Am. J. Hum. Genet. 59:905–911, 1996

Search for Unstable DNA in Schizophrenia Families with Evidence for Genetic Anticipation

Arturas Petronis,[1] Anne S. Bassett,[2] William G. Honer,[3] John B. Vincent,[1] Yuriy Tatuch,[1] Tsukasa Sasaki,[1] Da-Jun Ying,[1] Timothy A. Klempan,[1] and James L. Kennedy[1]

[1]Neurogenetics Section, Clarke Institute of Psychiatry, and [2]Schizophrenia Research Unit, Queen Street Mental Health Centre, Toronto; and [3]Jack Bell Research Centre, Department of Psychiatry, University of British Columbia, Vancouver

Summary

Evidence for genetic anticipation has recently become an important subject of research in clinical psychiatric genetics. Renewed interest in anticipation was evoked by molecular genetic findings of a novel type of mutation termed "unstable DNA." The unstable DNA model can be construed as the "best fit" for schizophrenia twin and family epidemiological data. We have performed a large-scale Southern blot hybridization, asymmetrical PCR-based, and repeat expansion–detection screening for $(CAG)_n/(CTG)_n$ and $(CCG)_n/(CGG)_n$ expansions in eastern Canadian schizophrenia multiplex families demonstrating genetic anticipation. There were no differences in $(CAG)_n/(CTG)_n$ and $(CCG)_n/(CGG)_n$ pattern distribution either between affected and unaffected individuals or across generations. Our findings do not support the hypothesis that large $(CAG)_n/(CTG)_n$ or $(CCG)_n/(CGG)_n$ expansions are the major etiologic factor in schizophrenia. A separate set of experiments directed to the analysis of small (30–130 trinucleotides), Huntington disease-type expansions in individual genes is required in order to fully exclude the presence of $(CAG)_n/(CTG)_n$- or $(CCG)_n/(CGG)_n$-type unstable mutation.

Introduction

Evidence for genetic anticipation has been an interesting development in clinical psychiatric genetics. Genetic anticipation, a phenomenon that exhibits increased disease severity and earlier age at onset (AAO) in younger generations, was first described in psychiatric disorders more than three quarters of a century ago (Mott 1911). No reasonable explanation for anticipation was available within the Mendelian paradigm until recently, and the majority of geneticists tended to interpret this finding as a statistical artifact—a result of a number of ascertainment biases (Penrose 1948).

Renewed interest in anticipation was evoked by molecular genetic findings of a novel type of mutation termed "unstable DNA." Unstable DNA, or an expansion of trinucleotide repeats, has been found to cause several neuropsychiatric disorders, including myotonic dystrophy, fragile X, spinal and bulbar muscular atrophy, spinal cerebellar ataxia 1, Huntington disease (reviewed in Caskey et al. 1992; Richards and Sutherland 1992; Sutherland and Richards 1992; Ross et al. 1993), dentatorubral pallidoluysian atrophy (Koide et al. 1994; Nagafuchi et al. 1994), and Machado-Joseph disease (Kawaguchi et al. 1994).

In psychiatric genetics, building upon several studies prior to the unstable-DNA era (Mott 1911; Penrose 1971; Gindilis 1979), there have been a number of recent investigations of anticipation in schizophrenia (Asherson et al. 1994; Bassett and Honer 1994; Sharma et al. 1994) and bipolar affective disorder (McInnis et al. 1993; O'Neil et al. 1993; Nylander et al. 1994). All of these studies have demonstrated an earlier AAO in younger generations, which is consistent with the anticipation hypothesis. Comparison of the intergenerational differences in disease severity, although relatively more difficult to measure than the AAO, is less sensitive to ascertainment biases in comparison with the AAO. In two studies (McInnis et al. 1993; Bassett and Honer 1994), disease severity was evaluated and determined to be more severe in younger generations, thus corroborating the anticipation hypothesis.

DNA instability provides a new opportunity to reevaluate a wide spectrum of controversial empirical findings in schizophrenia genetics. The unstable-DNA model is able to account for both polygenic and single-major-locus inheritance patterns of schizophrenia (Petronis and Kennedy 1995). Unstable trinucleotide repeats can simulate an additive effect of a large number of genes with small effect. On the other hand, unlike classical additive genes dispersed through the chromosomes, the pathogenic unstable trinucleotides are concentrated within

Received March 2, 1995; accepted for publication July 19, 1996.

Address for correspondence and reprints: Dr. James L. Kennedy, R-31, Neurogenetics Section, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario M5T 1R8, Canada. E-mail: kennedyj@cs.clarke-inst.on.ca

0002-9297/96/5904-0022$02.00

one genetic locus and therefore may segregate as a single gene.

