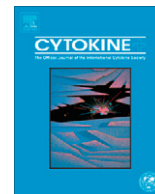




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Genetic variation in BDNF is associated with allergic asthma and allergic rhinitis in an ethnic Chinese population in Singapore

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ABSTRACT

Allergic diseases affect more than 25% of the world population and result from a complex interplay between genetic and environmental factors. Recent evidence has shown that BDNF (Brain Derived Neurotrophic Factor) could serve as an important marker of allergic disease. Increased levels of BDNF in blood, broncho-alveolar lavage fluid and nasal lavage fluid positively correlate with disease activity and severity in patients with allergic rhinitis (AR), asthma and atopic eczema. However, reports on the association between genetic variation in BDNF and allergic disease have been controversial. This study therefore aims to clarify the relationship between single nucleotide polymorphisms (SNPs) in BDNF and a genetic predisposition to AR and asthma in an ethnic Chinese population of Singapore. Volunteers with a self-reported history of asthma (718 subjects) or a history of AR as determined by a researcher-administered questionnaire (795 subjects) were used in this study, alongside controls with no personal or family history of allergy (717 subjects). The association results identified a significant association for the tagSNP rs10767664 with a significant $P_{\text{Dominant}} = 0.0007$ and $OR = 1.3$ for AR and $P_{\text{Dominant}} = 0.0005$ and $OR = 1.3$ for asthma (using a dominant model of association). The haplotype based analysis also identified a significant association further confirming the single SNP association. The SNP rs10767664 is strongly linked ($r^2 = 0.95$) to the functional polymorphism rs6265 (Val66Met), which has previously been reported to be associated to allergic phenotypes and also shown to affect BDNF expression. BDNF is a therefore a key molecular player in allergy. Further studies on polymorphisms within BDNF may shed light on its role in the pathogenesis of allergic diseases and potentially serve as biomarkers for allergic disease.

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1. Introduction

Allergic diseases such as asthma, allergic rhinitis (AR) and atopic dermatitis affect more than 25% of the world population and are the leading cause of illness in children [1–3]. The complex interplay between genetic, environmental and immunological risk factors results in the manifestation of allergic diseases [4–7]. The pathological presentation of allergic disease involves the activation of both the innate and adaptive immune systems, resulting in a multifaceted response in specific target tissues such as the airways [8–10]. This response results in the recruitment of inflammatory cells to target tissues and the production of specific IgE antibodies, cytokines and other inflammatory mediators [11,12]. It is well established that allergic inflammation triggers neuronal dysfunction, which activates specific inflammatory mechanisms, potentially leading to structural changes in the diseased tissue [11,13–15]. Neurotrophins are a family of structurally related proteins initially dis-

covered to be involved in regulating neuronal development and now known to govern both peripheral and central nerve growth [16–18]. Interestingly, the role of these proteins is not restricted to neuronal cells alone [19]. Various reports have shown that non-neuronal cells such as eosinophils, macrophages, mast cells, T and B cells produce neurotrophins [12,17]. These growth proteins form an integral part of the immune response during allergic inflammation [20]. Serum levels of neurotrophins have been observed to be increased in allergic diseases such as allergic rhinitis, chronic rhino sinusitis, atopic dermatitis and asthma [11,15,21–23].

BDNF is a secretory protein belonging to the neurotrophin family and is involved in a range of neural processes during human development [20,24]. In the early stages of development BDNF is essential for neurogenesis, survival and maturation of neuronal pathways. In the adult, alongside neurotransmitters, hormones and other neurotrophins, BDNF maintains synaptic plasticity, dendritic growth and the consolidation of long-term memory [25–27]. The biological effect of BDNF is mediated via its binding to the trkB receptor [28]. The activation of these receptors on eosinophils may be important in regulating the inflammatory cascade leading to

