gene, with 29 being the most commonly occurring repeat number [7]. The number of triplet repeats is increased in affected individuals to between 200 and 1000 repeats. Mothers of affected individuals carry between 50 and 200 repeats, which

lies between the number seen in normal individuals and the number seen in affected individuals; this intermediate number of repeats is termed a “premutation” [7]. Individuals with premutations in the *FMR1* gene may show mild cognitive deficits and behavioral abnormalities [8]. Women who are heterozygous for the mutation perform poorly in visuospatial and other memory subtests [9,10]. Furthermore, female premutation carriers show decreased total brain volumes and increased metabolic labeling within the hippocampus and cerebellum, as determined by MRI and positron emission tomography scans [11]. Mothers with the premutation are at a higher risk for producing offspring with expanded triplet repeats, and, hence, lead to the observed increased severity of the phenotype. Although it remains unclear as to why there is an initial expansion to a premutation, the molecular basis of anticipation can be explained by the increase in triplet repeats over the course of several generations.

Although it was originally believed that premutation carriers were mostly asymptomatic, two different phenotypes recently were described in these carriers. Early ovarian failure was reported in 16% to 24% of female carriers, whereas male premutation carriers older than 50 years develop fragile X–associated tremor and ataxia syndrome [12–15]. The molecular basis of these two disorders remains unclear, because it does not correlate directly with the size of triplet repeats or the extent of nonfunctional fragile X mental retardation protein (FMRP).

Cytosine-Guanine dinucleotide connected by phosphodiester bond (CpG) islands are stretches of DNA sequences larger than 200 base pairs, with greater than 50% cytosine and guanine nucleotide content and a high preponderance of CpG sequences. Usually, these sequences are found within the promoter sequences at the 5′ untranslated region of many genes [16]. The triplet repeat in FXS is CGG; it may be repeated many hundreds of times in affected individuals. Thus, patients who have FXS have a large increase in these CpG sequences. The cytosines in the CpG islands can be methylated, and this can regulate the expression of the gene negatively. Thus, the dramatic expansion of CGG repeats in FXS leads to an increase in methylated CpG islands [17]. The degree of methylation within this region is correlated directly to the extent of loss of functional FMRP [18].

What is the normal physiologic function of FMRP in neurons, and how does the lack of this protein lead to clinical symptoms? Shortly after the isolation of the gene in 1991, researchers noticed that the protein contained three conserved domains. These motifs have high homology to amino acid sequences known to be RNA-binding motifs. RNA-binding proteins play a role in regulating the processing, trafficking, or translation of mRNA transcripts within cells [19–21]. A mutation within the RNA-binding domain of these proteins can disrupt the normal binding to mRNAs, and, hence, affect the ability of the cells to produce mature messages or to translate those messages into protein.

The importance of the RNA-binding domain in FMRP was confirmed by the identification of a patient who had FXS and a point mutation that altered a conserved amino acid in one of the RNA-binding domains. This individual lacked the usual expansion of trinucleotide repeat [21–23]. The RNA-binding domain of FMRP is essential for protein function, and mutations within this region may affect the ability of FMRP to bind RNA and process them correctly. Although most cases of FXS are caused by the expansion of CGG repeats, the discovery of this case and others like it suggested that any mutation that interferes with the function of FMRP could lead to a similar clinical syndrome. It is a good example of allelic heterogeneity, where different types of mutations in a gene lead to the expression of the same phenotypes in affected individuals.

Characterization of the intracellular localization of the FMRP also has advanced our understanding of its function. Initially, the fragile X protein was found to associate with polyribosomes, the cellular organelles composed of ribosomal RNAs and various RNA-

binding proteins that function as an assembly line in the translation of mRNA transcripts into proteins [24]. The pattern of FMRP expression in various regions of the brain during development also has been studied in detail. The highest expression of the protein is observed in the basal forebrain and hippocampus [25]. Both of these brain regions are involved in the acquisition of short-term memory and sequential processing of information, and they are affected in some neurodegenerative disorders, such as Alzheimer's disease. Clinical neuroimaging studies have detected age-related changes in the brain volume of patients who have FXS, particularly in the cerebellar vermis, fourth ventricle, and hippocampus [26–28]. Finally, the development of animal models, such as the knockout mouse, has been useful to understand some of the molecular processes that are disrupted. FMRP knockout mice show many of the clinical symptoms noted in patients who have FXS [29].

Our understanding of the normal function of FMRP has been helped from two other lines of investigation: the site of protein translation and the mechanism by which mRNAs are transported. The first line of study was to identify the location of protein translation in neurons. According to older dogma, messages transcribed in the nucleus are transported rapidly to the cytoplasm where ribosomes are assembled onto the messages, and the translated proteins are deposited into the cytosol or into the endoplasmic reticulum for posttranslational modification. This mechanism is true for most messages; however, recent work suggested that a subpopulation of neuronal mRNAs is transported along dendrites to locations adjacent to spines. These messages that are shipped out await the arrival of signals, upon which they are translated locally near the sites of synaptic inputs to produce the proteins necessary for biochemical changes at the spine and the synaptic remodeling that must occur.

