Eur Arch Psychiatry Clin Neurosci (1998) 248 : 78–81

ORIGINAL PAPER

Dietmar Bengel · Ursula Balling · Gerald Stöber
Armin Heils · Shi-Hua Li · Cristopher A. Ross
Gerd Jungkunz · Ernst Franzek · Helmut Beckmann
Peter Riederer · Klaus-Peter Lesch

Distribution of the B33 CTG repeat polymorphism in a subtype of schizophrenia

Received: 31 January 1997 / Accepted: 16 October 1997

Abstract Clinical evidence for a dominant mode of inheritance and anticipation in periodic catatonia, a distinct subtype of schizophrenia, suggests that trinucleotide repeat expansions may be involved in the aetiology of this disorder. Since genes with triplet repeats are putative canditates for causing schizophrenia, we have analysed the polymorphic B33 CTG repeat locus on chromosome 3 in 45 patients with periodic catatonia and 43 control subjects. The B33 CTG repeat locus was highly polymorphic, but all alleles in both the patient and control groups had repeat lengths within the normal range. We conclude that susceptibility to periodic catatonia is not influenced by variation at the B33 CTG repeat locus. Nevertheless, that periodic catatonia displays dominant inheritance and anticipation, characteristic of genetic disorders involving trinucleotide repeats, justifies further screening for triplet repeat expansions in this illness.

Key words Anticipation · Association study · B33 CTG repeat locus · Schizophrenia · Periodic catatonia

Introduction

Human genes containing repetitive DNA elements, such as trinucleotide repeats, may be expanded, unstable and are potential cause for some neuropsychiatric disorders (Bassett and Honer 1994; Ross et al. 1993; Gorwood et al. 1996). Triplet repeat expansions may be the molecular correlate of the clinical phenomenon of anticipation observed in distinct subtypes of schizophrenia (Stöber et al. 1995). Anticipation describes an unusual pattern of inheritance in genetic disorders which increase in severity and/or have earlier onset in subsequent generations. The recent discovery of dynamic mutations due to trinucleotide repeat expansions has provided a molecular genetic explanation of several inherited neurodegenerative and neuropsychiatric disorders displaying anticipation (Mandel 1993), including Huntington's disease, myotonic dystrophy, fragile X syndromes, spinocerebellar ataxia types 1, 2 and 6, dentatorubral-pallidoluysian atrophy, X-linked spinal and bulbar muscular atrophy (Kennedy syndrome), Machado-Joseph disease and recently Friedreich's ataxia. Patients with these illnesses have unstable expansion of CAG, CTG, CGG and GAA repeats in coding as well as in noncoding regions of different genes. Repeat length and instability is directly associated with increased severity and earlier age of onset in successive generations (La Spada et al. 1994).

Recently, an investigation of familial aggregation of psychoses in pedigrees with schizophrenic disorders strongly suggested a dominant mode of inheritance in periodic catatonia, a distinct subtype of schizophrenia (Stöber et al. 1995). As compared with other forms of catatonic schizophrenia (age-corrected morbidity risk 4.6%) with a chronic progressive course and irreversible, well-defined defective states (Franzek and Beckmann 1992), periodic catatonia was characterized by an age corrected morbidity risk with homogenous psychoses of 26.9%. Moreover, pairwise comparison of patients and their parents revealed patterns of anticipation. The course of periodic catatonia is bipolar in both hyperkinetic as well as akinetic states.

Typically, symptoms of one pole dominate but are usually mixed with those of the other. The distortion of psychomotor activity leads to grimaces, parakinetic movements, stereotypies, impulsive actions with aggressiveness and negativistic behaviour. Although remissions occur after acute episodes, eventually residual states develop with increasing poverty of movements, blunted affect and lack of motivation.

D. Bengel (✉) · U. Balling · G. Stöber · A. Heils · E. Franzek
H. Beckmann · P. Riederer · K.-P. Lesch
Department of Psychiatry, University of Würzburg,
Füchsleinstrasse 15, 97080 D-Würzburg, Germany

S.-H. Li · C.A. Ross
Laboratory of Molecular Neurobiology,
Departments of Psychiatry and Neuroscience,
Johns Hopkins University, School of Medicine,
720 Rutland Avenue, Baltimore, MD 21205-2196, USA

G. Jungkunz
Nervenkrankenhaus, Bezirk Unterfranken,
D-97816 Lohr, Germany

Clinical evidence suggests a dominant mode of inheritance and anticipation in periodic catatonia. This raises the possibility that genes with trinucleotide repeat expansions or other unstable repetitive elements affecting gene expression may be involved in its aetiology (Lesch et al. 1994). Since genes with triplet repeats are candidates for causing schizophrenia, we have investigated the B33 CTG repeat locus on chromosome 3 in 45 patients with periodic catatonia and 43 control subjects. Although there is no linkage to this particular gene locus in combined samples of schizophrenic patients, highly polymorphic repeats expressed in the brain are putative candidates in a subtype of schizophrenia, in which dominant inheritance and anticipation have been observed.

