Brain-derived neurotrophic factor V66M polymorphism in childhood-onset obsessive–compulsive disorder

Received 24 February 2004; Reviewed 31 March 2004; Revised 17 October 2004; Accepted 19 October 2004

Obsessive–compulsive disorder (OCD) is characterized by recurrent, intrusive, and disturbing thoughts, as well as by repetitive stereotypic behaviour. Insight into the senseless nature of the symptoms is generally preserved. Patients try, albeit usually unsuccessfully, to suppress the obsessive thoughts and compulsive behaviours. Acting out the stereotypic behaviours reduces the anxiety generated by the obsessions and compulsions (APA, 2000). In 60% of patients OCD develops before the age of 25 yr, and onset of disease can already occur in childhood (Flament et al., 1990). Familial loading is higher in early-onset OCD, indicating that genetic factors may be of greater importance in OCD with early onset (Pauls et al., 1995).

Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophin superfamily and influences neuronal proliferation and survival as well as synaptic growth and memory formation (Hariri et al., 2003). Like other members of the neurotrophin superfamily, BDNF is synthesized as a larger precursor peptide, pro-BDNF, which is proteolytically cleaved to yield the mature protein (Seidah et al., 1996). A polymorphism in the BDNF gene leading to a valine→methionine (V66M) substitution in the prodomain of BDNF has been described (Cargill et al., 1999). The structure of the mature BDNF protein does not appear to be influenced by this polymorphism, since the amino-acid exchange is located in the prodomain of the precursor which is removed when mature BDNF is formed. However, it has recently been shown that this BDNF polymorphism influences the intracellular trafficking and packaging of pro-BDNF in cultured hippocampal neurons and thereby the activity-dependent secretion of mature BDNF (Egan et al., 2003). Genomic imaging studies have shown that carriers of the BDNF M66 variant exhibit diminished hippocampal activity during memory encoding and retrieval tasks than subjects with the V66/V66 genotype (Hariri et al., 2003). The reported frequency of the allele encoding V66 is 75% (Cargill et al., 1999; Sen et al., 2003).

A recent study has reported that the BDNF allele encoding V66 confers an increased risk for OCD (Hall et al., 2003). In that study, 44 patients with adult-onset OCD, 38 children with childhood-onset OCD, and 82 adults who reported OCD onset prior to the age of 18 yr, as well as their parents, were genotyped. The transmission rate of V66 in that study was 69% (58 transmissions, 26 non-transmissions; $p = 0.0005$). Since inhibition of the serotonin transporter (5-HTT) by serotonin re-uptake inhibitors such as clomipramine and fluoxetine is the best pharmacological treatment available for OCD (Zohar et al., 2000) and BDNF is a specific growth and differentiation factor for serotonergic neurons (Mamounas et al., 1995) and modulates 5-HTT expression (Mössner et al., 2000), we assessed the BDNF V66M polymorphism in childhood-onset OCD.

Methods

Study sample

Our study sample consisted of 67 (36 male, 31 female) patients (children and adolescents) with childhood-onset OCD (mean age 13.06 yr, s.d. = 2.6; mean age at onset: 11.52 yr, s.d. = 3.0) and both of their biological parents. All index patients had received in-patient treatment for OCD at the Departments of Child and Adolescent Psychiatry of the Universities of Würzburg, Marburg, Freiburg or Technical University of Aachen. The patients and their biological parents were all of German origin. All patients (children and adolescents) agreed to participate in the study and all participants (in case of minors their parents) gave written informed consent. The ethics committees of the Universities of Würzburg, Marburg, Aachen and Freiburg approved the study. Exclusion criteria for patients were: lifetime history of psychotic disorder, Tourette’s syndrome, autistic disorder, alcohol dependence and mental retardation (IQ <70). All patients fulfilled the diagnostic criteria for OCD according to DSM-IV (APA, 2000). To assess the criteria, all patients

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International Journal of Neuropsychopharmacology (2005), 8, 133–136. Copyright © 2004 CINP DOI: 10.1017/S146114570400495X

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were interviewed with (a) the Children’s Yale–Brown Obsessive–Compulsive Scale (C-YBOCS) (Goodman et al., 1989) and (b) ‘Diagnostisches Interview bei psychischen Störungen im Kindes- und Jugendalter’ (DIPS) children version and parents version (Unnewehr, 1995). Subjects with comorbid disorders were only included in the study if OCD predominated. In total, 65.7% of the patients had no comorbid diagnosis. Twenty-three of the children and adolescents with OCD had one comorbid diagnosis; the most frequent ones were attention deficit/hyperactivity disorder (ADHD) which was observed in six children (9%) and different anxiety disorders (n = 6). In the rest of the cases there were conduct disorders (n = 3), depressive disorders (one patient with single episode, one with recurrent episode and two patients had dysthymic disorders). Eating disorders occurred in two patients, dyslexia in one patient and one patient had complex motor tic disorders (but no Tourette’s syndrome).

