Trinucleotide Repeat Expansion and Neuropsychiatric Disease

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T rinucleotide, or triplet, repeats consist of 3 nucleotides consecutively repeated (eg, CCG CCG CCG CCG CCG) within a region of DNA, a not uncommon motif in the genome of humans and other species. In 1991, a new type of genetic mutation was discovered, known as a dynamic or expansion mutation, in which the number of triplets in a repeat increases and the length becomes unstable. During the past decade, nearly 20 diseases—including Huntington disease, 2 forms of the fragile X syndrome, and myotonic dystrophy—caused by trinucleotide repeat expansions have been identified. The unstable nature of the expanded repeat leads to remarkable patterns of inheritance in these diseases, distinctly at odds with traditional notions of mendelian genetics. We review the clinical and genetic features of these disorders, with a particular emphasis on their psychiatric manifestations. We also critically examine the hypothesis that expansion mutations may have an etiologic role in psychiatric diseases such as bipolar disorder, schizophrenia, and autism. *Arch Gen Psychiatry. 1999;56:1019-1031*

Genetic influences play an important etiologic role in many psychiatric disorders.[1] However, finding the specific genetic mutations involved in the etiology of these diseases has proved extremely difficult.[2-4] Many factors contribute to this difficulty, including a lack of biological findings to externally validate diagnostic constructs,[5] phenotypic heterogeneity,[6,7] patterns of inheritance that do not fit classic mendelian patterns,[8,9] incomplete penetrance,[10-13] and the presumed existence of phenocopies in which the same clinical syndrome arises from alternative genetic and nongenetic factors.[14]

The problem of finding genetic factors of etiologic significance for psychiatric disorders has been approached from 2 different directions. On the one hand, a number of large-scale projects have focused on finding genetic linkage of disease phenotype to anonymous genetic markers.[2,15-21] The goal is to determine the markers most consistently linked to the disease phenotype, and then gradually narrow down the suspect region until a specific etiologically relevant genetic mutation or variation is identified. These attempts have met with some success, although the regions of potential linkage remain quite large[2,18] and no mutations or etiologically significant variations have yet been identified. On the other hand, attempts have been made to demonstrate an association between disease and various candidate genes, chosen based on theories of disease pathophysiology or treatment pharmacology. In this case, the gene is chosen first, and then it is determined if affected subjects disproportionately inherit some marker within or near the gene. A number of associations have been reported with this approach, but replication and interpretation of findings have proved problematic.[2,22-25]

The recent discovery of trinucleotide repeat expansion has led to a third approach: a search for candidate genes with repeat expansions, a genetic hypothesis of etiology.[26-29] We review the currently known trinucleotide repeat diseases and the ratio-

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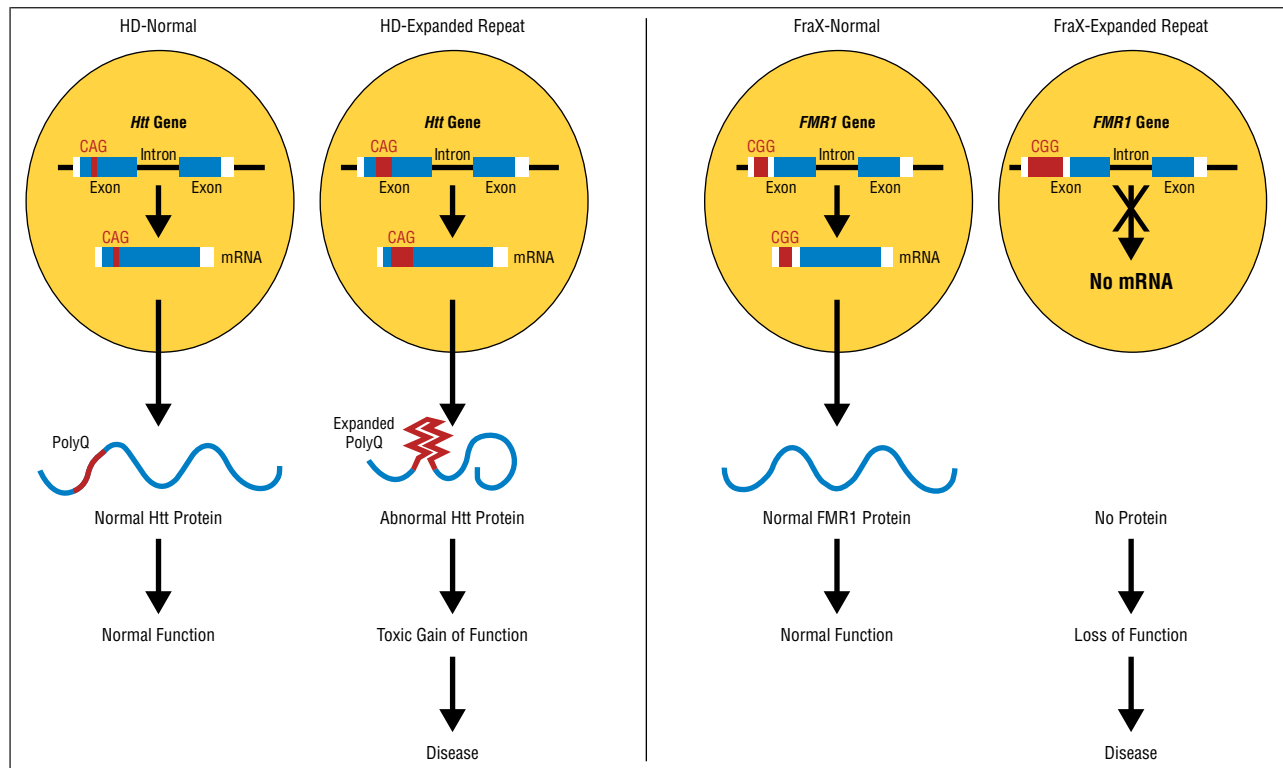
Figure 1. *Molecular pathogenesis of Huntington disease (HD) and the fragile X (FraX) syndrome. Left, The effect of a CAG repeat expansion in the* Htt *gene. Within the nucleus (yellow), genes with either a normal CAG repeat or an expanded CAG repeat are transcribed into messenger RNA (mRNA), with normal excision of introns and splicing together of exons. Outside the nucleus, mRNA with either a normal or a long CAG repeat is translated into protein. The CAG repeat itself, located within a protein coding region (blue), is translated into a stretch of the amino acid glutamine (Q). The mutant protein, containing an excessively long polyglutamine (polyQ) repeat, takes on an abnormal conformation that confers new and toxic properties to the protein. Right, The effect of an expansion of the CGG repeat in the FraX mental retardation type 1 (*FMR1*) gene. In* FMR1 *with a normal-length repeat, the gene is transcribed into mRNA, and the mRNA is translated into protein. The CGG repeat is located outside the protein coding region and, hence, is not translated into an amino acid repeat. In* FMR1 *with an expanded CGG repeat, the expansion prevents gene transcription into mRNA and therefore no protein is synthesized. Disease arises from a lack of the protein.*

