

Short communication

The effect of *BDNF* gene variants on asthma in German children

Background: Allergic inflammation can trigger neuronal dysfunction and structural changes in the airways and the skin. Levels of brain-derived neurotrophic factor (BDNF) are strongly up regulated at the location of allergic inflammation.

Aim: We systematically investigated whether polymorphisms in the *BDNF* gene influence the development or severity of asthma and atopic diseases.

Methods: The *BDNF* gene was screened for mutations in 80 chromosomes. Genotyping of six *BDNF* tagging polymorphisms was performed in a cross-sectional study population of 3099 children from Dresden and Munich (age 9–11 years, ISAAC II). Furthermore, polymorphisms were also investigated in an additional 655 asthma cases analysed with a random sample of 767 children selected from ISAAC II. Associations were calculated via chi-square test and ANOVA using SAS Genetics and SPSS.

Results: We identified nine polymorphisms with minor allele frequency ≥ 0.03 , one of them leading to an amino acid change from Valine to Methionine. In the cross-sectional study population, no significant association was found with asthma or any atopic disease. However, when more severe asthma cases from the MAGIC study were analysed, significant asthma effects were observed with rs6265 (odds ratio 1.37, 95% confidence interval 1.14–1.64, $P = 0.001$), rs11030101 (OR 0.82, 95%CI 0.70–0.95, $P = 0.009$) and rs11030100 (OR 1.19, 95%CI 1.00–1.42, $P = 0.05$).

Conclusions: As in previous studies, effects of *BDNF* polymorphisms on asthma remain controversial. The data may suggest that *BDNF* polymorphisms contribute to severe forms of asthma.

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Asthma is the most common chronic lung disease in childhood affecting approximately 10% of children worldwide. Asthma is characterized by reversible airflow obstruction and apart from local inflammation, neuronal signalling and neuropeptides seem to be involved in these mechanisms. Neurotrophins, such as brain-derived neurotrophic factor (BDNF) are important mediators of inflammation and linking immune cells and neurons. BDNF is released by mast cells, lymphocytes, monocytes and airway epithelial cells upon inflammation (1–3). Elevated levels of neurotrophins were observed in the blood (4) and bronchoalveolar lavage fluid during allergic inflammation (5), increasing even more after allergen provocation (6) and normalizing after anti-inflammatory treatment (7). In patients with atopic dermatitis BDNF blood levels correlate with disease severity (8). Mice treated with anti-BDNF antibodies showed reduced neuronal hyperreactivity in response to capsaicin (9).

Thus, we speculated that genetic variation that influences BDNF expression or function could also influence the development, perpetuation and severity of airway inflammation, bronchial hyperresponsiveness (BHR) and

asthma. Previous studies investigating *BDNF* polymorphisms in asthma lacked a systematic approach and were inconclusive (10, 11). We, therefore, systematically screened the *BDNF* gene for genetic alterations and investigated the effects of *BDNF* polymorphisms on asthma and atopic diseases in large German populations.

Methods

Mutation screening

DNA from 40 unrelated, randomly selected adult volunteers were sequenced for 11 509 bp in and around the *BDNF* gene using an ABI Prism 3730 sequencer (Applied Biosystems, Foster City, CA, USA). This included 832 bp of the 5' flanking region, exon 1 Isoform b and exon 1 isoform a4 (Chromosome 11: 27,700,704–27,698,220), exon 1 isoform a3, a2, c and a (chr11: 27,679,777–27,675,443) exon 2 isoform a, b and c and 468 bp of the 3' flanking region (chr11: 27,637,471–27,632,552). Thirty overlapping fragments covering the gene regions of interest were amplified by polymerase chain reaction (PCR) with specific primers designed using the NetPrimer software and obtained from Metabion GmbH (Planegg-Martinsried, Germany) as described in Table S1.

Association studies

Two study designs were used for association analyses: (i) cross-sectional ($n = 3099$, International Study of Asthma and Allergies in Childhood II, ISAAC II) and (ii) case-control [cases of MAGICs ($n = 655$) and stratified random sampling of reference children from ISAAC II: $n = 767$; $n = 73$ asthmatics and $n = 694$ healthy controls]. Children with asthma from the reference sample were treated as cases in the analysis. Written informed consent was obtained from all parents of children included in these studies. Study methods were approved by the respective ethics committees.