Methods

Recruitment of patients and diagnostic assessment

Forty-five unrelated German Caucasian (20 males and 25 females) meeting DSM-III-R criteria for schizophrenia, catatonic subtype and the criteria for periodic catatonia (Leonhard 1979; Leonhard 1980) were recruited from inpatients at the Department of Psychiatry, University of Würzburg, and from wards with chronically ill patients at the Lohr/Main Psychiatry State Hospital. The patients with periodic catatonia included in this study represent a subsample of a study of familial aggregation of psychoses in pedigrees with schizophrenic disorders published previously (Stöber et al. 1995). After an initial screening of the hospital records patients who fulfilled the DSM-III-R criteria for catatonic schizophrenia were diagnosed using Leonhard's nosology by personal examination by two independently working experienced clinical psychiatrists (E.F. and H.B.) on the basis of extensive case notes which did not contain information on familial affliction (Cohen's kappa 0.93). The mean age (± SD) of the patients with periodic catatonia was 50.5 ± 15.7 years, the duration of illness was 26.8 ± 12.6 years and the age on initial hospitalization was 22.7 ± 7.5 years. Forty-three unrelated German Caucasians (17 males and 26 females; mean age 25.6 ± 4.2 years) were included as controls. The study was approved by the local ethics committee.

PCR analysis of the C 33 CTG repeat

High molecular weight genomic DNA was extracted from EDTA-anticoagulated peripheral blood according to routine procedures. The B33 CTG repeat was amplified from genomic DNA (~50 ng) by PCR (30 s at 95 °C, 30 s at 58 °C, 1 min at 72 °C for 35 cycles) with the amplimers B33-1 (5′-CAAAAAAGCCACCTGGTAC-TAA- 3′) and B33-2 (5′-GGGCTGGAGCCTTTTTACTCGC- 3′; Fig. 1). The PCR products were analysed on a 2% agarose gel. For accurate determination of the number of CTG repeat units, PCR was performed with ~50 KBq of [α^{32}P]dATP and the size of the product estimated in comparison with a standard sequencing ladder by 6% denaturing polyacrylamide gel electrophoresis. The CTG trinucleotide length was determined by subtracting 77 bp of non-CTG-containing DNA from the PCR size.

401 GGCAGCTCTC CTGAAAGCTT CTCCCAAAAA AGCACCTGGT ACTAAGGGTA
B33-1
451 CTGCTGCTGC TGCTGCTGCT GCTGCTGCTG CTGCTGCTGC TAAAGTTCCA
501 GCAAAAAAGA TCACCGCCGC GAGTAAAAAG GCTCCAGCCC AGAAGGTTCC
B33-2

Fig. 1 Sequence and 3′ and 5′ flanking regions of the B33 repeat locus. PCR primers are *underlined*

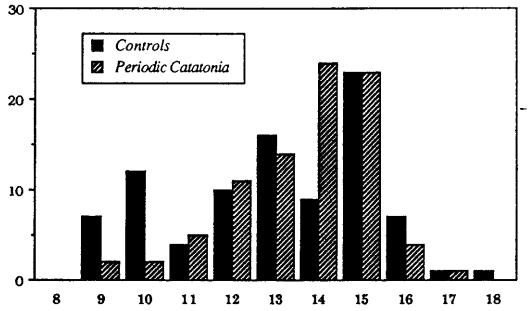

Fig. 2 Distribution of the B33 CTG repeat alleles in patients with periodic catatonia and controls

Results

The distribution of B33 CTG repeat alleles in patients with periodic catatonia and controls is depicted in Fig. 2. Ninety chromosomes were analysed in the patient sample and 86 in the controls. The B33 CTG repeat locus was highly polymorphic (10 alleles), but there was no significant difference between the patient and control groups in allele distribution. The heterozygosity of 81% in the control group and 95% in the patient group was not significantly different to that observed in a normal population. The modal repeat length was 13 repeats in the patients and 14 repeats in the control group, which corresponds to an allele size of 39 and 42 base pairs, respectively. There was no significant difference in the mean repeat number between the control and the patient samples (mean ± SD, 13.56 ± 1.63 vs 13.04 ± 2.25 CTG repeats; $t = -1.73$, $df = 174$, p-value 0.085, unpaired t-test).

