Increased BDNF levels and NTRK2 gene association suggest a disruption of BDNF/TrkB signaling in autism


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The brain-derived neurotrophic factor (BDNF), a neurotrophin fundamental for brain development and function, has previously been implicated in autism. In this study, the levels of BDNF in platelet-rich plasma were compared between autistic and control children, and the role of two genetic factors that might regulate this neurotrophin and contribute to autism etiology, BDNF and NTRK2, was examined. We found that BDNF levels in autistic children (n = 146) were significantly higher (t = 6.82; P < 0.0001) than in control children (n = 50) and were positively correlated with platelet serotonin distribution (r = 0.22; P = 0.004). Heritability of BDNF was estimated at 30% and therefore candidate genes BDNF and NTRK2 were tested for association with BDNF level distribution in this sample, and with autism in 469 trio families. Genetic association analysis provided no evidence for BDNF or NTRK2 as major determinants of the abnormally increased BDNF levels in autistic children. A significant association with autism was uncovered for six single nucleotide polymorphisms (SNPs) [0.004 (Z1 df = 2.85) < P < 0.039 (Z1 df = 2.06)] and multiple haplotypes [5×10⁻⁴ (X²df = 17.77) < P < 0.042 (X²df = 17.450)] in the NTRK2 gene. These results do not withstand correction for multiple comparisons, however, reflect a trend toward association that supports a role of NTRK2 as a susceptibility factor for the disorder. Genetic variation in the BDNF gene had no impact on autism risk. By substantiating the previously observed increase in BDNF levels in autistic children in a larger patient set, and suggesting a genetic association between NTRK2 and autism, this study integrates evidence from multiple levels supporting the hypothesis that alterations in BDNF/tyrosine kinase B (TrkB) signaling contribute to an increased vulnerability to autism.

Keywords: Autism, BDNF, genetic association, heritability, NTRK2

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Autism is a neurodevelopmental syndrome characterized by deficits in social interaction, impaired communication and restricted and stereotyped behaviors (Folstein & Rosen-Sheidley 2001). Although the etiology of autism is unclear, it may result from an abnormal development of the central nervous system (CNS) with alterations of neuronal organization, cortical connectivity and neurotransmitter pathways, and with a significant genetic influence (Pardo & Eberhart 2007).

Multiple lines of evidence directly or indirectly suggest an involvement in autism of the brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, which plays a pivotal role in the development and plasticity of the brain. Brain-derived neurotrophic factor has a trophic effect for specific neuronal populations during development and in the mature brain, influencing neuronal survival, morphology, differentiation and synaptic strength (Lang et al. 2004). Like other neurotrophins, BDNF acts through transmembrane receptors that modulate diverse biological actions, namely the specific high-affinity tyrosine kinase B (TrkB) receptor, encoded by the NTRK2 gene, and the non-specific low-affinity receptor p75. Direct evidence supporting an involvement of the BDNF/TrkB signaling pathway in autism comes from several studies in small population samples showing that BDNF levels in the blood, serum and brain are increased in autistic children compared with normal controls (Connolly et al. 2006; Miyazaki et al. 2004; Nelson et al. 2001; Perry et al. 2001). Genetic studies provide additional support for this hypothesis: in a family-based association study, specific BDNF single nucleotide polymorphism (SNP) haplotypes were associated with autism (Nishimura et al. 2007), and a whole-genome scan with dense SNP coverage, performed by the Autism Genome Project (AGP) consortium in a large set of autism families, identified a linkage peak with genome-wide significance on chromosome 11p12-13, where the BDNF gene maps (AGP et al. 2007). Variants of the BDNF and NTRK2 genes have been associated with several neuropsychiatric disorders, including bipolar disorder, schizophrenia, depression, attention deficit and hyperactivity.
disorder (ADHD) and obsessive compulsive disorder (OCD) (Alonso et al. 2008; Fanous et al. 2004; Neves-Pereira et al. 2002; Ribases et al. 2008). Other observations provide an indirect evidence for a role of BDNF in autism. Psychosomatomics and some antidepressant drugs commonly used for autism treatment, including tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs), modulate the expression of BDNF and its specific receptor TrkB (Duman 1998). Conditional Ntrk2 knockout mice (TrkB-CRE), showing reduced synaptic plasticity, display impaired behavioral flexibility, a trait that is frequently disrupted in individuals with autism (Vyssotski et al. 2002). Other mouse models in which the Ntrk2 gene has been disrupted show hyperlolocomotion, stereotyped behaviors and cognitive impairments, all behavioral problems typically associated with autism (Zorner et al. 2003).

