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Putative association of RUNX1 polymorphisms with IgE levels in a Korean population

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Abbreviations: BA, Bronchial asthma; IgE, immunoglobulin E; NC, normal control

Abstract

RUNX1, a member of the runt domain gene family of transcription factors, encodes a heterodimeric transcription factor and regulates the expression of various genes related to hematopoiesis and myeloid differentiation. RUNX1 has been one of the target genes for research into various autoimmune diseases due to its properties as a transcription factor and functional distribution for chromosomal translocation. In an effort to identify additional gene polymorphisms in which variants have been implicated in asthma, we investigated the genetic polymorphisms in RUNX1 to evaluate it as a potential candidate gene for a host genetic study of asthma and IgE production. We identified 19 sequence variants by direct DNA sequencing in 24 individuals of which four common variants were selected for genotyping in our asthma cohort (1,055 asthmatic patients, 384 normal controls). Using logistic regression analysis for association with the risk of asthma, while controlling for age, gender, and smoking status as covariates, no significant associations with the risk of asthma were detected. However, two polymorphisms in the promoter region (-2084G > C and -1282G > A) showed a marginal association with total IgE levels (0.03 and 0.03 in recessive models, respectively). Our findings suggest that polymorphisms in RUNX1 might be one of the genetic factors for the regulation of IgE production

Keywords: asthma; core binding factor alpha 2 subunit; immunoglobulin E; polymorphism, single nucleotide

Introduction

Asthma is a common and heterogeneous respiratory disease characterized by reversible airway obstruction caused by chronic inflammation of the airways. Asthma and related phenotypes are thought to be complex traits caused by an interaction of multiple disease susceptibility genes and environmental factors. Bronchial hyperresponsiveness is a characteristic feature of asthma, and serum IgE levels are closely associated with asthma development. The development of asthma is determined by an interaction between host genetic susceptibility and a variety of environmental factors (Burrows *et al.,* 1989; Kim *et al.,* 1999; Koh *et al.,* 2000; Ahmadi and Goldstein 2002).

Runt-related transcription factor 1 (RUNX1, MIM 151358) on chromosome 21q22 is a member of the runt domain gene family of transcription factors, and encodes a heterodimeric transcription factor containing an N-terminal DNA-binding domain (Kania *et al.,* 1990; Roumier *et al.,* 2003). RUNX1 is expressed in all hematopoietic lineages and regulates the expression of various genes specific for hematopoiesis and myeloid differentiation, such as IL3 and CSF2, as a key regulator of hematopoiesis through interactions with cofactors (Takahashi *et al.,* 1995; Uchida *et al.,* 1997; Lutterbach and Hiebert 2000; Roumier *et al.,* 2003). RUNX1 is one of the target genes for various autoimmune diseases.

Chromosomal translocation of *RUNX1* is most frequently found in leukemia, and different types of translocation of the RUNX1 gene corresponding to the production of different chimeric products and subtypes of acute myeloid leukemia have also been reported (Yamada *et al.,* 2004; Skubitz *et al.,* 2005). Thus, RUNX1 seems to be implicated in acute leukemia through various pathogenic mechanisms. Recent reports have also demonstrated that the RUNX1 gene is associated with several inflammatory and autoimmune disorders, such as systemic lupus erythematosus (SLE), psoriasis, and rheumatoid arthritis (RA) (Prokunina *et al.,* 2002; Helms *et al.,* 2003; Tokuhiro *et al.,* 2003). Additional aspects of the RUNX1 gene, such as point mutations, amplifications, and germ-line mutations, are also known to be factors for leukemia, platelet disorders, and myelodysplasia (Osato *et al.,* 1999; Song *et al.,* 1999; Niini *et al.,* 2000).

