# Association between a functional single nucleotide polymorphism in the brain-derived neurotrophic factor gene and risk of child asthma

**J.Y. Wang[1]*, A.L. Wang[2]*, W. Han[1] and Z.L. Mu[1]**

[1]The Third Ward, Xi'an Children's Hospital,
Shaanxi Province People's Hospital, Xi'an, China
[2]The Department of Pediatrics, Chinese Medicine Hospital, Baoji,
Shanxi Province, China

*These author contributed equally to this study.
Corresponding author: J.Y. Wang
E-mail: wangyanjurk@163.com

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**ABSTRACT.** Brain-derived neurotrophic factor (*BDNF*) promotes synaptic remodeling and modulates the function of other neurotransmitters. Allergic inflammation triggers neuronal dysfunction and structural changes in the airways. Genetic polymorphisms in functional regions of the *BDNF* gene have a plausible role in modulating the risk of child asthma (CA). This study examined the potential association between CA and three single nucleotide polymorphisms (SNPs) in *BDNF* (rs2030323, rs6265, and rs16917204 in the promoter, exon 4, and 3'-untranslated regions, respectively). The study was conducted in 350 children with asthma and 356 healthy controls. The genotype and allele frequencies and difference between groups were analyzed using HaploView 4.0 and SPSS 20.0 software platforms. The analysis revealed a strong association between the rs6265 genotype distribution and CA. The frequency of the G allele was significantly higher

 

in CA patients than in healthy controls (P = 0.0007, odds ratio = 1.323, 95% confidence interval = 1.073-1.632). Strong linkage disequilibrium was observed between rs16917204 and rs6265. A significantly higher number of G-G haplotypes were observed in CA patients than in controls (P = 0.024 after Bonferroni correction), while the G-A haplotypes were more significant in controls (P = 0.013 after Bonferroni correction). This suggested that *BDNF* gene polymorphisms confer susceptibility to CA, and also support the notion that BDNF dysfunction is involved in the pathophysiological process of CA.

**Key words:** Asthma in children; Brain-derived neurotrophic factor; Single nucleotide polymorphisms

## INTRODUCTION

Asthma is the most common chronic disease affecting children, and is characterized by reversible airflow obstruction and chronic inflammation of the airways (Quirce et al., 2015; Rix et al., 2015). Asthma is a heterogeneous disease involving both genetic and environmental factors (Custovic et al., 2012). Results from previous animal studies, as well as clinical observations, have suggested that brain-derived neurotrophic factor (BDNF) is involved in the etiology of asthma. Recent studies have also suggested that single nucleotide polymorphisms (SNPs) in the *BDNF* gene may be associated with child asthma (CA) (Muller et al., 2010; Yinli et al., 2013).

BDNF is the most abundant neurotrophin in the brain. BDNF regulates neuronal survival, differentiation, and synaptic remodeling (Ashe et al., 2001). Asthma patients exhibit airway inflammation, hyper-responsiveness, and remodeling, all of which are controlled by neurons (Nassenstein et al., 2006). The link between inflammation and neuronal dysfunction is mainly provided by neurotrophins, particularly the BDNF. BDNF is upregulated in cases of allergic airway inflammation, and induces airway hyper-responsiveness and airway obstruction in an animal model of allergic asthma, by increasing the neuronal sensitivity and activity in the airways (Lommatzsch et al., 2003; Braun et al., 2004; Hhna et al., 2006). Asthmatic patients show significantly higher serum BDNF levels, compared to healthy subjects. Allergic inflammation increases the local BDNF production, and its concentration is correlated to the clinical parameters of allergic airway dysfunction (Lommatzsch et al., 2005). Studies in mouse models have also suggested a post-inflammatory role for BDNF (Lommatzsch et al., 2003). These findings suggest that the *BDNF* gene is an excellent candidate for asthma.