These previous observations led us to hypothesize that BDNF/TrkB signaling alterations in the brain, with consequences to CNS structure and function, may contribute to an increased vulnerability to autism. To test this hypothesis, we first investigated whether BDNF levels in platelet-rich plasma (PRP), the main source of circulating BDNF, are altered in autistic patients relative to healthy control subjects in the Portuguese sample. Platelet- rich plasma dilution of 1/200 with sample buffer supplied by the kit was required to obtain adequate concentrations for quantification. The absorbance was recorded at 450 nm using a SpectraMax 340PC microplate reader (Molecular Devices, Sunnyvale, CA, USA). All BDNF measurements were performed in duplicate, and duplicate determinations of absorbance with a coefficient of variance >10% were excluded. To control for the interassay variance, patient and control samples were always assayed in the same plate, and PRP samples from a group of five subjects, with BDNF concentrations spread across the standard curve, were included in every plate as internal controls. The coefficient of variation for this group in all plates was <15%. In 99 subjects with BDNF measurements, platelet 5-hydroxytryptamine 5-HT had been previously quantified by high-performance liquid chromatography (HPLC) (Coutinho et al. 2004).

**Candidate gene genotyping**

To ensure full genetic coverage, we used information on the CEPH panel from the public release 22 of the HapMap project dataset and HAPLOVIEW to select BDNF and NTRK2 tagSNPs according to the default algorithm (R² > 0.8; minor allele frequency (MAF) >5%). In the BDNF region, 15 SNPs, including the 11 tagSNPs, were genotyped, and in the NTRK2 region, genotyping was carried out for 71 tagSNPs and 7 additional SNPs. Genotyping was carried out using the Sequenom® MassARRAY technology (Sequenom®, San Diego, CA, USA). Polymerase chain reaction (PCR) and MassExtend primers were designed using ASSAY DESIGN Version 3.1 software. The PCR primers were used to amplify 7.5 ng of genomic DNA using standard conditions for MassARRAY genotyping. The chip was scanned using a mass spectrometry workstation (Sequenom MassARRAY System) and the resulting spectra were analyzed using the Sequenom SPECTRO-TYPER-RT Version 3.4 software. HapMap samples were included in the genotyping assays. All markers with a call rate below 80% (1 in the BDNF gene and 3 in the NTRK2 gene) and not in Hardy–Weinberg equilibrium at the 5% significance level (2 in the BDNF gene and 10 in the NTRK2 gene) were excluded from further analysis. After the quality control, 12 markers in BDNF and 65 in NTRK2 were tested for association with autism and BDNF levels.

**Statistical analysis**

The correlation between BDNF levels and other parameters that could influence BDNF levels was ascertained using Spearman’s correlation coefficient or non-parametric tests. To evaluate the differences in BDNF levels between autistic and control children, a linear regression analysis was carried out, including the variables, as potential confounding factors, observed to be correlated with BDNF levels at a significance level of 0.1 (age and medication status). The same software was used for the calculations. Inconsistent Mendelian inheritance, non-paternity or other typing errors in the trio datasets were identified using PEDCHECK (O’Connell & Weeks 1998). Heritability of BDNF levels was estimated using the variance-components (VCE) analysis procedure implemented in Quantitative Transmission Disequilibrium Test (QDT) software Version 2.5.1. With QDT, estimates of the heritability of the trait are obtained by comparing...
We found that 25% of the autistic children have increased serum levels of various that could influence BDNF levels, namely serotonin (5-HT) and noradrenaline (NA). We found no correlation of BDNF levels with age (Spearman’s rank correlation coefficient, r = 0.139; P = 0.072) or gender (Z = 0.419; P = 0.675). We further conducted an association analysis with autism using the Bonferroni method or permutation analysis. To calculate the power of our transmission disequilibrium test (TDT), we used the QTDT program (Clayton & Jones 1999). Uncorrected P < 0.05 was regarded as significant. Multiple testing correction was carried out using the Bonferroni method or permutation analysis. To calculate the power of our transmission disequilibrium test (TDT), the QTDT program was used (Lange & Laird 2002). We assumed an additive model, a disease prevalence of 0.001 with a targeted significance level of 0.05 and marker and disease allele frequencies of 0.2.