The unstable-DNA hypothesis in schizophrenia can be tested by a set of independent molecular genetic methods for direct detection of trinucleotide-repeat expansion. Riggins et al. (1992) and Li et al. (1993) proposed the screening of cDNA libraries for genes containing GC-rich trinucleotide-repeat sequences. This strategy, although labor intensive, is useful for detecting relatively short (Huntington disease–type, 30–130 trinucleotide) expansions and already has proved to be efficient in cloning the gene of dentatorubral pallidoluysian atrophy (Koide et al. 1994; Nagafuchi et al. 1994).

A method for direct detection (repeat-expansion detection [RED]) was developed by Schalling et al. (1993). An advantage of this technique is that it does not require isolation of cDNA clones or information on regions flanking the unstable DNA. An application of this method to the screening of Danish schizophrenia kindred revealed several patients with a relatively large $(CTG)_{17}$ oligonucleotide ligation product (Sirugo et al. 1995). In association studies, a statistically significant shift toward larger $(CTG)_{10}$ and $(CTG)_{17}$ oligonucleotide ligation products was detected for unrelated schizophrenia and bipolar-affective-disorder patients in comparison with unaffected controls (Lindblad et al. 1995; Morris et al. 1995; O'Donovan et al. 1995). A similar trend, although not statistically significant, has been observed in our own study of unrelated schizophrenia and bipolar-affective-disorder patients compared with matched controls (Vincent et al. 1996). In addition to the RED technique, a set of methods that include PCR and two DNA hybridization–based methods for direct detection of unstable-DNA expansion in genomic DNA was developed recently by us for detection of large DNA expansions (Petronis et al. 1996). These direct-detection methods are able to unambiguously detect large, myotonic dystrophy range, trinucleotide-repeat expansions. The specific goal of this study was to search for large $(CAG)_n/(CTG)_n$ and $(CCG)_n/(CGG)_n$ expansions as the major etiologic factor in schizophrenia multiplex families demonstrating evidence for genetic anticipation.

Subjects and Methods

Subjects

The subjects were 81 members of five eastern Canadian schizophrenia multiplex families showing genetic anticipation (Bassett and Honer 1994). Thirty-nine individuals were from the index generation, 31 were from the parental generation, and 11 were from the grandparental generation. Ascertainment and diagnostic assessments have been described in detail elsewhere (Bassett et al. 1993; Bassett and Honer 1994). Research diagnostic–criteria diagnoses were assigned by consensus of two psychiatrists (A.S.B. and W.G.H.). Twenty-three individuals were affected with schizophrenia, schizoaffective disorder, or schizophrenia spectrum disorder, and 58 were unaffected.

Laboratory Techniques

Southern blotting and hybridization.—Given the proper conditions, our standard Southern blotting technique is able to detect a genomic DNA fragment containing $\geq$300 $(CTG)_n/(CAG)_n$ trinucleotide repeats (Petronis et al. 1996). Using this method, we screened genomic DNA samples from subjects as follows. Five micrograms of total genomic DNA were digested with 25–30 U of restriction enzyme overnight, under the manufacturer's (New England Biolabs) recommended conditions. The digested DNA was electrophoresed on 0.8% agarose gels at 50 V for 16–20 h. The gels were blotted to Hybond-N^+ membranes (Amersham), with 2 × SSC used as transfer buffer.

The large $(CAG)_n/(CTG)_n$ fragments that were employed as the DNA probe in the hybridizations were generated by PCR extension of complementary oligonucleotides $(CAG)_7$ and $(CTG)_7$. The PCR amplification was performed by use of 1.0 U of *Taq* polymerase in the presence of 0.05 μg each of $(CAG)_7$ and $(CTG)_7$ oligonucleotides, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 200 μM each of dATP, dCTP, and dTTP, 100 μM dGTP, 100 μM 7-deaza-dGTP, and dimethyl sulfoxide (DMSO) 10%. The cycling conditions consisted of 30 cycles at 95°C for 15 s, 54°C for 15 s, and 72°C for 15 s. Radioactive labeling of 50–100 ng (1–2 μl) of the PCR product was performed by use of the Multiprime labeling kit (Amersham) and 50–100 μCi of ^{32}P-α-dCTP (5,000 Ci/mmol). Incorporation of radioactive dCTP reached 30%–60%, and specific activities of the probes were 10^8–10^9 dpm/μg. Separation of unincorporated radioactive dCTP was performed on a Sephadex G-50 Column DNA grade (NICK TN Columns; Pharmacia).

