A R T I C L E

The Unstable Trinucleotide Repeat Story of Major Psychosis

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New hopes for cloning susceptibility genes for schizophrenia and bipolar affective disorder followed the discovery of a novel type of DNA mutation, namely unstable DNA. One class of unstable DNA, trinucleotide repeat expansion, is the causal mutation in myotonic dystrophy, fragile X mental retardation, Huntington disease and a number of other rare Mendelian neurological disorders. This finding has led researchers in psychiatric genetics to search for unstable DNA sites as susceptibility factors for schizophrenia and bipolar affective disorder. Increased severity and decreased age at onset of disease in successive generations, known as genetic anticipation, was reported for undifferentiated psychiatric diseases and for myotonic dystrophy early in the twentieth century, but was initially dismissed as the consequence of ascertainment bias. Because unstable DNA was demonstrated to be a molecular substrate for genetic anticipation in the majority of trinucleotide repeat diseases including myotonic dystrophy, many recent studies looking for genetic anticipation have been performed for schizophrenia and bipolar affective disorder with surprisingly consistent positive results. These studies are reviewed, with particular emphasis placed on relevant sampling and statistical considerations, and concerns are raised regarding the interpretation of such studies. In parallel, molecular genetic investigations looking for evidence of trinucleotide repeat expansion in both schizophrenia and bipolar disorder are reviewed. Initial studies of genome-wide trinucleotide repeats using the repeat expansion detection technique suggested possible association of large CAG/CTG repeat tracts with schizophrenia and bipolar affective disorder. More recently, three loci have been identified that contain large, unstable CAG/CTG repeats that occur frequently in the population and seem to account for the majority of large products identified using the repeat expansion detection method. These repeats localize to an intron in transcription factor gene SEF2-1B at 18q21, a site named ERDA1 on 17q21 with no associated coding region, and the 3′ end of a gene on 13q21, SCA8, that is believed to be responsible for a form of spinocerebellar ataxia. At present no strong evidence exists that large repeat alleles at either SEF2-1B or ERDA1 are involved in the etiology of schizophrenia or bipolar disorder. Preliminary evidence suggests that large repeat alleles at SCA8 that are non-penetrant for ataxia may be a susceptibility factor for major psychosis. A fourth, but much more infrequently unstable CAG/CTG repeat has been identified within the 5′ untranslated region of the gene, MAB21L1, on 13q13. A fifth CAG/CTG repeat locus has been identified within the coding region of an ion transporter, KCNN3 (hSKCa3), on 1q21. Although neither large alleles nor instability have been observed at KCNN3, this repeat locus has been extensively analyzed in association and family studies of major psychosis, with conflicting findings. Studies of polyglutamine containing genes in major psychosis have also shown some intriguing results. These findings, reviewed here, suggest that, although a major role for unstable trinucleotides in psychosis is unlikely, involvement at a more modest level in a minority of cases cannot be excluded, and warrants further investigation. Am. J. Med. Genet. (Semin. Med. Genet.) 97:77–97, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: schizophrenia; bipolar affective disorder; genetic anticipation; unstable DNA; repeat expansion detection; parent-of-origin effect; genetics

INTRODUCTION

General Introduction to the Genetics of Major Psychosis

Schizophrenia (SCZ) and bipolar affective disorder (BPAD) are the two major psychotic disorders, both with a lifetime prevalence of approximately 1%, and both having a substantial genetic contribution [Gottesman, 1991; McGuffin et al., 1994]. Elucidation of etiological factors for these two major psychoses (MP) remains the overwhelming challenge to psychiatric researchers, however, the most expeditious method for identification of genetic risk factors for MP is not clear. This has led to a number of diverse approaches being explored in attempts to clarify the genetic etiology. A potential new approach to the genetics of major psychosis was provided by the discovery of a new type of DNA mutation, unstable DNA, and we will briefly describe the genetic aspects of the known trinucleotide repeat diseases, focussing in particular on findings in Huntington disease (HD) as an example of unstable DNA.

Introduction to Trinucleotide Repeat Expansion Disease

Trinucleotide repeat expansion (TRE) mutations have been described in a number of rare, generally dominant Mendelian disorders. Only three of ten potential trinucleotide permutations have been shown to be associated with disease: CTG_n in myotonic dystrophy (DM) and spinocerebellar ataxia type 8 (SCA8) and its reverse strand complement CAG_n in SCA1, SCA2, SCA3, SCA6, SCA7, and SCA12, Machado-Joseph disease (MJD), and dentatorubral-pallidoluysian atrophy (DRPLA); AAG in Friedereich ataxia (FA) and CGG_n and its reverse strand complement CCG_n in Fragile X syndrome (FXS) and Fragile XE mental retardation (FRAXE) respectively [see Wilmont and Warren, 1998, for review; SCA8: Koob et al., 1999; SCA12: Holmes et al., 1999]. The actual pathogenic mechanism for repeat expansion in these diseases is not yet clear, however, because different trinucleotides and different ranges of repeat length for patho-genicity exist in these diseases, and because in some of the TRE diseases the repeats code for polyglutamine (HD, SBMA, DRPLA, SCA1, SCA2, SCA3, SCA6, SCA7) whereas in others the TRE occurs in the 3′ untranslated region (DM and SCA8) or 5′ untranslated region/promoter (FXS, FRAXE, SCA12), or in an intron (FA) there may be a variety of different molecular mechanisms. Furthermore, whereas many of these diseases share similar clinical features, the disease genes themselves encode proteins of very diverse function. In addition to the intergenerational expansion (and occasionally contraction) of triplets in TRE diseases, several other phenomena have been evidenced in the majority of these disorders, namely genetic anticipation and parental gender bias in transmission of pathogenic alleles. Genetic anticipation (GA) refers to increased severity and earlier age at onset (AAO) of disease in successive generations. TRE has been suggested to be a molecular substrate for GA, where length of repeats correlates strongly with severity and decreased AAO for many of these diseases, although other factors may also modulate GA [Snell et al., 1993; Andrew et al., 1993; Kehoe et al., 1999].

HD is a rare autosomal dominant neurodegenerative disorder characterized by progressive movement disorders and psychiatric symptoms. HD was noted to demonstrate two non-Mendelian features: genetic anticipation (GA) and parental origin effect [Ridley et al., 1988]. GA refers to increased disease severity and earlier age at onset in younger generations. The HD gene is located on chromosome 4p16.3 and there are up to 35 copies of a CAG repeat in unaffected individuals, whereas those with HD have 36 or more repeats. Penetrance is reduced for alleles with 36–39 repeats, whereas penetrance is complete for 40 repeats or more [Myers et al., 1998]. Transmission of disease alleles usually results in a larger number of repeats in the child, especially when transmitted paternally. In HD, trinucleotide repeat expansion (TRE) is the molecular mutation and has helped to clarify the genetic anticipation in this disorder. Parent-of-origin effect refers to the influence of the gender of the transmitting-parent. It was demonstrated in HD unstable CAG trinucleotide expansion occurs in both maternal and paternal transmissions. In paternal transmission, however, the sequence is expanded to a greater extent. Furthermore, in paternal transmissions the degree of genetic anticipation is on average 6–7 years, and is only 1 year in maternal transmissions [Ridley et al., 1988]. For maternal transmission, the genetic anticipation was positive for offspring of mothers with an AAO of >35, and was negative with the mother's AAO being <35 years of age. In paternal transmissions, such negative results were never observed.

The Unstable DNA Hypothesis of Major Psychosis

The unstable DNA hypothesis proposed that the presence of GA in a genetic disorder can be accounted for with unstable DNA as the underlying molecular cause. GA is a common feature of complex non-Mendelian genetic disorders. Other non-Mendelian features include incomplete penetrance, that is evident when individuals possessing the disease haplotype are unaffected. This could be explained by the unstable DNA hypothesis as a lack of sufficient expansion for the expression of the phenotype [Paterson et al., 1998]. Unstable DNA can also be used to explain variable expressivity, where variations in TRE above the threshold may be associated with alternate phenotypes. Monozygotic twins exhibiting discordance may be explained by the differential expansion of unstable DNA in the postzygotic period [reviewed by Petronis and Kennedy, 1995]. The effect of somatic mosaicism, shown to exist in fragile X [Devys et al., 1992], DM [Thornton et al., 1994], and HD [Telenius et al., 1994], may also add to incomplete penetrance, expressivity, and the partial discordance in monozygotic twins. The unstable DNA hypothesis may also account for sporadic cases in complex disorders that otherwise cannot easily be explained by traditional Mendelian genetics. Expansion of unstable DNA occurring in the germ cells

of one parent resulting in disease in the proband could be hypothesized. Conversely, the expansion may revert back to normal range in the probands' germ cells resulting in unaffected offspring of the proband [Paterson et al., 1998].

GENETIC ANTICIPATION: REVIEW OF MAJOR PSYCHOSIS STUDIES

Genetic anticipation (GA) was described as "degeneration" when it was first reported in the nineteenth century [Morel, 1857]. Other early studies of GA were reported at the beginning of the 20th century [Mott, 1910, 1911], but were generally dismissed as artifact due to ascertainment biases [Penrose, 1948]. A historical review of GA has been provided elsewhere [McInnis, 1996]. In the last five years, since the discovery of unstable DNA, a large number of researchers have studied GA in major psychosis.