nale for the hypothesis that repeat expansions may contribute to the genetic etiology of psychiatric disorders.

TRINUCLEOTIDE REPEATS

Trinucleotide, or triplet, repeats are 3 nucleotides consecutively repeated (eg, CGG CGG CGG CGG CGG) within a region of DNA. All possible combinations of nucleotides are known to exist as triplet repeats, though some, including CGG and CAG, are more common than others.[30,31] The repeats may be within genes or in intergenic DNA. In genes, repeats may be found in introns (gene segments transcribed into RNA but then excised from the primary RNA transcript) or in exons (gene segments that are transcribed and remain represented in mature RNA). If within exons, they may be present in a translated region and hence encode a series of identical amino acids, or they may occur in regions not translated into protein. Repeats are frequently found in genes that en-

code transcription factors (proteins that regulate the level of expression of other genes) and in genes that regulate development.[32-34]

In 1991, triplet repeats were found to undergo a new type of genetic mutation, termed a "dynamic" or "expansion mutation." In this type of mutation, through mechanisms during DNA replication that are only partly understood,[35] the number of triplets in a repeat increases. Unlike repeats of normal length, in which changes in length from one generation to the next are extremely rare, expanded repeats tend to be unstable—an expanded repeat passed from one generation to the next will usually vary in length, typically becoming longer. During the past decade, nearly 20 diseases caused by a trinucleotide repeat expansion have been identified, as well as other diseases caused by related mutations.[36,37] These disorders, with features of inheritance often at odds with the traditional teachings of classic mendelian genetics, have generated

considerable excitement among geneticists. This excitement has made an impact on psychiatric genetics, as aspects of expansion mutation genetics and the remarkable central nervous system manifestations of so many of these diseases force consideration of the potential role of repeat expansion in the etiology of idiopathic psychiatric disorders.

THE EXPANSION MUTATION DISEASES

Figure 1 depicts expansion mutations in Huntington disease (HD) and fragile X syndrome (A subtype), prototypical expansion disorders. In HD, the CAG expansion is in a protein coding region of an exon, so that the expansion results in an abnormal protein. In fragile X syndrome, the CGG repeat is in an untranslated region of an exon, and the expansion prevents gene transcription. **Figure 2** overviews the genetic locations of the repeats in the other expansion disorders. The
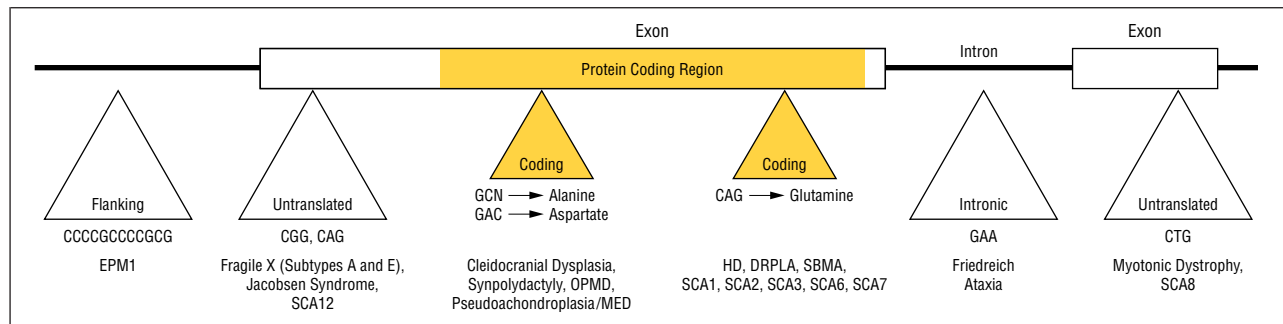
Figure 2. *Genetic locations of repeat expansions. Repeat expansions that cause disease have been detected in flanking and intronic regions, transcribed but untranslated regions, and protein coding regions (orange). Expansions within protein coding regions tend to be smaller than those in other genic regions. EPM1 indicates progressive myoclonic epilepsy type 1; SCA, spinocerebellar ataxia; OPMD, oculopharyngeal muscular dystrophy; MED, multiple epiphyseal dysplasia; HD, Huntington disease; DRPLA, dentatorubral-pallidoluysian atrophy; and SBMA, spinal and bulbar muscular atrophy.*