Between 1995 and 1996, a cross-sectional ISAAC phase II study was conducted in Germany (Munich: $n = 1159$, Dresden: $n = 1940$) to assess the prevalence of asthma and allergies in schoolchildren 9–11 years of age (12). Children were classified as having asthma if parents reported a physician's diagnosis of asthma at least once, or of spastic or asthmatic bronchitis more than once in self-administered questionnaires. Children's classification of hay fever or atopic eczema was based on a parental report of a physician's diagnosis. A child was considered atopic with a wheal reaction of ≥ 3 mm after skin prick test (SPT) to one or more of six common allergens after subtraction of the negative control. Standard baseline lung function was measured and unspecific bronchial reactivity was assessed in a random 50% sub-sample of the study population by inhalation of nebulized, hyperosmolar saline (4.5%). Children with a drop in FEV1 of 15% or more from baseline were classified as positive for BHR. Demographic characteristics have been summarized previously (13).

From 2001 to 2007, the Multicenter Asthma Genetic in Childhood (MAGIC) study recruited children in seven German and Austrian clinical asthma centres in Bochum, Cologne, Feldkirch, Freiburg, Munich/Rosenheim, Vienna and Wesel (14). Paediatric pulmonologist diagnosed asthma and allergy according to clinical guidelines and objective measures such as lung function tests, clinical examination, history and allergy testing in 865 children (mean age 11 years, range 3–22 years, 64% male). All 655 children of German origin with sufficient high-quality DNA available at the time this study started in 2006 were included in this analysis.

Genotyping

BDNF polymorphisms were genotyped using matrix-assisted laser desorption/ionization time-of flight (MALDI-TOF) mass spectrometry (Sequenom Inc., San Diego, CA, USA) (Table S2) except for SNPs rs6265 and rs11030101, which were genotyped by Sentrix HumanHap300 Genotyping BeadChips13 (Illumina, San Diego, CA, USA) in this case control study population only (14).

Statistical methods

Linkage disequilibrium (LD) patterns and the LD block structure were analysed by Haploview using all *BDNF* SNPs with a minor allele frequency (MAF) ≥ 0.03 . SNPs were tested for deviations from Hardy–Weinberg equilibrium (HWE). Association between SNPs and dichotomous outcomes were tested using chi-square tests in an allelic and dominant model. Differences in lung function parameters were tested by univariate variance analysis and *t*-tests in dominant or recessive models. All tests were two-sided and the differences were considered significant with $P < 0.05$. Power calculation had been performed using the PGA program (15). The power to detect significant asthma associations of a SNPs with an allele frequency of at least 0.1 and an effect size of 1.5 in a dominant disease model was close to 90 in the cross-sectional population and exceeded 90 in the case-control study. Calculations were carried out with SAS 9.1.3 or SAS Genetics.

Results

Results of mutation screening are summarized in Fig. 1 and Table 1. Nine polymorphisms with MAF ≥ 0.03 were identified. No polymorphisms were found in the putative promoter region, three were located in intronic regions and six in exons. The newly identified SNP G22025A previously not described in public SNP databases (dbSNP) was located in the coding region of exon 1, isoform c; SNP rs6265 in the coding region of exon 2 leading to an amino acid change from Valine to Methionine. In addition, seven rare mutations with MAF below 0.03 were identified but not included in further analyses (data not shown). A SNP labelled -270C/T in previous reports (10) was identified as C21227T in our screening population but not followed further due to a MAF of 0.025.

Based on SNPs with MAF ≥ 0.03 two LD blocks were identified with $r^2 > 0.8$ (Fig. 1/Table 1). SNPs rs7944119, rs61888800, rs7124442 were contained in block one, whereas rs11030100 and rs11030099 were found in block two. The other four polymorphisms could not be attributed to any LD block. Therefore, four single SNPs and one tagging SNP per LD block were genotyped in a cross-sectional study ($n = 3099$) and a case-control design for asthma ($n = 1422$). All polymorphisms were genotyped successfully in 94% to 97.5% of subjects and showed no significant deviation from HWE (Table S2).

BDNF SNPs showed no effects on doctor's diagnosis of asthma, BHR, atopic dermatitis, hay fever and atopy in the cross-sectional population. When the same SNPs were analysed in the case-control population SNPs rs11030101, rs6265 and rs11030100 were significantly associated with asthma (Table 1).

