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Screening for rare variants in TMEM132D: a candidate gene from genome-wide association studies in anxiety disorders

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Background

The aetiology of anxiety disorders is known to be influenced by genetic factors. Genome-wide association studies revealed common intronic SNPs within the 831942 bp long transmembrane protein 132D gene (TMEM132D) located on chromosome 12 to be associated with panic disorder and anxiety-related phenotypes (Erhardt et al., 2010). Recent studies in autism and schizophrenia have shown that a combination of common as well as rare functional disease variants is important in the genetic susceptibility to psychiatric disorders (Alaerts, Del-Favero, 2009; Uher, 2009). Therefore the exonic regions and exon-intron junctions of TMEM132D were investigated to identify both common and rare genetic variants which might be associated with changes in gene function in anxiety disorders.

Materials and methods

DNA from 300 patients suffering from anxiety disorders, especially panic disorder (66 %) and 300 healthy controls, matched for age and gender, was screened for polymorphisms using the ABI SOLID 3+ next-generation sequencing platform. Enrichment of all nine exons of the TMEM132D gene and the exon-intron boundaries, 40 kb in total, was done via long range PCR in a pooled approach. After amplification 4 pools of PCR products consisting of equal amounts of DNA from 150 patients or controls were prepared for the sequencing run using the standard protocol for an ABI SOLID fragment library with a read length of 50 bp. The pools were put on a quad-slide of the ABI SOLID 3+ and sequenced. The obtained raw reads underwent a quality control and only reads fulfilling the quality criteria were included in the following SNP calling analysis using vipR, an algorithm specifically designed to detect rare genetic variants in pooled samples (Altmann et al., submitted). To validate the putative variants MALDI-TOF (matrix-assisted laser desorption ionization time of flight) mass spectrometry on the Sequenom platform (San Diego, USA) was performed.

Results

Using the ABI SOLID 3+ system 328 million reads with a length of 50 bp were generated, approximate 82 million per DNA pool (table 1). The coverage was approximately 50,000 fold per base per DNA pool corresponding to 320 fold per person and 160 fold per haplotype.
With the VipR algorithm 371 SNPs could be identified. 258 of them (69.5%) have not been characterized so far and 113 (30.5%) previously identified SNPs reported in the public dbSNP130 database could be confirmed. Approximately one third of the detected SNPs are low frequency variants with a minor allele frequency (MAF) < 0.5% (figure 1).

pool	raw reads [mio]	reads after QC [mio]	mappable reads [mio]
Cases 1	82.2	48.9	45.1
Cases 2	77.5	48.6	45.4
Controls 1	86.5	53.3	50.1
Controls 2	81.7	49.2	45.8
total	327.9	200	186.4

QC = quality check

Table 1: Number of reads generated in the sequencing experiment.

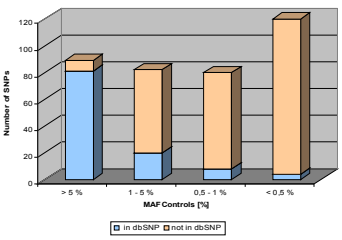

Figure 1: MAF distribution among all SNPs.

30 SNPs (8.1%) were in the coding region of the gene, 1 SNP (0.3%) in the 5'UTR, 15 SNPs (4.0%) in the 3'UTR, whereas the vast majority (325 SNPs, 87.6%) was located in the introns. Of the 30 coding SNPs, 17 are missense mutations leading to amino acid exchanges in the protein which might change its function, 15 of these have not been identified before (figure 2). Of the 72 putative genetic variants that were re-genotyped using MALDI-TOF, 59 (82.0%) could be validated. Within these the cross-methods correlation of genotype frequencies was *r = 0,866* in cases and *r = 0,925* in controls.

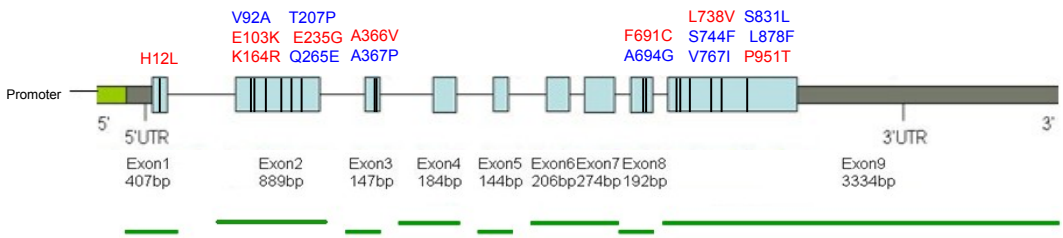

Figure 2: Mapping of the amplicons in TMEM132D and SNPs leading to amino acid exchanges in the protein. The green lines denote the 5 kb PCR amplicons. SNPs coloured in red are overrepresented in cases, blue SNPs in controls (note: amplicons and introns are not drawn to scale).

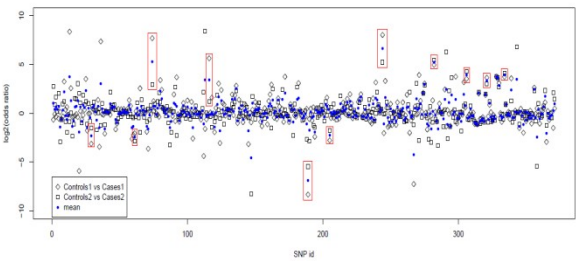

Figure 3: OR of all SNPs, sorted by base position. The red boxes highlight SNPs with high OR in both comparisons (Controls 1 vs Cases 1, Controls 2 vs Cases 2).

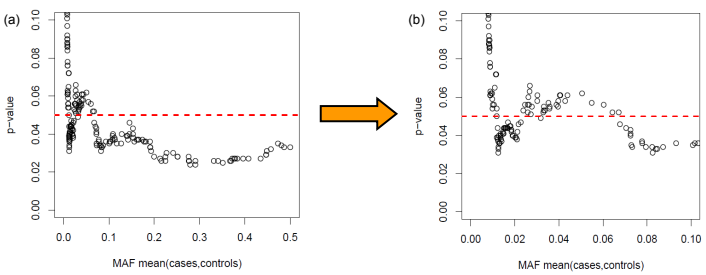

Figure 4: P-values of the additive log OR. (a) Whole MAF spectrum (b) MAF up to 10 %.

Conclusion

The variant screening approach using next-generation sequencing allowed identification of 371 genetic SNPs, 120 of these have frequencies lower than 0,5 %. 17 SNPs are missense mutations. There seems to be a significantly different SNP distribution in cases versus controls (MAF 1-3% and > 7%) in addition to the polymorphisms reported in the GWAS (Erhardt et al,. 2010). Further functional analysis of these SNPs and resequencing of relevant regulatory regions within TMEM132D is currently ongoing.

Disclosure

No potential conflict of interest.

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