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Screening of chromosomal region 21q22.3 for mutations in genes associated with neuronal Ca^{2+} signalling in bipolar affective disorder*

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The therapeutic effect of lithium in bipolar affective disorder may be connected with decreasing intracellular Ca^{2+} concentrations. Several linkage studies have identified a potential bipolar affective disorder susceptibility locus within chromosomal region 21q22.3. This locus contains two genes expressed in the brain — *ADARB1* and *TRPM2* — involved in regulating intracellular Ca^{2+} concentrations. The aim of this study was an identification of mutations in the coding sequences of *ADARB1* and *TRPM2* and their association with bipolar affective disorder. For that purpose we screened 60 patients with bipolar affective disorder and a control group of 66 subjects using single strand conformation polymorphism and sequence analysis. For rapid screening we performed restriction fragment length polymorphism analysis. Screening of bipolar affective disorder patients for mutations in *TRPM2* led to identification of three novel and four known transitions. Two transitions resulted in the substitutions: R755C and A890V. Screening of the coding sequence of *ADARB1* did not reveal any mutations except one already known transition. A comparison of the transition frequency in patients and controls does not support association of the detected mutations with bipolar affective disorder. According to our results, bipolar affective disorder may not be caused by mutations in *ADARB1*. However, this study does not exclude *TRPM2* as a candidate gene since we have screened only about 30 per cent of the entire coding sequence of this large gene.

Keywords: TRPM2 protein, AMPA glutamate receptor B, bipolar affective disorder

INTRODUCTION

Lithium, commonly used in the therapy of bipolar affective disorder (BPAD), decreases intracellular Ca^{2+} mobilisation (Iconomov *et al*., 1999). This observation led to the conclusion that high intracellular Ca^{2+} levels in neurones possibly due to mutations affecting the gene(s) responsible for Ca^{2+} signalling may contribute to the pathogenesis of BPAD. According to findings of Straub *et al*. (1994), confirmed later by different authors (Detera-Wadleigh *et al*., 1996; Vallada *et al*., 1996; Smyth *et al*., 1997; Aita *et al*., 1999), the susceptibility locus for BPAD may be present in the chromosomal region 21q22.3. There are two genes involved in the neuronal Ca^{2+} signalling – *ADARB1* and *TRPM2* – that map to this region. *ADARB1*, alternatively named *ADAR2* is expressed mainly in the brain, lungs and kidneys (Villard *et al*., 1997; Mittaz *et al*., 1997). The gene encodes an RNA editase (hRED1) whose function is editing of the pre-mRNA for glutamate receptor subunit B (GluR-B), by site-selective adenosine deamination. The GluR-B pre-mRNA editing at two adenosine residues results in amino acid changes that alter Ca^{2+} permeability of the glutamate AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-responsive receptor (Melcher *et al*., 1995).

*Accession numbers: ss49785909, ss49785910, ss49785911.

Abbreviations: BPAD, bipolar affective disorder; nt, nucleotide; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single strand conformation polymorphism; TRPM2, transient receptor potential cation channel subfamily M member 2.

TRPM2, previously termed *LTRPC2* or *TRPC7* (Nagamine *et al*., 1998), is expressed in the brain, lymphocytes, and human pancreatic islets (Qian *et al*., 2002). TRPM2 protein (transient receptor potential cation channel subfamily M, member 2) is linked to the phosphatidylinositol signal transduction pathway that causes Ca^{2+} influx into the cell (Clapham *et al*., 2001). Its specific function associated with cell death is not entirely clear (Hara *et al*., 2002). TRPM2 acts as a storage-operated calcium channel activated by intracellular Ca^{2+} depletion. The protein consists of six transmembrane domains (with the Ca^{2+} entry site located between the fifth and the sixth domain) and a large cytosolic, alternatively spliced domain, containing 80% of the protein sequence. Ca^{2+} entry into the cell requires intracellular ADP-ribose (Perraud *et al*., 2001) and provides positive feedback for activation of the channel (McHugh *et al*., 2003). The results of linkage studies and evidence for an altered Ca^{2+} homeostasis in lymphocytes of the patients with BPAD (Yoon *et al*., 2001) made these genes candidates for the causative agent of the disorder. To date, however, there is no evidence for *ADARB1* or *TRPM2* mutations in BPAD or any other disease.

The aim of the study was to detect mutations and/or polymorphisms in these genes and compare their frequencies in BPAD patients and in the healthy subjects. We screened the *ADARB1* coding sequence and exons of *TRPM2* that encode a putative transmembrane and a catalytic domain of the TRPM2 Ca^{2+} channel.

MATERIALS AND METHODS

Subjects included in the study (n=60) were unrelated patients with bipolar affective disorder diagnosed according to DSM-IV criteria and treated in the Department of Adult Psychiatry, University of Medical Sciences in Poznan (Poland). The control group consisted of 66 healthy persons. The Local Ethics Committee approved the study, and a written consent of all patients was obtained.

Single strand conformation polymorphism (SSCP). Genomic DNA was isolated from peripheral blood lymphocytes by a standard salt-out method. The designed primers corresponded to sequences flanking 10 exons: 3–12 of *ADARB1* (derived from human chromosome 21 genomic contig — accession No. NT_011515, nt 1807253...1959212) and 13 exons: 7, 8, 15–21, 29–32 of *TRPM2* (derived from human chromosome 21 genomic contig — accession No. NT_011515, nt 1085877...1175698).