Discussion

We performed a systematic mutation screening of the *BDNF* gene, including all exons, 5' and 3' flanking regions and intronic regions adjacent to exons. By screening 80 chromosomes we identified a number of common polymorphisms in *BDNF* exons with a low risk of missing common polymorphisms in the gene. One additional exonic SNP previously not reported in public databases was identified. Interestingly, *BDNF* is highly polymorphic in its exon sequence and strongly conserved in its proximal promoter region. Allele frequencies in our populations did not differ significantly from previous reported frequencies in Europeans, but from those reported in Asian and African populations (<http://www.hapmap.org>).

We did not find an association between *BDNF* SNPs and asthma or atopic phenotypes in a cross-sectional study population recruited through schools in two major German towns. No association with lung function measurements or BHR was found as in previous studies

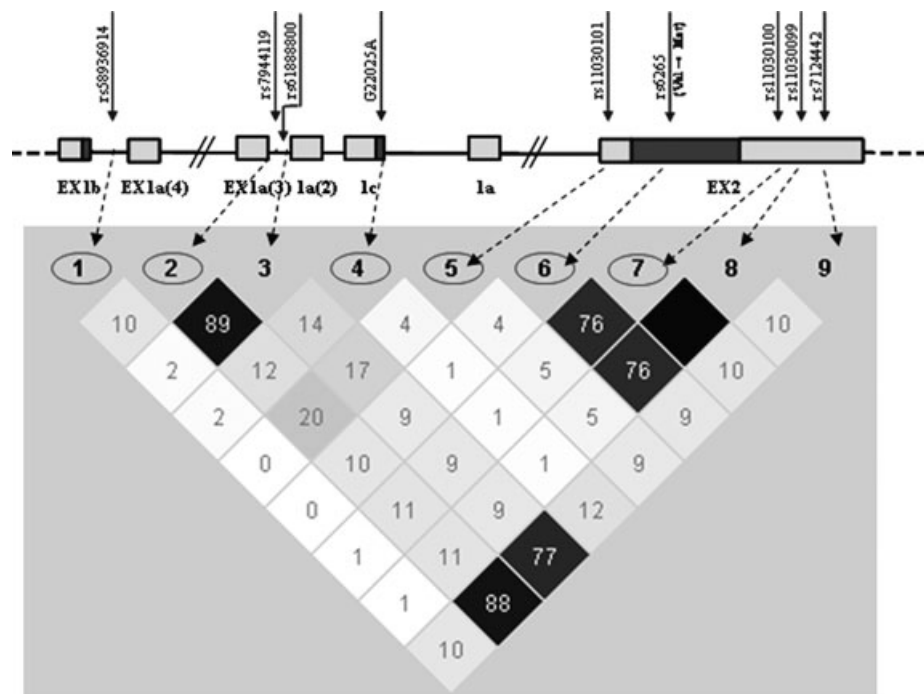


Figure 1. Depiction of the BDNF gene on chromosome 11, showing exon structure, positions of common SNPs, and linkage disequilibrium (r^2 plot) in the sequenced population sample ($n = 40$). LD Plot performed by Haploview with the given Colour code: white ($r^2 = 0$), shades of grey ($0 < r^2 < 1$) and black ($r^2 = 1$). Circles indicate the six polymorphisms genotyped.

Table 1. BDNF single nucleotide polymorphisms (SNPs) positions in relation to ATG (and rs numbers), position, minor allele frequencies (MAF) and association with asthma

SNP	SNP location	MAF	Case-control ($n = 1422$, 728 asthma cases)*		Cross-sectional ($n = 3099$, 267 asthma cases)*	
			OR (95% CI)	P-value	OR (95% CI)	P-value
G438C (rs58936914)	Intron	0.047	1.16 (0.80–1.68)	0.43	0.78 (0.48–1.25)	0.30
C20664A (rs7944119)	Intron	0.277	1.04 (0.88–1.22)	0.48	0.98 (0.80–1.19)	0.81
C20684A (rs61888800)	Intron	0.300†	Tagged by rs7944119		Tagged by rs7944119	
G22025A N/A	Exon 1 isoform c	0.051	0.95 (0.69–1.32)	0.73	0.96 (0.64–1.46)	0.86
T62218A (rs11030101)	Exon 2 isoform a	0.481	0.82 (0.70–0.95)	0.009	1.03 (0.86–1.24)	0.71
G63046A (rs6265)	Exon 2/CDS	0.195	1.37 (1.14–1.64)	0.001	1.01(0.81–1.27)	0.93
C65376A (rs11030100)	Exon 2/3'UTR	0.222	1.23 (1.02–1.47)	0.026	0.98 (0.79–1.22)	0.87
C65379T (rs11030099)	Exon 2/3'UTR	0.188†	Tagged by rs11030100		Tagged by rs11030100	
A65921G (rs7124442)	Exon 2/3'UTR	0.342†	Tagged by rs7944119		Tagged by rs7944119	

*Numbers may slightly vary due to differences in missing data per genotyping.
†MAF based on screening population, all other based on cross-sectional population.