BDNF is a major regulator of the phosphatidylinositol 3'-kinase (PI3K), mitogen activated protein kinase (MAPK), phospholipase C gamma (PLCγ), and nuclear factor kappa B (NFκB) signaling pathways, which influence the cellular survival, growth, differentiation, and structure (Russo et al., 2009). Although many polymorphisms have been identified in the *BDNF* gene, the exact role of these polymorphisms in asthma is unknown (Muller et al., 2010; Yinli et al., 2013). Neurons with at least one A allele of a common, non-conservative polymorphism rs6265 in the *BDNF* gene were characterized by lower depolarization-induced secretion (Egan et al., 2003; Hariri et al., 2003) and reduced intracellular trafficking and packaging of the BDNF precursor (pro-BDNF). A recent study has shown that the BDNF rs6265 polymorphism is associated with asthma in children (Yinli et al., 2013). The rs2030323 polymorphism occurs in the putative *BDNF*

promoter region. A computational analysis has suggested that this region is potentially a part of the eukaryotic polymerase II promoter binding site, and that the rs2030323 could disrupt the pattern of recognition; this implied a possible negative effect of rs2030323 on *BDNF* gene transcription (Zhang et al., 2006). The functional importance of this polymorphism in asthma, however, must be further analyzed. The rs16917204 polymorphism in the 3′-untranslated region (3′UTR) has been previously associated with Alzheimer's psychiatric disorders (Sartor et al., 2009; Muller et al., 2010). Further studies must be performed to determine whether these SNPs modulate the risk of asthma by themselves, and are repeated in other populations.

We hypothesized that the functional variants of the polymorphisms in the *BDNF* gene might contribute significantly to the predisposition of developing CA. In this study, we investigated 3 loci in a Chinese population from the Shaanxi province to verify the putative association between *BDNF* polymorphisms and CA.

## MATERIAL AND METHODS

### Subjects

A total of 350 unrelated patients with schizophrenia (194 males and 156 females, mean age of onset: 10.2 ± 1.6 years) were recruited from the Xi'an Children's Hospital of the Shaanxi Province People's Hospital. A diagnosis of asthma was made according to the recommendations of the Global Initiative for Asthma (GINA), based on the clinical symptoms of asthma and results of the test of lung function. The bronchodilator response was assessed 20 min after administration of 200 µg Salbutamol MDI via a holding chamber (Volumatic); a≥12% increase in FEV1 was diagnostic. The bronchial hyper-responsiveness was assessed by an exercise test using a treadmill (6 min run); a post-exercise fall of 15% in the FEV1 value was considered to be positive. Severe asthma was defined as follows: symptoms requiring daily therapy with high-dose inhaled corticosteroids despite regular therapy with long acting $\beta_2$-agonists and/or leukotriene antagonist, and 1 or more emergency care visit or oral steroid bursts per year. In all, 355 healthy blood donors (mean age: 11.1 ± 1.6) were recruited from the Shaanxi Province People's Hospital. Subjects who had participated in other studies, or suffered from chronic diseases were excluded. All participants were Han Chinese from the Shanxi Province, and not genetically related. Written informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of the Shaanxi Province People's Hospital Center, Xi'an, China.

### SNP selection and genotyping

SNPs in the promoter region, untranslated regions (UTRs), and exons of *BDNF* were systematically screened. Three SNPs with minor allele frequencies (MAF) greater than 0.05 were selected from the *BDNF* gene and nearby regions, based on a review of the published literature and the results of a HapMap and dbSNP (Han Chinese population) search (Figure 1). These SNPs were further analyzed in an association study. Peripheral blood (1-2 mL) was collected from each participant and placed in EDTA-containing specimen tubes. Genomic DNA was extracted using the TIANamp Blood DNA Kit (Tiangen, Beijing, China), and stored at -20°C until use. SNP genotyping was performed using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF; MassARRAY system; Sequenom Inc., San Diego, CA, USA) mass spectrometry. The probes

and primers were designed using the Assay Design Software (Sequenom Inc.) (Table 1). The completed genotyping reactions were spotted onto a 384-well spectroCHIP (Sequenom Inc.) using the MassARRAY Nanodispenser (Sequenom Inc.), and analyzed using the MALDI-TOF mass spectrometer. Real-time genotype calling was performed using the MassARRAY RT software (v.3.0.0.4), and analyzed using the MassARRAY Typer software (v.3.4; Sequenom Inc.).