This new model of local protein synthesis has helped to solve a major hurdle in our understanding of the development of synaptic plasticity. A typical neuron can have up to 10,000 spines. The extracellular signal probably arrives at a few hundreds of these spines. According to the previous model, this signal traveled to the nucleus, where it initiated gene transcription and subsequent translation in the perinuclear region. This brings up the obvious question of how these new synthesized proteins are transported to only a subset of spines that is activated by the original incoming signal. Based on the new model, the messages themselves are transported to the dendritic spines, ready to be translated when the signal arrives. Evidence for dendritic protein synthesis has accumulated over the past few years, with the finding of components of the translation machinery throughout dendrites and within the spines themselves [30]. The various components identified to date include mRNAs, ribosomes, and proteins that function as translation factors necessary for the synthesis of proteins [31].

Local protein synthesis does not preclude that the original signal also might be sent to the nucleus to trigger additional transcription of genes. In fact, the maintenance of long-term potentiation and the consolidation of long-lasting memories require that the incoming signal eventually reaches the nucleus. The initial burst of protein translation occurring near the spines is necessary for the induction of long-term potentiation (LTP), whereas the maintenance phase of LTP requires further mRNA transcription and protein synthesis. The importance of the induction and maintenance phase of LTP can be demonstrated by the infusion of transcriptional inhibitors into specific brain regions. For example, injection of these inhibitors into the amygdala blocks the consolidation of long-term fear memories, but does not affect short-term fear learning, which does not require gene transcription.

Only a portion of neuronal messages are transported along dendrites and out to the spines. The messages that are transported encode for proteins that are necessary for biochemical tagging and reorganization of synaptic structure [32,33]. FMRP probably is one of the many proteins necessary for this complex process. The transport of messages to the synapses, their inhibition or activation depending on the status of activity at the spines, as well as the scaffolding support

for protein synthesis require the interplay of many hundreds of proteins; current estimates are that approximately 400 mRNAs are actively transported to distal dendrites for translation. Based on this model, one could envision that mutations that alter or abolish the function of many proteins along this pathway could disrupt normal synaptic plasticity and produce cognitive deficits in affected individuals.

Having tackled the local translation mechanism, researchers next asked how these transported messages are kept dormant until a synaptic stimulus reaches the spine. Recent studies from various laboratories suggested that one function of FMRP is to inhibit the translation of mRNAs with which it associates [5]. FMRP binds to newly synthesized mRNA molecules in the nucleus and is transported along with these messages along dendrites, all the while preventing their translation into proteins. Two theories have been put forward for how FMRP associates with mRNAs, and each has experimental data to support it. The first showed that a subpopulation of the transported messages contains specific nucleotide sequences (G quartets) to which FMRP binds [33]. The second model showed that FMRP binds to a small RNA molecule (BC1), and, that BC1, in turn, binds to target messages. Together, this triad of proteins and RNAs forms a circular structure that prevents the initiation of translation [34]. How the FMRP-mediated inhibition of translation is removed is an area of active research, but it seems to involve the activation of extracellular signal-regulated protein kinase [35,36].

The model of synaptic translation allows us to make certain predictions about the molecular changes occurring in patients who have FXS. If FMRP plays a role in regulating protein translation at spines, then disruption of FMRP function should interfere with the normal structural remodeling of spines that accompanies synaptic plasticity. This is what Greenough and his colleagues found in anatomic studies in patients who had FXS as well as in animal models in which a mutated *FMR1* gene replaces the normal *FMR1* gene [37,38]. Compared with controls, humans with FXS and the transgenic mice had greater numbers of long, spindly, and immature-looking spines and reduced numbers of mature, short, and mushroom-shaped spines.

Although the expansion of trinucleotide repeats at the *FMR1* gene loci originally was considered to be a genetic aberration unique to FXS, more than a dozen other triplet repeat disorders have been identified. These include Huntington's chorea, Friedreich's ataxia, and myotonic dystrophy [39–41]. In most of these disorders, the phenomenon of anticipation is apparent, but it was explained away in earlier studies as being due to ascertainment bias; however, the discovery of triplet repeat expansions has provided the molecular explanation for its occurrence. The degree of anticipation observed between generations may be dramatic. For example, in muscular dystrophy, the expansion of repeats just above the threshold results in the development of cataracts late in adult life; however, further expansion of these long repeats over several generations results in fatal congenital disorder [42].

Prader-Willi and Angelman syndrome

We derive our genetic complement of 46 chromosomes from our parents, with the mother and father contributing 23 chromosomes each. It was assumed previously that the homologous genes on the maternal and paternal chromosomes were identical and produced comparable amounts of functional protein. It would follow that mutations in one copy of the gene might be overcome by the availability of functional protein obtained from the homologous gene.

Although this mechanism holds true for most genes, recent studies showed that some gene pairs are not functionally equivalent. In these cases, only one copy of the gene is active, whereas the other copy is repressed. Under normal conditions, whether a particular gene product is produced depends on whether the active gene locus was contributed by the mother or father. In certain instances, the maternal genes in a particular chromosomal region are expressed,

whereas in others it is the father's genes that are active. This phenomenon by which a subset of genes is expressed based on the parent of origin is called "genomic imprinting."