Summary of the Repeat Expansion Disorders*

Disease	Gene	Repeat	Amino Acid Encoded	Normal No. of Triplets	Expanded No. of Triplets	Inheritance	Effect of Expansion	Reference
CAG/Polyglutamine								
HD	*Huntington*	CAG	Gln	6-35	36-121	AD†	Abn prt	38
DRPLA	*Atrophin-1*	CAG	Gln	3-35	49-88	AD†	Abn prt	39, 40
SCA1	*Ataxin-1*	CAG	Gln	6-38	39-83	AD†	Abn prt	41
SCA2	*Ataxin-2*	CAG	Gln	14-31	32-77	AD†	Abn prt	42-44
SCA3 (MJD)	*Ataxin-3*	CAG	Gln	12-39	56-86	AD†	Abn prt	45
SCA7	*Ataxin-7*	CAG	Gln	7-35	38-200	AD†	Abn prt	46
SBMA	*Androgen receptor*	CAG	Gln	9-36	38-62	X-linked†	Abn prt	47
Short Expansions								
SCA6	*CACNA1A*	CAG	Gln	4-19	20-30	AD	Abn prt	48
CCD	*CBFA1*	GCN	Ala	17	27	AD	Abn prt	49
Synpolydactyly	*HOXD13*	GCN	Ala	15	22, 23, or 25	AD	Abn prt	50
OPMD	*PABP2*	GCG	Ala	6	7-13	AD or AR	Abn prt	51
Pseudoachondroplasia/ MED	*COMP*	GAC	Asp	5	4 (Contraction) 6-7 (Expansion)	AD	Abn prt	52
Untranslated								
Fragile X (A subtype)	*FMRI*	CGG	None (UTR)	6-54	200-2000+	X-linked recessive†	↓Express	53-55
Fragile X (E subtype)	*FMR2*	GCC	None (UTR)	6-35	>200	X-linked recessive	↓Express	56
Jacobsen syndrome	*CBL2*	CGG	None (UTR)	8-14	>100	Nonmendelian	Deletion	57
Myotonic dystrophy	*MDPK*	CTG	None (UTR)	5-38	50-2000+	AD†	Unknown	58-60
SCA8	*SCA8*	CTG	None (UTR)	16-37	107-127	AD	Unknown	61
SCA12	*PRB55β*	CAG	None (UTR)	7-28	66-78	AD	Unknown	62
Friedreich ataxia	*Frataxin*	GAA	None (intron)	8-22	120-1700	AR†	↓Express	63
EPM1	*Cystatin B*	Dodecamer	None (5' flanking)	2-3	30-75‡	AR	↓Express	64, 65

*HD indicates Huntington disease; DRPLA, dentatorubral-pallidoluysian atrophy; SCA, spinocerebellar ataxia; MJD, Machado-Joseph disease; SBMA, spinal and bulbar muscular atrophy; CCD, cleidocranial dysplasia; OPMD, oculopharyngeal muscular dystrophy; MED, multiple epiphyseal dysplasia; EPM1, progressive myoclonic epilepsy type 1; Gln, glutamine; Ala, alanine; Asp, aspartic acid; UTR, untranslated region; AD, autosomal dominant; AR, autosomal recessive; Abn prt, abnormal protein; and ↓Express, decreased gene expression. Data are derived in part from Andrew et al[66] and Ross et al.[67] The boundaries of the size of normal and abnormal repeats will likely change as additional subjects are tested.

†Anticipation.

‡EPM1 repeat is a dodecamer.

Table summarizes some of the genetic features of each disease.

Expansions of CAG repeats located within protein coding regions are currently known to cause 8 disorders, 7 of which are similar. CAG encodes the amino acid glutamine,[68] so that the mutant proteins contain extended stretches of glutamine residues. The number of glutamines in the normal range (below about 35) and abnormal range (above about 35) are similar in each disorder (with the exception of SCA6 [spinocerebellar ataxia type 6]; see below). The clinical phenotypes include abnormal voluntary and involuntary movements frequently accompanied by neuropsychiatric syndromes. Reliable diagnosis for most of these disorders is dependent on genetic testing.[69,70]

The pathological changes of the polyglutamine diseases are believed to stem from a toxic gain of function conferred by the abnormally long string of consecutive glutamine residues encoded by the expanded CAG repeat.[68] The pathological process involves neuronal degeneration[67,68,71] with selective neuronal vulnerability. Even though the genes are expressed ubiquitously, few abnormali-

ties have been detected outside the brain. The areas affected in the different disorders overlap to a considerable extent,[67,68,72,73] and include cerebral cortex, basal ganglia, brainstem nuclei, cerebellar dentate nucleus, Purkinje cells of the cerebellum, and spinal and bulbar motor neurons. In contrast with Alzheimer disease, abnormalities in the hippocampus and the basal forebrain are not prominent.