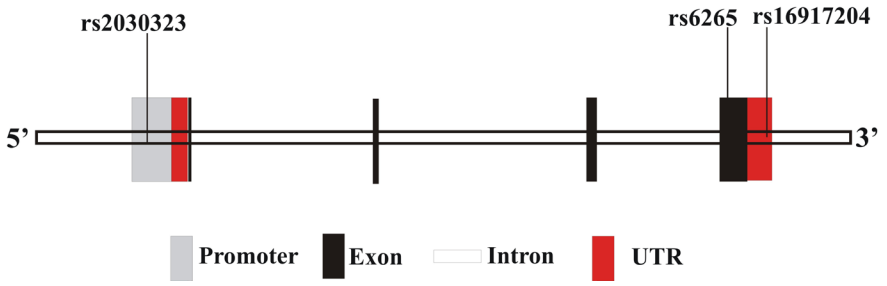


**Figure 1.** Structure of the human *BDNF* gene and 3 SNPs located on the gene.

**Table 1.** Primer sequences used for genotyping of the SNPs in the *BDNF* gene.

| SNPs | Forward primers (5'→3') | Reverse primers (5'→3') | Extension primers (5'→3') |
|---|---|---|---|
| rs2030323 | ACGTTGGATG TCTAACTGGATTTTGTGTGCAG | ACGTTGGATGAAGCTACGTCTTCTGTCCTTAGC | AACAGTGGCTTTTCTGTACTCC |
| rs6265 | ACGTTGGATGCATCATTGGCTGACACTTTC | ACGTTGGATGTTTTCTTCATTGGGCCGAAC | CTCCGCCAACAGCTCTTCTATCA |
| rs16917204 | ACGTTGGATGGTTTCTAATCACAGGGAATC | ACGTTGGATGGTTTCTAATCACAGGGAATC | TGAGCTCCTGAACGAGG |

## Statistical analysis

Allele and genotype frequencies for each individual polymorphism, and the Hardy-Weinberg equilibrium were evaluated by the chi-square test. The potential associations between CA and each polymorphism were analyzed using the Pearson chi-square test.

Unconditional logistic regression was used to calculate the odds ratio (OR) and 95% confidence interval (CI) of the independent association between each locus and the incidence of CA. The level of the test was adjusted during multiple comparisons by the Bonferroni correction, and the P value was divided by the total number of loci. Haplotype blocks were constructed using Haploview 4.0. All statistical analyses were carried out using SPSS (v.20.0; IBM, Armonk, NY, USA). P values <0.05 indicated a significant difference after Bonferroni correction.

The additive gene-gene interactions were detected using a binary logistic regression model to estimate the multiplicative interaction effect of the 3 SNPs.

## RESULTS

The cases and controls did not deviate significantly from the Hardy-Weinberg equilibrium for any of the SNPs. LD analyses of the patients and controls revealed that 2 of the SNPs (rs16917204 and rs6265) are located in a haplotype block (Figure 2).

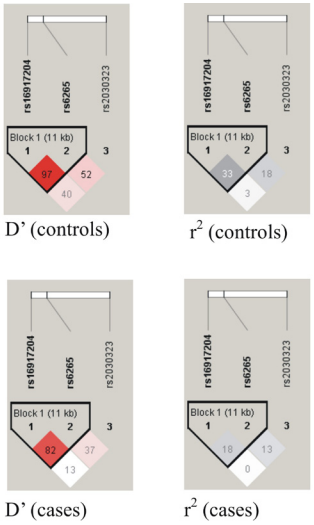


**Figure 2.** LD plot of the fifteen SNPs in the *BDNF* gene. Values in squares are the pair-wise calculation of $r^2$ (left) or D' (right).

The genotype distribution, allelic frequencies, and haplotypes of the patients with CA and healthy controls are summarized in Tables 2 and 3. A comparison of the genotype and allele frequency distribution of the rs6265 polymorphism revealed significant differences between the CA patients and controls. The frequency of the GG genotype was significantly higher in CA subjects than in the controls (P = 0.0002, OR = 1.908, 95%CI = 1.361-2.674). The frequency of the G allele was significantly higher in CA patients than in healthy controls (P = 0.0007, OR = 1.323, 95%CI = 1.073-1.632). The differences retained statistical significance after Bonferroni correction (P < 0.167).