Although the functional importance of the RUNX1 gene in immune disorders has been studied, the genetic associations of *RUNX1* polymorphisms are still obscure. Previous research has focused on genetic studies of the RUNX1 binding sites of interacting candidate genes, probably because of the functional features of *RUNX1* as a transcription factor. It has recently been demonstrated that the intronic polymorphism of RUNX1 is significantly associated with the susceptibility of RA in a Japanese population (Tokuhiro *et al.,* 2003). Based on the biological properties involved in the essential role of mediating various inflammatory responses, it is hypothesized that RUNX1 plays an important role in asthma development and the level of IgE.

We performed extensive screening of *RUNX1* by direct sequencing to detect polymorphisms and examined the genetic association with the risk of asthma and the level of IgE. Here, we present 19 genetic polymorphisms found in *RUNX1* and the results of an association study in a Korean asthma cohort.

Materials and Methods

Subjects and measures

Subjects were recruited from the Asthma Genome Research Center, which includes three hospitals in Korea (Soonchunhyang University, Seoul; Bucheon, and Chunan Hospitals). Ethical approvals were obtained from the institutional review board of Soonchunhyang University hospital. All patients exhibited clinical symptoms and physical examination results compatible with asthma. Each patient showed airway reversibility as documented by an inhalant bronchodilator-induced improvement of more than 15% of FEV1, and/or an airway hyperreactivity of less than 10 mg/ml of methacholine. No subject had used systemic or high dose inhaled steroids during the 4 weeks prior to the examination of the serum IgE. Normal subjects were recruited from patient spouses and from the general population by negative answers to a screening questionnaire for respiratory symptoms. Normal subjects exhibited normal findings on a simple chest radiogram and also had an FEV1 level greater than 75% predicted, the provocation concentration that causes a fall in the FEV1 level of 20% (PC_{20}) by methacholine greater than 10 mg/ml. Total IgE was measured using the UniCAP system (Pharmacia Diagnostics, Sweden). Atopy was defined as a wheal reaction by allergen extract equal to or greater than the reaction by histamine (1 mg/ml), or 3 mm in diameter. Patient clinical profiles are summarized in Table 1.

Table 1. Clinical profiles of asthmatic and normal subjects (n = 1,439).

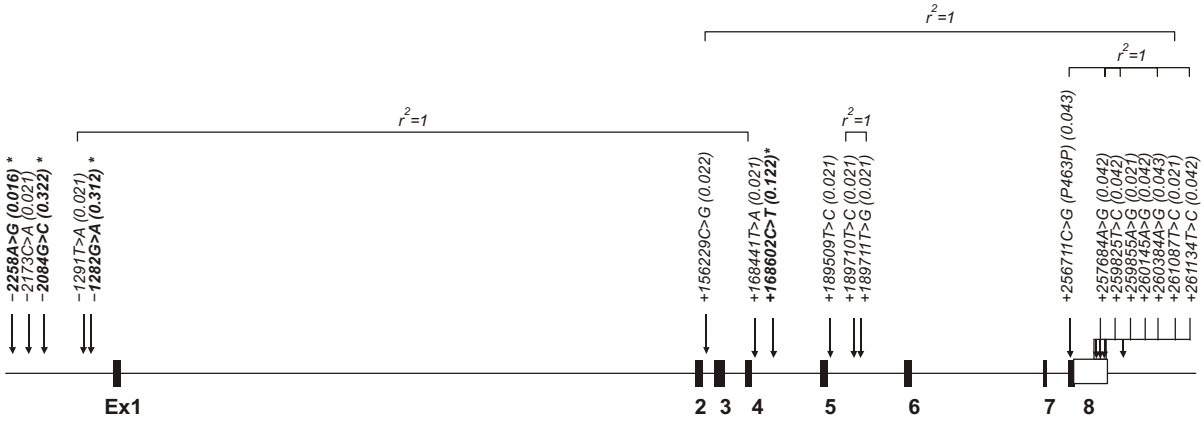
Clinical profiles	Normal controls	Asthmatics
Number of subjects	384	1055
Age [mean (range)]	44.8 (6-83)	48.7 (4-86)
Sex (male/female)	169/215	423/632
Current smoker (%)*	29.11%	34.27%
FVC1%, predicted	93.0 ± 11.9	83.5 ± 18.2
FEV1%, predicted*	102.1 ± 14.8	80.1 ± 22.7
PC_{20}, methacholine (mg/ml)*	23.4 ± 4.8	6.2 ± 8.6
Log [total IgE (IU/ml)]*	1.71 ± 0.63	2.13 ± 0.65
Peripheral blood eosinophil (%)*	2.55 ± 2.10	5.37 ± 5.37
Positive rate of atopy (%)*	34.85	56.87