Microscopic analysis of the affected regions indicates the presence of neuronal loss and gliosis without either inflammation or deposition of extracellular material. Aggregates of mutant protein forming inclusion bodies within neuronal nuclei and cytoplasm are a feature of all CAG/glutamine expansion diseases so far studied.[74-78] The role of these inclusions in disease pathogenesis, if any, remains to be determined.[71] Abnormalities of dendritic structure have also been described for HD.[79] It is likely that some of the manifestations of these diseases arise from neuronal dysfunction as well as neuronal death.[74]

The CAG expansion that causes SCA6 is much shorter than that of the other CAG-related diseases, suggesting a different form of pathogenesis. The glutamine repeat is in a variant of the neuronal P-type calcium channel, which is greatly enriched in cerebellar Purkinje cells.[48,80] Small increases in the length of the glutamine repeat in this protein might cause disease by altering channel function, with a consequent change in calcium fluxes resulting in damage to the Purkinje cells.

Three diseases can be caused by relatively small increases in number of consecutive GCN triplets (where N = any nucleotide), resulting in small elongations of repeats of the amino acid alanine. Cleidocranial dysplasia and synpolydactyly are both disorders of skeletal patterning. In the latter, the length of the mutant protein varies in different pedigrees, containing between 6 and 13 extra alanine residues. Pedigrees with the longer expansions tend to have a more severe phenotype. An increase from the normal 6 consecutive GCG triplets to between 8 and 13 triplets in the *PABP2* gene causes the autosomal dominant form of oculopharyngeal muscular dystrophy.[51] Remarkably, rare homozygotes in which both alleles contain $(GCG)_7$—a single triplet longer than normal—develop an autosomal recessive form of the disease, and a compound heterozygote with a $(GCG)_7$ allele and a $(GCG)_9$ allele has a particularly severe phenotype. Small differences in repeat length, therefore, can alter both disease phenotype and the mode of disease inheritance. Most recently, variations in the GAC repeat in the cartilage oligomeric matrix protein have been shown to cause forms of pseudoachondroplasia and multiple epiphyseal dysplasia.[52]

Other diseases are caused by trinucleotide repeat expansions occurring within portions of genes that are not transcribed into protein.[47,53-60,63] As listed in the Table, the expansions are generally quite long, but otherwise these diseases have little similarity to each other.

The fragile X syndrome is the most common form of hereditary mental retardation.[81] The fragile site detectable on cytogenetic analysis is now known to be a manifestation of a CGG expansion in the 5′ untranslated region of the fragile X mental retardation 1 (*FMR1*) gene.[53-55] The expansion leads to excessive attachment of methyl groups to the adjacent CpG island (a genomic region with a disproportionate number of the base pairs C and G in doublets) and consequent loss of gene transcription.[82,83] The complicated phenotype includes mental retardation, frequent psychiatric syndromes, and dysmorphic features.[84] Fewer than 1% of the cytogenetic cases of fragile X syndrome actually arise from a nearly identical mutation (fragile X subtype E) in a gene, *FMR2*, located near *FMR1*.[56,85-87] The phenotype tends to be milder.[85,88] Three other fragile sites are caused by repeat expansions. FRAXF (a CCG repeat),[89] FRA16A (a CCG repeat),[90] and FRA16B (an A-T rich repeat 33 nucleotides in length)[91] are not associated with a phenotype. FRA11B, resulting from a CGG expansion in the untranslated region of the proto-oncogene *CBL2*, is associated with in deletion of the distal portion of chromosome 11q. The consequent phenotype, known as 11q- or Jacobsen syndrome, includes abnormalities of cognition, facial structure, and the hematologic system.[57]

Myotonic dystrophy is characterized by myotonia, cataracts, cardiac conduction abnormalities, mental retardation in congenital cases, and marked anticipation (see below). It is caused by a CTG expansion in the 3′ untranslated portion of the gene encoding the enzyme myotonic dystrophy kinase.[58-60] The exact mode of pathogenesis remains controversial,[92,93] but may involve both myotonic dystrophy kinase and adjacent genes.

Expansions in repeats that do not encode amino acids can also result in neurodegenerative diseases. A CAG expansion in an untranslated region of a gene encoding a subunit of the enzyme phosphatase 2A appears to cause an autosomal dominant spinocerebellar ataxia, termed SCA12, perhaps by altering levels of gene expression.[62] A CTG expansion in an untranslated gene may cause another form of spinocerebellar ataxia, designated SCA8.[61] While inheritance of SCA8 is autosomal dominant, penetrance is incomplete and more likely when the expanded allele is maternally transmitted.

Friedreich ataxia is an autosomal recessive neurodegenerative disorder consisting of hyporeflexia, dysarthria, and sensory loss, among other more variable abnormalities.[94] The pathological changes usually involve atrophy of central sensory pathways, but may also include cell loss in the cerebellum, basal ganglia, and large pyramidal cells of motor cortex.[95] The cause is loss of function of both alleles of a gene termed *frataxin*, which encodes a protein involved in mitochondrial energy metabolism.[63,95] Most cases arise as a result of loss of gene expression consequent to long expansions of an intronic GAA repeat. A few affected individuals are compound heterozygotes, in which one allele contains a GAA repeat expansion mutation and the second allele contains an inactivating point mutation within the coding region of the gene.[96]

Progressive myoclonic epilepsy is another recessive neurodegenerative disorder, characterized by

childhood onset, seizures, myoclonus, ataxia, and dementia. Progressive myoclonic epilepsy is rare outside Finland, and is of historical interest as the first recessive human disease to be studied statistically and as one of the early disorders for which large-scale group genetic counseling was undertaken. Progressive myoclonic epilepsy is usually caused by a repeat expansion on both alleles in a region 5′ to the site of transcription initiation of the *cystatin B* gene.[64,65] The disease may also arise when a repeat expansion on one allele is inherited with a point mutation within the gene on the other allele. The repeating unit is a dodecamer (CCCCGCCCCGCG) rather than a triplet; thus, trinucleotides are not the only units of DNA subject to pathogenic repeat expansion.