Most genetic disorders are caused by mutations within the gene sequence that results in loss of protein function; however, in some disorders, chromosomal mutations are not involved. The production of functional protein in these cases is dictated by factors that regulate the pattern of gene expression, not the actual nucleotide sequence. This type of phenomenon is termed "epigenetic." Earlier, we reviewed an example of epigenetic factors when we discussed the methylation of CpG islands in FXS; we continue this discussion below with regards to Rett syndrome. Imprinting is another example of an epigenetic phenomenon.

Genomic imprinting has been identified in about 30 genes. Many of the protein products of these genes are necessary for growth and differentiation of various tissues. Disruption in the genetic imprinting of these genes has been implicated in a variety of cancers and developmental disorders. The latest advances in this field are discussed here related to Prader-Willi syndrome (PWS) and Angelman syndrome, two developmental disorders caused by imprinting defects [43].

PWS is a rare genetic disorder with a prevalence of 1 in 10,000 live births. Individuals with this defect manifest a plethora of symptoms at birth [44]. Newborns show pronounced hypotonia and failure to thrive. In addition, the dietary habits change dramatically within the first couple of years of life and are characterized by hyperphagia and food preoccupations, and the affected individual often become obese [45]. Mild to moderate mental retardation is present, as well as several other behavioral problems, including temper tantrums, aggressive behaviors, and obsessive-compulsive symptoms outside of the compulsive food-related behaviors [46–48].

Angelman syndrome is another rare neurologic disorder with a similar prevalence to PWS. Affected infants also are hypotonic after birth, develop motor delays, and show mild to moderate mental retardation. They have a characteristic facial appearance with a large mandible and an open-mouth expression, stiffened gait, and puppet-like limb movements. They rarely develop speech and exhibit severe learning disabilities and deficits in attention and hyperactivity. Most of the affected children develop abnormal electroencephalograms and epilepsy [49].

By the mid-1980s, the genetic locus associated with both syndromes was identified, and investigators mapped a deletion on chromosome 15 (15q11-13). Cytogenetic and molecular analysis showed that the same region of chromosome 15 was deleted in individuals with either disorder. It was difficult to comprehend how identical deletions could cause two completely different syndromes: Prader-Willi and Angelman [50,51]. Further studies showed that the clinical symptoms depend on whether the chromosome 15q deletion is derived from the mother or father [52]. It was then discovered that most cases of PWS resulted from deletions of the paternal chromosome, whereas Angelman was due to a deletion in the mother's chromosome 15.

The underlying mechanism was clarified further when a smaller segment within the chromosome 15 deletions was identified for each disorder. The segment of the DNA responsible for PWS is discrete, but maps close to the region that is implicated in Angelman syndrome. This explained how the identical chromosomal deletion could lead to the two syndromes. A large deletion of chromosome 15 spans genetic loci for both disorders; the child develops PWS when the paternal chromosome is deleted and Angelman syndrome when the maternal chromosome is deleted.

The genetic imprinting of these two closely inherited regions of chromosome 15 is different. Normally, the paternal chromosome expresses genes within the PWS region, whereas the nearby set of Angelman genes is imprinted or silenced. In contrast, the maternal chromosome expresses the genes within the Angelman region, whereas the adjacent PWS region is repressed [53,54]. Thus, a deletion of the paternal chromosome results in the deletion of the active PWS genes, and the corresponding region on the maternal chromosome does not compensate for the missing gene products: the result is PWS. When the deletion occurs on the maternal chromosome, the active Angelman genes are deleted, and the paternal chromosome remains imprinted and again cannot compensate for the missing genes.

Approximately 70% of cases of PWS and Angelman syndrome are attributed to chromosome 15 deletions; however, a second mechanism exists for these disorders. Occasionally, segregation defects during meiosis result in two copies of chromosome 15 being inherited from one of the parents [55]. This unusual mechanism is called uniparental disomy (UPD). UPD occurs when two copies of a chromosome or a part of the chromosome is inherited from one of the parents, with no contributions from the other. It usually arises because of a trisomy event, where two chromosomes from one parent are inappropriately passed on with one copy from the other parent, resulting in three copies of the chromosome. One of three chromosomes is lost during the formation of gametes. If the initial trisomy happened as a result of two copies of the maternal chromosome and one copy of the paternal chromosome, loss of the paternal chromosome results in maternal UPD with two maternal copies of the chromosome. Conversely, if the initial trisomy event involved two copies of the paternal chromosomes, the loss of the maternal chromosome during gamete formation leads to paternal UPD.

Inheritance of two maternal copies of chromosome 15 results in PWS [56]. In this case, the genes in the PWS region are present on both of the maternal chromosomes, but are repressed because of genomic imprinting; the converse applies for paternal UPD. The PWS genes present on both paternal copies of chromosome 15 are expressed. In contrast, the adjacent region critical for Angelman syndrome is imprinted, resulting in transcriptional silencing and lack of protein expression.

A third mechanism is observed in 2% to 3% of individuals with PWS or Angelman syndrome and involves mutations within the imprinting center [57,58]. The imprinting center regulates gene expression by controlling the level of methylation resulting in heterochromatin spreading for hundreds of kilobases on either side. One of the effects of methylation is to facilitate the compacting chromatin to be tightly packaged; however, a consequence is that this stretch of DNA is inaccessible for transcription. Imprinting of PWS and Angelman critical chromosomal region 15q11-13 is modulated by the same bipartite imprinting center. Mutation within this imprinting center results in a disruption of the normal patterns of paternal or maternal imprinting.