Discussion

Trinucleotide microsatellites are widespread in the human genome (Bates and Lehrach 1994). Many of these genes are expressed in different human brain regions and all available evidence suggests that unstable trinucleotide repeats are the biological basis of the clinical phenomenon of anticipation. Thus far, expansions of unstable trinucleotide repeats have been associated with several different neurological and neuropsychiatric diseases. The experience with all diseases thus far known to be caused by triplet repeat expansion suggests that repeats that are highly polymorphic in the normal population may be more likely to expand with pathological consequences (Li et al. 1993). Although ten possible trinucleotides can occur at the DNA level, only CTG, CAG, CGG and GAA repeats are associated with the disorders described thus far. Because the aetiology of schizophrenia is likely to be heterogeneous and the strength of the genetic contribution to schizophrenia varies considerably among its subtypes, we have screened the polymorhic B33 CTG repeat locus in a specific clinical subtype of schizophrenia with strong evidence for a dominant pattern of inheritance and anticipation. Previous studies already emphasized an increased

familial loading in catatonic psychosis compared with paranoid or disorganized schizophrenia (Slater and Cowie 1971; Leonhard 1979; Scharfetter and Nuesperli 1980; Propping 1989).

In accordance with Leonhard's findings (Leonhard 1980), we have previously demonstrated that periodic catatonia exhibits a morbidity risk of 26.9% among first-degree relatives and accounts for approximately 60% of schizophrenia, catatonic subtype, diagnosed according to DSM-III-R (Stöber et al. 1995). High familial loading and pronounced vertical transmission are consistent with a dominant mode of inheritance. In addition, anticipation was observed in 27 of 29 pedigrees with periodic catatonia and occurred equally with maternal or paternal transmission. All alleles in both the patients and the control group had repeat sizes within the normal range. Previous studies failed to detect abnormalities in the DRPLA (B37 CAG repeat) or other repeat loci in patients with schizophrenic disorder (Lesch et al.1994; Rubinsztein et al. 1994a; Gaitonde et al. 1997).

Strong evidence for anticipation has been previously reported in an unselected sample of patients with familial schizophrenia (Bassett and Honer 1994; Thibaut et al. 1995; Yaw et al. 1996). Whereas the repeat expansion detection (Schalling et al. 1993) sometimes fails to detect smaller expansions in the human genome, a newly developed fluorescence in situ hybridization revealed an expansion of 306 CTGs on chromosome 18q21 in a patient suffering from paranoid schizophrenia (Haaf et al. 1996). Furthermore, a few recent studies report association between schizophrenia, meeting DSM-III-R or DSM-IV criteria, and different repeat expansions (Morris-Rosendahl et al. 1997; Morris et al. 1995; O'Donovan et al. 1996). Another repeat expansion at the Huntington's disease locus has been reported in a chronic schizophrenic patient without any evidence for movement abnormalities and normal striatal histology (St. Clair 1994). Rubinsztein and colleagues, however, failed to detect CAG-repeat expansion of the Huntington locus in 71 unrelated schizophrenic patients and 18 patients with schizoaffective disorder (Rubinsztein et al. 1994b).

Trinucleotide repeat expansion has become a well-established mutation underlying human genetic disease. Since additional disorders, such as schizophrenia, bipolar affective disorder, and possibly others, show features of anticipation, reanalysis of the genetics of major psychosis from the perspective of the unstable DNA is of significant interest (McInnis et al. 1993; Petronis and Kennedy 1995; Sasaki et al. 1996; Gorwood et al. 1996). Many deviations from a single-gene mode of inheritance can be easily explained by the non-Mendelian behaviour of unstable DNA and suggest that some subtypes of schizophrenia and major affective disorders may be the result of a similar mutation mechanism.

The evidence for anticipation in families with periodic catatonia indicates that genes with triplet repeat expansions or other unstable repetitive elements affecting gene expression may also be involved in the aetiology of this well-defined subtype of schizophrenia. Although no abnormality was found in the B33 CTG locus in these patients, high prevalence of human brain genes (Li et al. 1993) containing trinucleotide repeats clearly justifies further screening for abnormalities in genes with tri- and oligonucleotide repeats.