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allergic disease [15,29]. In patients with allergic asthma, segmental allergen provocation resulted in increased BDNF levels, as well as other neurotrophins, in bronchoalveolar lavage fluid (BAL) [30]. Release of these neurotrophins upon allergen challenge may explain the increase in bronchial hyper responsiveness [31]. Production of BDNF was also up regulated in macrophages and T cells which infiltrate the asthmatic airways in an animal model for allergic inflammation [19]. Mouse models have also suggested a post inflammatory role for BDNF [32]. Christina et al., have demonstrated a functional role for BDNF in eosinophil viability and activation [31]. This is significant because a high influx of activated eosinophils in the bronchial lumen is characteristic of the late phase reaction in asthma, which corresponds both to the release of eosinophil products into the airways and disease severity as denoted by bronchial hyper responsiveness [31,33,34]. Neurotrophin mediated activation of bronchial eosinophils might therefore play a role in the regulation of eosinophilic inflammation in allergic asthma [31].

The BDNF gene is located on chromosome 11p13 and is alternatively spliced resulting in several different transcripts [35]. Genetic polymorphisms in BDNF have been associated with allergic phenotypes such atopic dermatitis [36] and asthma [37–40] in different populations. The functional polymorphism rs6265 (Val66Met) has been shown to regulate intracellular trafficking and affect the secretion of BDNF [41]. This polymorphism, along with other genetic variants in BDNF, has been associated with asthma in German children [37]. In contrast, other studies have suggested that though BDNF is a significant biomarker for allergic phenotypes, genetic variations in BDNF are not associated to allergic diseases [36,39]. Thus the association of genetic polymorphisms in the BDNF gene with allergic diseases remains controversial. We therefore conducted a population genetic study to evaluate the association of polymorphisms in the BDNF gene with allergic rhinitis and allergic asthma in an ethnic Chinese cohort in Singapore.

2. Materials and methods

2.1. Ethics statement

This study has been performed with the approval of the Institutional Review Board (IRB, Reference – NUS07-023) of National University of Singapore and is also in compliance with the Helsinki declaration. DNA samples used in this study were collected from ethnic Chinese participants following standard protocols of informed consent.

2.2. Subjects

The DNA samples used in this study were collected from ethnic Chinese participants following standard protocols of informed consent, as part of an on-going whole-genome asthma and allergic disease case-control association study in Singapore [42]. Experimental research reported in the manuscript has been performed with the approval of NUS Institutional Review Board (IRB, Reference – NUS07-023) and is also in compliance with the Helsinki declaration. Genomic DNA was extracted from buccal cells obtained from a mouthwash of 0.9% saline solution following a standardized protocol [43]. Samples were quantified in triplicate on a Nanodrop (ND-1000). Phenotypic characteristics of the cohort have been described in Table 1. Recruited subjects were classified as asthma cases, AR cases and healthy controls according to their disease condition. Their disease condition was determined by a questionnaire based on ARIA and ISAAC-derived questionnaires [2,44] and a skin prick test (SPT) using a panel of common allergens in Singapore such as *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Elaeis guineensis*

Table 1
Demographic and clinical characteristics of the samples used in the study.

Category	Allergic rhinitis ^b	Asthma ^a	Controls ^c
Subjects, n	795	718	717
Mean age, years	20.24	21.06	22
Gender, %Female	51.6	47.5	72.6
Skin prick test positive reaction (allergen tested)			
<i>Dermatophagoides pteronyssinus</i>	92%	90.2%	N.A
<i>Blomia tropicalis</i>	85%	81.5%	N.A
<i>Elaeis guineensis</i>	6.4%	7.2%	N.A
<i>Curvularia lunata</i>	3.3%	2.8%	N.A
Smoking status			
Current	0.9%	1.5%	N.A
Ex-smoker	0.4%	0.2%	N.A
Co-morbidity	43%	47%	N.A

^a Asthma case was classified based on doctor diagnosis of the disease with a positive skin prick test reaction to one of the allergens tested. (Based on WHO and GINA guidelines for asthma).