*P value $<$ 0.001 for difference between asthma patients and normal controls

Sequencing analysis of the human *RUNX1*

We sequenced all exons, their boundaries, and the promoter region (~1.5 kb), to discover single-nucleotide polymorphisms (SNPs) in 24 Korean DNA samples using an ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA). Eighteen primer sets for the amplification and sequencing analysis were designed based on GenBank sequences (Ref. Seq. of *RUNX1* mRNA: NM_001754 and contig: NT_011512).

A Map of *RUNX1* (runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)) on chromosome 21q22.3 (262 kb)



B Haplotypes of *RUNX1*

Hap.	−2258A>G	−2084G>C	−1282G>A	+168602C>T	Freq.
ht1	A	G	G	C	0.577
ht2	A	C	A	C	0.266
ht3	A	G	G	T	0.078
ht4	A	C	A	T	0.040
ht5	G	G	G	C	0.014
ht6	A	C	G	C	0.014

C LD (|*D*'| and r^2) measures among *RUNX1* SNPs

		|*D*'|			
		−2258A>G	−2084G>C	−1282G>A	+168602C>T
r^2	−2258A>G	−	1	1	0.024
	−2084G>C	0.008	−	0.965	0.031
	−1282G>A	0.007	0.896	−	0.024
	+168602C>T	0	0	0	−

Figure 1. Gene maps and haplotypes of *RUNX1*. Coding exons are marked by black blocks, and 5' and 3' UTRs by white blocks. The first base of the translation start site is denoted as nucleotide +1. Asterisks indicate polymorphisms genotyped in a larger population (n = 1,439). The frequencies of polymorphisms not subjected to larger-scale genotyping were based on sequence data (n = 24). (A) Polymorphisms identified in *RUNX1* on chromosome 21q22.3 (Ref. Genome Seq. NT_011512). (B) Haplotypes of *RUNX1*. (C) Linkage disequilibrium coefficients (|*D*'| and r^2) among *RUNX1* polymorphisms.

Table 2. Logistic analysis of *RUNX1* polymorphisms with the risk of asthma while controlling for age and sex as covariates among asthmatic and normal subjects.

Loci	Position	rs#	BA (n = 997)	NC (n = 353)	Co-dominant	
					OR (95%CI)	*P*
-2258A > G	Promoter	rs9977916	0.016	0.016	0.82 (0.43-1.56)	0.55
-2084G > C	Promoter	rs12626613	0.317	0.330	0.96 (0.80-1.14)	0.62
-1282G > A	Promoter	rs2071029	0.309	0.330	0.91 (0.75-1.11)	0.37
+168602C > T	Intron4	rs2298351	0.129	0.123	1.03 (0.81-1.31)	0.82
h1 (AGGC)	-	-	0.580	0.546	-0.611	0.54
h2 (ACAC)	-	-	0.260	0.288	0.984	0.33
h3 (AGGT)	-	-	0.076	0.089	0.204	0.84

Genotype distributions and *P*-values for logistic analyses of three alternative models (co-dominant, dominant, and recessive models) controlling for age and sex as covariates are shown. *P*-values of haplotype associations were calculated by the algorithm developed by Schaid *et al*., (Haplo.Score), while controlling for age and sex as covariates. Haplotypes with a frequency > 0.05 are presented.

Table 3. Regression analyses of log-transformed total IgE as a function of *RUNX1* polymorphisms in asthmatics.