Two myotonic dystrophy DNA samples with 1.0-kb and 2.2-kb $(CAG)_n/(CTG)_n$ expansions were used as positive controls. The size of the expansions was evaluated by standard Southern blotting hybridization with a specific DNA probe for the myotonic dystrophy gene (Petronis et al. 1996).

Hybridization for the restriction enzyme–digested genomic DNA was performed at 63°C for 14–20 h in 1.5 × SSPE, 10% SDS, 10% PEG, 100 μg of herring sperm DNA/ml, and 2–3 × 10^7 dpm of DNA probe/20 ml of hybridization solution (one 22-cm × 22-cm membrane). Washing of the membranes containing genomic DNA was performed three times in 0.3 × SSC and 0.1% SDS, at 20°C for 40 min each time and then twice at 65°C for 40 min. The membranes were exposed to Kodak X-ray film for 1–3 d at −70°C, with intensifying screens.

In order to avoid a false-negative result due to potential somatic mosaicism of the expansion site (see the Discussion section in Petronis et al. 1996), a variety of different restriction enzymes was applied. For detection of $(CAG)_n/(CTG)_n$ expansion, we tested eight restriction enzymes in the genomic digests: *Apa*L1 (rare cutter); *Bst*EII (quite-rare cutter); *Hin*dIII, *Eco*RI, and *Hinc*II/*Pst*I (average cutters); and the relatively frequent cutters *Taq*I, *Msp*I, and *Bst*NI. The total number of genotypings performed was 648. For detection of $(CCG)_n/(CGG)_n$ expansion a total of five enzymes (*Bst*EII, *Pst*I *Taq*I, *Msp*I, and *Bst*NI) were used, and a total of 405 genotypings were performed.

Asymmetrical PCR.—The asymmetrical PCR–based method can be used as an additional test for large (>0.5-kb) trinucleotide RED (Petronis et al. 1996). An asymmetrical extension of a $(CAG)_7$ primer was performed with 0.5 μg of total genomic DNA from each subject. In order to reduce the DNA-template variability across samples, equal amounts of DNA template are required. We double-checked DNA concentrations by using spectrophotometer measurement of optical density and ethidium bromide visualization on agarose gels. The other components in the PCR mixture were 2.0 U of *Taq* polymerase (Perkin Elmer Cetus), 0.05 μg of $(CAG)_7$ oligonucleotides, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 200 μM each of dATP, dCTP, 100 μM dGTP, 100 μM 7-deaza-dGTP, and 10% DMSO in a total volume of 25 μl (Perkin Elmer Cetus 9600 thermocycler). The cycling conditions consisted of 150 cycles at 95°C for 30 s, 56°C for 60 s, and 72°C for 60 s. After the first 75 cycles an additional 2.0 U of *Taq* polymerase were added. The PCR product was loaded onto a 1.5% agarose gel and electrophoresed for 3 h at 100 V in a standard Pharmacia LKB gel tank. The gel was blotted on a Nylon-N^+ membrane (Amersham) overnight. Hybridization with the $(CAG)_n/(CTG)_n$ probe was performed as described above in the discussion of Southern blot technique. The membranes with the asymmetrical PCR products were washed three times in 0.1 × SSC and 0.1% SDS at 20°C for 40 min, then twice at 65°C for 40 min.

RED analysis.—RED was performed as described by Schalling et al. (1993), with minor alterations (Sirugo and Kidd 1995), on a subsample of 50 individuals from four families. Two micrograms of genomic DNA and 100 ng of the phosphorylated oligonucleotide $(CTG)_{10}$ were used in the ligase chain reaction. After denaturation at 98°C for 5 min, 5 U of the thermostable DNA ligase, Ampligase (Epicentre Technologies), were added, and the reactions were cycled at 70°C for 30 s and at 94°C for 10 s, for 396 cycles. The same DNAs from two myotonic dystrophy individuals as were used in the Southern blotting approach were used as positive controls. Electrophoresis was performed as described by Schalling et al. (1993), and then the product was capillary blotted overnight onto Hybond-N^+ soaked in 1 × Tris-borate EDTA and covered with four layers of 3M paper. The DNA was UV cross-linked to the membrane by means of Stratalinker (Stratagene). The membranes were prehybridized for 1 h in Amasino buffer (7% SDS, 10% polyethylene glycol, 0.25 M NaCl, and 0.13 M phosphate buffer, pH 7.2) at 65°C and then hybridized for 16 h in the same buffer with $(CAG)_{10}$ 3′ end-labeled with ^{32}P-αdCTP (NEN-Dupont), with terminal deoxynucleotidyl transferase (Gibco-BRL). The membranes were washed twice for 30 min in 1 × SSC and 0.1% SDS at room temperature and then twice for 30 min in the same solution at 64°C. Autoradiography was performed for 16 h–3 d, with intensifying screens at −80°C and NEN/Dupont X-ray film.