^b Allergic rhinitis (AR) was classified based on two or more major symptoms which include (nasal congestion, rhinorrhea, nasal itching, sneezing) and a positive skin prick test reaction to one of the allergens tested. (Based on 2008 guidelines set by Allergic rhinitis Impact on Asthma (ARIA) consortium).

^c Healthy controls are individuals classified based on no symptoms and history of allergic disease and a negative skin prick test reaction.

and *Curvularia lunata*. SPT response is considered positive when the wheal diameter is 3 mm or greater. SPT tests used positive (histamine) and negative (saline) controls. AR is diagnosed based on the presence of a positive SPT response to either *D. pteronyssinus* or *B. tropicalis* and typical AR symptoms as defined by the ARIA 2008 guidelines [2,44] i.e., two or more AR symptoms (nasal congestion, rhinorrhea, nasal itching, sneezing) persisting for four or more days a week during the past year. Asthma subjects were identified based on self-reported physician-diagnoses, with wheezing symptoms and a positive skin prick reaction to either of the dust mite allergens. Conversely, control subjects were confirmed with no positive skin prick reaction to any of the allergens tested and no history of allergy.

2.3. Selection of tagSNPs

TagSNPs for the BDNF gene were identified using the Hapmap Han Chinese from Beijing (CHB) dataset as a reference. The CHB population consists of 45 unrelated individuals from Beijing, China genotyped in the Hapmap project to develop the common haplotypes that exist in the Chinese population. We have previously shown that the Hapmap CHB population can be used as a reference population for our Singaporean Chinese cohort [41]. All genetic variants described in the Hapmap [45] for the CHB population were retrieved and tagging was performed in Haploview using the Gabriel et al., algorithm [46]. Of the 21 polymorphisms identified within BDNF in Hapmap CHB, four tagSNPs were found to represent all the variation within the gene at a Linkage Disequilibrium (LD) threshold of $r^2 = 0.8$. The LD bins for the BDNF gene have been described in Supplementary Table 1. These four tagSNPs were targeted for genotyping of BDNF.

2.4. Genotyping

Genotyping was performed on purified DNA samples using the Illumina BeadXpress Assay (Illumina Inc., San Diego, CA, USA) at the University of Utah Genomics Core Facility (Salt Lake City, UT, USA) according to manufacturer recommendations.

2.5. Statistical analysis

This study was well powered to detect associations consistent with complex genetic traits, due to the large sample sizes utilized,

with a combined total of 1176 allergic rhinitis and allergic asthma subjects and 717 non-allergic control subjects. This resulted in a power of over 80% to detect effect sizes with odds ratio of 1.3–1.5 under the dominant model of association at minor allele frequencies ranging from 0.05–0.3. Hardy–Weinberg equilibrium (HWE) was measured in control subjects as a quality control measure. The primary test of allelic association was performed using the Cochran Armitage trend test which evaluates the association of the minor allele to the disease phenotype. We then performed a two d.f global test of genotypic association. If the results showed significant p -values, we then applied the dominant model as it was well powered to detect the association. All p -values, odds ratios with 95% confidence intervals, were estimated using the PLINK program version 1.06. Linkage disequilibrium was calculated as defined by Gabriel et al., [47] with the program Haploview [48]. An LD block thus generated is described in [Supplementary Fig. 1](#). Two-SNP haplotype analysis was completed using the PLINK program version 1.06. A P value less than 0.05 was considered significant for all tests.

3. Results

3.1. Association of BDNF SNPs to allergic rhinitis

All SNPs considered in the analysis were in Hardy Weinberg Equilibrium (HWE). A total of 795 AR cases and 717 non-allergic healthy controls were genotyped for the four tagSNPs of BDNF. SNPs were then subjected to clustering analysis to evaluate if there were any bad clusters using the Genome Studio software (Illumina). Samples which fell out of the cluster were removed to ensure the genotypes were called with precision. This resulted in an overall genotyping efficiency of 96.28%. The allele based association analysis using the Cochran Armitage trend (CAT) test identified a strong association of the SNP rs10767664 with AR at a $P_{\text{Allele}} = 0.00097$ ([Table 2](#)) and OR = 1.3 (95% CI: 1.114–1.527). The other three tagSNPs showed no association. We then performed a genotype based association analysis using the Chi-squared (χ^2) test. A significant association was observed using the dominant model with $P_{\text{Dominant}} = 0.0007$ ([Table 4\(a\)](#)). These results remained