This section provides a summary of studies that have been published featuring GA in SCZ and BPAD. Published articles were obtained from a MEDLINE search performed using the keyword 'genetic anticipation' and SCZ or BPAD covering the period from 1966 to October 1999, and from references cited in publications relating to GA. Ascertainment criteria, analysis and interpretation are covered, and pre-sented in Table I for SCZ, and in Table II for affective disorders. There are a number of major issues concerning both ascertainment and statistical analysis related to the study of GA. This has led some researchers to conclude that it is a matter of opinion whether or not GA is genuine for a specific disease [Ashizawa et al., 1992].

Ascertainment Issues

Significant potential ascertainment biases in the analysis of data for anticipation have been discussed in detail elsewhere [Penrose, 1948; Ridley et al., 1988], and include:

1. Preferential ascertainment of parents with later onset (due to decreased reproductive fitness of earlier onset individuals).
2. Preferential ascertainment of offspring with early onset, as later onset individuals may not be affected at time of study.
3. Decreased memory of earlier episodes of illness in older generation.
4. Increased awareness of illness by family members and physician.

It has now become clear that to overcome these biases, population-based random samples of families are necessary for anticipation studies [Heiman et al., 1996; Huang and Vieland, 1997]. It has been suggested that the ideal design for the study of anticipation requires ascertainment of probands independent of family history and to then follow them prospectively for many years until both parents and children are through the age of risk [Heiman et al., 1996]. Unfortunately, relatively few epidemiological studies contain information relating to the age at onset (AAO) and age at diagnosis (AAD) of affected relatives, and therefore most of these studies cannot be used for population-based studies of anticipation. As a compromise to the proposed ideal design, it has been suggested that other types of data should be used for anticipation, specifically studies either of severity, or studies involving whole families where unaffected siblings of both the parents' and children's generation are included [Heiman et al., 1996]. These authors suggest that it is possible to overcome the bias due to differential timing of the diagnostic assessment by employing survival analysis in this situation. An alternative statistically appropriate approach requires ascertainment of families at only a single time-point and analysis is performed that takes into account the differential timing of diagnosis between generations [Huang and Vieland, 1997].

Other factors also need to be considered when contemplating GA in complex, multifactorial or polygenic disorders. For instance, although many

TABLE I. Genetic Anticipation in Schizophrenia*

Reference	Ascertainment	No. families	Anticipation	Genomic imprinting
Bassett and Honer, 1994	Linkage study	8 Families	+	NS
Asherson et al., 1994	Linkage study	28–40 Relative pairs	11.1 years	NS
Thibaut et al., 1995	Linkage study	26 Families	+	NS
Yaw et al., 1996	Linkage study	15 Families	+	NS
Gorwood et al., 1996	Population based	24 Families	+	NS
Ohara et al., 1997a	Clinic-based	24 Families	+	NS
Bassett and Husted, 1997	Retrospective registry	137 Pairs	+	ND
Johnson et al., 1997	Linkage study	33 Families	+	ND
Imamura et al., 1998	Clinic-based	37 Pairs	+	NS
Valero et al., 1998	Linkage study	25 Families	+	NS
Heiden et al., 1999	Clinic-based	15 Families	+	ND

*NS, not significant; ND, not done; +, evidence for anticipation.

TABLE II. Genetic Anticipation in Bipolar Affective Disorder*

Reference	Ascertainment	No. families	Anticipation	Imprinting
McInnis et al., 1993	Unilineal linkage	34	$P = 0.013$ to <0.001	NS
Nylander et al., 1994	Lithium clinic patients and family histories	14	10 years	NS
Engstrom et al., 1995	Northern Swedish families		+	NS
Kumar et al., 1996	Unilineal families	19	+	ND
Grigoroiu-Serbansescu et al., 1997	Hospitalised patients	36 Parent-child pairs	+	Paternal delta 12 years, maternal delta 3 years
Ohara et al., 1998	Clinic-based	26 Families	+	NS

*NS, not significant; ND, not done; +, evidence for anticipation.

studies of genetic anticipation have selected families for unilineal transmission, this certainly does not mean that risk alleles have not been transmitted to the child from the clinically unaffected parent. If, as could be imagined, there is a relationship between the number of risk alleles and age of onset, then if the child inherited risk alleles from both parents, then one could expect to see an earlier onset in the child, which would be consistent with GA. Assortative mating for psychological traits would make this situation even more relevant.

Statistical Considerations

Pairwise statistical tests

A further important consideration, in addition to issues of ascertainment and sampling, is the choice of appropriate statistical tests. As can be seen from Tables I and II, many studies on anticipation have employed some form of pairwise statistical tests to compare the age at diagnosis in parents and offspring. It has recently been demonstrated, however, that using such an approach may lead to erroneous results [Heiman et al., 1996]. The crux of the matter relates to the difference in ages between generations, so that a proportion of children have not reached the AAO for the disease at the time of ascertainment, thus producing a so-called right-truncation in the distribution of the children's AAO. In brief, the simulation study described by Heiman et al. [1996] generated parent-child pairs, their AAO, and an age at interview for each parent-child pair. The presence of children unaffected at the time of simulated interview resulted in rejection of that pair, and further pairs were simulated until a total of 100 pairs were obtained where both parent and child were affected. This study revealed that when parent-child pairs are interviewed at a relatively early age (i.e., before the majority of children have reached the mean AAO) the result is an elevation of the type I error, that in some cases can be as high as 100% [Heiman et al., 1996]. A correlation between AAO in parents and the degree of anticipation has led some workers to reject anticipation [Asherson et al., 1994]. They attributed the observed anticipation to ascertainment biases where the children of young parents have not yet reached the age of risk. This contention has been used previously [Penrose, 1948], but it has been convincingly argued that the presence of such a correlation does not exclude anticipation and is in fact a separate phenomenon [Hodge and Wickrameratne, 1995].

New statistical approaches

A new test recently developed by Huang and Vieland [1997] takes into consideration the right truncation of the children's generation age at onset distribution, thus reducing the elevated type I error that may result from the use of traditional pair-wise statistics [Heiman et al., 1996]. Thus, this new method defines a conditional likelihood that takes into account the right trun-cation of the data, uses iterative likelihood methods to find estimates for means and variances of the AAO for parents and children, and then uses those adjusted estimates to test for anticipation. This approach [Huang and Vieland, 1997] has been tested on BPAD families ascertained for linkage analysis, that have previously been reported to demonstrate evidence for anticipation using simple paired statistics [McInnis et al., 1993]. The evidence for anticipation is reduced from $P = 0.0001$ using simple paired statistics to a P-value of 0.014 using the new test [Huang and Vieland, 1997]. Simulations have shown this new test to have an empirical type I error rate of 5% when performed at a nominal value of $\alpha = 5\%$, even when samples as small as 25 pairs are used [Huang and Vieland, 1997]. Further work investigating this test indicates, however, that its power to detect GA is low, resulting in the suggestion that studies of GA cannot be recommended [Vieland and Huang, 1998].

Parent-of-Origin Effect in Diseases Demonstrating Genetic Anticipation

Parent-of-origin effect, as described for HD, is a common feature of unstable trinucleotide repeat diseases, and is influenced largely by differences in repeat instability during male and female transmission of gametes. It has been suggested that the influence of ascertainment biases in maternal and paternal

transmissions should be the same, indicating that a difference in the degree of anticipation depending on the gender of the disease-transmitting parent supports the idea of true anticipation [Ridley et al., 1988]. Evidence for anticipation, however, should not be rejected if parental origin effects are absent [Petronis et al., 1995]. In complex disorders, various susceptibility loci may be differentially imprinted and mask parental effects. Clinical and molecular parental origin effects may act in opposite ways. For example, in probands with type 1 diabetes a paternal history of type 1 diabetes is about five times more common than a maternal history [reviewed in Bain et al., 1994]. At one of the susceptibility loci for type 1 diabetes (insulin gene VNTR), certain alleles confer risk for disease when transmitted maternally, but seem to have no effect on risk when transmitted from the father [Bennett et al., 1997].

The issue of parent-of-origin effect is further complicated by differences in the AAO distribution for males and females, that may result in an apparent parent-of-origin effect when the data are separated into mother-offspring and father-offspring pairs. Division of the data depending on the gender of the disease-transmitting parent as well as offspring is required in such a situation. Despite potential heuristic advantage of studying parent-of-origin effect in diseases with evidence for anticipation in such a situation, such analyses have not been undertaken universally (Tables I and II).

Conclusions

Studies of GA in major psychosis have produced strikingly consistent results across a number of different analyses. These findings, however, should be viewed with caution for a number of reasons, such as the fact that a population-based approach has not been used for ascertainment in the vast majority of these studies. Also, regarding statistical approaches, it is clear that simple pairwise analysis in most cases is not appropriate because this tends to elevate the type I error [Heiman et al., 1996]. In addition, anticipation has been reported

for many diseases, some of which have a clear molecular cause other than unstable DNA [for review see Paterson et al., 1998]. Thus there are two possible ways to view this—either GA is a strong, consistent effect, more so than any other findings in major psychosis, or there are fundamental flaws with the practicalities for testing for GA in a complex disease. We believe that there are a number of overwhelming methodological issues that confound the positive findings of GA in complex diseases, and it is likely that the degree of complexity is too great for clear answers to be obtained [Vieland and Huang, 1998; Paterson et al., 1998].