Finally, a fourth molecular mechanism for Angelman syndrome is responsible for about 10% of cases; mutations occur in a specific gene (*UBE3A*) that lies within the Angelman region [59–61]. *UBE3A* encodes a protein called ubiquitin protein ligase that normally is required for regulating the turnover of cellular proteins by ubiquitin-mediated proteolysis. The removal of proteins from the intracellular environment by protein degradation is essential for maintaining proper cellular function. These include signaling proteins that need to be sequestered and degraded quickly. It also is vital to remove damaged proteins before they disrupt the normal cellular processes. Several copies of a small protein molecule (ubiquitin) are added to the protein targeted for proteasomal degradation. The attachment of several ubiquitin molecules requires the function of *UBE3A*, because it one of the enzymes that mediates the addition of ubiquitin tags. Intracellular organelles called proteasomes recognize the ubiquitinated proteins, bind to them, and activate proteases necessary to degrade these proteins into constitutive amino

acids. Mutation in *UBE3A* leads to improper accumulation of damaging molecules within the central nervous system (CNS) that interferes with normal synaptic functioning.

In addition to *UBE3A*, several other enzymes are required to catalyze the intermediary steps in the attachment of ubiquitin molecules to protein targeted for proteolysis. It is possible that mutations in these other proteins could result in Angelman syndrome. This would be an excellent example of locus heterogeneity, where mutations in distinctly different genes of the same pathway result in the expression of identical phenotype. Several laboratories are pursuing this line of study. In the case of PWS, mutations in a single gene have not been identified.

Two final points should be made about Angelman syndrome. Recent studies suggested that the maternal chromosomes are differentially imprinted in a brain region-specific pattern [62]. For example, the maternal copy of *UBE3A* is expressed exclusively in the hippocampus and cerebellum. This adds an interesting twist to the imprinting story, because it suggests that some genes are expressed depending on whether they lie on the paternal or maternal copy and that specific brain areas may contribute to expression levels through activation of imprinting mechanisms. Finally, one of the genes encoded by the Angelman critical region is a subunit of the γ -amino-butyric acid (GABA)_A receptor [63]. The aberrant GABA transmission as a result of this mutation is believed to be responsible for the epilepsy that develops in many affected individuals. Patients who have cases of Angelman syndrome in which only a point mutation exists in the *UBE3A* gene do not develop epilepsy.

Rett syndrome

Rett syndrome is characterized by progressive neurologic decline [64]. Children affected by this syndrome develop normally and achieve age-appropriate milestones during the first year of life; parents of these children do not report developmental abnormalities. One of the first observable clinical signs is the loss of voluntary hand movements and the development of stereotypical repetitive behaviors, such as hand wringing. Other symptoms of the disorder soon emerge, including progressive loss of speech, growth retardation that leads eventually to microcephaly, ataxia, and a severe disruption of higher brain functions. The clinical symptoms reach a plateau and stabilize over the next few decades of life [65].

An unusual aspect of this syndrome is that most of the affected individuals are female. The female prevalence was explained by the fact that the mutated gene was on the X-chromosome, and, hence, would cause embryonic lethality in males. There are numerous examples of X-linked disorders in which the males die in utero because they have only one copy of the X-chromosome. In females, the second normal X-chromosome seems to generate enough gene products to provide protection during embryonic development and into the initial phase of postnatal life. Nevertheless, symptoms eventually emerge as a result of haploinsufficiency, because a single copy of the gene is unable to impart lasting protection.

Analysis of affected female siblings led to the mapping of the gene to Xq28, and candidate genes in this region were studied carefully [66]. A systematic comparison of nucleotide sequences from affected and normal individuals excluded a number of genes as possible candidates; however, more recent studies identified a gene that is mutated in several patients who have Rett syndrome [67]. The gene encodes a methyl CpG-binding protein 2 (MeCP2), and mutations in two of its functional domains have been reported.

What is the relevance of MeCP2 mutations to the clinical symptoms? Of an estimated 25,000 to 35,000 genes in the human genome, a third of them are expressed solely in the CNS. A portion of these genes and their gene products are essential for the normal development of the brain, whereas others are required during postnatal development, and the rest are expressed constitutively to perform housekeeping functions. Therefore, it is important to regulate gene

expression carefully, such that only those genes are expressed that are required in a particular tissue and during a specific time period.

The protein that is implicated in Rett syndrome plays an important role in regulating gene expression. The accessibility of DNA to transcription factors is determined by the degree of methylation of the regulatory sequences [68]. Methylation is a chemical modification of the DNA, where a methyl group is attached to the cytosines, especially when it occurs within CpG sequences. These CpG islands are found predominantly within the regulatory region of genes termed the “promoter.” One of the approaches to identify transcriptional start sites is to look for CpG islands, because they are found often adjacent to transcriptional initiation sites.