Results

**PRP BDNF levels in autistic vs. control children**

The distribution of BDNF PRP levels in 146 autistic and 50 control Portuguese children is shown in Fig. 1. Mean BDNF levels in autistic patients and in control children were 40.44 ± 13.87 ng/ml and 23.26 ± 12.34 ng/ml, respectively. We found that 25% of the autistic children have increased BDNF levels, defined as values above the control mean ± 2 SD (47.95 ng/ml). We found no correlation of BDNF levels with several clinical, demographic and experimental variables that could influence BDNF levels, namely gender (Z = 0.419; P = 0.675), age (Spearman’s rank correlation coefficient, r = 0.139; P = 0.094), medication status (χ²(1) = 3.614; P = 0.057), developmental level (assessed using the autism severity (assessed using the Childhood Autism Rating Scale CARS) (Spearman’s rank correlation coefficient, r = 0.016; P = 0.851) and date of sample collection (χ²(3) = 1.169; P = 0.761). Linear regression analysis showed significant effects of medication and age (t = 2.84; P = 0.005 for age; t = 2.58; P = 0.011 for medication) and, after adjusting for these confounding factors, we found a highly significant difference in BDNF levels between autistic and control children (t = 6.82; P < 0.0001).

Because platelet hyperserotonemia has consistently been associated with autism and several findings indicate that there is a positive, reciprocal interaction between 5-HT and BDNF (Duman 1998; Mattson et al. 2004), with 5-HT stimulating the expression of BDNF and BDNF enhancing the growth and survival of 5-HT neurons (Zetterstrom et al. 1999), we analyzed the correlation between PRP BDNF and platelet serotonin levels in the affected children. The distribution of PRP BDNF in this population sample showed a significant positive correlation with platelet serotonin levels (Spearman’s rank correlation coefficient, r = 0.222; P = 0.004).

**Heritability of BDNF levels and investigation of candidate genes**

The polygenic heritability of PRP BDNF was estimated at 29.7%, indicating that almost one third of the total variance in BDNF levels can be explained by a genetic component. This observation prompted us to investigate the role of two candidate genes involved in the regulation of BDNF, BDNF and NTRK2, which might contribute to its increase in about 25% of the autistic children in this study population.

Using a family-based association test for quantitative traits, the QTDT, we could not find any evidence for an association of the BDNF or NTRK2 genes with the distribution of PRP BDNF.

We further conducted an association analysis with autism in a larger trio set. The tested population sample consisted of 469 trios with 87% powered to detect association at a significance level of 5%. Results of FBAT showed a significant transmission disequilibrium for several SNPs in the NTRK2 gene: rs1187321, located 5′ of the gene, rs1211166, rs93584, rs1187352 located in intron 5-6 and rs4144550 in intron 15-16 and a nominal association with the rs4578034 marker in intron 22-23 (0.004 < P < 0.039, uncorrected for multiple testing).

Table 1: Transmission disequilibrium test results of significantly associated NTRK2 markers with autism in the 469 trio dataset

<table>
<thead>
<tr>
<th>Marker</th>
<th>Over transmitted allele</th>
<th>Afreq</th>
<th>Fam#</th>
<th>S − E(S)</th>
<th>Z</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs1187321</td>
<td>A</td>
<td>0.79</td>
<td>211</td>
<td>23.0</td>
<td>2.85</td>
<td>0.004</td>
</tr>
<tr>
<td>Rs1211166</td>
<td>A</td>
<td>0.79</td>
<td>208</td>
<td>21.5</td>
<td>2.70</td>
<td>0.007</td>
</tr>
<tr>
<td>Rs93584</td>
<td>G</td>
<td>0.58</td>
<td>271</td>
<td>23.5</td>
<td>2.43</td>
<td>0.015</td>
</tr>
<tr>
<td>Rs1187352</td>
<td>C</td>
<td>0.67</td>
<td>268</td>
<td>26.0</td>
<td>2.74</td>
<td>0.006</td>
</tr>
<tr>
<td>Rs4144550</td>
<td>G</td>
<td>0.87</td>
<td>156</td>
<td>16.0</td>
<td>2.41</td>
<td>0.016</td>
</tr>
<tr>
<td>Rs4578034</td>
<td>C</td>
<td>0.43</td>
<td>279</td>
<td>20.0</td>
<td>2.06</td>
<td>0.039</td>
</tr>
</tbody>
</table>

afreq, allele frequency; E(S), expected value under the null hypothesis; fam#, the number of informative families; S, test statistic; Z, S normalized. The P-values shown are not corrected for multiple testing.