**Table 2.** Genotypic and allelic frequencies of BDNF polymorphisms in controls and patients with child asthma.

| Variable | Position | MAF | Controls | | Child asthma | | P value[a] | OR | 95%CI |
|---|---|---|---|---|---|---|---|---|---|
| | | | N | % | N | % | | | |
| rs2030323 | Promoter | 0.486 | | | | | | | |
| GG | | | 101 | 28.37 | 102 | 29.14 | 0.883 | 1.025 | 0.740-1.418 |
| GT | | | 163 | 45.79 | 180 | 51.43 | 0.117 | 1.267 | 0.943-1.704 |
| TT | | | 92 | 25.84 | 68 | 19.43 | 0.685 | 0.930 | 0.654-1.321 |
| G | | | 365 | 51.26 | 384 | 54.86 | 0.176 | 1.155 | 0.937-1.424 |
| T | | | 347 | 48.74 | 316 | 45.14 | | | |
| rs6265 | Exon 4 | 0.487 | | | | | | | |
| GG | | | 75 | 21.07 | 118 | 33.71 | 0.0002 | 1.908 | 1.361-2.674 |
| GA | | | 197 | 55.34 | 154 | 44.00 | 0.003 | 0.633 | 0.470-0.852 |
| AA | | | 84 | 23.60 | 78 | 22.29 | 0.685 | 0.930 | 0.654-1.321 |
| G | | | 347 | 48.74 | 390 | 55.71 | 0.009 | 1.323 | 1.073-1.632 |
| A | | | 365 | 51.26 | 310 | 44.29 | | | |
| rs16917204 | 3'-UTR | 0.221 | | | | | | | |
| GG | | | 212 | 59.55 | 219 | 62.57 | 0.412 | 1.135 | 0.839-1.536 |
| GC | | | 131 | 36.80 | 108 | 30.86 | 0.080 | 0.730 | 0.513-1.038 |
| CC | | | 13 | 3.65 | 23 | 6.57 | 0.082 | 1.855 | 0.925-3.731 |
| G | | | 555 | 77.95 | 546 | 78.00 | 0.982 | 1.003 | 0.780-1.290 |
| C | | | 157 | 22.05 | 154 | 22.00 | | | |

MAF = minor allele frequency in controls; OR = odds ratio; CI = confidence interval. [a]P value was calculated using the codominant, dominant (for the rare allele), heterosis, and recessive for the rare allele models of inheritance. Alpha value is adjusted by Bonferroni correction and statistically significant results (P < 0.167).

**Table 3.** *BDNF* haplotype in block 1 frequencies and the results of their associations with risk of child asthma.

| Haplotype | Cases [N (%)] | Controls [N (%)] | Statistics | | | |
|---|---|---|---|---|---|---|
| | | | $\chi^2$ | P | OR | 95%CI |
| G-G | 194 (55.43) | 167 (46.91) | 5.125 | 0.024 | 1.407 | 1.047-1.893 |
| G-A | 80 (22.86) | 111 (31.18) | 6.193 | 0.013 | 0.654 | 0.468-0.915 |
| C-A | 76 (21.71) | 72 (20.22) | 0.236 | 0.627 | 1.094 | 0.761-1.572 |

*P value is adjusted by Bonferroni correction; P < 0.025 indicates statistically significant results.

Furthermore, the results of the linkage disequilibrium tests demonstrated that the rs16917204 and rs6265 polymorphisms in the *BDNF* gene were in strong linkage disequilibrium (D' > 0.9). CA patients exhibited a significantly higher number of G-G haplotypes than the controls (P = 0.024, OR = 1.407, 95%CI = 1.047-1.893 after Bonferroni correction). In addition, a significantly higher number of G-A haplotypes were seen in the controls than in the cases (P = 0.013, OR = 0.654, 95%CI = 0.468-0.915 after Bonferroni correction). The differences retained statistical significance after Bonferroni correction (P < 0.025).

We observed no correlations among the rs16917204, rs6265, and rs2030323 SNPs (P > 0.05) (Table 4).