NEUROPSYCHIATRIC SYNDROMES IN EXPANSION MUTATION DISEASES

Almost all of the trinucleotide repeat expansion disorders involve the brain, and the central nervous system is often the primary organ system affected. As a result, either mental retardation or dementia is a feature of most of the repeat expansion diseases, and classic psychiatric syndromes are common in several disorders.

In HD, dementia is of the subcortical type, with losses in cognitive speed, attention, and flexibility typically more prominent at early stages than aphasia or agnosia, a pattern opposite that observed in such cortical dementias as Alzheimer disease.[97,98] Systematic studies suggest that 70% to 80% of all patients with HD develop some form of psychiatric disorder in addition to dementia.[99] The incidence of affective disorder in HD is about 40%, including a 10% rate of bipolar disorder[100] and a suicide rate estimated as high as 12.7%.[99] In addition, irritability, apathy, and preoccupations with particular ideas are common findings, while syndromic obsessive-compulsive disorder and schizophrenia are less common.[69]

The prevalence of psychiatric disorder in dentatorubral-pallidoluysian atrophy is similarly high; the most systematic study of the psychopathology of this disease found that 74% of patients had psychiatric syndromes, including mania, depression, and schizophrenia.[101] There is also a suggestion that psychiatric syndromes are more common in the SCAs than in control populations,[102] particularly affective disturbance[103] and frontal-executive dysfunction.[104-106]

Fragile X syndrome is primarily a disorder of neurodevelopment, although other organ systems are also involved.[84] In addition to mental retardation, the mutation also predisposes affected individuals to a variety of psychiatric syndromes. A substantial number of males with the fragile X mutation have autism. Nearly 100% have 1 or more behaviors commonly observed in autism, such as hand flapping and biting, poor eye contact, or tactile defensiveness. The symptom rate decreases in males who are mosaic for the expansion or in whom methylation is only partial.[84] Females with the full mutation (heterozygotes) not only have high rates of cognitive deficits, but also high rates of anxiety symptoms, affective syndromes, and schizotypal and schizoid personality traits.[107-109] Carriers of the premutation (about 50-200 repeats, unstable on transmission) manifest anxiety and obsessive symptoms, and mood lability that may not meet the full criteria for an affective disorder.[84,110] The rare E subtype of the fragile X syndrome appears to result in a similar though milder spectrum of cognitive and psychiatric syndromes.[111-113]

Neuropsychiatric manifestations may also be present in other expansion disorders, although the available evidence derives from clinical observation rather than systematic study. For instance, Jacobsen syndrome includes mental retardation and psychomotor slowing.[57] A high rate of suicide has been reported in patients with progressive myoclonic epilepsy,[114] and psychiatric syndromes may be common in Friedreich ataxia.[115]

ANTICIPATION

Anticipation is defined as decreasing age at onset of a disease (or increasing disease severity) in affected members of successive generations of a pedigree (see McInnis[116] and Asherson et al[117] for a full discussion). The concept dates back to the 19th century,[118] but the term "anticipation" was first used by Sir Frederick Mott in 1910. Mott[119,120] referred to the "law of anticipation" after he studied 420 mentally ill parent-offspring pairs from the asylums of London, England, and reported an earlier age at onset in the offspring compared with their parents. In 1948, Penrose[121] largely discredited the notion of anticipation by persuasively arguing that the phenomenon was an artifact of ascertainment. He pointed out that genetic studies would selectively ascertain (1) late-onset parents (since individuals with early onset of a serious illness tend to have reduced fertility), (2) offspring with early onset (since early-onset cases tend to be more severe and hence attract disproportionate clinical attention), and (3) families in which onset in parents and offspring occurred simultaneously (a bias of the limited time frame of most studies). The influence of Penrose was such that anticipation was not seriously investigated for 40 years, when systematically ascertained patient samples yielded clear evidence of anticipation in myotonic dystrophy[122] and HD.[123] Even so, the biological validity of anticipation remained in dispute until the discovery of the trinucleotide repeat expansions.

Two aspects of trinucleotide repeat expansion explain anticipation. First, expanded (but not normal sized) repeats are often unstably transmitted, and repeat length tends to increase in successive generations (**Figure 3**, left). This trend is often modified by a parent of origin effect: in the CAG repeat expansion diseases with anticipation, expansion is primarily observed during paternal transmission. In fragile X syndrome, Friedreich ataxia, and myotonic dystrophy, expansion is primarily observed in maternal transmission.[124,125]