We also tested a $(CTG)_{17}$ oligonucleotide, as suggested in Schalling et al.'s (1993) original paper. DNA from 51 subjects, including 24 members of five new schizophrenia families showing anticipation, was examined under the same experimental conditions as were used for the $(CTG)_{10}$ oligonucleotide. Scoring of Southern blot hybridization, asymmetrical PCR, and RED autoradiograms was independently performed by two investigators who were blind to affected status of tested individuals.

Statistical Analysis

Means of ligation-product size for affected and unaffected groups were assessed by the *t*-test. The Wilcoxon rank-sum test was used to compare parental and proband generation ligation-product size; a Kruskal-Wallis test also was employed as a χ^2 approximation.

Results

Southern Blot Hybridization–Based Screening

Southern blot hybridization–based screening showed no evidence for large $(CAG)_n/(CTG)_n$ and $(CCG)_n/(CGG)_n$ expansions. In the case of the $(CAG)_n/(CTG)_n$ hybridization, 10–25 DNA fragments were revealed on the autoradiogram. In most cases these hybridization bands were monomorphic in the affected and unaffected individuals of all families. The highest degree of DNA-fragment-length variation was detected for the frequently cutting enzymes, such as *Msp*I and *Bst*NI. None of these polymorphisms correlated with affected status. Possibly the variation of the bands was due to the presence of a polymorphic restriction site in the trinucleotide repeat–flanking region, because, in comparison with other DNA hybridization bands, there was no difference in the hybridization signal intensity. Such difference in hybridization signal intensities was clearly detected in the analysis of myotonic dystrophy DNA (fig. 1).

In the case of $(CCG)_n/(CGG)_n$ blot hybridization, the

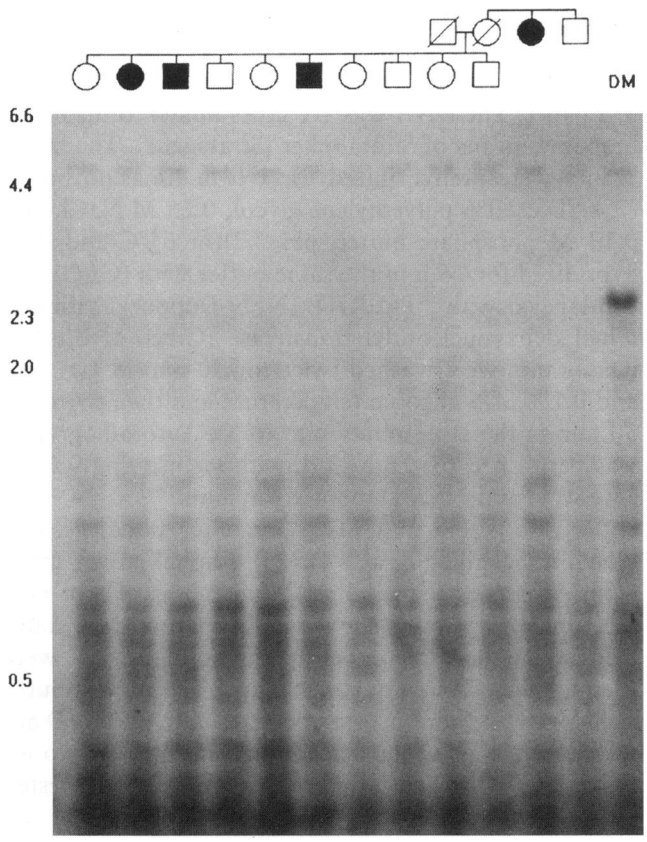

Figure 1 Southern blot analysis of eastern Canadian schizophrenia family members, using a $(CTG)_n/(CAG)_n$ repeat probe. DNA samples from family members were digested with *Bst*NI, electrophoresed on a 1.0% agarose gel, and blotted onto nylon membranes. Hybridization with the PCR-generated t-repeat probe revealed the presence of $(CTG)_n/(CAG)_n$-containing bands but no evidence for large $(CTG)_n/(CAG)_n$ trinucleotide expansions in the affected individuals. The 2.2-kb expansion in the myotonic dystrophy DNA (lane DM) was used as a positive control and produced the strong hybridization signal at the level of 2.3 kb. Sizes (in kb) are indicated in the left-hand margin (λ/*Hin*dIII). The family-member diagnoses have been altered in order to preserve confidentiality of the study.