It was believed previously that methylation of DNA sequences was sufficient for repressing gene transcription. RNA polymerase II, which is the enzyme that transcribes most DNA to RNA, was believed to be incapable of binding to promoter regions with methylated CpG islands, and, hence, could not initiate transcription of these genes. The mechanism of gene activation and repression turns out to be more complicated.

MeCP2, the protein mutated in Rett syndrome, consists of different functional domains [69]. The amino-terminal methyl CpG binding domain recognizes methylated cytosines and binds to them. The transcriptional repressor domain (TRD) is then activated, leading to the recruitment of other proteins to form a regulatory complex that works together to repress transcription. One of the proteins in this complex is a histone deacetylase. Histones are a family of nuclear proteins that play a role in packaging DNA into higher-order chromatin structures. Modification of histones by methylation and acetylation determines whether the secondary structure of DNA is accessible to transcription factors. The histone deacetylases remove the acetyl groups from histones, resulting in the compaction of DNA around the promoter such that the transcriptional machinery is no longer able to access it. This heterochromatic region effectively represses gene expression. In summary, MeCP2 functions to silence genes to which it binds, and mutations that inactivate it presumably lead to inappropriate transcriptional activation [70].

The first report on MeCP2 mutations identified six types [67]. Several of these were missense mutations that replaced critical amino acids and led to aberrant protein function. Most of the mutations of this kind were mapped to the methyl CpG binding domain, and, thereby, disrupt the ability of MeCP2 to recognize and bind methylated DNA. Other mutations were found in the second critical domain of the protein: the transcriptional repressor domain that is necessary for recruiting histone deacetylases. These mutations included a single nucleotide insertion that leads to a frame shift of the downstream codons. A shift in the codon reading frame results in an altered amino acid sequence downstream of the point of mutation. The second mutation found within the TRD domain was an inappropriate change to a stop codon. This type of mutation leads to the production of shortened or truncated proteins. The proteins produced as a result of these mutations have reduced function or are completely nonfunctional [67].

In the initial study, only certain regions of the MeCP2 locus was sequenced. Only the DNA sequence that encodes for amino acids was sequenced. Generally, these regions of the DNA are analyzed first because they require far fewer nucleotides to be sequenced as compared with the large noncoding sequences that include introns and regulatory elements. For this reason, mutations within the coding regions of the gene are identified earlier, although mutations may occur within the regulatory sequences disrupting the gene function. As additional noncoding sequences were analyzed, more than 80% of the individuals who had Rett syndrome were found to carry mutations in the MeCP2 gene.

Different mutations within the same gene that result in similar phenotypic expression are examples of allelic heterogeneity. In many disorders, identical or closely related clinical

phenotypes are observed when different functional domains of the same protein are mutated. This phenomenon of allelic heterogeneity was found in the initial Rett syndrome study in which six mutations in the MeCP2 gene produced individuals with the same clinical disorder [67]. It is likely that additional mutations identified in the MeCP2 gene will produce similar phenotype. In certain cases, mutations to a single gene can produce different clinical manifestations. One example would be mutations that occur in one of the fibroblast growth factor receptors, where several independent mutations result in distinctly different skeletal and growth abnormalities [71].

Conversely, locus heterogeneity refers to the phenomenon by which mutations in different genes produce similar clinical symptoms among affected individuals. This can happen when several proteins are necessary for a series of signaling events. MeCP2, for example, is one member of a large family of proteins that play a role in gene silencing. Two other members of this protein family have been reported to bind DNA and recruit histone deacetylase complex. Various laboratories are examining the function of these proteins in gene repression and whether mutations in them can lead to Rett syndrome or related disorders. Several proteins besides MeCP2 are necessary for maintaining specific genes in a repressed state. The clinical phenotype produced by mutations in these other genes may result in symptoms that are similar to those seen in Rett syndrome. Recently, mutations in two other genes, cyclin-dependent kinase like 5 and netrin G, were reported to result in similar clinical phenotype [72–74].

Recent developments in the field of Rett syndrome have raised several interesting questions. One of the questions relates to the preponderance of neurologic problems in the clinical assessment. MeCP2 expression is not restricted to the brain, but is found in many other tissues; however, symptoms other than the neurologic deficits are not observed commonly. This suggests that the CNS may be especially sensitive to the disruption of MeCP2 protein function. Another disorder with a similar situation is Huntington's chorea, in which the neurologic disruptions are a central component of the disease but the mutated gene is expressed in multiple tissue types.

The second question relates to the normal development that occurs early in life. In many neurodegenerative disorders, the development of clinical signs is delayed and not detected until the fourth or fifth decade of life. One possible explanation for this delay is that toxic molecules need to accumulate over a long period of time before they cause neuronal damage. The neuronal loss due to free radical damage, for example, has been implicated in neurodegenerative disorders, including Huntington's chorea, Parkinson's disease, and Alzheimer's disease. In normal cells, enzymes are present to neutralize the free radicals. Loss of enzymatic activity or reduced amounts of the protein because of mutations can cause toxic compounds to accumulate over time and eventually disrupt the normal neuronal function.