Involvement of BDNF/TrkB signaling in autism

![Figure 1: Distribution of BDNF levels in the platelet-rich plasma of autistic and control children.](image)

The number of individuals with autism is represented on the Y-axis, plotted in a decimal metric scale, and on the X-axis is shown each PRP-PDNF levels interval (measured in ng/ml). The bars in dark grey represent the control sample, whereas the bars in light grey represent the individuals with autism.
Evidence of association with autism was also found for two-, three-, four- and five-marker haplotypes containing the associated SNPs in the 5′ region and intron 5-6 (Table 2), with the strongest association observed for haplotype rs11897952-rs1659409 [\(\chi^2(3) = 17.77; P = 0.0005\), uncorrected P-value]. Significant two- and three-marker haplotypes including SNPs not individually associated were also identified in intron 22-23, with haplotype rs4271046-rs4578034-rs12001219 being the strongest finding [\(\chi^2(5) = 17.41; P = 0.0038\), uncorrected P-value]. No haplotype association remained significant after correction for multiple comparisons. Pair-wise D′ values across the association regions were determined (Fig. 2) and the LD pattern shows that the association results are driven by both populations. Evidence of association with autism was also found for three- and four-marker haplotypes without SNPs individually associated (Table 2), with the strongest finding being for haplotype rs42710249-rs1659409-rs12001219-rs1189752-rs1659409 [\(\chi^2(9) = 20.230; P = 0.0061\). These observations therefore indicate that the association results are driven by both populations.}

There was no evidence for association of BDNF markers with autism in the population tested.

**Discussion**

In the present study, we examined the involvement of the BDNF signaling pathway in autism using various approaches. First, PRP BDNF levels were compared in autistic and control children, in the largest population sample thus far reported. The observed BDNF increase in 25% of our autistic patients extended previous evidence for quantitative BDNF anomalies in autism, obtained in smaller cohorts and in diverse tissues (Connolly et al. 2006; Hashimoto et al. 2006; Katoh-Semba et al. 2007; Miyazaki et al. 2004; Nelson et al. 2001; Perry et al. 2001). A significant correlation with age was detected, corroborating previous studies showing that serum or plasma BDNF levels in rats (Karege et al. 2002) and healthy human subjects (Katoh-Semba et al. 2007; Lommatsch et al. 2005; Nelson et al. 2006). The low number of medicated patients precluded the analysis of the effect of different drug groups separately. No significant difference in BDNF levels between males and females was observed, in agreement with previous reports (Katoh-Semba et al. 2007; Lommatsch et al. 2005; Nelson et al. 2006).

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**Table 2**: Significant NTRK2 haplotype association results for the 469 trio dataset