Genotyping

Genomic DNA was extracted from whole blood according to standard protocols. For the BDNF gene, the G→A single nucleotide polymorphism (SNP) coding for the V66M substitution was genotyped employing a modification of a protocol described by Sen and co-workers (Sen et al., 2003). A 274-bp PCR product containing the SNP was amplified by polymerase chain reaction (PCR) using the following re-action mix: 20 ng of genomic DNA in 75 mM Tris–HCl (pH 9.0), 20 mM ammonium sulphate, 0.01% Tween-20, 1.5 mM magnesium chloride, 0.4 μM of each of the primers, BDNF-for (5’-AAA GAA GCA AAC ATC CGA GGA CAA G) and BDNF-rev (5’-ATT CCT CCA GCA GAA AGA GAA GAG G), 0.4 mM dNTP, and 1 U Taq polymerase. After an initial denaturation for 5 min at 95°C, 35 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 40 s and extension of 72°C for 50 s were performed, followed by a final extension of 72°C for 5 min. PCR products were digested with BseGI. The undigested PCR product carries the A variant, whereas the digested product with three fragments of 57, 77 and 140 bp contains the G allele.

Statistics

The transmission disequilibrium test (TDT) was used to assess the transmission of BDNF alleles from heterozygous parents to affected offspring (Spielman et al., 1993).

Results

In our study sample of 67 patients with childhood-onset OCD, 16 patients (23.9%) had obsessions only, 17 patients (25.4%) had compulsions only and 34 (50.7%) had both obsessions and compulsions. Severity of OCD was assessed by C-YBOCS. The average score of overall severity of OCD symptoms according to C-YBOCS was 22.04 points (S.D. = 8.33 points, median: 23 points, range of C-YBOCS scores: 5–38 points). Our C-YBOCS scale scores suggest that the patients had moderate severity of OCD symptoms. Our data are comparable with other recorded C-YBOCS scores in children and adolescents with OCD (Geller et al., 2001; Hanna et al., 1995). It should be noted that in childhood the recognition of obsessions and compulsions as excessive and unreasonable may be reduced due to insufficient cognitive awareness to make this judgement (APA, 2000).

No preferential transmission of the alleles encoding the M66 or V66 BDNF variants was observed. The transmission rate of V66 was 46% (17 transmissions, 20 non-transmissions; p = 0.62). We, therefore, did not observe an increased risk to develop OCD for carriers of the V66 allele.

Discussion

We have analysed the functional V66M polymorphism of the BDNF gene in a sample of 67 childhood-onset OCD patients and their parents, employing the TDT. We did not find a preferential transmission of the V66 BDNF variant, in contrast to the finding of a previous study (Hall et al., 2003). In the study by Hall and co-workers, the transmission rate of V66 was 69% (58 transmissions, 26 non-transmissions; p = 0.0005). The observed number of 37 heterozygous parents in our study provides a power of 77% for a one-sided test of a preferential transmission of V66 if the transmission rate is indeed 69%.

Our sample consisted of children with a very early onset of OCD, with a mean age of onset of 11.52 years. In this group, which due to its very early onset is thought to represent a more severe form of OCD with a greater importance of genetic factors, the V66M polymorphism does not appear to be relevant for the development of OCD. In contrast to our investigation, where the severity of OCD was measured by the C-YBOCS, the study of Hall and co-workers have not reported direct measurements of the severity of OCD at the time of investigation, thus making a direct comparison difficult. Moreover, the latter study suggests that their sample is quite heterogeneous and
may represent a wide range of severity levels and clinical phenomenology (Hall et al., 2003). Furthermore, in the study of Hall and co-workers most of the probands were recruited through advocate newspapers (Hall et al., 2003), while in our study patients who had received in-patient treatment for OCD at a Department of Child and Adolescent Psychiatry were included.

In conclusion, the V66M polymorphism, which influences intracellular packaging of pro-BDNF and activity-dependent secretion of BDNF, does not appear to be a risk factor for very early-onset OCD in our study group.

Acknowledgements

We thank all probands for their participation. We also thank T. Elpel, G. Ortega, and N. Steigerwald for excellent technical assistance. The Bundesministerium für Bildung und Forschung (BMBF, 01KW006; 01GS0118) and the Deutsche Forschungsgemeinschaft (SFB 581; KFO 125/1-1) supported the molecular genetic and biometrical analyses.

Statement of Interest

None.

References


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