number of bands also varied for different restriction enzymes. The autoradiograms of *Msp*I blots revealed 10–15 hybridization signals with three to five polymorphic bands. As in the case of the $(CAG)_n/(CTG)_n$ hybridization, all these polymorphic bands exhibited similar intensity, and therefore they were unlikely to be caused by a larger or smaller number of $(CCG)_n/(CGG)_n$ repeats within the *Msp*I restriction fragment. In the case of *Bst*NI digestion, three very intensive hybridization signals plus a large number of weak ones were detected. It is likely that the number of $(CCG)_n/(CGG)_n$ loci is higher in comparison with that of $(CAG)_n/(CTG)_n$ loci but that the $(CCG)_n/(CGG)_n$ regions, or tracts, are shorter than $(CAG)_n/(CTG)_n$ ones in the human genome and that therefore the majority of them do not provide a strong separate band of hybridization. The absence of multiple long tracts of the $(CCG)_n/(CGG)_n$ repeats makes it easier to detect a new expansion of this type. In summary, none of the five enzymes testing for $(CCG)_n/(CGG)_n$ unstable sites detected any evidence of a large expansion.

Asymmetrical PCR–Based Screening

In order to verify the Southern blot–hybridization results, the asymmetrical PCR for the same sample of 81 individuals was performed with a $(CAG)_7$ primer. A myotonic dystrophy DNA sample containing a 1.0-kb $(CTG)_n$ expansion was used as a positive control. No difference in the PCR-product hybridization pattern was detected for affected and unaffected individuals.

In contrast to the case for $(CAG)_n/(CTG)_n$ expansions, asymmetrical PCR could not be applied for detection of $(CCG)_n/(CGG)_n$ repeats (Petronis et al. 1996). Because of imperfect $(CCG)_7$ oligonucleotide dimerization in the presence of dCTP and dGTP, the oligonucleotide extension dominates over the asymmetrical synthesis from the expanded trinucleotide region. This is valid for all "xyy"- or "xyx"-type trinucleotides, whereas the combination of "xyz" is free from the self-annealing process during extension.

RED-Based Screening

In the RED technique with the $(CTG)_{10}$ oligonucleotide, the majority of individuals tested ($n = 40$) showed a ligation product of $4 \times (CTG)_{10}$; $5 \times (CTG)_{10}$ was identified in one individual, $6 \times (CTG)_{10}$ was detected in four, $7 \times (CTG)_{10}$ in three, $8 \times (CTG)_{10}$ in one, and $11 \times (CTG)_{10}$ in one. Two positive controls with large $(CAG)_n/(CTG)_n$ repeats at the myotonic dystrophy locus produced ladders of ligated $(CTG)_{10}$ products. An illustrative autoradiograph for $(CAG)_n/(CTG)_n$ trinucleotide-repeat analysis by RED in the eastern Canadian schizophrenia families is provided in figure 2.

Fifty-one individuals who also were tested with $(CTG)_{17}$ oligonucleotide demonstrated a pattern of ligation products very similar to that seen with $(CTG)_{10}$. In the majority of cases the number of ligated oligonucleotides was half that for $(CTG)_{10}$; that is, $4 \times (CTG)_{10}$ corresponded to $2 \times (CTG)_{17}$.

The distribution of $(CTG)_{10}$ RED ligation products was similar for affected and unaffected family members. The mean size of the ligation product was 4.64 (SD = 0.93) in 14 affected individuals and 4.53 (SD = 1.50) in 36 unaffected individuals ($t = -0.27$; $P = .79$). There were no significant differences, in ligation-product size, between 19 parental generation and 30 proband-generation subjects ($z = -.64$; $P = .52$; χ^2 approximation = 0.43; df = 1; $P = .51$). Similar results were found for $(CTG)_{17}$ ligation products. Confirming the data of our other methods, no evidence for $(CAG)_n/(CTG)_n$ repeat expansion in schizophrenia was detected by RED.

