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ARTICLE

European combined analysis of the CTG18.1 and the ERDA1 CAG/CTG repeats in bipolar disorder

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Several groups have reported association between large CAG/CTG repeats in the genome and BP disorder using the Repeat Expansion Detection (RED) method. Molecular interpretation studies demonstrated that around 90% of the large CAG/CTG repeats detected by RED can by explained by repeat size at either the CTG18.1 or ERDA-1 locus. In this study we report the findings on a large European BP case-control sample analysed for these two frequently expanded repeats. The frequency of expanded alleles (>40 repeats) at the CTG18.1 locus was significantly higher in the subgroup of patients with a more severe phenotype BPI and a positive first degree family history than in a group of matched controls (9% *vs* 5%). No difference in ERDA-1 expansion frequency was seen between BP cases and matched controls. We conclude that the ERDA-1 locus is not related to the BP phenotype while expanded alleles at the CTG18.1 locus cannot be excluded as a vulnerability factor for BP disorder.
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Introduction

Bipolar (BP) disorder is a severe psychiatric condition affecting about 1% of the population and is characterised by disturbances in mood, ranging from an extreme state of mania to a severe state of depression. BP illness type I (BPI) is characterised by major depressive episodes alternated with phases of mania, and type II (BPII) is characterised by major depressive episodes alternating with phases of hypomania. Relatives of BP probands have an increased risk for BP spectrum disorders, comprising of BP and unipolar disorder, cyclothymia and schizoaffective disorders.

Anticipation – an increase in disease severity in successive generations – has been described in BP disorder[1–5] and gained renewed attention with the demonstration of a causal relationship between anticipation and triplet repeat expansions in several neurodegenerative diseases.[6] A number of groups have tested this hypothesis using the Repeat Expansion Detection (RED) method, which identifies the size of the largest trinucleotide repeat present in the genome of an individual.[7] Of eight independent studies four reported an association between expanded RED products and BP disorder[5–10] while another four did not find a significant

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association.[11–14] Unfortunately, the RED method cannot identify the specific repeat(s) responsible for these findings. Recently it was shown that about 90% of the large CAG/CTG repeats (repeat size $\geqslant$40 units) detected by RED can be explained by two loci.[15–18] The first locus is CTG18.1, which maps to 18q21.1 and is located in the third intron of the SEF2-1 gene. SEF2-1 encodes one of a family of basic helix–loop–helix proteins that bind to promoter sequences to regulate transcription of genes.[19] The CTG18.1 locus is also of particular interest for bipolar disorder since linkage has been reported in the immediate genetic vicinity.[20,21] The second locus is called ERDA-1, also known as Dir-I and maps to 17q21.3.[22,23] Although this repeat may be transcribed, its function, if any, remains unknown. [23]

Previously, we reported association between expanded RED CAG/CTG products and bipolar disorder in a sample of 51 unrelated BP patients and 99 matched controls of Belgian ancestry.[8] In the same study, a Swedish BP sample showed similar results. The association was strongest in BPI patients with a familial history of affective disorders. Our group also replicated this finding in a BP case-control sample from Croatia.[10] Subsequent analysis of both the Belgian and Swedish case-control samples with the two known CAG/CTG repeat loci showed a significant association between expanded ERDA1 alleles and BP disorder (P=0.04) in a Belgian sample and between CTG18.1 alleles and BP disorder ($P<0.03$) in the Swedish sample.[16,18] These data suggest that the associations between BP disorder and large CAG/CTG RED products may be explained at least in part by a specific association between BP disorder and CTG18.1 and/or ERDA-1. To test this hypothesis we determined the frequency of the CTG18.1 and ERDA-1 alleles in a large combined European case-control sample composed of BP subjects and age, gender and ethnicity matched controls from Belgium, Croatia, Denmark, Scotland and Sweden.

Material and methods

Subjects

The patient and control subjects for this study were consecutively recruited in the five participating centres.[24] Whole venous blood was used for the extraction of DNA by standard methods. Clinical evaluation of all patients was performed by a semi-structured interview using the Schedule for Affective Disorders and Schizophrenia–Lifetime Version (SADS–LA). Diagnosis of BP was made according to the Research Diagnostic Criteria (RDC). Control subjects were drawn from the respective populations and matched for age and gender. The Belgian sample in this study contains an overlap of 33 patients and 25 control individuals with a previous study.[16]

Genotyping

CTG18.1 and ERDA-1 primer sequences and the respective PCR conditions were as described[18] and were used in all participating laboratories.