The mechanism for repeat length instability is as yet incompletely understood. For most short repeats found in the human ge-
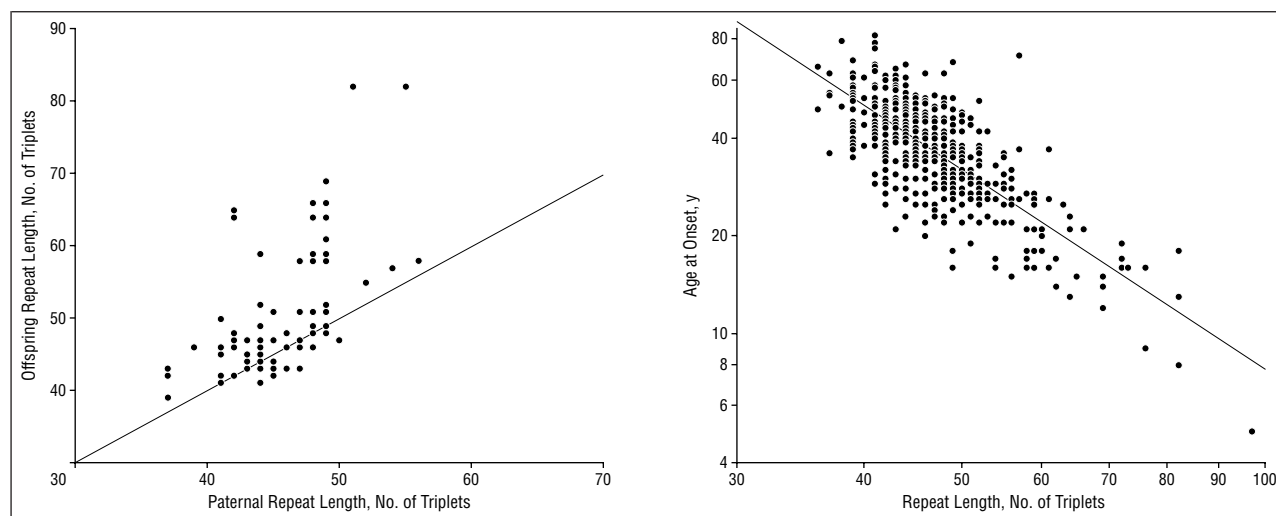
Figure 3. The molecular basis for anticipation in repeat expansion diseases. Data are from the Baltimore (Md) Huntington's Disease Center. Left, Increase in repeat length with paternal transmission of Huntington disease (HD). Points above the diagonal line represent cases in which the repeat length increased during transmission from father to child (N = 84 pairs, mean ± SD increase of repeat length = 4.2 ± 0.8 triplets). Right, Correlation of repeat length with age at onset in HD. As repeat length increases, age at onset of disease decreases (N = 480, r^2 = 0.57).

nome, including triplet repeats, mutations (which are rare) usually involve an expansion (and less frequently a contraction) by 1 or 2 units of the repeat.[126] At a length sufficient to cause disease, however, most repeats become markedly unstable. The length may increase by dozens or even hundreds of triplets from one generation to the next, with a correlation between repeat length and instability. At the molecular level, it is believed that long triplet repeats may form into hairpin loops and other abnormal secondary structures. The abnormal structures may then lead to repeat expansion through errors in DNA replication, abnormal patterns of recombination between 2 sections of DNA, or misguided application of the DNA repair mechanisms.[35]

Second, longer repeat expansions are correlated with a younger age of disease onset (Figure 3, right). In HD, repeat length explains about 50% to 60% of the variance in age at onset.[66,127-129] Repeat lengths of more than 60 or 70 CAG triplets frequently result in a juvenile-onset form of the disease.[130-132] However, the age at onset is highly variable for all but these longest repeat lengths. The most common repeat length is in the range of 40 to 50 triplets; for these individuals the age at onset varies from the early 20s to the mid-60s or even later.

The variability in repeat expansion during intergenerational trans-

mission, coupled with the modest correlation between repeat length and age at onset, explains why it was difficult to definitively establish the presence of anticipation in HD. Definitive proof only emerged with the discovery of the molecular mechanism. In myotonic dystrophy the relationship between repeat length and age at onset (and disease phenotype) is substantially more robust. An expansion in the 50- to 80-triplet range may result only in cataract formation in old age, while a large expansion (typically greater than 1000 triplets) generally leads to a life-threatening congenital disorder of muscle and brain.[97] Even with this dramatic progression of disease phenotype from one generation to the next, the presence of anticipation in myotonic dystrophy remained controversial until the discovery of the molecular etiology.[79]

In 1993, among the first of a growing number of contemporary studies of anticipation in psychiatric disease, our group examined the possibility of anticipation in affective disorder.[133] In 34 bipolar pedigrees ascertained for a genetic linkage study,[134] age at onset and disease severity (measured by episodes of illness per year) were compared between generations, using several sampling schemes to minimize ascertainment bias. All sampling schemes revealed significantly earlier age at onset and an increase in the frequency of disease episodes in

the younger generation, with a pattern resembling that seen in triplet repeat expansion disorders such as HD (**Figure 4**). Controlling for other possible biases, including substance abuse and decreased fertility or premature death of the most severely affected individuals, did not affect the results. Anticipation remained significant even after controlling for the cohort effect (an observation that the age at onset of mood disorders has progressively declined during the past century[135,136]). Several analyses of bipolar pedigrees by other investigators have been consistent with this initial finding.[137-140] The available evidence suggests, on average, a 6- to 10-year advance in the age at onset from the older generation to the younger.