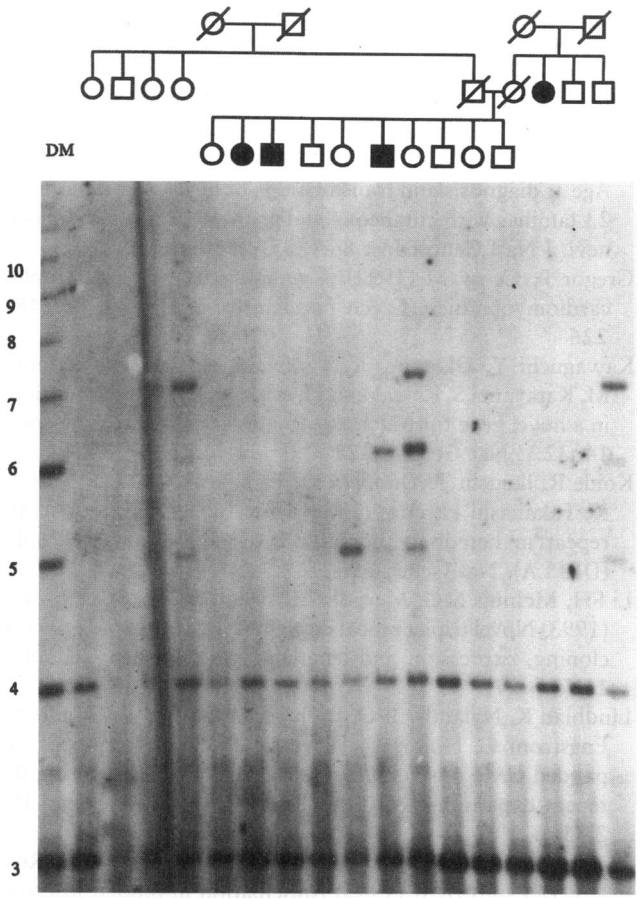

Figure 2 RED in eastern Canadian schizophrenia families. A $(CTG)_{10}$ oligonucleotide was used to search for DNA expansion in 17 affected and unaffected members of a schizophrenia multiplex family. Myotonic dystrophy DNA was used as a positive control (lane DM). The number of ligated oligonucleotides is provided on the left-hand side. As in figure 1, the diagnoses have been altered.

Discussion

A large-scale search for unstable-DNA loci was performed in the eastern Canadian schizophrenia multiplex families. All of these families have demonstrated strong evidence for anticipation, suggesting that unstable trinucleotide repeats might be the cause or one of the predisposing factors in the etiology of schizophrenia. Three independent laboratory approaches (Southern blot hybridization, asymmetrical PCR, and RED) were applied, and no evidence for $(CAG)_n/(CTG)_n$-repeat expansions was detected. For $(CCG)_n/(CGG)_n$ repeats, Southern blot hybridization was applied, and, again, no trinucleotide-repeat expansion was identified. These results suggest that large $(CAG)_n/(CTG)_n$ expansions with very high probability—and $(CCG)_n/(CGG)_n$ with lower probability—can be excluded as the major cause of schizophrenia in our sample. Our results are consistent with other studies searching for unstable DNA in familial schizophrenia (Sirugo et al. 1994, 1995). Although several individuals affected with major psychosis in these kindreds demonstrated the presence of relatively large $(CTG)_n$ ligation products, the overwhelming majority of affected individuals showed no difference in comparison with unaffected family members.

The presence of shorter (30–130 trinucleotides) cannot yet be excluded as an etiologic factor. There are no clear clinical criteria to differentiate between an unstable-DNA disease with a small expansion versus one with a large expansion. All the techniques applied in the current study detected background trinucleotide signal in both affected and unaffected individuals. For example, the RED approach showed that the majority of ligation patterns fall within the limits of ~30–100 trinucleotides. If the size of the ligation products can be extrapolated directly to the size of trinucleotide tracts in the genome, background trinucleotide tracts could not be distinguished from pathogenic expansions and therefore could mask them. For the complete exclusion of the etiologic role of $(CAG)_n/(CTG)_n$ or $(CCG)_n/(CGG)_n$, testing of individual genes that contain trinucleotide repeats is indicated.

In schizophrenia association studies using unrelated subjects, several independent groups have demonstrated evidence for the presence of longer $(CAG)_n/(CTG)_n$ repeat tracts in DNA of affected individuals in comparison with unaffected controls (Morris et al. 1995; O'Donovan et al. 1995; Vincent et al. 1996). Such a consistent shift found in three different samples argues strongly for the presence of some functionally important triplet tracts that are longer in schizophrenia patients in comparison with unaffected controls, although the difference is not specific. It is important to note that these findings do not provide any evidence for either DNA expansion per se or other intergenerational trinucleotide-repeat instability. The origin of such a shift may result from prevalence of genetically stable alleles with a larger number of repeats in the schizophrenia group. The analysis of dynamics of short trinucleotide repeats across generations in schizophrenia families is indicated, in order to address this issue.

Another explanation for the negative results in our multiplex families is that the evidence for anticipation in schizophrenia is actually a statistical artifact. Despite replicated evidence for anticipation in schizophrenia, there is no unanimous opinion regarding the validity of this phenomenon (reviewed in Petronis et al. 1995). Numerous ascertainment biases may simulate genetic anticipation, and it is rather difficult to perform an epidemiological study that would be free of ascertainment biases.