This mechanism may explain what is happening in the brain of patients who have Rett syndrome. The normal function of MeCP2 is to repress the expression of certain genes. The downstream target genes, as well as their function, have not been discovered; however, it is reasonable to propose that mutations in MeCP2 result in inappropriate expression of these genes. These gene products may be toxic and disrupt the functioning of normal cellular proteins over time; however, several laboratories have searched for up-regulation of genes using microarray techniques and none have been found.

Summary

The focus of this special issue is on neuropsychiatric disorders, and this article has reviewed several childhood neuropsychiatry disorders with established mutations or deletions. The molecular basis for most common child and adolescent mental disorders has not been determined. Although the reasons for this failure are beyond the scope of this article, one of

the major stumbling blocks has been genetic complexity. Many of the genetic disorders discussed, such as Rett syndrome or FXS, exhibit Mendelian patterns of inheritance; however, pedigree analysis of most child and adolescent psychiatric disorders fails to reveal a clear vertical pattern of transmission across generations.

The presence of non-Mendelian patterns of inheritance does not exclude the involvement of genetic factors; rather, it suggests that their role in the transmission or expression of the clinical symptoms is complex. Polygenic disorders are illness in which multiple genes and environmental factors contribute to the expression of an illness. Autism, childhood-onset anxiety disorders, and attention deficit hyperactivity disorder are examples of disorders that likely fall into this category. Estimates for autism, for example, suggest that up to 10 genes may contribute to the etiology of the disorder, with any one of them making only a small contribution. Significant progress has been made in understanding the genetics of complex disorders in several other fields, including breast cancer and hypertension [75,76]. Child psychiatry should take advantage of the knowledge that has become available from these successes.

Technical advances in genomics, molecular genetics, and developmental neurobiology has given us a remarkable list of accomplishments over the past decade, and these advances lay the foundation for further studies into the molecular basis of these disorders. Success in the field ultimately will lead to the genes that cause more complex diseases, such as autism and pervasive developmental disorders. Identification of cellular and molecular components that are responsible for these disorders will prompt advances in drug development, and the promise of gene therapy may be realized for monogenic disorders.

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References

1. Kandel ER. A new intellectual framework for psychiatry. *Am J Psychiatry* 1998;155:457–469. [PubMed: 9545989]
2. Neslter EJ, Barrot M, DiLeone RJ, et al. Neurobiology of depression. *Neuron* 2002;34:13–25. [PubMed: 11931738]
3. Abelson JF, Kwan KY, O’Roak BJ, et al. Sequence variants in *SLITRK1* are associated with Tourette’s syndrome. *Science* 2005;310:317–320. [PubMed: 16224024]
4. Gupta AR, State MW. Recent advances in the genetics of autism. *Biol Psychiatry* 2007;61:429–437. [PubMed: 16996486]
5. Bagni C, Greenough WT. From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. *Nat Rev Neurosci* 2005;6:376–387. [PubMed: 15861180]
6. Lubs H. A marker X chromosome. *Am J Hum Gen* 1969;21:231–244.
7. Fu YH, Kuhl DP, Pizzuti A, et al. Variation of the CGG repeat at the fragile site results in genetic instability: resolution of the Sherman paradox. *Cell* 1991;67:1047–1058. [PubMed: 1760838]
8. Hagerman RJ, Staley LW, O’Conner R, et al. Learning-disabled males with a fragile X CGG expansion in the upper premutation size range. *Pediatrics* 1996;97:122–126. [PubMed: 8545206]
9. de von Flindt R, Bybel B, Chudley AE, et al. Short-term memory and cognitive variability in adult fragile X females. *Am J Med Genet* 1991;38:488–492. [PubMed: 2018091]
10. Kemper M, Hagerman R, Ahmad R, et al. Cognitive profiles and the spectrum of clinical manifestations in heterozygous *fra(X)* females. *Am J Med Genet* 1986;23:139–156. [PubMed: 3953643]