A series of studies similar to those performed in bipolar disorder, incorporating strategies to minimize ascertainment and other biases, has suggested the presence of anticipation in schizophrenia. For instance, Bassett and Husted,[141] analyzing data (from Penrose[121]) on all first admissions to psychiatric hospitals in Ontario, Canada, between 1926 and 1943, found clear evidence of anticipation; 88% of 137 pairs showed an intergenerational age-at-onset difference, with a median onset 15 years earlier in the younger generation. At least 7 other studies[142-148] have also found evidence for anticipation in schizophrenia.
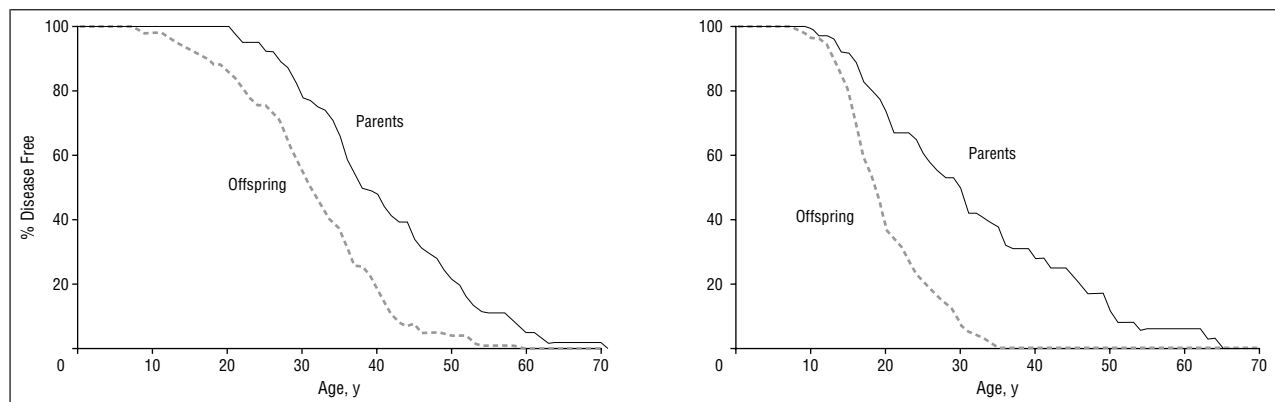
Figure 4. *Anticipation in Huntington disease (HD) (left) and affective disorder (right). The age at which affected parents and their affected offspring first manifest disease symptoms is depicted as a survival curve. In both HD and affective disorder, the younger generation is affected at a substantially earlier age than their parents. (Subjects with affective disorder are from the Johns Hopkins Bipolar Project [Baltimore, Md]: parents, N = 36; offspring, N = 97. Subjects with HD are from the Baltimore Huntington's Disease Center: parents, N = 61; offspring, N = 82.)*

Other psychiatric disorders may show anticipation. Using methods similar to those used in the initial study of anticipation in bipolar disorder, anticipation has been detected in panic disorder.[149] Autism presents a more complicated picture, in that affected individuals only rarely have children. As a consequence, there have been no formal studies of anticipation in autism. However, a compelling body of work indicates that the parents of autistic children tend to have a higher than expected prevalence of behavioral and emotional symptoms generally similar to (but milder than) those seen in their offspring with autism. These abnormalities include anxiety disorder, social phobia, and depression,[150,151] and, most strikingly, social deficits such as limited friendships, diminished displays of affection, odd behavioral stereotypies, rigidity, and perfectionism.[152]

Despite the large amount of data consistent with the presence of anticipation in psychiatric disorders, the case is far from proven. In some populations, such as schizophrenia in the genetically isolated Palau islanders, no anticipation has been detected.[153] The role of the cohort effect, in which the onset age, severity, or frequency of a disease changes for an entire population, as well as reductions in fertility in the most severely affected individuals (a strong factor in schizophrenia[154]), may confound analysis of anticipation. Another bias might stem from increasing familiarity with a disease in successive generations of an affected family, leading to earlier rec-

ognition of symptoms. There is wide agreement that it is nearly impossible to eliminate the ascertainment biases described by Penrose,[121] and such biases appear to account for apparent anticipation in at least some studies.[155] There has also been disagreement about the statistical methods used in some studies to determine the significance of differences in onset age between generations.[156,157] Finally, anticipation could derive from genetic factors other than trinucleotide repeats, such as increasing accumulation of multiple genetic vulnerability factors in successive generations, or perhaps unstable deletions.[158]

In sum, there is a large amount of data supporting a younger age at onset of psychiatric disorders in later generations of affected pedigrees. The problem is how the data are best interpreted. Given the often subtle nature of genetically validated anticipation, such as in HD, the meaning of the data will only become clear when the molecular causes of these diseases are known. Until then, the possibility of anticipation is one hint as to the type of mutations that may be present in psychiatric diseases.

EXPANSION MUTATION AND NONMENDELIAN GENETICS

The repeat expansion diseases are characterized by patterns of inheritance, in addition to anticipation, that fall outside traditional mendelian genetics. The explanation again resides with the unique phenomenon of a mutation that quantitatively varies among affected indi-

viduals with the same disease and is unstably transmitted from one generation to the next.