<table>
<thead>
<tr>
<th>Haplotype Location</th>
<th>Over transmitted</th>
<th>Under transmitted</th>
<th>Global P</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3 5′ Region 11.218 (3) 0.0106</td>
<td></td>
<td>CT/TT</td>
<td>4.453/6.604</td>
<td>0.0350/0.010</td>
<td></td>
</tr>
<tr>
<td>3,4 5′ Region/intron 5-6 10.220 (3) 0.0168</td>
<td></td>
<td>TC/TT</td>
<td>4.140/6.211</td>
<td>0.0420/0.0130</td>
<td></td>
</tr>
<tr>
<td>4,5 Intron 5-6 13.088 (3) 0.0045</td>
<td></td>
<td>CG/TG</td>
<td>4.427/9.087</td>
<td>0.0350/0.0026</td>
<td></td>
</tr>
<tr>
<td>8,9 Intron 5-6 10.058 (3) 0.0181</td>
<td>GC 5.837 0.0157</td>
<td>AT 7.910 0.0049</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,10 Intron 5-6/intron 7-8 17.765 (3) 0.0005</td>
<td>CA 7.076 0.0078</td>
<td>TA/TG 6.296/8.445</td>
<td>0.0121/0.0037</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43,44 Intron 19-20 11.640 (3) 0.0087</td>
<td></td>
<td>AC 6.397 0.0114</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61,62 Intron 22-23 8.379 (3) 0.0388</td>
<td></td>
<td>TG 4.510 0.0337</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63,64 Intron 22-23 13.355 (3) 0.0039</td>
<td>GC 5.668 0.0173</td>
<td>AT 9.090 0.0026</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64,65 Intron 22-23 8.761 (3) 0.0326</td>
<td></td>
<td>TT/TC 4.280/4.106</td>
<td>0.0396/0.0430</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,5,6 5′ Region/intron 5-6 16.170 (6) 0.0129</td>
<td>CTTGT/TTTGT 7.410/8.695</td>
<td>0.0061/0.0032</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4,5 Intron 5-6 14.925 (6) 0.0208</td>
<td></td>
<td>TCG/TTG 4.395/8.917</td>
<td>0.0360/0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41,42 Intron 6-6 16.387 (6) 0.0118</td>
<td></td>
<td>TTGT 8.883 0.0029</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,3,4,5 5′ Region/intron 5-6 13.361 (6) 0.0376</td>
<td></td>
<td>CTGT/TTGT 7.534/8.230</td>
<td>0.0061/0.0032</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,3,4,5,6 5′ Region/intron 5-6 13.881 (5) 0.0164</td>
<td></td>
<td>CTGT/TTGT 7.410/8.695</td>
<td>0.0061/0.0032</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8,9,10 Intron 5-6/intron 7-8 22.287 (7) 0.0023</td>
<td>GCA 5.268 0.0217</td>
<td>ATA/ATG 5.545/9.387</td>
<td>0.0185/0.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61,62,63 Intron 22-23 11.955 (7) 0.0122</td>
<td></td>
<td>TAA 9.850 0.0017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62,63,64 Intron 22-23 19.027 (7) 0.0081</td>
<td></td>
<td>AAT/GGT 9.083/4.381</td>
<td>0.0026/0.0360</td>
<td></td>
<td></td>
</tr>
<tr>
<td>63,64,65 Intron 22-23 17.412 (5) 0.0038</td>
<td>GCC 5.003 0.0253</td>
<td>ATC/GTT 9.063/4.228</td>
<td>0.0026/0.040</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The SNP numbers used here are as shown in Fig. 2. The P-values shown are not corrected for multiple testing.
Figure 2: Schematic diagram of the NTRK2 gene showing the location of the 24 exons (vertical lines), the functional domains of the encoded protein (horizontal gray bars) and genotyped SNP marker positions. Linkage disequilibrium plots for the two NTRK2 regions associated with autism are shown. Markers showing association with autism in the dataset are boxed. LRR, leucine-rich region; Ig-like, immunoglobulin-like domain.
The strong positive correlation found between BDNF PRP levels and platelet serotonin levels is consistent with the described crossregulation between BDNF and serotonin (5-HT) (Mattson et al. 2004) and with the platelet hyperserotonemia found in subgroups of autistic individuals (Coutinho et al. 2004).

The origin of increased BDNF in PRP, and its relation to brain BDNF levels, is not clear. Although BDNF is highly concentrated in the nervous system, it is also found in other tissues including blood, where it is essentially stored in platelets and thought to be involved in platelet activation and associated events (Fujimura et al. 2002). This is not unexpected, as platelets and neurons develop from a common embryonic precursor in the neural crest (Pearse 1980). Karege et al. (2002) have shown that the variation profile of BDNF protein in serum and brain is similar during postnatal development in rats, with a positive correlation between serum and cortical BDNF levels (Karege et al. 2002). In humans, the developmental curve of serum BDNF has been shown to be similar to that of the neurotrophin in the rat neocortex (Katoh-Semba et al. 2007; Nelson et al. 2006). These observations suggest that BDNF levels in the blood may reflect BDNF levels in the brain. This parallelism between brain and blood variation is similar to what has been postulated for the serotonergic system. A fully functional serotonin pathway is required for platelet aggregation, and many of the molecules involved in this system are present both in brain and platelets. Platelet hyperserotonemia has been a hallmark of autism, with variation in serotonin-related genes associated with this trait thought to functionally disturb the serotonin pathway both in the CNS and in platelets. Although the origin and function of BDNF in blood are less clear, our finding of a strong correlation between platelet serotonin and BDNF levels suggests a parallel variation between serotonin and BDNF, both in brain and in the periphery. On the other hand, the estimation of BDNF heritability close to 30% supported the hypothesis that the observed BDNF increase in autistic individuals results, at least in part, from a genetic primary defect that may have an effect on BDNF signaling pathways in every tissue where it is present, namely blood and brain.