An alternative interpretation—absence of trinucleotide-repeat expansion does not imply absence of genuine anticipation—should also be considered. Anticipation

in schizophrenia may result from other, nontrinucleotide repeat–based mechanisms. A number of diseases—such as Parkinson disease (Payami et al. 1995), hypertrophic cardiomyopathy (Gregor and Cerny 1992), olivopontocerebellar atrophy (Dai 1991), paraganglioma (van der Mey et al. 1989), cutaneous malignant melanoma (Goldstein et al. 1994), rheumatoid arthritis (Deighton et al. 1994), Blau syndrome (Raphael et al. 1993), colon and breast cancer, Alzheimer disease, and diabetes mellitus (Paterson et al. 1996)—have shown evidence of genetic anticipation. Can genetic anticipation in these diseases be rejected on the basis of either phenomenological dissimilarities with currently known unstable-DNA diseases or absence of trinucleotide-repeat expansion in the diseases' genes? Other genetic factors related to the disease gene may control the timing of the pathogenic process. Even in unstable-DNA diseases, the causal relationship between DNA expansion and AAO remains to be proved. Experimental data show that the degree of expansion correlates inversely with AAO, but there is no evidence that larger expansions directly *cause* earlier AAO. New mechanisms of genetic anticipation may be beyond our imagination now, as was the existence of unstable DNA several years ago.

Acknowledgments

We would like to acknowledge Kathryn Tzimika and Kevin Lowther for their editorial assistance, Dr. Robin Sherrington (Tanz Neuroscience Center, Toronto) for his useful insights, and Dr. G. Sirugo (Yale University, New Haven) for methodological assistance. We also thank Jackie McAlduff for assistance with the clinical work. This work was supported by a Scottish Rite Schizophrenia Research Program grant to J.L.K. and A.P., by grants from the Medical Research Council of Canada and the Ontario Mental Health Foundation to A.S.B., and by a grant from Ontario Friends of Schizophrenics and the Ontario Mental Health Foundation to A.P. A.P. holds an award from the Schizophrenia Society of Canada and the Medical Research Council of Canada.

References

Asherson P, Walsh C, Williams J, Sargeant M, Taylor C, Clements A, Gill M, et al (1994) Imprinting and anticipation: are they relevant to genetic studies of schizophrenia? Br J Psychiatry 164:619–624

Bassett AS, Collins EJ, Nuttall S, Honer WG (1993) Positive and negative symptoms in families with schizophrenia. Schizophr Res 11:9–19

Bassett AS, Honer WG (1994) Evidence for anticipation in schizophrenia. Am J Hum Genet 54:864–870

Caskey TC, Pizzuti A, Fu Y-H, Fenwick RG, Nelson D (1992) Triplet repeat mutations in human disease. Science 256: 784–789

Dai ZH (1991) Olivopontocerebellar atrophy: clinical analysis of 100 cases. Chin J Neurol Psychiatry 24:111–113

Deighton C, Heslop P, McDonagh J, Walker D, Thomsom G (1994) Does genetic anticipation occur in familial rheumatoid arthritis? Ann Rheum Dis 53:833–835

Gindilis VM (1979) The genetics of schizophrenia psychosis. DSc thesis, Institute of Medical Genetics, Moscow

Goldstein AM, Fraser MC, Clark WH, Tucker MA (1994) Age at diagnosis and transmissions of invasive melanoma in 23 families with cutaneous malignant-melanoma dysplastic nevi. J Natl Cancer Inst 86:1385–1390

Gregor P, Cerny M (1992) A genetic study in hypertrophic cardiomyopathies (Czech population). Cor Vasa 34:218–226

Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S, Kawakami H, et al (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8:221–228

Koide R, Ikeuchi T, Onodera O, Tanaka H, Igarashi S, Endo K, Takahashi H, et al (1994) Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). Nat Genet 6:9–13

Li SH, McInnis MG, Margolis RL, Antonarakis SE, Ross CA (1993) Novel triplet repeat containing genes in human brain: cloning, expression and length polymorphisms. Genomics 16:572–579

Lindblad K, Nylander P-O, De bryen A, Sourey D, Zander C, Engstrom C, Holmgren G, et al (1995) Detection of expanded CAG repeats in bipolar affective disorder using the repeat expansion detection (RED) method. Neurobiol Dis 2:55–62

McInnis MG, McMahon FJ, Chase GA, Simpson SG, Ross CA, DePaulo JR Jr (1993) Anticipation in bipolar affective disorder. Am J Hum Genet 53:385–390

Morris AG, Gaitonde E, McKenna PJ, Mollon JD, Hunt DM (1995) CAG repeat expansions and schizophrenia: association with disease in females and with early age-at-onset. Hum Mol Genet 4:1957–1961