In fragile X syndrome, in which genotype-phenotype correlations have been thoroughly analyzed, at least 5 factors are known to affect the phenotypic expression of the mutation: sex of the affected individual, repeat length (including repeat contractions[159-161]), the pattern of X-inactivation (for females), somatic cell mosaicism, and methylation status of the CpG island adjacent to the repeat.[162] These factors have a profound affect on the clinical phenotype and patterns of inheritance. There are cases in both myotonic dystrophy and fragile X syndrome of repeat contraction to normal or near normal length,[163,164] and at least 3 pairs of monozygotic twins fully or partially discordant for fragile X syndrome have been reported in the literature.[162,165]

Apparent sporadic cases of several repeat expansion diseases can be explained by expansion of a repeat of intermediate length, unstable but not long enough to cause disease itself, to a full mutation in the subsequent generation.[166-168] Similarly, for unclear reasons, a full CGG expansion in the *CBL2* gene only occasionally causes chromosomal deletion, also leading to sporadic cases.[57]

Some of the unusual genetics of the repeat disorders are reminiscent of the genetics of psychiatric disorders, in which inheritance is also characterized by apparently nonfamilial cases, skipped generations, monozygotic twin discordance, and marked variability of phe-

notype within a pedigree.[169-171] However, these unusual patterns of inheritance are more frequent in the psychiatric disorders than in the repeat expansion diseases, suggesting the presence of a number of interacting etiologic factors.

ATTEMPTS TO DETECT TRINUCLEOTIDE REPEAT EXPANSION IN PSYCHIATRIC DISORDERS

Several different strategies have been used to search for repeat expansions in DNA from individuals with psychiatric disorders. One of these strategies involves testing the length of repeats present in known genes or fragments of genes. The only requirement is that a small region of the DNA sequence flanking both sides of the repeat is known. Several criteria have been used to select individual repeats for testing in psychiatric disorders. Repeats that expand to cause one disease have frequently been examined in other diseases,[172,173] leading to the finding that perhaps 5% of cases of autism in males result from the fragile X mutation.[84] Other repeats are of interest because of the function of the gene in which they reside. The CAG repeats in the gene encoding the calcium-gated potassium channel hSKCA3[174-178]and in the gene encoding mab21L1, a regulator of neuronal development,[179] have been studied; psychiatric disease has not been consistently associated with long or expanded repeats in either gene. Repeats have also been selected for testing in psychiatric disorders based on their length, the variability of their length in the normal population, and their location in a chromosomal region to which psychiatric disorders have been linked.[180] No marked association with a psychiatric disease has yet been demonstrated, although only a small fraction of all repeats has been examined.[179,181-187]

A second strategy, a search for expansions at the protein level, takes advantage of an antibody that detects expanded, but not normal length, glutamine repeats.[188] Reports of expanded glutamine repeats in schizophrenia have been inconsistent.[189-191]

A third strategy, known as RED (repeat expansion detection), was developed by Schalling and colleagues[192] to screen genomic DNA for expansion mutations. The advantage of the technique is that no prior knowledge of repeat location or flanking sequence is required. The disadvantage is that RED only indicates the presence of a longer than normal repeat; it does not specify which repeat, of the hundreds present in the human genome, is abnormally long. Also, for most types of repeats, RED is only able to detect expansions beyond about 40 to 50 triplets. Smaller expansions, such as those that cause SCA6 or oculopharyngeal muscular dystrophy, would not be detectable. Nonetheless, RED has proved valuable in isolating the causative genes of 3 of the CAG expansion disorders.[61,62,193]

At least 15 independent studies have used RED to compare CAG repeat lengths of subjects with bipolar affective disorder and/or schizophrenia with those of controls, with mixed results. Of 8 studies examining bipolar disorder, 4 reported an association between bipolar disorder and longer CAG repeats,[194-197] while another 4 did not find a significant association.[198-201] In schizophrenia, an association with long CAG repeats was found in 3 studies[194,202,203] but not in 7 others.[198-200,204-207]

Two CAG expansions that occur frequently in the normal population, located in an intron of the *SEF2-1* gene on chromosome 18[181] and in the *ERDA1/Dir1* locus on chromosome 17,[208,209] are now known to account for most of the long CAG repeats detected by RED.[195,210] Variations in the frequency of these expansions in different populations may explain some of the discrepant findings in RED studies of psychiatric disorders, and there are inconsistent data concerning the role of these expansions themselves as risk factors for psychiatric disease.[195,199] However, the most important implication of their discovery is that it will now be possible to focus on families with CAG expansions not accounted for by these 2 repeats. The use of RED, or alternative techniques,[211] to search for expansions of other types of re-peats, such as CGG or GAA, has received relatively little attention,[206,207] and remains a promising approach.

CONCLUSIONS

The unique phenotypic and genotypic characteristics of the repeat expansion disorders present a new perspective from which to view human disease. Previously enigmatic phenomena, such as anticipation, qualitative and quantitative differences in disease phenotype among individuals within the same pedigree, and monozygotic twin discordance, can be understood at the molecular level as consequences of repeat length variation. Equally intriguing is the frequency with which the central nervous system is affected in these disorders, with psychiatric syndromes not uncommon and at times the presenting manifestation of the disease. The psychiatrist, neurologist, or other clinician has the chance to make a specific diagnosis based on a genetic test (widely available for most expansion diseases), to provide a specific prognosis, and, when appropriate, to offer predictive testing for at risk individuals.

Might repeat expansion account for some portion of the genetic susceptibility to idiopathic psychiatric disorders? Diseases such as bipolar affective disorder, autism, and schizophrenia certainly have similarities to the known repeat expansion diseases, including prominent brain involvement and nonmendelian modes of inheritance, most notably anticipation. At present, the evidence supporting a role of repeat expansion in the psychiatric disorders remains preliminary and inconclusive. Over the next few years, in conjunction with other genetic studies of psychiatric disorders and with advances in the Human Genome Project and related endeavors, it should be possible to reach a more definitive conclusion.

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