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SHORT REPORT

No evidence for the involvement of CAG/CTG repeats from within 18q21.33–q23 in bipolar disorder

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We previously identified 18q21.33–q23 as a candidate region in one BP family and constructed a yeast artificial chromosome (YAC) contig map. Here, we mapped eight known CAG/CTG repeats relative to 18q21.33–q23. We also isolated four CAG/CTG repeats from within the region using CAG/CTG YAC fragmentation, one of which is located in the 5' untranslated region of the *CAP2* gene coding for a brain-expressed serine proteinase inhibitor. The triplet repeats located in the 18q21.33–q23 BP candidate region showed no expanded alleles in the linked BP family nor in a BP case-control sample. Moreover, only the *CAP2* triplet repeat was polymorphic but no genetic association with BP disorder was observed.
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Introduction

Bipolar disorder (BP) is a severe psychiatric condition affecting about 1% of the population. The clinical signs of BP are alternating episodes of depression and mania (BP type I) or hypomania (BP type II). Genetic studies have identified several potential disease loci of which chromosome 18 is of particular interest since several independent studies have identified candidate regions for BP disorder.[1] We identified a 8.9 cM region between D18S68 and D18S979 at 18q21.33–q23 by linkage analysis studies and constructed a physical map using yeast artificial chromosomes (YACs).[2]

Several studies have described anticipation in families transmitting BP disorder[3,4] suggesting the involvement of trinucleotide repeat expansions. Significantly larger CAG/CTG repeats were detected in BP patients compared to controls using the repeat expansion detection method (RED).[5] More recent studies have shown that most (86–89%), but not all, RED expansions (>120 bp) in BP cases and controls can be explained by two highly polymorphic CAG/CTG loci, ie ERDA1[6] located on chromosome 17q21.3 and CTG18.1[7] on chromosome 18q21.1.[8,9] Here we have analysed known as well as novel CAG/CTG repeats located at chromosome 18q21.33–q23 for their involvement in BP disorder.

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Materials and methods

Subjects

The pedigree and the clinical diagnoses in family MAD31 have been described in detail elsewhere.[2] Case-control studies were performed on a Belgian sample of 75 BP patients and 75 controls matched for age, sex and ethnicity. All individuals were interviewed using the SADS-LA after written informed consent.

Triplet repeat isolation

CAG/CTG repeats were isolated by YAC fragmentation as described.[10] The sequences of fragmented ends were obtained by plasmid based end rescue.[10,11] When BLASTN analysis did not result in the opposite flanking sequence, primers based on the YAC end sequence were designed and used to screen a cosmid library of the parent YAC by radioactive hybridisation. DNA from the resulting cosmids was isolated for direct sequencing and primer pairs for the CAG/CTG repeats were designed.

YACs were subcloned in sCOGH-2 as described[12] and CAG-positive cosmids were identified by $(CAG)_{10}$ hybridisation and arrayed in a 96-well plate. Replica filters were screened separately with primers flanking CAG/CTG repeats isolated by YAC fragmentation. DNA of cosmids containing unknown CAG/CTG repeats was isolated and processed as described.[12]

For sequencing, plasmids or cosmids were grown overnight in liquid LB medium supplemented with 100 μg/ml ampicillin and DNA was prepared with the Wizard DNA purification system (Promega, Madison, WI, USA) and sequenced on an automated DNA sequencer model ABI 377 (PE Biosystems, Foster City, CA, USA).

PCR amplification, mapping and genotying

Mapping experiments were performed on a chromosome 18 mapping panel available through the NIGMS Human Genetic Mutant Cell Repository (http://locus.umdnj.edu/nigms/ideograms/18.html). CAG/CTG repeats were PCR amplified and genotyped as described[10] using the following primers Cap2F: ATCGAACGGTTCTGAGTCATCT and Cap2R: CGCTCTGATTCCTGCTCTG for the *CAP2* repeat, 11A4F: AGAAGGAAGCACAGCAAATTTG and 11A4R: GCATGGTGCTGGAGATCAAT for 11A4 and 11B3F: GGCTGAGATGTTCCTTGACTGC and 11B3R: CCTTCCCATGCCACCACTACTA for 11B3.

Results

Mapping of known CAG/CTG repeats

We selected eight chromosome 18 CAG/CTG repeat markers through the CHLC database and determined their location relative to the BP candidate region 18q21.33–q23[2] by PCR screening of a chromosome 18 somatic cell hybrid mapping panel (data not shown). Since GCT3E06, GCT6F12 and GCT3G01 were assigned to 18q22.2–qter, we verified whether they localised to 18q21.33–q23 by PCR analysis of YACs 961h9, 766f12 and 907e1, representing the minimal YAC tiling path (Figure 1). GCT3G01 mapped on YAC 907e1, whilst GCT3E06 and GCT6F12 are located outside the BP candidate region. The size and stability of the three YACs had previously been confirmed by PFGE.[2] Metaphase FISH and fiber FISH experiments confirmed that the three YACs are non-chimeric and overlapping (Figure 2).