Mott FW (1911) A lecture on heredity and insanity. Lancet 1:1251–1259

Nagafuchi S, Yanagisawa H, Sato K, Shirayama T, Ohsaki E, Bundo M, Takeda T, et al (1994) Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. Nat Genet 6:14–18

Nylander P-O, Engstrom C, Chotai J, Walstrom J, Adolfson R (1994) Anticipation in Swedish families with bipolar affective disorder. J Med Genet 31:686–689

O'Donovan MC, Guy C, Craddock N, Murphy KC, Cardno AG, Jones LA, Owen MJ, et al (1995) Expanded CAG repeats in schizophrenia and bipolar disorder. Nat Genet 10: 380–381

O'Neil J, Bryjolfsson J, Curtis D, Rifkin L, Maloney E, Murphy P, Petursson H, et al (1993) Testing the hypothesis for genomic imprinting and anticipation in bipolar and related unipolar affective disorder. Psychiatr Genet 3:151

Paterson AD, Kennedy JL, Petronis A (1996) Evidence for genetic anticipation in non-Mendelian diseases. Am J Hum Genet 59:264–268 Payami H, Bernard S, Larsen K, Kaye J, Nutt J (1995) Genetic anticipation in Parkinson's disease. Neurology 45:135–138

Penrose LS (1948) The problem of anticipation in pedigrees of dystrophia myotonica. Ann Eugenics (Lond) 14:125–132

——— (1971) Critical survey of schizophrenia genetics. In: Howells JG (ed) Modern perspectives in world psychiatry. Olivier & Boyd, Edinburgh, pp 3–19

Petronis A, Heng HHQ, Tatuch Y, Shi X-M, Tsui L-C, Ashizawa T, Surh LC, et al (1996) Direct detection of expanded trincuelotide repeats using DNA hybridization techniques. Am J Med Genet 67:85–91

Petronis A, Kennedy JL (1995) Unstable genes—unstable mind? Am J Psychiatry 152:164–172

Petronis A, Sherrington RP, Paterson AD, Kennedy JL (1995) Genetic anticipation in schizophrenia: *pro* and *con*. Clin Neurosci 3:76–80

Raphael SA, Blau EB, Zhang WH, Hsu SH (1993) Analysis of a large kindred with Blau syndrome for HLA, autoimmunity, and sarcoidosis. Am J Dis Child 147:842–848

Richards RI, Sutherland GR (1992) Heritable unstable DNA sequences. Nat Genet 1:7–9

Riggins GJ, Lokey LK, Chastain JL, Leiner HA, Sherman SL, Wilkinson KD, Warren ST (1992) Human genes containing trinucleotide repeats. Nat Genet 2:186–191

Ross CA, McInnis MG, Margolis RL, Li S-H (1993) Genes with triplet repeats: candidate mediators of neuropsychiatric disorders. Trends Neurosci 16:254–260

Schalling M, Hudson TJ, Buetow KH, Housman DE (1993) Direct detection of novel expanded trinucleotide repeats in the human genome. Nat Genet 4:135–139

Sharma T, Brynjolfsson J, Butler R, Murphy P, Petursson H, Read T, Sigmundsson T, et al (1994) Anticipation in schizophrenia. Schizophr Res 11:141

Sirugo G, Haaf T, Parnas J, Matthysse S, Levy D, Holzman P, Kidd KK (1995) A (CTG)n expansion detected by RED in schizophrenia kindred maps to 18q21 by FISH with a CTG/CAG polymer. Am J Hum Genet Suppl 57:A36

Sirugo G, Kidd KK (1995) Repeat expansion detection using Ampligase thermostable DNA ligase. Epicentre Forum 2:1–3

Sirugo G, Parnas J, Haaf T, Holzman P, Wetterberg L, Matthysse S, Kidd KK (1994) Scanning for unstable trinucleotide repeats in neuropsychiatric disorders: detection of a large CTG expansion in a schizophrenic patient. Am J Hum Genet Suppl 55:A203

Sutherland GR, Richards RI (1992) Dynamic mutations: a new class of mutations causing human disease. Cell 70:709–712

van der Mey AGL, Maaswinkel-Mooy PD, Cornelisse CJ, Schmidt PH, van de Kamp JJP (1989) Genomic imprinting in hereditary glomus tumours: evidence for new genetic theory. Lancet 2:1291–1294

Vincent JB, Klempan T, Parikh SS, Sasaki T, Meltzer HY, Sirugo G, Cola P, et al (1996) Frequency analysis of large CAG/CTG trinucleotide repeats in schizophrenia and bipolar affective disorder. Mol Psychiatry 1:141–148