11. Murphy DG, Mentis MJ, Pietrini P, et al. Premutation female carriers of fragile X syndrome: a pilot study on brain anatomy and metabolism. *J Am Acad Child Adolesc Psychiatry* 1999;38:1294–1301. [PubMed: 10517063]
12. Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, et al. Fragile X premutation is a significant risk factor for premature ovarian failure: The International Collaborative POF in Fragile X Study-preliminary data. *Am J Med Genet* 1999;83:322–325. [PubMed: 10208170]
13. Jacquemont S, Hagerman RJ, Leehey MA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. *J Am Med Assoc* 2004;291:460–469.
14. Jacquemont S, Leehey MA, Hagerman RJ, et al. Size bias of fragile X premutation alleles in late-onset movement disorders. *J Med Genet* 2006;43:804–809. [PubMed: 16723388]
15. Hagerman PJ, Hagerman RJ. Fragile X-associated tremor/ataxia syndrome (FXTAS). *Ment Retard Dev Disabil Res Rev* 2004;10:25–30. [PubMed: 14994285]
16. Nelson D. The fragile X syndromes. *Semin Cell Biol* 1995;6:5–11. [PubMed: 7620122]
17. Oberle I, Rousseau F, Heitz D, et al. Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science* 1991;252:1097–1110.
18. Pieretti M, Zhang F, Fu Y-H, et al. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell* 1991;66:817–822. [PubMed: 1878973]
19. Eichler EE, Richards S, Gibbs RA, et al. Fine structure of the human FMR1 gene. *Hum Mol Genet* 1993;2:1147–1153. [PubMed: 8401496]
20. Siomi H, Siomi M, Nussbaum R, et al. The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. *Cell* 1993;74:291–298. [PubMed: 7688265]
21. Siomi H, Choi M, Siomi MC, et al. Essential role for KH domains in RNA binding: impaired RNA binding mutation in the KH domain of FMR1 that causes fragile X syndrome. *Cell* 1994;77:33–39. [PubMed: 8156595]
22. De Boulle K, Verkerk AJ, Reyniers E, et al. A point mutation in the FMR-1 gene associated with fragile X mental retardation. *Nat Genet* 1992;3:31–35. [PubMed: 8490650]
23. Musco G, Stier G, Joseph C, et al. Three dimensional structure and stability of the KH domain: molecular insights into the fragile X syndrome. *Cell* 1996;85:237–245. [PubMed: 8612276]
24. Khandjan EW, Corbin F, Woerly S, et al. The fragile X mental retardation protein is associated with ribosomes. *Nat Genet* 1996;12:91–93. [PubMed: 8528261]
25. Abitbol M, Menini C, Delezoide AL, et al. Nucleus basalis magno cellularis and hippocampus are the major sites of FMR-1 expression in human fetal brain. *Nat Genet* 1993;4:147–152. [PubMed: 8348153]
26. Mostofsky SH, Mazzocco MM, Aakalu G, et al. Decreased cerebellar posterior vermis size in fragile X syndrome: correlation with neurocognitive performance. *Neurology* 1998;50:121–130. [PubMed: 9443468]
27. Mostofsky SH, Reiss AL, Lockhart P, et al. Evaluation of cerebellar size in attention-deficit hyperactivity disorder. *J Child Neurol* 1998;13:434–439. [PubMed: 9733289]
28. Reiss AL, Lee J, Freund L. Neuroanatomy of fragile X syndrome: the temporal lobe. *Neurology* 1994;44:1317–1324. [PubMed: 8035938]
29. Dutch-Belgian Fragile X Consortium. FMR-1 knock out mice: a model to study fragile X mental retardation. *Cell* 1994;78:23–33. [PubMed: 8033209]
30. Steward O, Schuman EM. Protein synthesis at synaptic sites on dendrites. *Annu Rev Neurosci* 2001;24:299–325. [PubMed: 11283313]
31. Ostroff LE, Fiala JC, Allwardt B, et al. Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* 2002;35:535–545. [PubMed: 12165474]
32. Brown V, Jin P, Ceman S, et al. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell* 2001;107:477–487. [PubMed: 11719188]
33. Darnell JC, Jensen KB, Jin P, et al. Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 2001;107:489–499. [PubMed: 11719189]

34. Zalfa F, Giorgi M, Primerano B, et al. The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. *Cell* 2003;112:317–327. [PubMed: 12581522]
35. Kelleher RJ, Govindarajan A, Tonegawa S. Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* 2004;44:59–73. [PubMed: 15450160]
36. Kelleher RJ, Govindarajan A, Jung HY, et al. Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* 2004;116:467–479. [PubMed: 15016380]
37. Irwin SA, Patel B, Idupulapati M, et al. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. *Am J Med Genet* 2001;98:161–167. [PubMed: 11223852]
38. Comery TA, Harris JB, Willems PJ, et al. Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proc Natl Acad Sci USA* 1997;94:5401–5404. [PubMed: 9144249]
39. Caskey CT, Pizzuti A, Fu YH, et al. Triplet repeat mutations in human disease. *Science* 1992;256:784–789. [PubMed: 1589758]
40. Nelson D, Warren S. Trinucleotide repeat instability: when and where? *Nat Genet* 1993;4:107–108. [PubMed: 8348143]
41. Warren S. The expanding world of trinucleotide repeats. *Science* 1996;271:1374–1375. [PubMed: 8596908]
42. Fu YH, Pizzuti A, Fenwick RG Jr, et al. An unstable triplet repeat in a gene related to myotonic dystrophy. *Science* 1992;255:1256–1258. [PubMed: 1546326]
43. Isles AR, Humby T. Modes of imprinted gene action in learning disability. *J Intellect Disabil Res* 2006;50:318–325. [PubMed: 16629925]
44. Prader A, Labhart A, Willi H. A syndrome of adiposity, small stature, cryptorchism, mental retardation and hypotonia in new-born infants [German]. *Schweiz Med Wschenschr* 1956;86:1260–1261.
45. Holm VA, Cassidy SB, Butler MG, et al. Prader-Willi syndrome: consensus diagnostic criteria. *Pediatrics* 1993;91:398–402. [PubMed: 8424017]
46. Dykens EM, Cassidy SB. Correlates of maladaptive behavior in children and adults with Prader-Willi syndrome. *Am J Med Genet* 1995;60:546–549. [PubMed: 8825893]
47. Dykens EM, Leckman JF, Cassidy SB. Obsessions and compulsions in Prader-Willi syndrome. *J Child Psychol Psychiatry* 1996;37:995–1002. [PubMed: 9119946]
48. State MW, Dykens EM, Rosner B, et al. Obsessive-compulsive symptoms in Prader-Willi and “Prader-Willi-like” patients. *J Am Acad Child Adolesc Psychiatry* 1999;38:329–334. [PubMed: 10087695]
49. Angelman H. ‘Puppet children’ a report of three cases. *Dev Med Child Neurol* 1965;7:681–688.
50. Ledbetter DH, Riccardi VM, Airhart SD, et al. Deletions of chromosome 15 as a cause of the Prader-Willi syndrome. *N Engl J Med* 1981;304:325–329. [PubMed: 7442771]
51. Magenis RE, Brown MG, Lacy DA, et al. Is Angelman syndrome an alternate result of del(15)(q11q13)? *Am J Med Genet* 1987;28:829–838. [PubMed: 3688021]
52. Cassidy SB, Schwartz S. Prader-Willi and Angelman syndromes: disorders of genomic imprinting. *Medicine* 1998;77:140–151. [PubMed: 9556704]
53. Cassidy SB. Prader-Willi syndrome. *J Med Genet* 1997;34:917–923. [PubMed: 9391886]
54. Nicholls RD, Saitoh S, Horsthemke B. Imprinting in Prader-Willi and Angelman syndromes. *Trends Genet* 1998;14:194–200. [PubMed: 9613204]
55. Ledbetter D, Engel E. Uniparental disomy in humans: development of an imprinting map and its implications for prenatal diagnosis. *Hum Mol Genet* 1995;4:1757–1764. [PubMed: 8541876]
56. Nicholls RD, Knoll JH, Butler MG, et al. Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. *Nature* 1989;342:281–285. [PubMed: 2812027]
57. Ohta T, Gray TA, Rogan PK, et al. Imprinting-mutation mechanisms in Prader-Willi syndrome. *Am J Hum Genet* 1999;64:397–413. [PubMed: 9973278]
58. Buiting K, Barnicoat A, Lich C, et al. Disruption of the bipartite imprinting center in a family with Angelman syndrome. *Am J Hum Genet* 2001;68:1290–1294. [PubMed: 11283796]
59. Kishino T, Lalande M, Wagstaff J. UBE3A/E6-AP mutations cause Angelman syndrome. *Nat Genet* 1997;15:70–73. [PubMed: 8988171]