CAG/CTG YAC fragmentation

CAG/CTG YAC fragmentation[10] was applied to YACs 961h9, 766f12 and 907e1. Size determination by PFGE and Southern blot hybridisation resulted in seven sets of equally sized fragmented YAC clones. Sequencing identified five (out of seven) sets of fragmented YACs with identical end sequences resulting from a specific homologous recombination event.

BLASTN analysis[13] of the CAG/CTG flanking sequence showed that two were novel CAG/CTG repeats (11A4 and 11B3). 6A4 matched to part of the cytoplasmic antiproteinase *CAP2* cDNA (GenBank acc. No L40377) confirming that the fragmentation occurred at the $(CAG)_6$ sequence in the 5' UTR of this gene. Clone 26C1 corresponds to GCT3G01 (GenBank acc. No G09484) mapping to YAC 907e1 (Figure 1). 11F2 was identical to a yeast sequence flanking a $(CAG)_8$ repeat (GenBank acc. No Z38059) and was excluded from further studies. Sequencing showed that 11A4 and 11B3 resulted from fragmentation at a $(CAG)_5$ and a $(CAG)_3$ repeat, respectively. Primer pairs for the *CAP2* repeat, 11A4 and 11B3 were used to position them on the YAC contig map (Figure 1).

To exclude the possibility of CAG/CTG repeats from within the BP candidate region being missed by the YAC fragmentation method, we screened cosmid sublibraries of YACs 961h9, 766f12 and 907e1 for the presence of CAG/CTG repeats. Hybridisation showed that 62 (84%) of 74 CAG/CTG positive cosmids contained either the *CAP2* triplet repeat, 11A4, 11B3, GCT3G01 or yeast CAG/CTG repeats. The remaining

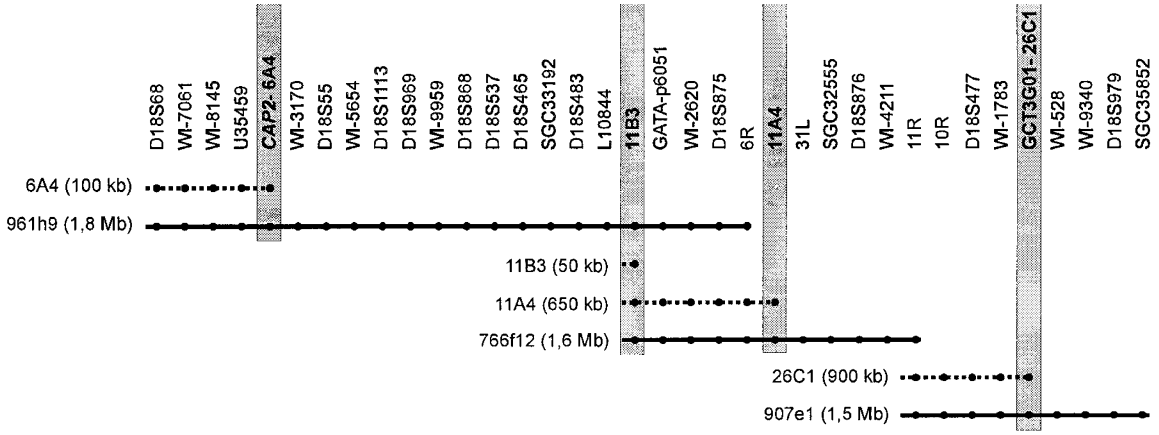

Figure 1 Minimal YAC tiling path of the 18q21.33–q23 BP candidate region.[2] The YACs are represented by solid lines, the CAG/CTG fragmentation products by dotted lines. YAC sizes, between brackets, are estimated by PFGE analysis. Solid circles indicate positive STS/STR hits. Shaded boxes highlight the four CAG/CTG repeats isolated by YAC fragmentation.