(10), although one has to keep in mind that a histamine challenge would have been a more appropriate neuronal stimuli than the challenge with hypertonine saline we had performed. These data are in accordance with a recent report where also no association between BDNF SNPs and asthma was observed for four SNPs which were all

covered by our study (11). In contrast, strongly significant but modest effects, influencing asthma risk by not more than 40%, were observed in our large asthma population where asthma cases were recruited from tertiary referral centres for childhood asthma. Those two SNPs that showed the strongest effects were located within exon 2 of

the *BDNF* gene. The SNP rs6265 associated with asthma in our study and neurological disorders in other studies leads to an amino acid change from Val66Met in the prodomain of BDNF. Previously, this SNP had been shown to alter intracellular trafficking and packing of pro-BDNF, with the met allele showing lower inducible BDNF secretion (16). It had been discussed that this polymorphism effect could explain altered BDNF activity and airway reactivity (10).

The results in the case-control study remain significant even when corrected for the number of test performed (0.05 divided by six SNPs and four main outcomes = 0.002). These data may indicate that *BDNF* SNPs may play a role in more severe forms of childhood asthma or, that *BDNF* SNP effects are so modest that very large populations are needed to observe these effects. Indeed, our case-control design, having the best power of all studies on the role of genetic variants in asthma so far, comprises three times more asthmatics than our cross-sectional study population and the asthma phenotype was assessed by clinical experts.

In contrast to modest genetic effects, there is some evidence for a role of *BDNF* in allergic inflammation of the lung and the skin (17, 18). While BDNF may very well be involved in disease mechanisms of asthma and allergy, this does not necessarily translate into a major role of the gene in disease susceptibility.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1. Description of the primers used for PCR amplification and re-sequencing.

Table S2. *BDNF* SNPs, primers used in cross sectional (A) and case-control (B) study designs, minor allele frequency (MAF), successful genotyping call rate (Call R in %) and *P*-values for deviation from Hardy-Weinberg equilibrium (pHWE).

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ONLINE SUPPLEMENT

Table S1

Description of the primers used for PCR amplification and re-sequencing

Fragment	Length	1st 5' - 3'	2nd 3' - 5'	Sequ.pr fwd/ 5' - 3'
1	501	gcaggatgaggaagcggc	CACTGTGACTCTCTGGGTAAAAAAG	
2	696	c[t->a]cggcttacacaggttcc	AATGAGCGAGGTTACCAATG	ccccccagtccccatttg
3	553	ttcagtgccaggaccagtaac	ACTGTGTAAAAACCCGATTCCC	
3 a I	415	ggcacaatggtgagtagcaataaaac	CCCCTAACCCCAACCTCTAACAGC	
3 a II	436	ctttcctt[a->g]atgtgtatggtgtatg	AATGGACTCCTATCGCCCCG	
4	774	cgtgacaaaccgtaaggaag	GGAAAAGAGGGAGGTGAAGAG	
5	499	cgaggcagcggaggtatc	CAGGAAGCCCAGGCAATGAC	
5a	574	ggtt[a->g]ctttgacaagtagtgac	GCTT[C->A]GAGGGGTGTTCCAG	
5 d	318	ggtgcgggaaggtgtgtctgtg	GCCTGTCCTCACCTCCTCCCC	
6	469	cgtttgaccaa[t->c]cgaagc	GGGAGGAAAGAAGGAGACTG	
6a	477	gctgggaatgggcttggtg	GGGAAGGGATGCGGGCTG	
7	598	cccagcccaggagtagtgc	CAGTTCACCTCGCAGCC	
7a	453	gaagagggaatgggtgagtgag	ATAGAAGCCAAACAAGAACGAG	
7b	424	cttagag[a->g]gctggtaggagg	TGAGTCAGTAGGGAGGGGTC	
7b II	426	tttgttaaggtca[a->g]aagcc	TTCCAGTAAAGGTTATGGT[T->C]AC	
7c	403	aatcacta[a->c]actcctgttcctg	CGTAAGCCTTGTTTATCTGG	
8	578	catacctttggagccttcatag	TACTATCAGGTGGCGTGC	
9	551	ccccaggcatagacacatc	TTCTCCTTCTTCCCACTTTAGC	
10	430	tgctgttcctgtcacacattcc	AACTGGTTAGGGCTTTCTTTCAC	
11	461	tacagcacactactgacactgatttg	GTCTGCGTCCTTATTGTTTTCTTC	
12	487	caaggcaggttcaagaggc	GGTAGTTCGGCACTGGGAG	
13	494	tgaaaagggtccctgtatcaaaag	GGCAACAAACCACAACATTATC	
14	896	aaggggaagatagtggtattatgtg	AAGAGGAGACCAGAGGGGGAG	
14 a	436	ttaggggatgg[g->a]ggatgg	GTGTTCACTTGTTTCACAGCAG	
15	431	gaacaaaaaatgctcacactcc	CTGGGTTGATACAGGGCTCTAC	
16	453	aatgaagattattttatgaggagtg	CAGAGGTGGGATGGTGGG	
17	501	gctgcttgacctcgtgc	CCATGAACAGACAGGATGGGC	
18	540	gtccagagacaagacttcggg	TCAAACAACACAAAACAATCTACAC	
19	521	ggataagcaaaagaggatgttcacc	ACACTTCTGCCACAAAGAGACCAC	
20	968	ttttaccaataagatacatcacagtcac	GCACCACTTCCAACCGAGG	taaccgcatcagaagcctattg