60. Matsuura T, Sutcliffe JS, Fang P, et al. Novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. *Nat Genet* 1997;15:74–77. [PubMed: 8988172]
61. Nicholls RD. Strange bedfellows? Protein degradation and neurological dysfunction. *Neuron* 1998;21:647–649. [PubMed: 9808447]
62. Albrecht U, Sutcliffe JS, Cattanach BM, et al. Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. *Nat Genet* 1997;17:75–78. [PubMed: 9288101]
63. DeLorey TM, Handforth A, Anagnostaras SG, et al. Mice lacking the beta3 subunit of the GABAA receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. *J Neurosci* 1998;18:8505–8514. [PubMed: 9763493]
64. Rett A. On a unusual brain atrophy syndrome in hperammonemia in childhood [German]. *Wien Med Wochenschr* 1966;116:723–726. [PubMed: 5300597]
65. Naidu S. Rett syndrome: natural history and underlying disease mechanisms. *Eur Child Adolesc Psychiatry* 1997;6:14–17. [PubMed: 9452913]
66. Webb T, Clarke A, Hanefeld F, et al. Linkage analysis in Rett syndrome families suggests that there may be a critical region at Xq28. *J Med Genet* 1998;35:997–1003. [PubMed: 9863596]
67. Amir RE, Van den Veyver IB, Wan M, et al. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 1999;23:185–188. [PubMed: 10508514]
68. Kass SU, Pruss D, Wolffe AP. How does DNA methylation repress transcription? *Trends Genet* 1997;13:444–449. [PubMed: 9385841]
69. Neul JL, Zoghbi HY. Rett syndrome: a prototypical neurodevelopmental disorder. *Neuroscientist* 2004;10:118–128. [PubMed: 15070486]
70. Nan X, Campoy FJ, Bird A. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. *Cell* 1997;88:471–481. [PubMed: 9038338]
71. Park WJ, Meyers GA, Li X, et al. Novel FGFR2 mutations in Crouzon and Jackson-Weiss syndromes show allelic heterogeneity and phenotypic variability. *Hum Mol Genet* 1995;4:1229–1233. [PubMed: 8528214]
72. Weaving LS, Christodoulou J, Williamson SL, et al. Mutations of CDKL5 cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. *Am J Hum Genet* 2004;75:1079–1093. [PubMed: 15492925]
73. Evans JC, Archer HL, Colley JP, et al. Early onset seizures and Rett-like features associated with mutations in CDKL5. *Eur J Hum Genet* 2005;13:1113–1120. [PubMed: 16015284]
74. Borg I, Freude K, Kubart S, et al. Disruption of Netrin G1 by a balanced chromosome translocation in girl with Rett syndrome. *Eur J Hum Genet* 2005;13:921–927. [PubMed: 15870826]
75. Lifton RP. Molecular genetics of human blood pressure variation. *Science* 1996;272:676–680. [PubMed: 8614826]
76. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;226:66–71. [PubMed: 7545954]