Genotyping of the Croatian, Belgian and Swedish samples was performed by a two-stage procedure. First, PCR was carried out for each locus with one of the primers labelled with a fluorescent dye. One microlitre of the PCR product was denaturated, run on a 6% denaturing polyacrylamide gel and analysed on an ABI 377 DNA sequencer using the Genescan and Genotyper 2.0 software. Second, all PCR products were reanalysed to allow detection of expanded alleles that might not be identified during the fluorescent analysis. Ten μl of the PCR product was separated on a 2% agarose gel, blotted to Hybond N^+ membranes, fixed and hybridised at 58°C for at least 2 h with a γ-^{32}P-ATP end-labelled $(CAG)_{10}$ oligonucleotide. Membranes were washed and autoradiographed for 0.5 to 4 h.

Genotyping of the Scottish and Danish sample was performed by a one-stage procedure. PCR products were heat denatured and subsequently separated on a 6% denaturing polyacrylamide gel. DNA from the polyacrylamide gel was blotted to Hybond N^+ membranes, fixed and hybridised at 58°C for at least 2 h with a γ-^{32}P-ATP end-labelled $(CAG)_{10}$ oligonucleotide. Membranes were washed and autoradiographed for 0.5 to 12 h.

Two raters performed sizing of the alleles independently and blind with respect to case/control status. An error checking procedure was implemented to insure standardisation of genotyping. For this purpose, each analysis included 3 CEPH individuals to correct for inter-gel variation and five random samples from each participating group were analysed and scored by the reference laboratory in Antwerp.

Statistical analysis

Statistical analysis (allelic and genotypic) was performed by dichotomous analysis, classifying the alleles at each locus in a group of 'long' alleles in the case the repeat number was $\geqslant$40 and a group of 'short' alleles if the number of repeats was less than 40. This classification has no biological validity but is based upon the RED method, which yields a lower allele size of 40 repeats. The hypothesis of association between BP disorder and expansion at the ERDA-1 or CTG18.1 locus was tested by a χ^2 analysis. Secondary analyses were performed after stratification for family history of affective disorder in first-degree relatives and/or severity of the disease reflected by BPI diagnosis. In case stratification was used, the significance level of allelic and genotypic association was adjusted according to the number of comparisons used (Bonferroni approach). The significance level after stratification for disease severity was set at 0.025 (i.e. 0.05/2) while the significance level after stratification for disease severity and family history was set at 0.017 (i.e. 0.05/3).

Results

The total sample consisted of 889 subjects, 403 BP patients and 486 controls. A total of 889 CTG18.1 and 884 ERDA-1

typings were generated for analysis (Table 1). Statistical analysis of expanded allele frequencies between the respective control groups showed no deviation from Hardy–Weinberg equilibrium among the five control groups. The dichotomized frequencies for the individual as well as combined control populations and affected populations are shown in Table 2.

In the individual samples the two repeat loci ERDA-1 and CTG18.1 showed no significant difference at either locus, in the frequency of large alleles in the BP group compared with the controls under the dichotomous model (Table 2). However, a borderline statistical significant difference was observed for the CTG18.1 locus in the Croatian sample after stratification for first-degree family history and BPI (χ^2=4.42, P=0.04). Analysis of the combined BP sample did not show a significant difference at either of the two CAG/CTG repeat loci. After stratification for first-degree family history and disease severity (BPI), a borderline significant difference was found (χ^2=4.54, P=0.03).

Genotypic analysis of the two repeat loci in the individual samples showed no significant difference at either locus nor in the combined sample (all P-values >0.1). However, a borderline statistical significant difference was observed for the CTG18.1 locus in the combined sample after stratification for first-degree family history and BPI (χ^2=6.33, P=0.02). For BPI familial cases, the relative risk of the CTG18.1 homozygous expanded genotype is 2.43 (95% CI: 1.37–4.33).

Discussion

We genotyped a large combined BP case-control sample of individuals derived from populations of European descent with two frequently expanded CAG/CTG containing loci in normal individuals: ERDA-1 and CTG18.1. In the controls we observed expansion frequencies of 26% (range 20–32%) for ERDA-1 and 5% (range 3–7%) for CTG18.1 (Table 2), which is in agreement with previously published repeat frequencies.[16,18,19,22,23,25]

Dichotomous analysis of the combined sample for ERDA-1 does not support the involvement of this repeat locus in BP disorder, except for a borderline effect in the Belgian familial BPI cases (P=0.07). In a previous study by our group,[18] we found a larger proportion of BP patients carrying an expanded ERDA-1 allele than controls (P=0.03). This was not replicated in other studies.[14,16]

