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The number of triplet repeats in five brain-expressed loci with CAG repeats is not associated with schizophrenia

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Abstract

We have previously shown that large expansions of CAG (CTG) triplets are associated both with schizophrenia itself and with an early age-at-onset of the disease. However, the repeat expansion detection (RED) method used did not provide a chromosomal location for the expanded region(s) (Morris et al., 1995). In a further study of our schizophrenic and control patients, we have now examined the length of the repeated sequence in five loci that are expressed in brain and are known to contain CAG repeat regions (Li et al., 1993). No enlarged repeat regions were identified; it is unlikely therefore that expansions at any of these five loci can account for expansions of up to 136 triplets identified by the RED method. © 1997 Elsevier Science B.V.

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1. Introduction

Six neurological disorders have been shown to result from an increase in the number of CAG triplet repeats within a gene (LaSpada et al., 1991; Huntington's Disease Collaborative Research Group, 1993; Orr et al., 1993; Kawaguchi et al., 1994; Koide et al., 1994; Nagafuchi et al., 1994). These repeats may be either in a non-coding region, as is the case for myotonic dystrophy (Brook et al., 1992; Fu et al., 1992; Mahadevan et al., 1992), or in a coding region, as in Huntington's disease, where the expanded region encodes a polyglutamine tract in the resulting protein. When the CAG repeat is in a coding region, expansion above 37 repeats appears to cause disease, and expansions of over 100 repeats are rare. When the repeat is in a non-coding region, extremely large expansions of thousands of repeats can occur.

Five of the disorders show anticipation, that is, an increase in severity and/or earlier age-at-onset through successive generations in affected families. The number of CAG repeats in the expanded region has been shown to increase dramatically between generations in those diseases showing

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anticipation, and the increase in severity and reduction in age of onset have been shown to correlate with the increase in the number of repeats (Brook et al., 1992; Mahadevan et al., 1992; Andrew et al., 1993; Matilla et al., 1993; Orr et al., 1993; Koide et al., 1994). The mechanism of action of these expanded regions on the activity of the gene or the gene product remains unclear, although there would appear to be a toxic gain of function. This may be mediated via interactions with other proteins, as suggested by the increase in the affinity of the Huntington's associated protein (HAP-1) for the Huntington gene product, huntingtin (Li et al., 1995). Long stretches of polyglutamine present in the DRPLA (dentato-rubral and pallido-luysian atrophy) protein and in huntingtin have also been found to interact selectively with glyceraldehyde-3-phosphate dehydrogenase (Burke et al., 1996).

The disorders already known to be associated with moderately-sized CAG (CTG) repeats show movement disorders and psychoses among their clinical manifestations. This has led to the hypothesis that triplet repeat expansions may play a role in the pathology of schizophrenia. By using a method that screens the whole genome (Schalling et al., 1993), large CAG repeat regions have been shown to be more common in schizophrenics than in controls (Morris et al., 1995; O'Donovan et al., 1995). In addition, patients with 69–136 CAG repeats in their genome appear to have a significantly earlier age-at-onset than patients with fewer CAG repeats (Morris et al., 1995) and patients with 35–68 CAG repeats in their genome were found to have significantly higher negative symptom scores than those with no CAG expansion (Morris et al., 1996). This approach does not, however, identify any particular gene or chromosomal region.

Schizophrenia may also exhibit the phenomenon of anticipation in common with other CAG diseases, and indeed reports of anticipation in schizophrenia (Morel, 1857; Mott, 1911; Gottesman, 1991; Bassett and Honer, 1994; Sharma et al., 1994) prompted the recent search for repeat expansions in this condition. However, there is in fact no secure evidence for anticipation in schizophrenia (Petronis et al., 1995). All studies of anticipation, however carefully performed, suffer from the problem that patients with onset of disease at a younger age are less likely to have children. Patients who do have children will therefore have a later age at onset of disease than average, although their children may not. A spurious decrease in age at onset will then appear to occur between the two generations (Penrose, 1968). This problem is especially acute for a disease like schizophrenia, which typically arises in early adulthood and is characterised by much reduced fecundity.