References

American Psychiatric Association (1987) Diagnostic and statistical manual of mental disorders (DSM-III-R), 3rd edn, revised. American Psychiatric Press, Washington DC

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

Bates G, Lehrach H (1994) Trinucleotide repeat expansions and human genetic disease. BioEssays 16: 277–284

Franzek E, Beckmann H (1992) Schizophrenia: not a disease entity? A study of 57 long-term hospitalized chronic schizophrenics. Eur J Psychiatry 6: 97–108

Gaitonde EJ, Sivagnanasundaram S, Morris AG, McKenna PJ, Mollon JD, Hunt DM (1997) The number of triplet repeats in five brain-expressed loci with CAG repeats is not associated with schizophrenia. Schizophr Res 25: 111–116

Gorwood P, Leboyer M, Falissard B, Jay M, Rouillon F, Feingold J (1996) Anticipation in schizophrenia: new light on a controversial problem. Am J Psychiatry 153: 1173–1177

Haaf T, Sirugo G, Kidd KK, Ward DC (1996) Chromosomal localization of long trinucleotide repeats in the human genome by fluorescence in situ hybridization. Nat Genet 12: 183–185

La Spada AR, Paulson HL, Fischbeck KH (1994) Trinucleotide repeat expansion in neurological disease. Ann Neurol 36: 814–822

Leonhard K (1979) The classification of endogenous psychoses. Irvington, New York

Leonhard K (1980) Contradictory issues in the origin of schizophrenia. Br J Psychiatry 136: 437–444

Lesch KP, Stöber G, Balling U, Franzek E, Li SH, Ross CA, Newman M, Beckmann H, Riederer P (1994) Triplet repeats in clinical subtypes of schizophrenia: variation at the DRPLA (B37 CAG repeat) locus is not associated with periodic catatonia. J Neural Transm Genet 198: 153–157

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

Mandel JL (1993) Questions of expansion. Nat Genet 4: 8–9

McInnis MG, McMahon FJ, Chase GH, Simpson SG, Ross CA, DePaulo JR (1993) Anticipation of 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

Morris-Rosendahl DJ, Burgert E, Uyanik G, Mayerova A, Duval F, Macher JP, Crocq MA (1997) Analysis of the CAG repeats in the SCA1 and B37 genes in schizophrenic and bipolar disorder patients: tentative association between B37 and schizophrenia. Am J Med Genet 74: 324–330

O'Donovan MC, Guy C, Craddock N, Bowen T, McKeon P, Macedo A, Maier W, Wildenauer D, Aschauer HN, Sorbi S, Feldman E, Mynett-Johnson L, Claffey E, Nacmias B, Valente J, Dourado A, Grassi E, Lenzinger E, Heiden AM, Moorhead S, Harrison D, Williams J, McGuffin P, Owen MJ (1996) Confirmation of association between expanded CAG/CTG repeats and both schizophrenia and bipolar disorder. Psychol Med 26: 1145–1153

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

Propping P (1989) Psychiatrische Genetik. Springer, Berlin Heidelberg New York

Ross CA, McInnis MG, Margolis RL, Li SH (1993) Genes with triplet repeats: candidate mediators of neuropsychiatric disorders. Trends Neurosci 16: 264–272

Rubinsztein DC, Leggo J, Goodburn S, Barton DE, Ferguson-Smith MA, Ross CA, Li SH, Lofthouse R, Crow TH, Delisi TH (1994a) B 37 repeats are normal in most schizophrenic patients (letter). Br J Psychiatry 164: 851–852

Rubinsztein DC, Leggo J, Goodburn S, Crow TJ, Lofthouse R, Barton DE, Ferguson-Smith MA (1994b) Study of the Huntington disease (HD) gene CAG repeats in schizophrenic patients shows overlap of the normal and HD affected ranges but absence of correlation with schizophrenia. J Med Genet 31: 690–693

Sasaki T, Billet E, Petronis A, Ying D, Parsons T, Macciardi FM, Meltzer HY, Lieberman J, Joffe RT, Ross CA, McInnis MG (1996) Psychosis and genes with trinucleotide repeat polymorphism. Hum Genet 97: 244–246

Schalling M, Hudson TJ, Buetow KW, Housman DE (1993) Direct detection of novel expanded trinucleotide repeats in the human genome. Nat Genet 7: 135–139

Scharfetter C, Nuesperli M (1980) The group of schizophrenias, schizoaffective psychoses, and affective disorders. Schizophr Bull 6: 586–591

Slater E, Cowie V (1971) The genetics of mental disorders. Oxford Press, London

St. Clair D (1994) Expanded CAG trinucleotide repeat of Huntington′s disease gene in a patient with schizophrenia and normal striatal histology. J Med Genet 31: 658–659

Stöber G, Franzek E, Lesch KP, Beckmann H (1995) Periodic catatonia: a schizophrenic subtype with major gene effect and anticipation. Eur Arch Psychiatry Neurol Sci 245: 135–141

Thibaut F, Martinez M, Petit M, Jay M, Campion D (1995) Further evidence for anticipation in schizophrenia. Psychiatry Res 59: 25–33

Yaw J, Myles-Worsley M, Hoff M, Holik J, Freedman R, Byerley W, Coon H (1996) Anticipation in multiplex schizophrenia pedigrees. Psychiatr Genet 6: 7–11