None of the tested markers in the NTRK2 and BDNF genes, fully covering genetic variation in these regions, were associated with BDNF levels, including the described BDNF functional polymorphism Val66Met (rs6265), associated with abnormal intracellular trafficking and regulated secretion of BDNF (Egan et al. 2003). The lack of association of the Val66Met polymorphism with BDNF levels was consistent with previous reports in other neuropsychiatric conditions or healthy subjects (Duncan et al. 2008; Mercader et al. 2007; Trajkovska et al. 2007; Yu et al. 2008), while previous studies examining the association of BDNF with autism report discrepant results (Nishimura et al. 2007). We suggest that the increase in levels of BDNF is not due to variants of the BDNF gene or its receptor, which means that other genes that affect BDNF transcription and secretion are likely responsible for this change. However, we cannot rule out the hypothesis that association with BDNF levels could not be detected because of the low power of the tested sample.

The current study provided for the first time evidence for an association between the NTRK2 gene and autism. Two main regions of susceptibility in the NTRK2 gene were identified (from the 5’ end to intron 7-8 and in intron 22-23), and should have high priority in searching for functional variants. Exons 6, 7 and 8 together encode a leucine-rich domain that has a crucial role in ligand binding, while exons neighboring intron 22-23 encode a tyrosine kinase domain (Stoilov et al. 2002). A bioinformatics investigation of the putative functional effects of the associated SNP in the 5′ region, using GENOMATIX, showed that this SNP lies in the core of a sequence with high similarity to the matrix of the homeomain transcription factors (HOMF) family and of the factors with moderate activity to homeomain consensus sequence HOXF family, and that allele T in this SNP disrupts the binding of these factors. Many of these homeobox genes, such as PITX1, HOXA1 and the DLX family, have been associated with autism (Ingram et al. 2000; Filippi et al. 2007) and, remarkably, a recent functional study found that NTRK2 is a transcriptional target of DLX2 during mouse retinal development (de Melo et al. 2007).

The genetic association results were not significant after correction for multiple comparisons. Working with a relatively small population sample, this result is not unexpected, as the increase in risk conveyed by common genetic factors will likely be subtle for autism, as is the case for most complex disorders. As there is no consensus regarding the most appropriate method for multiple testing correction, it is generally accepted that the replication of a genetic association in independent populations is considered the best evidence of a true association. We observe in this study that the two datasets included, with distinct genetic ancestries, show the same pattern of allelic transmissions for the significantly associated SNPs and haplotypes, indicating that the association in the combined sample was driven by both populations. Further genetic studies in larger datasets are required to fully validate this hypothesis.

In the absence of definitive statistical evidence or a method for appropriately correcting for multiple testing, approaches that integrate evidence from multiple levels, including biological observations, are also valuable tools for assessing the validity of results. In this study, we provide two lines of evidence which, taken together, suggest that an alteration in the BDNF/TrkB signaling is involved in autism etiology, because of the presence of risk genetic variants of the receptor, or to a genetically determined increased level of the ligand. Because we found no evidence for a role of NTRK2 in determining the abnormally increased BDNF, we suggest that these two processes are separate. Other genetic factors would be expected to contribute to the increase in BDNF, while NTRK2 risk variants operate downstream in this pathway. Interestingly, there is increasing evidence that several syndromic disorders co-morbid with autism, rare mutations and common variants associated with autism converge on dysfunctional signaling of the Phosphatidylinositol-3-kinase (PI3K)-Akt pathway, one of the three signaling pathways activated by BDNF (Levitt & Campbell 2009). A genetically determined disruption of BDNF/TrkB signaling in autism may therefore occur at different levels: in the availability of the ligand, in the catalytic or binding affinity of the receptor or in alterations of the signaling cascades. Abnormalities in various neurotransmitter systems have been implicated in autism,
including the glutamatergic, GABAergic and serotonergic systems (Lam et al. 2006), all of which are regulated by the BDNF/TrkB signaling system. It is therefore a plausible hypothesis that altered BDNF/TrkB signaling may be an upstream event contributing to abnormalities in these neurotransmitter systems, frequently reported in autism.

In conclusion, our study provides evidence for a dysregulation of the BDNF/TrkB signaling system in autism. We report an increase in BDNF PRP levels in 25% of the autistic children in this population sample, the largest tested thus far. We further provide evidence that the NTRK2 gene is a susceptibility factor for autism, although we did not find any evidence for a role of the NTRK2 or BDNF genes in mediating the BDNF increase in autistic children. We therefore propose that a disruption of the BDNF/TrkB signaling pathway is associated with autism and, consistent with the etiological heterogeneity expected for autism, can occur at different levels, including the availability of the ligand, the catalytic or binding affinity of the receptor and the subsequent signaling cascades.

References


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