Table S2

***BDNF* SNPs, primers used in cross sectional (A) and case control (B) study designs, minor allele frequency (MAF), successful genotyping call rate (Call R in %) and p-values for deviation from Hardy-Weinberg Equilibrium (pHWE).**

(A)	1st PCR Primer 2nd PCR Primer Extension Primer	MAF	Call R%	pHWE
rs number				
rs58936914	ACGTTGGATGCCCCCATCTACACCTAAAC ACGTTGGATGTAATGCTGTTAGAGGTTGGG GGAGTGGGGTAAGGGTGGGGTAAA	0.0474	94.38	0.8592
rs7944119	ACGTTGGATGACATCGCCCTGCGAGTCCT ACGTTGGATGTGAGGCTGGGGCTGGAACAC CCGAGAACCCCCTCGAAGC	0.2771	96.12	0.2861
N/A	ACGTTGGATGTCCAGGAGTAACTCACTCAC ACGTTGGATGGGATTCTTGACTCGGGTTAG TGGTGATGCAGAGCCGGGAA	0.0505	97.18	0.1030
rs11030101	ACGTTGGATGCCTCTTCCACAGAGATAC ACGTTGGATGCCTGTAAACAGGATGGCTC GGATGGCTCAATGAAATTATC	0.4809	97.18	0.7372
rs6265	ACGTTGGATGTTTTCTTCATTGGGCCGAAC ACGTTGGATGGCTTGACATCATTGGCTGAC TCTGACACTTTCGAACAC	0.1945	96.94	0.1237
rs11030100	ACGTTGGATGTCTTCCATTGAGCAAGGCAC ACGTTGGATGTGTCCAGAGACAAGACTTCG CTGTACATTCAAGACCAAGA	0.2215	97.24	0.1576

(B)	GWA/MAGICS	1st PCR Primer 2nd PCR Primer Extension Primer	MAF	Call R%	pHWE
rs number					
rs58936914	ACGTTGGATGCCCCCATCTACACCTAAAC ACGTTGGATGTAATGCTGTTAGAGGTTGGG GGGGTAAGGGTGGGGTAAA		0.0445	97.05	0.6668
rs7944119	ACGTTGGATGACATCGCCCTGCGAGTCCT ACGTTGGATGTGAGGCTGGGGCTGGAACAC GAACACCCCCTCGAAGC		0.2748	97.4	0.1758
N/A	ACGTTGGATGTCCAGGAGTAACTCACTCAC ACGTTGGATGGGATTCTTGACTCGGGTTAG ggcGGGGATGCAGAGCCGGGAA		0.0568	97.12	0.7376
rs11030101			0.49	100.00	0.2627
rs6265			0.2057	99.86	0.8065
rs11030100	ACGTTGGATGTCTTCCATTGAGCAAGGCAC ACGTTGGATGTGTCCAGAGACAAGACTTCG ggTGGACATTCAAGACCAAGA		0.2319	97.26	0.5854