Statistical analysis of the genotypes from the CTG18.1 locus showed a higher frequency of expanded CTG18.1 repeats in BP individuals as compared to controls, after stratification for first degree family history and BPI in the Croatian sample (P=0.04). The results from the Croatian sample are in agreement with a previous study by our group, which showed that expanded RED products are associated with familial BPI subjects.[10]

There is no difference in the distribution of expanded alleles in the BP cases versus controls in the overall sample, which is in agreement with the data reported by McInnes *et al*.[25] However, a difference in frequencies of expanded CTG18.1 alleles is present between BPI cases with a first degree family history and controls (P=0.03). These findings support those of Lindblad *et al*.[16] who reported an association with enlarged alleles at the CTG18.1 locus in a sample of BP disorder ($P<0.03$). The replication of this finding in the present study which is much larger (403 BP cases) than the Lindblad *et al*.[16] study (60 BP cases), renders further support to the specific hypothesis that SEF2-1 cannot be excluded as a susceptibility gene for bipolar disorder. Moreover, genotypic analyses showed that there is a relative risk of 2.4 of developing BP disorder in BPI familial cases homozygous for the expanded allele.

Our finding is of particular interest since at least two studies showed linkage in the immediate vicinity of CTG18.1.[20,21] CTG18.1 was of particular interest in the Belgian BP family MAD31, since this family was previously shown to segregate with markers from chromosome 18q21.3–18q23.[21] However, CTG18.1 is not expanded in family MAD31[18] and we recently located CTG18.1 outside the BP candidate region defined in MAD31.[17] Nevertheless, the possibility exists that CTG18.1 is in linkage disequilibrium with a causal gene for BP disorder.

Also, CTG18.1 is located within the third intron of the SEF2-1 gene, coding for a transcription factor that is widely expressed, including in brain.[19] Altered expression of the SEF2-1 gene as a direct consequence of the presence of expanded CTG18.1 alleles can have profound effects on cellular processes in cells expressing the SEF2-1 gene. Furthermore, the repeat is transcribed into a prepro-mRNA CTG sequence and could potentially play a role similar to

Table 1 Number of individuals and typings for the European combined sample

	Numbers			*Tyings*	
	Co	*BP*	*Total*	*CTG18.1*	*ERDA-1*
Belgium	64	63	127	127	125
Sweden	91	78	169	169	167
Croatia	87	81	168	168	167
Denmark	146	81	227	227	227
Scotland	98	100	198	198	198
Total	486	403	889	889	884

Table 2 Results of the statistical analysis on the individual samples and the combined sample

		CTG18.1				*ERDA-1*			
	Type	*Number of individuals*	*Expanded alleles*	*%*	P	*Number of individuals*	*Expanded alleles*	*%*	P
Belgium	BP	63	5	8	0.98	61	20	33	0.11
	BPI	46	3	7	0.80	44	14	32	0.17
	BPI fam	29	1	3	0.43	29	11	38	0.07
	CO	64	5	8		64	13	20	
Sweden	BP	78	2	3	0.57	76	22	29	0.30
	BPI	63	2	3	0.67	62	19	31	0.23
	BPI fam	33	1	3	0.71	33	10	30	0.34
	CO	91	3	3		91	20	22	
Croatia	BPI	81	7	9	0.47	80	18	23	0.80
	BPI fam	40	7	18	0.04	39	14	36	0.17
	CO	87	5	6		87	21	24	
Denmark	BPI	81	3	4	0.69	81	20	25	0.28
	BPI fam	61	2	3	0.83	61	16	26	0.45
	CO	146	4	3		146	46	32	
Scotland	BP	100	11	11	0.35	100	25	25	0.93
	BPI	92	10	11	0.37	92	24	26	0.93
	BPI fam	56	9	16	0.08	56	15	27	0.86
	CO	98	7	7		98	25	26	
Combined	BP	403	28	7	0.20	398	105	26	0.93
	BPI	363	25	7	0.23	359	95	26	0.86
	BPI fam	219	20	9	0.03	218	66	30	0.23
	CO	486	24	5		486	125	26	

that of the untranslated CTG repeat in myotonic dystrophy.[26]

In summary, we have shown in a large combined BP case-control sample of European origin that CTG18.1 expansions are over-represented in familial BPI cases as compared to age, gender and ethnicity matched controls. The exact nature of this finding is not clear at this time but indicates that the expanded CTG18.1 repeats may predispose to affective disorder.

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