MOLECULAR STUDIES OF TRINUCLEOTIDE REPEATS IN MAJOR PSYCHOSIS

Introduction to the Identification and Analysis of Trinucleotide Repeats in Major Psychosis

Several methods have been developed to locate TRE without any prior data regarding genomic location. These involve four main screening approaches:

1. DNA libraries and databases, i.e., cDNA library screening [Li et al., 1993; Margolis et al., 1997; Bulle et al., 1997; Albanese et al., 1998], genomic library screening [Gastier et al., 1996; Philibert et al., 1998; Kleiderlein et al., 1998; Mangel et al., 1998], cDNA sequences in databases [Neri et al., 1996]; also more focussed strategies involving screening patient-specific genomic libraries, such as Direct Identification of Repeat Expansion and Cloning Technique (DIRECT) [Sanpei et al., 1996], Rapid Analysis, Pooled Isolation and Detection (RAPID) [Koob et al., 1998], di- or trinucleotide polymerase reaction and capture (DTC) [Inoue et al., 1999] and including a variation of DIRECT and RAPID [Vincent et al., in press].
2. Total cDNA, i.e., identification of cDNAs containing CAG/CTG repeats using a PCR-based approach [Kaushik et al., 1998]; a modification or the RACE technique, ran-

dom rapid amplification of cDNA ends (RRACE) [Carney et al., 1995]; oligo-capture method [Reddy et al., 1997].
3. Total genomic DNA, i.e., repeat expansion detection (RED) [Schalling et al., 1993], Southern blot hybridization, asymmetric PCR [Petronis et al., 1996a], as well as fluorescent in situ hybridization (FISH) [Petronis et al., 1996a; Haaf et al., 1996].
4. Identification of polyglutamine-containing proteins. This approach may be useful for detecting the presence of a polyglutamine-containing protein that is expanded in patients but not controls [Stevanin et al., 1996].

The most widely used approach, the RED technique [Schalling et al., 1993], has been used to detect the presence of large repeat tracts in the human genome. This method is based on the ligase chain reaction (LCR), and uses a thermostable DNA ligase enzyme to covalently link adjacent trinucleotide repeat oligonucleotides annealed on single stranded template genomic DNA. It does not require the determination of flanking sequence or the use of single-copy probes. RED presumably detects the largest given repeat located anywhere in the genome, and therefore detects both stable as well as unstable repeats. RED typically uses a ligation oligonucleotide such as either $(CTG)_{17}$ [Schalling et al., 1993], $(CTG)_{10}$ [O'Donovan et al., 1995] or $(CTG)_8$ [Martorell et al., 1997]. RED reaction conditions have been optimized for $(CTG)_{10}$ oligonucleotide to 500 cycles of 20 sec ligation at 77°C and 10 sec denaturing at 94°C, using 1 μg of template DNA, 100 ng of ligation oligonucleotide and 15 units of enzyme [Zander et al., 1998a]. After cycling, the products are fractionated by denaturing polyacrylamide gel electrophoresis and transferred onto a nylon membrane and hybridized with a radiolabeled probe complimentary to the oligonucleotide used in the LCR. RED may be useful for detecting large repeats in the genome for single gene disorders (i.e., MJD [Lindblad et al., 1996a;

SCA7 [Linblad et al., 1996b]; HD [Hofferbert, 1997]), with the caveat that a repeat region may not be detected due to masking by large repeats at one or more of several loci that are frequently expanded in the background population (*ERDA1, SEF2-1B, SCA8*). It is debatable, however, whether large repeats could be detected above this background for diseases that demonstrate locus heterogeneity, such as SCZ and BPAD. Furthermore, a TRE in the same repeat range as *SCA6* would not be detected using RED. One approach for maximizing the likelihood of identifying etiologic TRE above this background is to screen sample populations of the severest and earliest onset cases who, if the unstable DNA hypothesis is correct, should have longer repeat tracts. Once the presence of large repeats has been established using RED, to isolate the specific locus, one of the above screening-based approaches is then required.

Major Psychosis and Known Trinucleotide Repeat Disease Genes

Psychosis is known to be a common feature of a number of neurodegenerative diseases, some of which have TRE as the molecular mutation. Although it is unclear whether the psychiatric symptoms are secondary to the neurodegeneration, or a result of increased environmental loading, or whether there is genetic linkage between these disorders, it seems appropriate that such cases should be considered as phenocopies of major psychosis [Propping, 1983]. Some studies have, however, investigated whether expansion mutations may occur in psychosis patients, who may be non-penetrant for the neurodegenerative disorder, or whether there may be linkage disequilibrium with other, uncharacterized mutations/polymorphisms at these genes.

HD

An initial case study of the CAG repeat at the Huntington locus in 38 SCZ patients found alleles sizes between 16–25 repeats, and in 16 patients with major depression, alleles sizes between 17–24 repeats, showing no overlap with alleles in the HD patients [Kremer et al., 1994]. A study of 71 schizophrenia and 18 schizoaffective disorder individuals revealed no disease range alleles [Rubinsztein et al., 1994], also no evidence of involvement of HD in schizophrenia (n = 20–97) or bipolar affective disorder (n = 23–30) compared to controls (n = 43–146; Jain et al., 1996). A single case-report was presented of a SCZ patient with *HD* expansion mutation but no *HD* phenotype [St. Clair, 1994].

SCA1

In French Alsatian schizophrenia (n = 76) and bipolar affective disorder I (n = 35) patients and matched controls (n = 73) there were no large alleles and no association of alleles or genotypes with disease [Morris-Rosendahl et al., 1997]. Significant association was, however, observed between 49 Caucasian SCZ patients and 88 Caucasian controls ($P = 0.018$), and the 31 repeat allele was significantly more frequent in patients ($P = 0.002$ [Joo et al., 1999]). It is worth noting that in a number of genome-wide searches a number of positive parametric and non-parametric linkage findings have been observed for schizophrenia on 6p, spanning the *SCA1* locus [Schizophrenia Linkage Collaborative Group for Chromosomes 3, 6 and 8, 1996], however, the numbers of patients in the *SCA1* studies are too small to qualify as a corroboration of the 6p linkage findings. Further evidence for linkage disequilibrium of the *SCA1* CAG repeat with schizophrenia, however, has been provided by analysis of the *SCA1* CAG repeat in 55 multiplex SCZ families, where linkage disequilibrium was observed using TDT between SCZ and the 29 repeat allele ($P = 0.0016$ [Wang et al., 1996]).

SCA6

Two-hundred twenty-fine Scottish schizophrenia patients and 198 Scottish controls showed no association with *SCA6* ($P = 0.72$) and no longer alleles in patients versus controls ($P = 0.45$ [Breen et al., 1999]).

DRPLA

In 53–74 North American and Italian schizophrenics and matched controls, different allele frequencies were observed ($P = 0.086$ [Sasaki et al., 1996]). Also, no evidence of involvement of *DRPLA* in schizophrenia (n = 20–97) or bipolar affective disorder (n = 23–30) compared to controls (n = 43–146 [Jain et al., 1996]). In a study of French Alsatian schizophrenia (n = 83) and bipolar affective disorder I (n = 33) patients and matched controls (n = 65) no large alleles were found, however, association with alleles ($P = 0.019$) but not with genotypes ($P = 0.49$) for schizophrenia and for a sub-group of schizophrenia with positive family history was noted ($P = 0.0001$ [Morris-Rosendahl et al., 1997]).

FRAXA

No evidence of linkage of schizophrenia to the *FMR1* locus was found in 10 multiplex families [DeLisi et al., 1991] or 23 multiplex families [Ashworth et al., 1996], although in one family in the latter study a schizophrenia individual with developmental delay was found to have expansion/methylation mutation. Another study screened 128 individuals with SCZ or other related disorders, and found no full mutations, but one premutation was detected [Jonsson et al., 1995]. An association study showed no variation in repeat distribution between 79 BPAD individuals and 77 controls, and no expansion alleles [Craddock et al., 1994]. A single case of an adult psychosis male has been reported where there was methylation mosaicism at *FMR1* [Khin et al., 1998].

Molecular Studies Using Repeat Expansion Detection (RED) and Other Genome-Wide Approaches

The first study performed using RED demonstrated the presence of large CAG/CTG repeat tracts in three Centre d'Etude Polymorphisme Humaine (CEPH) pedigrees, 1334, 1344 and 1420 [Schalling et al., 1993]. Apparent linkage of RED "alleles" in these three

families to chromosome 18 was reported. It has since emerged that, although families 1344 and 1420 do show very large alleles at *SEF2-1B* on 18q21 [Breschel et al., 1997], very large alleles are present for family 1334 at the *SCA8* locus on 13q21 [Vincent et al., 2000]. Other trinucleotide repeat combinations were attempted using RED in the Schalling et al. [1993] study, such as CGG, TGG, CCT and CGT, but no positive controls were available to assess the ligation efficiency for the last three of these. Studies using RED to detect CAG/CTG repeats are summarized in Table IIIa for SCZ and Table IIIb for BPAD.