significant even after applying the Bonferroni correction, with P values $P_{\text{Bonferroni}} = 0.0039$ for the allele and $P_{\text{Bonferroni}} = 0.0028$ for the genotype based association, respectively. Association analysis stratified by gender identified similar minor allele frequencies for cases and controls in both males and females (Data not shown).

3.2. Association of BDNF SNPs to asthma phenotype

A total of 718 asthma cases and 717 non-allergic healthy controls were genotyped for the four tagSNPs of BDNF. The CAT test identified a strong association of the SNP rs10767664 with asthma at a $P_{\text{Allele}} = 0.0018$ ([Table 3](#)) and OR = 1.3 (95% CI: 1.101–1.526) at the allele level. The other three tagSNPs, similar to the analysis for AR, showed no association. Association to asthma was also observed at the genotype level ($P_{\text{Dominant}} = 0.0005$) ([Table 4\(b\)](#)). These results remained significant after applying the Bonferroni correction with P value $P_{\text{Bonferroni}} = 0.0072$ for the allele and $P_{\text{Bonferroni}} = 0.002$ for the genotype based association, respectively. Gender-stratified association testing showed similar minor allele frequencies for cases and controls in both males and females (Data not shown).

3.3. Haplotype based association analysis

The LD measures for our population have been summarized in [Supplementary Fig. 1](#) and [Supplementary Table 1](#). We then performed two-locus based haplotype association analysis using the sliding window algorithm as implemented in the PLINK software. Haplotype analysis results using two SNP sliding windows are displayed in [Table 5](#) for association with AR and [Table 6](#) for asthma. The analysis identified significant associations between SNP haplotypes within the BDNF gene with both AR and asthma phenotypes. Two specific haplotypes, H2 (rs12273539 and rs10767664) and H3 (rs10767664 and rs962369), were significantly different for both AR and asthma phenotypes when compared to controls. However, the SNP driving the associations within these haplotypes was the same SNP identified in the single SNP analysis and the magnitude of the haplotype associations was similar to that found in single SNP analysis.

Table 2
Allele based association test for allergic rhinitis (AR) phenotype.

SNP	Position	Minor allele	Allele frequency		Major allele	P_{trend}	OR	L95	U95
			Cases	Controls					
rs11030101	27637320	T	0.248	0.262	A	0.4108	0.9334	0.792	1.1
rs12273539	27639887	T	0.164	0.163	C	0.9779	1.003	0.826	1.217
rs10767664	27682562	A	0.485	0.420	T	0.00097*	1.304	1.114	1.527
rs962369	27690996	G	0.037	0.043	A	0.4567	0.8706	0.6042	1.254

P_{trend} is calculated by the Cochran Armitage trend test using the PLINK v 1.06 software.

OR – Odds ratio; CI – Confidence Interval.

* $P_{\text{trend}} < 0.05$ for the association test for AR.

Table 3
Allele based association test for asthma phenotype.

SNP	Position	Minor allele	Allele frequency		Major allele	P_{trend}	OR	L95	U95
			Cases	Controls					
rs11030101	27637320	T	0.248	0.262	A	0.4281	0.934	0.789	1.106
rs12273539	27639887	T	0.15	0.163	C	0.3358	0.905	0.739	1.109
rs10767664	27682562	A	0.484	0.420	T	0.0018*	1.30	1.101	1.526
rs962369	27690996	G	0.039	0.043	A	0.58	0.90	0.621	1.306

P_{trend} is calculated by the Cochran Armitage trend test using the PLINK v 1.06 software.