So far, CAG is the only type of trinucleotide repeat that has been shown to be involved in the diseases that are caused by exonic expansions (Willems, 1994). It is estimated that there are well over 1000 $(CAG/CTG)_n$-repeat-containing loci present in the human genome (Gastier et al., 1996). A computer search for CAG repeat regions showed that they are concentrated in exons, where they would encode stretches of polyglutamine (Stallings, 1994). In a study of brain-expressed genes containing triplet repeats, Li et al. (1993) recovered 38 confirmed positives from an initial screen of a frontal cortex cDNA library with a CTG-repeat probe. Of these, one was identified as the gene for myotonic dystrophy (Brook et al., 1992), 12 coded for 28S ribosomal RNA, nine were from other known genes and three could not be sequenced. The remaining 14 were confirmed as novel cDNAs and eight were fully sequenced. Since one of these has been subsequently identified as the gene altered in DRPLA (Koide et al., 1994), only seven have been considered in this study. Two contain less than eight contiguous repeats of either CAG or CTG and were therefore discarded. The corresponding gene sequences for the remaining five cDNAs are potential sites for additional triplet expansions and are candidates therefore for the expanded region(s) identified in schizophrenic patients (Morris et al., 1995; O'Donovan et al., 1995). Accordingly, we have examined these CAG- and CTG-repeat-containing genes in all members of the population of schizophrenics in which an increase in the frequency of large CAG (or CTG) expansions has been reported (Morris et al., 1995). Three of these five genes are already known to be polymorphic (Li et al., 1993).

2. Materials and methods

2.1. Selection and assessment of patients and controls

Eighty-four schizophrenic subjects of Caucasian origin (46 men and 39 women) had already been recruited for genetic studies from the rehabilitation and acute services at Fulbourn Hospital, Cambridge. The majority of the subjects suffered from chronic schizophrenia and attended the clozapine clinic. All the probands were unrelated and were not selected for family history. Linkage analysis has not been performed on the families of those probands with affected relatives. Informed consent was obtained. All patients satisfied DSM-III-R criteria for schizophrenia.

Seventy-seven unrelated control subjects of Caucasian origin (41 men and 36 women) were recruited from oral surgery and ophthalmology clinics at Addenbrooke's Hospital, Cambridge. A semi-structured interview was administered and subjects with any history of major mental illness in either themselves or a first-degree relative were excluded. Only one male and one female subject were excluded on this basis. This control sample was an extension of the sample described previously (Morris et al., 1995). The age range of the control and patient groups is not significantly different. The case and control groups were matched therefore for ethnicity, sex and age.

2.2. Isolation of DNA

DNA was extracted from 10 ml of venous blood using the Nucleon II kit and dissolved in 0.1 ml of sterile distilled water.

2.3. PCR amplification

A radioactive PCR technique was used to amplify fragments containing the CAG-repeat regions of the selected sequences, using the primers listed in Table 1. Amplification was carried out using 0.4 mM of each primer pair, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 1.5 mM $MgCl_2$, 0.5 units of *Taq* polymerase, 50–100 ng genomic DNA and reaction buffer in a total volume of 25 μl. Cycling consisted of a 2-min denaturation at 96°C, followed by 32 cycles of 96°C for 1 min, annealing at either 55 or 58°C for 30 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 5 min.

2.4. Statistical analysis

The $2 \times k$ χ^2 statistic was used to compare allele frequencies between the control and patient groups (Siegel and Castellan, 1988). Chi-square was also used to compare the numbers of homozygotes between the control and patient groups for each locus. The Bonferroni correction was applied to allow for multiple testing.

3. Results and discussion

The five brain-expressed loci with CAG repeats examined in this study are listed in Table 2. The different alleles identified are arranged in size order, and are numbered to reflect the number of CAG repeats. Each locus lies on a different chromosome (Li et al., 1993) and, with the exception of B33, the CAG repeat region is contained in a long open reading frame. The number of samples amplified for each locus is given in the legend.