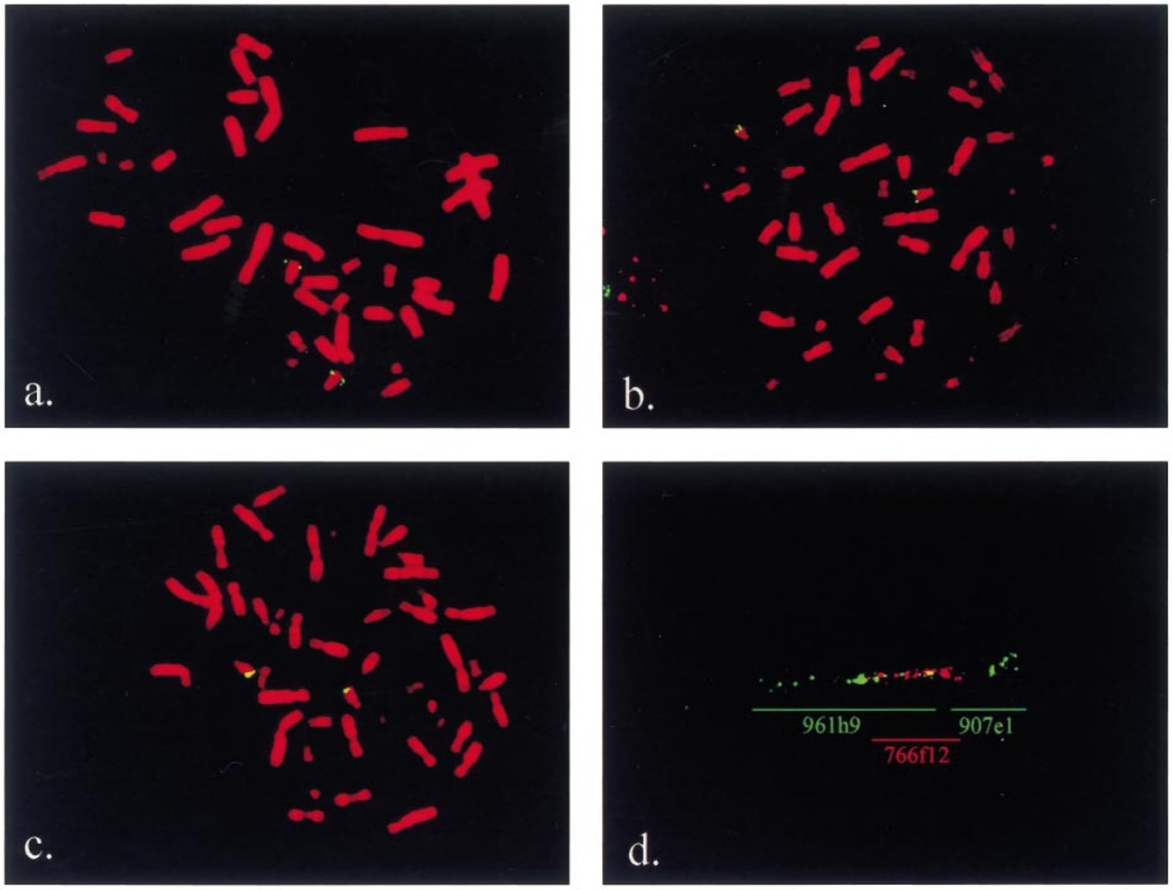

Figure 2 Metaphase and fiber FISH analyses of the YACs of the minimal tiling path. **a**–**c**: Metaphase FISH of respectively 961h9, 766f12 and 907el, **d**: Fiber FISH.

cosmids were plasmid subcloned and sequenced but no new CAG/CTG repeats were identified.

Genetic analysis of the CAG/CTG repeats

We analysed the four 18q21.33–q23 triplet repeats *CAP2*, GCT3G01, 11A4 and 11B3 in family MAD31 and in 75 unrelated BP cases and 75 matched controls. No expanded alleles were detected for any of the four triplet repeats. Only the *CAP2* repeat was polymorphic with the common allele (257 bp) co-segregating with the disease in family MAD31 (Table 1). For this marker no deviations from the Hardy-Weinberg equilibrium were observed in the control sample nor in the BP sample. Comparison of the allele and genotype distribution of the *CAP2* repeat between BP cases and controls using Genepop[14] did not reveal significant differences (Table 1).

Discussion

To analyse the involvement of CAG/CTG repeats in the aetiology of BP disorder linked to 18q21.33–q23, we determined the localisation of eight known CAG/CTG repeats

Table 1 Allele and genotype distribution of the *CAP2* repeat in a case–control study. Genepop was used to compare both distributions. The allele present in the parental YAC is indicated by an asterisk and was determined by PCR amplification of YAC 961h9 DNA