Molecular studies of SCZ families

No evidence for CAG/CTG or CGG/CCG repeat expansion or co-segregation of large repeats with disease was found in five large Eastern Canadian multiplex SCZ families [Petronis et al., 1996b] using Southern blot hybridization, asymmetrical PCR–based, and RED. The families contained 81 members of whom 23 had SCZ, schizoaffective disorder or SCZ spectrum

disorder, and had previously been shown to exhibit GA [Bassett and Honer 1994]. The distribution of $(CTG)_{10}$ RED ligation products was similar in 14 affected and 36 unaffected family members ($P = 0.79$ [Petronis et al., 1996b]). There was no significant difference in repeat sizes between 19 parental generation and 30 proband generation subjects ($P = 0.52$). Also, using RED and Southern hybridization techniques, no evidence was observed of larger CAG/CTG or AAG/CTT repeats in 23 (11 Icelandic and 12 UK pedigrees) multiplex SCZ families

TABLE IIIa.   Studies Using RED in Cases With Schizophrenia Versus Controls

Group	Source/ethnicity	Diagnostic criteria	Controls source/ethnicity	RED $(CTG)_n$ oligo	Matched for	Test used	*P* value
O'Donovan et al., 1995	52 UK Caucasian	DSM III-R SCZ	74 UK Caucasian	(CTG)10	Ethnicity	Wilcoxon rank sum	0.024
O'Donovan et al., 1996[a]	152 European Caucasian	DSM III-R SCZ	160 European Caucasian	(CTG)10	Ethnicity	Wilcoxon rank sum	0.0025
Petronis et al., 1996	5 E. Canadian peds, 14 affected members	RDC SCZ	36 Unaffected members	(CTG)10		*t*-test	0.79
Morris et al., 1995	84 England	DSM III-R SCZ	70 England	(CTG)17	Ethnicity	Statistic U	0.048
Vincent et al., 1996	54 Mixed ethnicity	DSM III-R SCZ	54 Mixed ethnicity	(CTG)10	Pairwise: age, gender, ethnicity	Mann–Whitney U	0.09
	55 Mixed ethnicity		81 Mixed ethnicity	(CTG)17	Not matched	Mann–Whitney U	0.22
Macedo et al., 1997	43 Portuguese	DSM IV SCV	43 Portuguese	(CTG)10	Age, ethnicity	Mann–Whitney U	0.08
Li et al., 1998a	82 Han Chinese	DSM III-R SCZ	61 Han Chinese	(CTG)10	Ethnicity	Wilcoxon rank sum	0.45
Burgess et al., 1998	36 Childhood onset, mixed ethnicity	DSM III-R SCZ	44 US mixed ethnicity	(CTG)10	Age, gender	Wilcoxon rank sum	0.036
Laurent et al., 1998	59 French	DSM III SCZ	59 French	(CTG)10	Age, gender, ethnicity	Mann–Whitney U	0.25
	21 French peds						0.23
Vincent et al., 1998	9 Affected sibs from 9 UK/ Iceland peds	RDC SCZ	9 Unaffected sibs	(CTG)10		paired *t*-test	0.31
Vincent et al., 1999a[b]	95 US mixed	DSM III-R/ DSM IV SCZ	95 US/Canada mixed	(CTG)10	Pairwise: age, gender, ethnicity	Mann–Whitney U	0.23

★RDC, research diagnostic criteria.

[a]O'Donovan et al., 1996 samples include O'Donovan et al., 1995 samples.

[b]Vincent et al., 1999a samples include Vincent et al., 1996 samples.

TABLE IIIb.   Studies Using RED in Bipolar Affective Disorder Cases Versus Controls

Group	BPAD patients, source/ethnicity	Diagnostic criteria	Controls, source/ethnicity	RED oligo	Matched for	Test used	P value
O'Donovan et al., 1995	49 UK Caucasian	DSM III-R BPAD I	74 UK Caucasian	(CTG)10	Ethnicity	Wilcoxon rank sum	0.003
O'Donovan et al., 1996[a]	143 European Caucasian	DSM III-R BPAD I	160 European Caucasian	(CTG)10	Ethnicity	Wilcoxon rank sum	0.0006
Lindblad et al., 1995	123 Belgium/ Sweden	DSM III-R/ RDC BPAD I/II	274 Belgium/ Sweden	(CTG)10	Ethnicity	Wilcoxon rank sum	0.0006
Vincent et al., 1996	52 Mixed	DSM III-R BPAD I	52 Mixed	(CTG)10	Pairwise: age, gender, ethnicity	Mann-Whitney U	0.21
	58 Mixed	DSM III-R BPAD I	81 Mixed	(CTG)17	Not matched	Mann-Whitney U	0.36
Oruc et al., 1997	40 Croatians	RDC BPAD I	79 Croatians	(CTG)10	Age, gender, ethnicity	Wilcoxon rank sum	>0.30
	15 Familial Croatians	RDC BPAD I	79 Croatians	(CTG)10		Wilcoxon rank sum	0.0075
Li et al., 1997	43 Han Chinese	DSM III-R BPAD I/II	61 Han Chinese	(CTG)10	Ethnicity	Wilcoxon rank sum	0.37
Lindblad et al., 1998	12 Families, N. Sweden, 53 affected relatives	DSM III-R BPAD I/II	123 Unaffected relatives	(CTG)10		Wilcoxon rank sum	<0.0007
Zander et al., 1998b	119 French Caucasian	DSM III-R BPAD I/II	88 French Caucasian	(CTG)10	Age, ethnicity	Mann-Whitney U	0.38
Vincent et al., 1999a[b]	91 Canada, mixed ethnicity	DSM III-R BPAD I	91 Canada, mixed ethnicity	(CTG)10	Pairwise: age, gender, ethnicity	Mann-Whitney U	0.98
Verheyen et al., 1999	10 Belgian families	RDC BPAD, UPR, SA, MDD		(CTG)10			no segregation

RDC, Research Diagnostic Criteria; UPR, unipolar recurrent major depression; SA, schizoaffective; MDD, major depressive disorder.
[a]O'Donovan et al., 1996 samples include O'Donovan et al., 1995 samples.
[b]Vincent et al. 1999a samples include Vincent et al., 1996 samples.

[Vincent et al., 1998a] that seemed to demonstrate anticipation [Sharma et al., 1994]. Another study looking at 21 SCZ families that had previously been reported to show anticipation [Thibaut et al., 1995] also failed to show larger CAG/CTG repeats in affected versus unaffected individuals or between parental and offspring generations [Laurent et al., 1998].

BPAD families

Eighty-seven two-generation pairs recruited from 29 (17 Belgian, 12 Swedish) BPAD families showed significant changes in age at onset and episode frequency in successive generations [Mendlewicz et al., 1997]. RED using a $(CTG)_{10}$ oligonucleotide was used to look for differences in CAG repeat length between generation one (G1) and generation two (G2), and mean CAG repeat sizes increased significantly between parental and offspring generations when the phenotype increased in severity ($P = 0.019$). A significant increase in median length CAG in 63 pairs with maternal inheritance was observed ($P = 0.025$), and a decrease in CAG repeat length was observed with paternal inheritance (24 pairs) ($P = 0.04$).

Molecular studies of SCZ in twins

RED has been used to study trinucleotide repeats in monozygotic twins (MZ) discordant for SCZ. At the post zygotic stage, various degrees of expansion may occur in an individual. It has been suggested that different degrees of expansion in critical tissues may occur in MZ co-twins [Petronis and Kennedy, 1995; Vincent et al., 1998]. The unaffected twin may contain a repeat below the threshold, whereas the affected twin contains a repeat above the threshold. TREs may also be larger in concordant MZ twins than in discordant MZ twins,

both concordant twins would be predicted to have expansions beyond the threshold and have the disease. Vincent et al., [1998] performed a study looking at differences in trinucleotide repeat sizes in discordant and concordant monozygotic (MZ) twins with SCZ. Southern blotting and the RED technique was used to examine for variations in CAG/CTG repeat sizes in MZ twins. Thirteen pairs were fully discordant for SCZ, nine pairs were fully concordant and five pairs showed partial concordance. The concordant twins showed an overall larger mean repeat size than the discordant twins. In two out of three instances where a difference in ligation product was observed in discordant co-twins, however, the unaffected twin had a larger ligation product than the affected twin.

Molecular studies in cases and controls

Tables III and IV outline the studies performed using RED in SCZ and BPAD cases and controls. Although several of the studies observed a significant gender-specific effect, for female schizophrenics only ($P = 0.0023$ [Morris et al., 1995]) and for male childhood-onset schizophrenics ($P = 0.002$ [Burgess et al., 1998]), the samples studied were very small, and other groups observed no such gender difference either in controls or affected patients, [Lindblad et al., 1995; O'Donovan et al., 1996; Vincent et al., 1996], suggesting that the initial findings may have been a type I error. For the unstable DNA hypothesis, it is actually gender-of-transmitting-parent effect rather than a gender effect within the offspring that is expected. Analysis of CAG/CTG repeats in offspring/parent trios may be necessary to test the hypothesis of parental effect.