The amplification was conducted under standard conditions with annealing temperatures varying from 59.5°C to 63.8°C. Denatured PCR fragments were analysed by SSCP on 8% polyacrylamide gel with silver staining.

Sequencing. The PCR products showing altered migration were purified on 1.5% agarose gel and subjected to direct sequencing with the use of specific Cy5-labelled dideoxy nucleotides (Thermo Sequenase Cy5 Dye Termination Kit, Amersham Biosciences) or Cy5-labelled primers (AmpliCycle Sequencing Kit, Applied Biosystems). The sequenced fragments were analysed on an ALFexpress sequencer (Amersham-Pharmacia Biotech).

Restriction fragment length polymorphism (RFLP). For rapid detection of the c.2769C>T transition in the control group, digestion of 185-base pair (bp) PCR products with the *Msl*I restriction endonuclease (New England Biolabs) was carried out at 37°C for 16 h. The digested PCR products harbouring the c.2769C>T transition showed two bands (111 bp and 53 bp) on 3% agarose gel.

RESULTS AND DISCUSSION

With the use of SSCP and sequence analysis we detected one known heterozygous transition — c.2234A>G in the coding sequence of *ADARB1* (GenBank mRNA BC065545.1). This transition resulted in synonymous substitution K694K. Four known: c.2363C>T, c.2890^{+55}A>G, c.3246^{+68}C>T, c.3205C>T and three novel: c.2421^{+23}C>T (ss49785911), c.2769C>T (ss49785909), c.3010C>T (ss49785910) transitions were detected in the coding sequence of *TRPM2* (GeneBank mRNA NM_003307). The c.2363C>T transition resulted in the substitution R755C while the c.2769C>T transition resulted in the A890V substitution. The c.3010C>T and the c.3205C>T transitions resulted in the synonymous substitutions A970A and F1035F, respectively. The other transitions: c.2421^{+23}C>T, c.2890^{+55}G>A and c.3246^{+68}C>T were intronic substitutions. We compared the frequency of the transitions in BPAD patients and controls but the difference was statistically insignificant. The highest χ^2 values were: 1.96 for c.2234G>A in *ADARB1* and 1.02 for c.3205C>T in *TRPM2* (Table 1).

We screened the coding sequence of *ADARB1* comprising 10 exons, and part (13 of 32 exons) of the *TRPM2* sequence which encodes a putative transmembrane domain with the channel pore (permeable to Ca^{2+}) as well as a catalytic region (involved in ADP-ribose hydrolysis) containing a highly conserved NUDIX domain. We detected three novel c.C>T transitions in *TRPM2* and one of them (c.2769C>T) led to a rare substitution A890V. However, our findings do not support an association of the transitions with the disorder.

The role of *TRPM2* as a candidate gene was confirmed by the recent finding of three single nu-

Table 1. Transitions detected in BPAD patients in comparison with controls

GENE Transition	Forward primer ...mutation site... Reverse primer	Localisation	Consequence	Frequency: patients/controls
ADARB1 c.2234A>G	TTACAGCGTCAACAGTTGC ...AGATTACCAA**A/G**CCCAACGTGT... CAGTTCAGATGTGAGGTTC	exon 10	synonymous K694K	0.20/0.11 χ^2=1.96
TRPM2 c.2363C>T	GCCTTCCTGACCAAGGTGT ...TGGGCTGTGG**C/T**GTGTGACCCT... CTAGGGAAGATCCTGAGCC	exon 15	nonsynonymous R755C	0.038/0.042
c.2421^{+23}C>T*	GCCTTCCTGACCAAGGTGT ...tgcggggctg**C/T**gggactgtgg... CTAGGGAAGATCCTGAGCC	intron 15	–	0.008/0.007
c.2769C>T*	TGTGAGCAGGTGGCTGAGA ...GCTCATCCCGG**C/T**GACGCTGT... CCCTTACCATCCGCTTCAC	exon 18	nonsynonymous A890V	0.007/0.006
c.2890^{+55}A>G	TGTGAGCAGGTGGCTGAGA ...ggtgggcctc**A/G**gggagggcag... GTACAGGCAAAGATACGAG	intron 18	–	0.24/0.20
c.3010C>T*	TGTCCTGCAGATGAAGGAC ...TCCGAGGGGC**C/T**GTCTACCACT... CGAAGATGGTGAGGTAGGA	exon 19	synonymous A970A	0.016/0.00
c.3205C>T	GTGTGAACTTCAACCCGGA ...ACCTGCTCTT**C/T**ACCAACATC... CATCATCCACACTGTCCAC	exon 20	synonymous F1035F	0.13/0.07 χ^2=1.02
c.3246^{+68}C>T	GTGTGAACTTCAACCCGGA ...gcagtggcca**C/T**gctgtctcca... CATCATCCACACTGTCCAC	intron 20	–	0.22/018

*novel transition

cleotide polymorphisms strongly associated with BPAD (McQuillin *et al*., 2006; Xiu *et al*., 2006). It also remains possible that in some families BPAD is associated with an as yet unknown mutation(s) in *TRPM2*. As *TRPM2* is expressed also in pancreatic islets (Qian *et al*., 2002) some mutations could affect not only neuronal but also pancreatic Ca^{2+} homeostasis. Bipolar disorder often coincides with type 2 diabetes (Regenold *et al*., 2002), which may appear as a result of an altered pancreatic intracellular Ca^{2+} homeostasis. Further screening of patients affected with both diseases for mutations in the *TRPM2* coding sequence might help to resolve this problem.

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