OR – Odds ratio; CI – Confidence Interval.

* $P_{\text{trend}} < 0.05$ for the association test for AR.

Table 4

Genotype based association test .

SNP id	Minor allele	Major allele	Model	Genotype	Case (%)	Control (%)	χ^2	P
<i>(a) Allergic rhinitis (AR) phenotype</i>								
rs10767664	A	T	Full genotype	AA	162 (22.81)	103 (18.43)	12.17	0.0023 ⁺
				TA	365 (51.41)	263 (47.05)		
				TT	183 (25.78)	193 (34.52)		
			Dominant	AA + TA	527 (74.22)	366 (65.47)	11.49	0.0007 [#]
				TT	183 (25.78)	193 (34.53)		
<i>(b) Asthma phenotype</i>								
rs10767664	A	T	Full genotype	AA	135 (21.95)	103 (18.43)	12.17	0.0021 ⁺
				TA	325 (52.84)	263 (47.05)		
				TT	155 (25.21)	193 (34.52)		
			Dominant	AA + TA	460 (74.80)	366 (65.47)	11.49	0.0005 [#]
				TT	155 (25.20)	193 (34.53)		

* P value calculated using χ^2 test of association for all the three genotypes.[#] P value calculated using χ^2 test of association for a dominant model.**Table 5**

Association of haplotypes in the BDNF gene to AR.

Haplotype	Haplotype frequency			Chi-Sq value	P value
	Entire dataset	795 AR cases	717 controls		
<i>H1 (rs11030101 and rs12273539)</i>					
AA	0.163	0.163	0.163	0.00	0.988
CC	0.255	0.248	0.262	0.84	0.360
AC	0.582	0.589	0.574	0.68	0.410
<i>H2 (rs12273539 and rs10767664)</i>					
CC	0.450	0.481	0.420	9.30	0.002*
AA	0.170	0.164	0.175	0.58	0.446
CA	0.380	0.355	0.405	6.47	0.011*
<i>H3 (rs10767664 and rs962369)</i>					
AC	0.045	0.040	0.051	1.92	0.166
CA	0.453	0.486	0.420	10.82	0.001*
AA	0.502	0.475	0.529	7.31	0.007*

Haplotypes are constructed using the sliding window method and haplotype frequencies estimated by PLINK software.

* P<0.05 for the association test.

Table 6

Association of haplotypes in the BDNF gene to asthma.

Haplotype	Haplotype frequency			Chi-sq value	P value
	Entire dataset	718 Asthma cases	717 controls		
<i>H1 (rs11030101 and rs12273539)</i>					
AA	0.15635	0.1496	0.1631	0.9745	0.323
CC	0.25545	0.2482	0.2627	0.7793	0.377
AC	0.58815	0.6021	0.5742	2.283	0.130
<i>H2 (rs12273539 and rs10767664)</i>					
CC	0.4511	0.4825	0.4197	9.193	0.002*
AA	0.1654	0.1554	0.1754	1.684	0.194
CA	0.3835	0.3621	0.4049	4.471	0.034*
<i>H3 (rs10767664 and rs962369)</i>					
AC	0.04719	0.0433	0.05108	0.7876	0.374
CA	0.4524	0.4845	0.4203	9.715	0.002*
AA	0.50045	0.4722	0.5287	7.441	0.006*

Haplotypes are constructed using the sliding window method and haplotype frequencies estimated by PLINK software.

* P<0.05 for the association test.

4. Discussion

Our study has identified a significant association of a BDNF SNP to AR. To the best of our knowledge this is the first evidence for the genetic association of BDNF with AR. A similar association was observed for allergic asthma in our population, suggesting an important role for BDNF variations in asthma susceptibility. The same tagSNP rs10767664 had a significant $P_{\text{Dominant}} = 0.0007$ and OR = 1.3 for AR and $P_{\text{Dominant}} = 0.0005$ and OR = 1.3 for allergic asthma.