Some of the RED results have

TABLE IVa. Studies at *ERDA1* in Cases With Schizophrenia Versus Controls

Group	SCZ patients source/ethnicity	Diagnostic criteria	Controls source/ethnicity	Matched for	Test used	*P*-value
Vincent et al., 1998						
Vincent et al., 1998a	151 US, mixed	DSM III-R	151 Canada, mixed ethnicity	Pairwise: age, gender, ethnicity	Mann–Whitney U	0.05[a]
Bowen et al., *submitted*	206 UK/Irish Caucasian	DSM III-R	219 UK Caucasian	Age, gender, ethnicitiy	Mann–Whitney U	0.46
Sidranksy et al., 1998	36 Childhood onset	DSM III-R	51	Non-matched	Chi square (≤150 nt)	<0.568

TABLE IVb. Studies at *ERDA1* in Cases with Bipolar Affective Disorder Versus Controls

Group	BPAD patients source/ethnicity	Diagnostic criteria	Controls source/ethnicity	Matched for	Test used	*P*-value
Vincent et al., 1999a	101 Canada, mixed ethnicity	DSM III-R BPAD I	101 Canada, mixed ethnicity	Pairwise: age, gender, ethnicity	Mann–Whitney U	0.4
Guy et al., 1999	210 UK/Irish Caucasian	DSM IV BPAD I	203 UK Caucasian	Age, gender, ethnicity	Wilcoxon rank sum	0.522
Verheyen et al., 1999	51 Swedish/Belgian	RDC BPAD I/II	99 Belgian	No matching	Wilcoxon rank sum	0.038
Verheyen et al., 1999	10 Belgian families	RDC BPAD, SA, UPR, MDD				No segrn.
Lindblad et al., 1998	60		114		Wilcoxon rank sum[b]	<0.25
Lindblad et al., 1998	12 Peds N. Sweden, 53 Affected		123 Unaffected		Wilcoxon rank sum[b]	<0.17

★RDC, research diagnostic criteria; UPR, unipolar recurrent major depression; SA, schizoaffective; MDD, major depressive disorder.
[a]Larger alleles more frequent in control group.
[b]Dichotomized sample (>120/≤120 nt).

been analyzed for clinical evidence that would support the unstable DNA hypothesis, such as correlation of severity with RED product size and inverse correlation with AAD. In Parikh et al. [1999], a number of severity indicators (such as age of onset, number of hospitalizations, presence of psychotic episodes, rapid cycling and attempted suicides) were noted and compared for BPAD individuals whose DNA produced large CAG/CTG RED products (>240 nt) compared with matched BPAD individuals with small RED products (<180 nt). No differences between groups were observed, although small sample size (n = 8 per group) makes interpretation of results difficult. In a similar study by the same group, 14 SCZ individuals with large RED products (>210 nt) were compared with 42 age- and gender-matched SCZ individuals with small RED products (<180 nt) for several clinical correlates (Brief Psychiatric Rating Scale: BPRS, BPOS, BNEG baseline scores; Global Assessment Scale: GAS baseline scores) also for age at onset and clozapine response. No significant differences were observed, although trends toward increased severity were seen for $BPRS_{base}$ and $BPOS_{base}$ for the small RED product group, contrary to the hypothesis [Vincent et al., 1999a]. In another study clinical correlates were related to RED product size for a combined group of 114 Portuguese and UK SCZ patients. OPCRIT factor scores were compared for 14 variables, but no significant relationship with CAG/CTG repeat length was found [Cardno et al., 1996].

Molecular Studies at Specific CAG/CTG Repeat Loci in Major Psychosis
ERDA1

The ERDA1 CAG expansion was cloned using a genomic library from an individual with a large RED product [Nakamoto et al., 1997]. The number of repeats ranged from 10–92 in 75 normal Japanese individuals. Large alleles (>51 repeats) were found in 13% of Caucasian individuals and in 43% of Japanese individuals, making it the commonest long CAG repeat reported in normal populations. FISH experiments localized the expansion to chromosome 17q21.3, but thus far investigations have not identified any nearby transcript. Preliminary molecular experiments revealed no instability between generations during either maternal or paternal transmission. This repeat was also independently cloned by others (Dir1 [Ikeuchi et al., 1998]) where they described expression of a 5.8 Kb band in brain, heart, skeletal muscle, ovary and spleen in northern analysis. Furthermore, based on 24 parent-offspring pairs, they describe instability of larger alleles (52–92 repeats) in both maternal and paternal transmission, with significantly higher instability in maternal transmissions.

A comparison of alleles at ERDA1 in 60 Swedish BPAD individuals and 114 Swedish controls showed no significant difference in distribution ($P < 0.25$) and no difference in frequency of large alleles ($P < 0.41$ [Lindblad et al., 1998]). A study of allele distribution at ERDA1 in 151 SCZ individuals and 151 controls matched pairwise for age, gender and ethnicity, showed a slight trend toward larger repeats in controls ($P = 0.05$) contrary to the expected direction of effect from the unstable hypothesis, but no difference between 101 BPAD and 101 pairwise-matched control individuals ($P = 0.4$ [Vincent et al., 1999a]). In a study of 210 UK and Irish BPAD individuals and 203 UK group-matched controls, similar allele distribution was reported ($P = 0.19$) and similar frequency of large repeat alleles ($P = 0.68$ [Guy et al., 1999]). In a study of 186 UK and Irish SCZ individuals and 203 UK controls, similar allele distribution was reported ($P = 0.46$) and similar frequency of large repeat alleles ($P = 0.41$ [Bowen et al., submitted]). Similar frequency of large alleles (>50 repeats) was reported in 36 childhood onset SCZ individuals in comparison to 51 controls ($P < 0.568$ [Sidransky et al., 1998]). Significant difference of ERDA1 allele distribution was reported in one study of 49 Belgian BPAD individuals and 99 Belgian controls, with a trend toward larger repeats in affected individuals ($P = 0.038$ [Verheyen et al., 1999]). It is not clear whether the controls were from the same ethnic group as affected individuals in these last two studies. Because there are clearly inter-ethnic differences in allele distribution and frequencies of large repeat alleles at ERDA1 [Nakamoto et al., 1997], as is the case for many highly polymorphic repeat regions, it is difficult to reach any conclusions from these data.

The reported range of repeat size is 10–92 repeats [Nakamoto et al., 1997; Ikeuchi et al., 1998]. One family has been reported to demonstrate expansion at ERDA1 above this range, where the proband with a diagnosis of severe childhood onset depression inherits an allele with ≈115 repeats expanded from her father, in whom the allele was 102 repeats long [Vincent et al., 1999b]. No repeats larger than 92 were detected amongst 780 other alleles. Although the expansion observed in this family fits with the unstable hypothesis, it is unclear whether there is any relationship between this expansion and disease in this family. Screening of large numbers of cases, controls and families may be necessary to exclude a role for ERDA1 in rare cases of major psychosis.

Large repeats at ERDA1 are estimated to be responsible for as few as 28% [Guy et al., 1999] or as much as 74% of RED products >120 nt long [Verheyen et al., 1999]. Studies of CAG/CTG repeats at ERDA1 are summarized in Table IVa for SCZ and Table IVb for BPAD.

SEF2-1B

A large CAG repeat was initially mapped to chromosome 18 by fluorescent in situ hybridization using a large CAG/CTG repeat probe [Haaf et al., 1996]. The large CTG/CAG repeat had been detected previously, using RED on a Danish SCZ kindred [Sirugo et al., 1997]. Although the large repeat tracts were present in affected cousins, the repeats were absent in other affected pedigree members. Pairwise linkage analysis showed no linkage of the large RED product to SCZ in this family. Interestingly, linkage of certain chromosome 18 markers to families with

BPAD has been described [Stine et al., 1995; Van Broeckhoven, 1999; Rice, 1997]. A search for CAG/CTG repeat sequences on chromosome 18 was carried out with the aim of identifying candidate genes with large repeats [Breschel et al., 1997]. A polymorphic CTG repeat, termed CTG18.1, was cloned by screening a human chromosome 18 specific genomic library using a $(CAG)_{15}$ probe, and specifically localized to chromosome 18q21.1. This CTG repeat localizes within an intron of a transcription factor gene, *SEF2-1B*. The 1.6 Kb clone contains a stable $(CTC)_{13}$ repeat flanking a polymorphic $(CTG)_n$ repeat. Alleles ranged from $(CTG)_{10}$ to $(CTG)_{2100}$. Alleles ranging from $(CTG)_{10-37}$ are common and generally stable whereas alleles larger than $(CTG)_{37}$ are frequently unstable. The allele frequency of moderately enlarged alleles in CEPH reference pedigrees and BPAD populations is 3% [Breschel et al., 1997]. The $(CTG)_n$ repeat is 114 bp from the beginning of the clone and is located 803 bp upstream from a 73 bp region that has 100% identity with human *SEF2-1B* cDNA. *SEF2-1B* is expressed in muscle, brain, hematopoietic cells and liver tissue. *SEF2-1B* encodes a basic helix-loop-helix DNA binding protein thought to be involved in transcriptional regulation [Breschel et al., 1997]. PCR analysis in 56 unrelated individuals from CEPH pedigrees resulted in highly polymorphic allele lengths with heterozygosity of 84%. Forty-eight BPAD families were analyzed at the CTG18.1 locus and revealed no evidence for linkage. In these pedigrees, larger alleles at *SEF2-1B* showed random segregation with BPAD [Breschel et al., 1997]. A sub-set of the families used in the study by Breschel et al. [1997] had previously been reported to show linkage to chromosome 18q21 [Stine et al., 1995]. Moderately enlarged alleles $(CTG)_{53-250}$ were not associated with the BPAD. The "super" expansions at SEF2-1B, $(CTG)_{800-2100}$, although not segregating with BPAD or SCZ in families [Breschel et al., 1997; Sirugo et al., 1997], have not been reported in unaffected families or individuals. Two out of three of the CEPH pedigrees iden-

tified by Schalling et al. [1993] as *RED1* had large alleles at *SEF2-1B* (CEPH pedigrees 1344 and 1420).