	BP cases *Fraction*	*Controls* *Fraction*	*Genepop* *Exact value*
Alleles (bp)			
248	0.01	0.01	*P*=0.51
257*	0.78	0.77	
260	0.02	0.00	
263	0.17	0.20	
274	0.03	0.03	
Genotypes (bp)			
248–257	0.01	0.01	*P*=0.46
257–257	0.60	0.61	
257–260	0.03	0.00	
257–263	0.28	0.28	
257–274	0.04	0.03	
260–263	0.01	0.00	
263–263	0.01	0.04	
263–274	0.01	0.03	

relative to the candidate region and isolated all CAG/CTG repeats from within a non-chimeric YAC minimal tiling path spanning this region using CAG/CTG YAC fragmentation. This resulted in four region-specific CAG/CTG repeats corresponding to an average repeat frequency of 1 per 1.23 Mb based on the YAC insert sizes.[2] The possibility that CAG/CTG repeats were missed by the YAC fragmentation was excluded by $(CAG)_{10}$ screening of cosmid sublibraries. No additional CAG/CTG repeats were isolated confirming that the CAG/CTG YAC fragmentation is a sensitive method.[10]

Next we analysed the CAG/CTG repeats from within the 18q21.33–q23 candidate region in a case control sample of 75 BP patients and 75 matched controls. The only polymorphic triplet repeat was contained in the 5' UTR of the *CAP2* gene, a potential candidate gene for BP disorder since it codes for a serine proteinase inhibitor expressed in brain.[15] No significant differences were observed between the allele and genotype distributions of this repeat in a BP case and control sample and expanded alleles were absent. Analysis of family MAD31 showed that the *CAP2* repeat segregated with the disease but no expanded alleles were observed.

Together, our data indicate that at least in family MAD31 expanded CAG/CTG repeats are unlikely to cause BP disorder. However, we cannot exclude that expanded CAG/CTG repeats do explain the pathogenesis in some BP cases linked to other regions on chromosome 18 or elsewhere in the genome. Also, it remains possible that the BP disorder in family MAD31 is due to expansions of triplet repeats of a different nature such as CCG/CGG or GAA/TTC.

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References

1 Van Broeckhoven C, Verheyen G: Report of the chromosome 18 workshop. *Am J Med Genet* 1999; **88**: 263–270.
2 Verheyen GR, Villafuerte SM, Del-Favero J *et al*: Genetic refinement and physical mapping of a chromosome 18q candidate region for bipolar disorder. *Eur J Hum Genet* 1999; **7**: 427–434.
3 McInnes MG, McMahon FJ, Chase GA, Simpson SG, Ross CA, DePaulo JRJ: Anticipation in bipolar affective disorder. *Am J Hum Genet* 1993; **53**: 385–390.
4 Nylander PO, Engstrom C, Chotai J, Wahlstrom J, Adolfsson R: Anticipation in Swedish families with bipolar affective disorder. *J Med Genet* 1994; **31**: 686–689.
5 Lindblad K, Nylander P-O, De bruyn A *et al*: Detection of extended CAG repeats in bipolar affective disorder using the repeat expansion detection (RED) method. *Neurobiol Dis* 1995; **2**: 55–62.
6 Nakamoto M, Takebayashi H, Kawaguchi Y *et al*: A CAG/CTG expansion in the normal population. *Nat Genet* 1997; **17**: 385–386.
7 Breschel TS, McInnes MG, Margolis RL *et al*: A novel, heritable, expanding CTG repeat in an intron of the SEF2-1 gene on chromosome 18q21.1. *Hum Mol Genet* 1997; **6**: 1855–1863.
8 Lindblad K, Nylander PO, Zander C *et al*: Two commonly expanded CAG/CTG repeat loci: involvement in affective disorders? *Mol Psychiatr* 1998; **3**: 405–410.
9 Verheyen GR, Del-Favero J, Mendlewicz J *et al*: Molecular interpretation of expanded RED products in bipolar disorder by CAG/CTG repeats located at chromosomes 17q and 18q. *Neurobiol Dis* 1999; **6**: 424–432.
10 Del-Favero J, Goossens D, De Jonghe P *et al*: Isolation of CAG/CTG repeats from within the chromosome 2p21–p24 locus for autosomal dominant spastic paraplegia (SPG4) by YAC fragmentation. *Hum Genet* 1999; **105**: 217–225.
11 Del-Favero J, Goossens D, Van den Bossche D, Van Broeckhoven C: YAC fragmentation with repetitive and single-copy sequences: detailed physical mapping of the presenilin 1 gene on chromosome 14. *Gene* 1999; **229**: 193–201.
12 Del-Favero J, Krols L, Michalik A *et al*: Molecular genetic analysis of autosomal dominant cerebellar ataxia with retinal degeneration (ADCA type II) caused by CAG triplet repeat expansion. *Hum Mol Genet* 1998; **7**: 177–186.
13 Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. *J Mol Biol* 1990; **215**: 403–410.
14 Raymond M, Rousset F: GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. *J Hered* 1995; **86**: 248–249.
15 Sprecher CA, Morgenstern KA, Mathewes S *et al*: Molecular cloning, expression, and partial characterization of two novel members of the ovalbumin family of serine proteinase inhibitors. *J Biol Chem* 1995; **270**: 29854–29861.