All of the five loci showed size polymorphisms in both our patient and control groups. Two of these loci, B43 and B45d, were previously reported not to be polymorphic (Li et al., 1993), but in this study (see Table 2) they were found to have four and five alleles, respectively. This discrepancy may be due to the larger size of our sample, or may reflect the different origin of our population. However, none of the loci exhibited a substantially increased number of CAG repeats. In fact, the frequency and size range of alleles was similar for cases and controls in all five loci, with no statistically significant differences in allele frequencies between the control and patient groups, including those of B33, after correction for multiple testing. Similarly, no significant differences were obtained between schizophrenic patients and controls in other studies for B33 (Jain et al., 1996; Sasaki et al., 1996) or for B1 (Sasaki et al., 1996).

Two of the loci examined here, A4 and B33,

Table 1
Primer pairs used for candidate loci

Locus	Chromosome	Primer sequences	Annealing temp. (°C)
A4	13	Forward: 5′-TGA TCA CTT GTG GTT CTG CG Reverse: 5′-GAG AGG AGT CTG TGT GCC TG	58
B1	1	Forward: 5′-AAG CTC CGG AAT GTT GTC C Reverse: 5′-CTT GAT GTG CTG AGA CCT GC	58
B33	3	Forward: 5′-ACCTGG TAC TAA GGG TAC TGCT Reverse: 5′-GCC ACT TAT GCT TTC TTG CC	55
B43	16	Forward: 5′-AGA CCT ACA CGG TAC GCG TC Reverse: 5′-GAA GCA GAT ACC AGG TTC GG	55
B45d	10	Forward: 5′-CGT CAA TGA GCG CAA AGT AG Reverse: 5′-GGA AGC AGA TAC CAG GTT CG	58

Table 2
Allele distributions for candidate loci

Locus	Allele	Number of CAG repeats	Patients		Controls		χ^2 P value (alleles, patients vs. controls)
			No. of chromosomes	Percentage	No. of chromosomes	Percentage	
A4	1	16	4	2.5	5	4.6	0.437
	2	17	4	2.5	3	2.8	
	3	19	148	92.5	94	87	
	4	20	4	2.5	6	5.6	
B1	1	12	35	25.4	40	27.4	>0.75
	2	14	96	69.6	99	67.8	
	3	15	6	4.3	5	3.4	
	4	16	1	0.7	2	1.4	
B33	1	9	1	0.6	4	2.8	0.03
	2	10	21	13.7	7	4.9	
	3	11	1	0.6	1	0.7	
	4	12	31	20.1	24	16.9	
	5	13	30	19.5	37	26.1	
	6	14	0	0	4	2.81	
	7	15	47	30.5	54	38.0	
	8	16	18	11.7	6	4.2	
	9	17	3	2.0	3	2.1	
	10	18	0	0	0	0	
	11	19	2	1.3	2	1.4	
B43	1	24	2	1.2	0	0	0.08
	2	30	32	19.3	30	21.4	
	3	31	132	79.5	106	75.7	
	4	32	0	0	4	2.9	
B45d	1	0	2	1.3	0	0	0.284
	2	8	25	16.0	31	20.6	
	3	9	127	81.4	114	76	
	4	10	1	0.64	4	2.7	
	5	14	1	0.64	1	0.7	

Allele frequencies are shown for each locus studied. Patient numbers: A4, $n=80$; B1, $n=69$; B33, $n=77$; B43, $n=83$; B45d, $n=79$. Control numbers: A4, $n=54$; B1, $n=73$; B33, $n=71$; B43, $n=70$; B45d, $n=75$. P values are before correction for multiple testing.

localize to chromosomes 13 and 3, respectively, which have previously been implicated in linkage to schizophrenia (Antonarakis et al., 1996; Pulver et al., 1996). However, since the localization of the expanded genes has not been refined to particular regions of these chromosomes, the failure to find any association with schizophrenia does not contradict the finding of linkage.

No significant differences in homozygosity frequencies were seen between controls and patients at any of the loci.

In conclusion, no expansions of sufficient size were seen that could account for expansions of up to 136 repeats previously reported in our RED study (Morris et al., 1995). It is unlikely therefore that any of these loci contribute to the aetiology of schizophrenia in the population of patients studied.

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