In a study by Vincent et al., [1998], the *SEF2-1B* locus was analyzed for MZ twins and families with SCZ. Of a sample of discordant, concordant, and partially concordant twin pairs, none showed any intra-twin variation in allele size. No alleles within the range $(CTG)_{800-2100}$ were observed. Alleles greater than $(CTG)_{50}$ were observed for three concordant twin-pairs and one partially concordant twin pair. These twin-pairs all had large RED products. In an analysis of affected/unaffected sib-pairs from SCZ families, a comparison of mean size of the larger allele showed no significant difference between 18 affected and 18 unaffected sibs ($P = 0.5$ [Vincent et al., 1998]).

A study of *SEF2-1B* in 97 SCZ individuals and 97 controls pairwise-matched for age, gender and ethnic background, also 100 BPAD individuals and 100 pairwise-matched controls, no difference in allele distribution and no increase in large alleles (>37 repeats) was observed in affected individuals ($P = 0.61$ and 0.95 respectively [Vincent et al., 1999a]). In a study of 206 UK and Irish BPAD individuals and 206 UK group-matched controls, similar allele distribution was reported ($P = 0.52$) and similar frequency of large repeat alleles ($P = 0.56$ [Guy et al., 1999]). In a study by the same group of 183 UK and Irish SCZ individuals and 190 group-matched controls, allele distribution and frequency of large repeat alleles were similar ($P = 0.43$; $P = 0.36$, respectively [Bowen et al., submitted]). No significant differences were observed for 49 Belgian BPAD individuals and 99 Belgian controls ($P = 0.73$ [Verheyen et al., 1999]), but in a sample of 60 Swedish BPAD and 114 Swedish control individuals expansion above 40 repeats were more common in affected individuals (13% in BPAD, 5% in controls; $P < 0.07$ [Lindblad et al., 1998]).

In the study by Vincent et al. [1999a], one interesting, albeit not statistically significant, observation was that 9% of BPAD individuals with large (≥ 180 bp) RED products had large (≥ 50 repeats) alleles for both *SEF2-1B*

and *ERDA1*, in comparison to 2% of SCZ and none of the controls individuals. Thus an epistatic model of predisposition to BPAD cannot be excluded. Large repeats at *SEF2-1B* are estimated to be responsible for between 7% [Guy et al., 1999] and 17% of RED products >120 nt long [Verheyen et al., 1999]. Studies of CAG/CTG repeats at *SEF2-1B* are summarized in Table Va for SCZ and Table Vb for BPAD.

SCA8

A large CAG/CTG trinucleotide repeat was identified using RED in a family segregating for an undefined form of dominantly inherited spinocerebellar ataxia, and was subsequently cloned from total genomic DNA from an affected female using the RAPID cloning procedure [Koob et al., 1999]. The repeat region localizes to 13q21 and is believed to be at the 3′ end of a gene expressed at low levels in cerebellum for which no open reading frame exists. The repeat, that is a $[CTA]_n[CTG]_n$ repeat, with the majority of total repeats lying between 18 and 30 repeats, seems to be highly unstable, with expansions generally occurring through maternal transmissions, and paternal transmissions of large repeat alleles often leading to very large contractions. The pathogenic repeat range seems to be relatively narrow in the large ataxia kindred, with 107–127 CTG repeats leading to disease. Although penetrance is clearly incomplete in this pedigree, linkage analysis of this repeat locus produces a maximum lod score of 6.8, with $\Theta = 0.0$. PCR analysis detected expansion at SCA8 in a further 7 out of 102 families with undefined hereditary ataxias.

This repeat region was also independently cloned from a woman with BPAD with very large CAG/CTG RED products [Vincent et al., 2000], and shows sequence and localization identity with the Koob et al. [1999] repeat. Screening of 1120 unrelated patients affected with SCZ, BPAD, schizoaffective disorder, childhood onset psychosis or childhood onset depression and 710 unrelated unaffected controls (screened for lack of psychiatric illness) using PCR and genomic Southern hy-

TABLE Va.   Studies at *SEF2–1B* in Cases With Schizophrenia Versus Controls

Group	SCZ patients source/ethnicity	Diagnostic criteria	Controls source/ethnicity	Matched for	Test used	*P* value
Vincent et al., 1998	18 UK/Iceland sibpairs	RDC SCZ	18 Unaffected sibs		Paired *t*-test	0.5
Vincent et al., 1999a	97 US, mixed	DSM III-R SCZ	97 US/Canada, mixed ethnicity	Pairwise: age, gender, ethnicity	Mann-Whitney U	0.61
Bowen et al., submitted	206 UK/Irish	DSM III-R SCZ	219 UK Caucasian	Age, sex, ethnicity	Mann-Whitney U	0.43

TABLE Vb.   Studies at *SEF2–1B* in Cases With BPAD Versus Controls

Group	BPAD patients source/ethnicity	Diagnostic criteria	Controls source/ethnicity	Matched for	Test used:	*P*-value
Breschel et al., 1997	48 Affected sib pairs	RDC BPAD			chi square[a]	0.64
Lindblad et al., 1998	60		114			
Lindblad et al., 1998	12 Families N Sweden, 53 Affected		123 Unaffected		Wilcoxon rank sum[a]	<0.03
Vincent et al., 1999a	100 Canada, mixed ethnicity	DSM III-R BPAD	100 Canada, mixed ethnicity	Pairwise: age, gender, ethnicity	Mann-Whitney U	0.95
Guy et al., 1999	206 UK/Irish Caucasian	DSM IV BPAD I	206 UK Caucasian	Age, gender, ethnicity	Wilcoxon rank sum	0.522
Verheyen et al., 1999	51 Swedish/ Belgian	RDC BPAD I/II	99 Belgian		Wilcoxon rank sum	0.072
Verheyen et al., 1999	10 Belgian families	RDC BPAD, UPR, SA, MDD				No segregation

[a]Dichotomized sample (>120/≤120 nt).

bridization (for very large repeats that fail to amplify by PCR) showed that large alleles in and above the suggested pathogenic range at *SCA8* are, in fact, common in the general population (ranging from 100 to 1300 repeats: 0.7% in 710 controls, 1.25% in 1120 major psychosis and childhood onset depression). Subdivision of the large patient group revealed that the highest clustering of expansions was in SCZ individuals (2.1%; n = 390). In another study, 1 out of 206 UK and Irish unrelated SCZ individuals had a *SCA8* allele with above 100 repeats (approximately 180 repeats) and none out of 219 controls [Bowen et al., submitted]. Large alleles at *SCA8* are present in members

of CEPH reference pedigree number 1334 [Vincent et al., 2000], for which large RED products were reported by Schalling et al. [1993]. This relatively high frequency of large repeats, spanning the entire range from 100 to 1300 repeats and including a number within the expected pathogenic range for *SCA8*, is inconsistent with the rarity of spinocerebellar ataxias (≈1/10,000 for all ataxias [Koob et al., 1999]), let alone the SCA8 subtype. This suggests that either the observations of repeat expansion at *SCA8* are purely coincidental, or more likely that expansion at *SCA8* alone is insufficient to precipitate the neurodegenerative disorder and another mutation or allelic variant at this

locus is required for pathogenicity. It seems more than probable that a proportion of the ataxia families with expansion mutations at *SCA8* detected by Koob et al. [1999] are, in fact, non-pathogenic at this locus and have disease-causing mutations elsewhere in the genome. Until this second factor for *SCA8* is delineated, diagnostic analysis at this locus would be ill-advised. What remains, however, is an intriguing over-abundance of large alleles in the major psychosis patients, suggesting that this may be a susceptibility factor with a small risk ratio, 1.8–3.0. To reach statistical significance, however, screening samples in excess of 10,000 cases and controls may be required. Family stud–

ies for *SCA8* in psychosis would be useful. Preliminary studies of *SCA8* amongst 36 Azorean pedigrees ascertained for major psychosis shows three of the families (8.3%) segregate expanded alleles [Kennedy et al., 1999].

Large repeats at *SCA8* are probably responsible for around 8% of large RED products (>180 bp [Vincent et al., unpublished results]. It is unlikely that the differences in RED distribution for major psychosis and controls observed by several groups [O'Donovan et al., 1995, 1996; Lindblad et al., 1995; Morris et al., 1995] could be accounted for by the differences reported between patient and control groups for *SCA8*, because these expansions are relatively rare (≈1%), and generally lie above the size range where the significant RED differences were observed.

MAB21L1

A trinucleotide repeat containing cDNA clone, termed *CAGR1*, was isolated from a retinal cDNA library and mapped to 13q13 [Margolis et al., 1996]. This cDNA sequence encodes a protein (*MAB21L1*) that is homologous to the *C. elegans* protein mab-21. The CAG repeat is highly polymorphic, and is situated within the 5′ untranslated region. No significant differences in allelic distribution were seen between schizophrenics (n = 77), bipolar affective disorder individuals (n = 46) and control individuals, and no large alleles in either disease group. Almost all alleles lie within the 6–31 repeat range, but very rare large alleles of 46 and 50 repeats have been reported [Potter, 1996; Margolis et al., 1999], and are unstably transmitted. Although there is no clear correlation of any phenotype with expansion at *CAGR1*, this trinucleotide repeat is a further addition to the growing family of unstable repeats, and an etiological role in a small fraction of major psychosis cannot be excluded until much larger studies are performed.