Furthermore the association of single SNP analysis was confirmed by significant associations of two-SNP haplotypes with both AR and asthma. These results therefore confirm that BDNF gene variants do contribute to disease predisposition and progression in allergic diseases. The SNP identified to be associated, rs10767664, is one of the tagSNPs used in this study. We therefore need to investigate other SNPs linked to rs10767664 to understand which of them might be causal. The SNP rs6265 displays a highly significant LD of $r^2 = 0.95$ with the associated SNP (Supplementary

Fig. 1). The polymorphism rs6265 commonly referred to as the “Val66Met” is a missense mutation which alters the amino acid at position 66 in the protein from valine to methionine and is in a recombination hotspot (Supplementary Table 1). The recombination rate was estimated using the NextBio web-based analysis portal using the Hapmap CHB population as the reference. Petryshen et al., have recently reported that the region containing this functional variant might be different in various populations and hence estimated recombination rates with correction using the Hapmap population data [49]. This functional polymorphism affects intracellular trafficking and regulates the activity-dependent secretion of BDNF, although it does not affect severity in neurodegenerative diseases [41,50]. The SNP rs10767664 has previously been reported to be significantly associated to serotonin reporter availability [51]. Notably, the authors suggest that the association could be observed due to the strong linkage between rs10767664 and rs6265. Similar to our results they have also reported evidence for a haplotype block containing both of these SNPs. In an animal model for anxiety and stress related disorders, a transgenic mouse engineered to reproduce the human Val66Met SNP resulted in defective secretion of BDNF from neurons, though overall BDNF expression remained unaltered [52]. Much of this evidence does support the causality of the identified association might be controlled by the rs6265 variant, however the other polymorphisms linked to the tagSNP (Supplementary Table 1) cannot be deemed not significant. Non-neuronal cells such as eosinophils, macrophages and mast cells constitutively express BDNF, suggesting a potential bidirectional link between the neurosensory system and the immune response, coordinated by neurotrophins such as BDNF [15,21]. Further evidence linking the role of neurotrophins to inflammation and in particular allergic disease is building, with several studies documenting increased levels of BDNF, NGF and other neurotrophins such as NT-3 in allergic patients [11,53]. In a study on allergic rhinitis, the increased nasal expression of BDNF positively correlated with the AR score in patients, while there was no difference in expression for controls [54].

Hence a common, single point mutation identified through a genetic association study has been shown to produce a phenotype in transgenic mice similar to that seen in humans. There are other intronic SNPs linked to the associated SNP and the effect of these SNPs cannot be discounted. Further functional studies are required, including *in vitro* studies analyzing the effect of polymorphisms on BDNF expression in order to elucidate its role and significance in allergic diseases and their severity. Thus BDNF could serve as a potential biomarker for allergic phenotypes such as asthma and allergic rhinitis and also could provide avenues for therapeutic intervention in the future.

5. Conclusion

Several studies have suggested that BDNF has great therapeutic potential to treat a wide variety of neurodegenerative diseases [55,56]. A recent paper by Xu et al., has demonstrated that the combined effects of genetic variants in BDNF do influence the response to therapy with risperidone in a Chinese Han population [57]. In allergic diseases such as asthma, allergic rhinitis and atopic dermatitis altered expression of BDNF has been positively correlated to the increased symptomology or severity of the disease [54,58,59]. In our study we identified a tagSNP, rs10767664, which shows strong linkage to another functional variant rs6265 (Val66Met) which is known to alter the expression of BDNF. Taken together these results and current literature suggests that the study of genetic variants in BDNF and other neurotrophins warrants further research to elucidate their role in airway pathophysiology and thereby provide new therapeutic options in the future.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

AKA, WSY, RA, PNP and BKS were involved in the design of the study, recruitment of participants for the study, and extraction of DNA samples. AKA and WSY performed the statistical analysis and drafted the manuscript. PNP performed the resequencing. DYW edited the manuscript. FTC conceived, designed and planned the study and edited the manuscript. All authors have read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cyto.2011.05.008.

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