**Table 4.** Multiplicative interaction analyses of gene-gene interactions involved in child asthma by logistic regression.

| Interaction | Chi-square statistic | P value | OR (95%CI) |
|---|---|---|---|
| rs2030323 x rs6265 | 3.296 | 0.069 | 0.563 (0.303-1.047) |
| rs2030323 x rs16917204 | 0.128 | 0.720 | 0.968 (0.808-1.159) |
| rs6265 x rs16917204 | 3.822 | 0.051 | 0.854 (0.730-1..000) |
| rs2030323 x rs6265 x rs16917204 | 2.132 | 0.144 | 1.326 (0.908-1.937) |

## DISCUSSION

The results of recent research have shown that *BDNF* could serve as an important marker of allergic disease. Increased levels of BDNF in blood, broncho-alveolar lavage fluid, and nasal lavage fluid have been positively correlated with increased disease activity and severity in patients with asthma (Muller et al., 2010). Our results provide direct evidence of a link between a genetic change in BDNF and CA incidence, and proposed the variants that may affect the development of CA (Jesenak et al., 2014).

Through genotyping of individuals (with and without CA), we found that the rs6265 exonic polymorphism was associated with CA. CA patients with the GG and GA genotypes at rs6265 exhibited significantly higher frequencies of the disease compared to those with the AA genotype. Furthermore, our results showed that the G allele of rs6265 is overrepresented in CA subjects, compared to the healthy controls. Similarly, a recent study demonstrated an association between this polymorphism and CA in a Slovakian population, and established the G allele as a risk factor for CA (Jesenak et al., 2014). This study confirmed the association of this putative functional polymorphism with CA in a Chinese Han population. However, Zeilinger et al. (2009) reported that CA was not associated with the rs6265 polymorphisms in the *BDNF* gene. The contradictory results obtained in this study might be attributed to the sampling method. Association studies are affected by the sample size and genetic heterogeneity. The Val (G) allele of rs6265 displayed increased

activity in this respect (Egan et al., 2003; Hariri et al., 2003; Chen et al., 2004); in addition, the homozygous Val/Val genotype may exhibit higher BDNF activity and be related to enhanced airflow limitation and airway hyper-responsiveness. Therefore, these results confirm that the *BDNF* gene variants do contribute to the predisposition for and progression of asthma in children.

The SNP rs6265, associated with asthma in this study, results in an amino acid change from valine to methionine (Val66Met) in the prodomain of BDNF. This SNP has been previously shown to alter the intracellular trafficking and packing of pro-BDNF, with the Met allele showing lower inducible BDNF secretion (Zhang et al., 2006). It has also been theorized that the effect of this polymorphism could explain altered BDNF activity and airway reactivity (Muller et al., 2010). This indicated that BDNF SNPs may play a role in severe forms of childhood asthma. Cao et al. observed that the frequency of the G allele was significantly higher in asthmatic children than that in the healthy controls (Cao et al., 2013). The rs6265 SNP could affect susceptibility to CA by affecting the function of the BDNF protein rather than its expression.

Strong linkage disequilibrium was observed between the rs16917204 and rs6265 SNPs. CA patients showed a significantly higher number of G-G haplotypes compared to the controls, who exhibited a significantly larger G-A haplotype frequency. These results indicated that people with the G-G haplotype of the *BDNF* gene were more prone to CA. The G-A haplotype of the *BDNF* gene exerted a protective effect against CA. The results of our study support the findings of other recent studies that the *BDNF* polymorphisms (rs6265, rs11030101, and rs56164415) are associated with the incidence of severe forms of asthma (Szczepankiewicz et al., 2007; Zeilinger et al., 2009). Moreover, a significantly higher number of T-T-G-C (rs12273363, rs7124442, rs6265, and rs2030324) haplotypes were found in asthmatic patients (Szczepankiewicz et al., 2010).

In conclusion, *BDNF* gene rs6265 polymorphisms (especially the G allele) were found to be associated with heroin dependence in a study involving a relatively large sample size and a homogeneous sampling population (Han Chinese). In summary, the results of this study show that the *BDNF* gene polymorphism (rs6265) must be analyzed further using different population samples, because of its complex patterns of association in complex disorders like CA.

## Conflicts of interest

The authors declare no conflict of interest.

## ACKNOWLEDGMENTS

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