KCNN3/hSKCa3

This CAG/CTG repeat locus is the exception from the previous four in that it shows no allelic instability during pa-

rental transmission, and no sufficiently large alleles have been found that could explain the initial findings of larger CAG/CTG RED products among SCZ and BPAD affected individuals compared to control individuals (for which the lower end of the repeat range starts at around 50 repeats for BPAD and 60 repeats for SCZ [O'Donovan et al., 1996]). The reason that we have included studies of *HSKCA3/KCNN3* in this section is that this gene was initially considered by some to be such a good candidate for major psychosis, and as a result has become widely studied, with the unstable DNA hypothesis cited as part of the rationale. To date, at least 15 peer-reviewed studies have been published, and many more presented as abstracts at meetings. The gene encodes a novel neuron-specific calcium-activated potassium channel protein. The gene was initially incorrectly mapped to 22q11–13, a region of utmost interest for major psychosis and SCZ in particular, where several positive linkage results have been found. The linkage findings at 22q11 overlap with a deletion region for velocardiofacial syndrome (VCFS), that occurs at a significantly high rate among SCZ individuals [Bassett et al., 1998]. The gene has, however, been re-mapped by many researchers to 1q21.3. There are, in fact, two stretches of trinucleotide repeats, encoding two separate polyglutamine stretches in the protein, the first that shows low polymorphic variability, and the second that is highly polymorphic, with heterozygosity of 77% [Joober et al., 1999a].

The repeat length polymorphism at the second of the two repeats has been widely studied. The repeat sizes range from 12–28 repeats. Results for family-based and association studies at *HSKCA3/KCNN3* are summarized in Tables VIa (SCZ) and VIb (BPAD). The initial study by Chandy et al. [1998] reported a significant difference in distribution ($P = 0.024$), and in particular an increase in frequency of alleles with more than 19 CAG repeats in 141 SCZ probands compared with 158 unmatched controls ($P = 0.0035$). Several other studies corroborate this finding for SCZ, albeit very weakly ($P = 0.05$

[Bowen et al., 1998]; $P = 0.06$ [Austin et al., 1999]), and one study comparing alleles in 84 SCZ Ashkenazi Jews and 102 matched controls showed strong differences in frequencies with larger alleles more common in the patient sample ($P = 0.00017$ [Dror et al., 1999]). The majority of studies, however, find no significant effect [Li et al., 1998b; Tsai et al., 1999; Joober et al., 1999a; Hawi et al., 1999; Meissner et al., 1999; Antonarakis et al., 1999; Bonnet-Brilhaut et al., 1999]. A study by Stober et al. [1998] reports significant increased transmission of alleles with 19 or fewer repeats in 59 SCZ trios ($P = 0.014$), thus contradicting the initial report.

It is worth noting that of the four studies that gave positive results for *KCNN3* and psychosis, three of the studies, including the initial study, were population-based rather than family-based, and are thus more likely to be prone to population stratification, leading to elevation of type I error. Given the lack of any repeat instability to justify claims of anticipation, lack of any large repeats to support the RED association of CAG/CTG repeats and psychosis, the re-localization of the gene to a genomic region not yet implicated in psychosis by linkage findings, and finally the wide failure to replicate the initial findings, the value of *KCNN3* as a candidate gene for SCZ should perhaps be reconsidered.

Polyglutamine-Containing Protein Detection

A useful approach for detecting patients who have expanded CAG/CTG genes involves using a monoclonal antibody (mAb1C2) specific for large polyglutamine tracts to detect polyglutamine-proteins derived from lymphoblastoid cell lines from patients. Schurhoff et al. [1997] were unable to detect proteins with expanded polyglutamine in a sample of early onset schizophrenia (n = 3) and bipolar disorder (n = 4). Similarly, Jones et al. [1997] found no expanded polyglutamine proteins associated with schizophrenia (n = 18) or bipolar disorder patients (n = 18), and Turecki et al. [1999] found no different polyglutamine bands in 70 bipolar patients and 73 controls. Several more re-

TABLE VIa. Studies at hSKCa3/KCNN3 in Cases With Schizophrenia Versus Controls★

Group	SCZ patients source/ethnicity	Diagnostic criteria	Controls source/ethnicity	Matched for	Test used	*P*-value
Chandy et al., 1998	43 US and 98 French/Alsace	DSM-IV SCZ	159.5(!) US and French/Alsace	Ethnicity	Wilcoxon rank sum	0.024
					chi square[a]	0.0035
Li et al., 1998b	97 Han Chinese trios	DSM III-R/ IV SCZ	Parents		ETDT	0.31
					TDT[a]	0.066
Bowen et al., 1998	194 UK/Irish	DSM III-R SCZ	183 UK/Irish	Age, gender, ethnicity	Mann–Whitney rank sum	0.11
					chi square[a]	0.047
Dror et al., 1999	84 Israeli Ashkenazi	DSM IV SCZ	102 Israeli Ashkenazi	Ethnicity	Wilcoxon rank sum	0.00017
Austin et al., 1999	186 Mixed	DSM III-R SCZ	54 Unaffected relatives	None	chi square[a]	0.063
Hawi et al., 1999	265 Irish trios	DSM III-R SCZ	Parents		ETDT	NS
	170 Irish	DSM III-R SCZ	217 Irish	Ethnicity	chi square[a]	NS
Bonnet-Brilhault et al., 1999	151 French	DSM III-R SCZ	153 French	Age, gender	Mann–Whitney rank sum	0.53
	20 Families: 7 France/ 13 Reunion: 62 affected	DSM III-R SCZ	60 Unaffected		Mann–Whitney rank sum	0.32
Wittekindt et al., 1999	193 German/ Israeli trios	DSM III			TDT[a]	0.424
Antonarakis et al., 1999	54 Multiplex families	DSM IV SCZ			TDT-LIKE	0.07
					SIBPAIR	0.102
Meissner et al., 1999	12 German multiplex families	DSM III-R SCZ	Parents		ETDT	0.67
					GENEHUNTER: NPL	0.218
Joober et al., 1999a	63 Neuroleptic non-responders and 43 Neuroleptic responders	DSM IV SCZ	93 Healthy	50% for ethnicity	chi square	0.97
Stober et al., 1998	59 Trios		Parents		chi square[a]	0.014[b]
Tsai et al., 1999	92 Chinese	DSM IV SCZ	100 Chinese	Age, ethnicity	Wilcoxon rank sum	0.664
					Fisher's exact[a]	0.739

★NS, not significant.
[a]Dichotomized around modal value (19 repeats).
[b]Short repeats increased.

cent studies have, however, detected large polyglutamine bands: first, in two amongst 59 schizophrenia patients and in a sibling diagnosed with major depression, but not in controls (n = 73 [Joober et al., 1999b]); second, in 8 out of 32 childhood onset schizophrenia patients (all 8 positives were Afro-American), but not in 38 Afro-American or 53 American healthy controls [Moriniere et al., 1999]. Ethnic backgrounds of samples in the other studies was not reported, hence inter-study comparison of findings is difficult. The earlier studies also used very small sample sizes, and little can be con-cluded. The findings presented by Joober et al. [1999b] and especially Moriniere et al. [1999], however, are particularly intriguing, and further investigation and replication is crucial.

The limitations of this technique are the length of polyglutamine required for detection by 1C2 (>32 glu-

TABLE VIb.   Studies at hSKCa3/KCNN3 in Cases With Bipolar Affective Disorder Versus Controls★

Group	BPAD patients source/ethnicity	Diagnostic criteria	Controls source/ethnicity	Matched for	Test used	P-value
Chandy et al., 1998	USA and French/ Alsace, n not shown	DSM IV, BPAD I	USA and French/ Alsace, n not shown	Ethnicity	Wilcoxon rank sum chi-square[a]	NS NS
Guy et al., 1999	203 UK/Irish	DSM IV BPAD I	206 UK/Irish	Group matched: age, gender Ethnicity	Mann–Whitney rank chi square[a]	0.36 0.23
Hawi et al., 1999	91	DSM III-R BPAD	217 Irish	Ethnicity	chi square	NS
McInnis et al., 1999	69 Families	BPI, II and SA			ETDT chi square[a]	0.47 0.49

★NS, not significant; SA, schizoaffective.
[a]Dichotomized around modal value (19 repeats).
[b]Short repeats increased.

tamine residues or 28 with gross over-exposure [Trottier et al., 1998]), the requirement of strong expression of the gene in the (lymphoblastoid) cells used, and for the trinucleotide repeat to be in the coding region, and in the correct reading frame. Alteration of methylation state at expanded trinucleotide repeats during Epstein–Barr virus (EBV)-transformation has been observed [Petronis et al., in press], and may alter expression of expanded polyglutamine genes. The assumption that polyglutamine expansion is involved in the disease mechanism is also central to this approach.

Screening Other CAG/CTG Repeat Loci in Major Psychosis: SCZ

A study by Jain et al. [1996] compared 20 to 97 SCZ patients with 43 to 146 controls not matched for age or gender but matched for ethnic background. Eleven polymorphic trinucleotide repeat-containing genes expressed in the brain, that had previously been identified by screening cDNA libraries [Li et al., 1993; Margolis et al., 1995] were analyzed, but no differences in the allele distributions and no expansions were observed. Forty-five CAG repeat loci were studied for association with SCZ using 11 patients shown by RED

screening to have 60–90 repeats [Bowen et al., 1996]. The loci were identified using the CAG/CTG repeat genomic PCR screening set [Gastier et al., 1996]. All loci described to be polymorphic by Gastier et al. [1996] were used in the study. Nine markers expected to be polymorphic were non-polymorphic among the sample population. The largest allele size detected was 30 repeats found in marker *GCT5E11*. No evidence for large alleles (>35 repeats) was detected. Eight CAG trinucleotide repeat loci from the Gastier screening set [Gastier et al., 1996] mapping to chromosome 4 were studied using a sample of ten SCZ patients and ten BPAD patients with RED products between 60 and 90 repeats [Speight et al., 1997]. Chromosome 4 was chosen because of linkage of BPAD in a single pedigree to D4S394 (Z_{max} = 4.09 [Blackwood et al., 1996]), and weaker evidence for linkage in a pedigree for schizoaffective disorder (Z_{max} = 1.86 [Asherson et al., 1995]). Only one of the markers used was found to be polymorphic in the sample, and did not contain alleles with greater than 35 repeats. Five CAG/CTG loci were studied in 17 families with SCZ patients from Japan and China [Ohara et al., 1997b]. The loci were identified using a human brain cDNA library as described by Li et al.,

[1993]. Two of the five loci were polymorphic. The patients and controls were matched for ethnicity but not age or gender. The allele frequencies of the two polymorphic loci were not significantly different between the patients and the controls. Also, no evidence for expansion was observed in the affected subjects. Four trinucleotide polymorphisms (DRPLA, N-cadherin, B1 and B33) were investigated in 53 to 74 North American and Italian schizophrenics with matched controls, as well as two polymorphisms in 49 to 63 BPAD patients. No marked differences in allelic distribution were observed [Sasaki et al., 1996].

SCZ families

Following evidence of linkage disequilibrium to SCZ on 6p [Wang et al., 1995], three polymorphic CAG/CTG loci from the Gastier set on chromosome 6 [Gastier et al., 1996] were tested in 30 SCZ families from Japan and China. No evidence for CAG trinucleotide repeats of more than 35 repeats was found [Ohara et al., 1997c].

BPAD

In a study of thirteen trinucleotide repeat loci by Jain et al. [1996] 23–30 East Anglian BPAD patients with 43–146

Anglian controls not matched for age or gender but matched for ethnicity were compared. Two of the 13 loci include CAG repeats in the gene for HD and DRPLA. No difference in allele distribution was observed between the BPAD patient sample and the controls. Speight et al. [1997] also studied 12 CAG repeat loci in ten BPAD subjects. GCT10B09 was the only marker that was polymorphic in the sample, and no evidence for large alleles (>35 repeats) was detected. There was no evidence of large CAG/CTG repeats (>35 repeats) at 43 polymorphic autosomal loci and 7 X-chromosomal loci taken from the Gastier screening set [Gastier et al., 1996] in 9 Caucasians with BPAD who were observed to have between 60–90 (CAG/CTG) repeat units by RED [Guy et al., 1997]. Secondary screening at a locus (GCT5E11 on chromosome 3) containing a large allele (37 repeats) was pursued using 122 British BPAD individuals and 109 British controls matched for age and gender, however, no difference in allele distribution was found ($P = 0.46$ [Guy et al., 1997]).

Non-CAG/CTG Repeats in Major Psychosis

Very few studies have been reported that seriously address the possibility that trinucleotide repeats other than CAG/CTG could play an etiological role in major psychosis, or cryptic triplet repeats that may encode long, even expanded polyglutamine stretches (TATA-binding protein [Koide et al., 1999]), or indeed repeats other than trinucleotides, such as the dodecamer repeat in the cystatin B gene that is expanded in progressive myoclonus epilepsy, EPM1 [Lafreniere et al., 1997]. Apart from obvious reasons for focussing on CAG/CTG, such as the relative abundance of known diseases caused by expansion at CAG/CTG repeats, there may be a number of other, more subtle biases, such as the relative abundance of CAG/CTG in exons compared to other trinucleotides [O'Donovan and Guy, 1997], and the CpG-rich and CNG-rich nature of coding sequence vs non-coding, and in particular at the 5′ end of genes

[Gardiner-Garden and Frommer, 1987].

Hofferbert et al. [1997] attempted RED analysis in unrelated controls for all ten possible triplet combinations. RED for CGG/CCG repeats gave poor results. AAG/CTT repeat RED analysis showed the presence of very large repeats (252–540 nt), close to the upper limits of resolution on 6% polyacrylamide gels. For AGG/CCT, ATG/CAT and GGT/ACC the product sizes ranged from 60–90 nt, 60–240 nt, and 120–210 nt, respectively. For AGT/ACT, AAT/ATT and ACG/CGT only single ligation products were observed for at least 85% of individuals tested.

Vincent et al. [1998] used Southern hybridization and RED analyses to investigate the triplet AAG/CTT in discordant SCZ MZ twins, and in SCZ families showing anticipation. RED results suggested the presence in the genome of a common, large AAG/CTT repeat stretch, at least 390 to 600 bp in length. Accurate size of maximum ligation product was difficult to ascertain [see Hofferbert et al., 1997], as signal strength diminished with increasing size of band, unlike RED for CAG/CTG where strong signal for the largest band was generally observed. Southern hybridization experiments showed no band variations.

RED analysis for non-CAG/CTG repeats maybe worthwhile, although without prior evidence that the repeat may demonstrate instability such studies may seem unattractive. Also, although the involvement of other unstable repeat motifs in psychosis cannot be excluded, at present there is no clear rationale for testing other repeat-containing candidate loci (such as the initial RED findings for SCZ and BPAD that provided a rationale for the testing of CAG/CTG repeats). It is recommended that each repeat locus should be evaluated for study according to its merits, such as whether or not it lies within a gene, whether the gene is within a region of suggestive linkage, or whether the gene function fits with a good a priori hypothesis for the disease, whether the repeats are highly polymorphic and long (although there are

several exceptions to this such as *SCA6* and *CBL2*). Much of this is clearly subjective, however, and open to wide interpretation.

CONCLUSIONS

Despite the initial findings of larger CAG/CTG RED ligation products among SCZ and BPAD individuals, a number of studies failed to replicate these results (Tables IIIa and IIIb). The reported shift toward larger RED products does not necessarily infer instability. Using a $(CTG)_{10}$ oligonucleotide gives genomic repeat size representations in 30 bp intervals. The difference in base pairs between controls and patients at a specific locus may be smaller than this interval. RED reading errors, variation in template DNA quantity and quality may influence the results, in addition to inter-experiment variation such as enzyme efficiency, oligonucleotide phosphorylation efficiency, DNA transfer and hybridization efficiency and evaporation of reaction mix. Although RED may be capable of discerning large expanded repeats for single gene disorders, i.e., MJD [Lindblad et al., 1996a] SCA7 [Lindblad et al., 1996b] and HD [Hofferbert et al., 1997], smaller expansions such as *SCA6* would not be seen above the background repeats. Also, it is not clear whether RED could detect expansions for a complex disorder with locus heterogeneity and possible involvement of numerous susceptibility genes, or what size odds ratio could be detected and what size of sample would be required.

Because completion of the sequencing of the human genome is imminent, database analysis and PCR-based detection for potential unstable repeats may become the method of choice for concluding the trinucleotide repeat story in major psychosis. RED may still provide an efficient screening procedure for identifying individuals with large repeats to locate expanded sequences that can then be tested.

In the analyses of specific unstable repeat loci, it seems that a large proportion of RED products ≥180 nt have large alleles at *SEF2-1B, ERDA1* or *SCA8*. Some suggestions of association

at these loci have been reported (*ERDA1* and BPAD [Verheyen et al., 1999]; *SEF2-1B* and BPAD [Lindblad et al., 1998]; *SCA8* [Vincent et al., 2000]), however, the *ERDA1* and *SEF2-1B* findings were not corroborated in other studies (see Tables IV and V). Also, the suggestion that expansion at both *ERDA1* and *SEF2-1B* may be associated with BPAD [Vincent et al., 1999a] warrants further investigation. Much larger, family-based studies may be required to implicate or eliminate these genes.

The discovery of polyglutamine protein bands specific to lymphoblastoid cells from SCZ patients, and Afro-American SCZ individuals in particular [Joober et al., 1999b; Moriniere et al., 1999] also adds fuel to the unstable DNA theory for major psychosis. Identification of the gene or genes encoding the polyglutamine protein(s) would provide the basis for large scale genetic analysis, and verification of these findings.

In summary, the RED method has been unable to prove or disprove a role for CAG/CTG repeats in SCZ and BPAD, partly due to: a) the likely genetic heterogeneity in affected populations; b) the high background of large repeats such as *SCA8, SEF2-1B* and in particular *ERDA1* in the population; and c) the lack of sensitivity of this technique to discern smaller, potentially disease-causing repeats that may be concealed by the high level of background repeat DNA. After eliminating all large RED products thought to be due to *SCA8, SEF2-1B* and *ERDA1*, the remainder represents a highly enriched sample of individuals with CAG/CTG repeats within (or above) the pathogenic size-range of other trinucleotide repeat diseases, but at unknown, possibly etiologic loci. The localization and characterization of these unknown repeats for SCZ and BPAD may clarify the issue of unstable DNA, anticipation and major psychosis, at least as far as CAG/CTG repeats are concerned. Analysis of other triplets, cryptic repeats and minisatellites is also necessary to conclude the unstable DNA chapter, but would demand expansion of effort and resources.

Although initial reports suggested that unstable DNA may have an etiological role in psychosis, it now seems that this theory fairs no better than linkage or association studies in being unable to clarify what is a true effect, and it is unable to refute small effects. Results of case-control studies raise concerns as to its usefulness in admixed populations.

ACKNOWLEDGMENTS

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