Loci	Position	rs#	C/C	C/R	R/R	*Pa*	*Pb*	*Pc*
-2258A > G	Promoter	rs9977916	944 (2.14 ± 0.65)	32 (2.06 ± 0.74)	1 (1.81)	0.30	0.37	0.33
-2084G > C	Promoter	rs12626613	**446 (2.16 ± 0.63)**	**447 (2.07 ± 0.67)**	**89 (2.26 ± 0.67)**	0.71	0.08	**0.03**
-1282G > A	Promoter	rs2071029	**462 (2.15 ± 0.63)**	**429 (2.07 ± 0.68)**	**88 (2.28 ± 0.66)**	0.86	0.13	**0.03**
+168602C > T	Intron4	rs2298351	740 (2.13 ± 0.66)	213 (2.10 ± 0.63)	17 (2.23 ± 0.54)	0.98	0.76	0.29
h1 (AGGC)	-	-	0.739	-	-	0.46	-	-
h2 (ACAC)	-	-	0.019	-	-	0.98	-	-
h3 (AGGT)	-	-	0.287	-	-	0.77	-	-

Genotype distributions, means, and standard deviations (SD) of Log (total IgE), and *P*-values for regression analyses of three alternative models (co-dominant, dominant, and recessive models) are shown. *P*-values of haplotype associations were calculated by the algorithm developed by Schaid *et al.*, (Haplo.Score), while controlling for age and sex as covariates. Haplotypes with a frequency > 0.05 are presented.

Genotyping with fluorescence polarization detection

For genotyping of polymorphic sites, amplifying primers and probes were designed for TaqMan (Livak 1999). Primer Express (Applied Biosystems) was used to design both the PCR primers and the MGB TaqMan probes. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye.

Statistics

χ^2 tests were used to determine whether individual variants were in equilibrium at each locus in the population (Hardy-Weinberg equilibrium). We used both Lewontin's D' (|*D*'|) (Hedrick 1987) and r^2 as measures of linkage disequilibrium between all pairs of biallelic loci. Haplotypes of each individual were inferred using software (PHASE) based on the algorithm developed by Stephens *et al.* (2001), which uses a Bayesian approach that incorporates a priori expectations of haplotypic structures from population genetics and coalescent theory. The genotype distribution of *RUNX1* SNPs and haplotypes among the asthmatics and the normal subjects was analyzed using logistic regression models controlling for age (continuous value), sex (male = 0, female = 1), and smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) as covariates. Multiple regressions, while adjusting for age, sex, and smoking status, were used for association analyses of total IgE levels. *P* values of haplotype associations were calculated using the algorithm developed by Schaid *et al.* (2002) (Haplo.Score).

Results and Discussion

RUNX1, a member of the runt domain gene family of transcription factors, encodes a heterodimeric transcription factor and regulates the expression of various genes related to hematopoiesis and myeloid differentiation (Kania *et al.,* 1990; Takahashi *et al.,* 1995; Uchida *et al.,* 1997; Lutterbach and Hiebert 2000; Roumier *et al.,* 2003). RUNX1 proteins contain a highly evolutionary conserved runt domain, which is responsible both for heterodimerization with the CBF (Core Binding Factor) and for binding with DNA (Downing 2001). *RUNX1* has been a target gene for research into various autoimmune diseases due to its properties as a transcription factor and functional distribution for chromosomal translocation. Translocation of the RUNX1 gene corresponds to production of different chimeric products and subtypes of acute myeloid leukemia (Yamada *et al.,* 2004; Skubitz *et al.,* 2005).

We performed direct DNA sequencing of the RUNX1 gene in 24 unrelated individuals, and identified 19 sequence variants of *RUNX1* within exons and their flanking regions. Five in the 5' region, seven in exons, six in introns, and one in the 3' region (Figure 1A). Four polymorphisms were selected for larger-scale genotyping based on locations, LDs, frequencies, and, haplotype tagging status. The frequencies of the four SNPs were 0.016 (*-2258A > G*), 0.322 (*-2084G > C*), 0.312 (*-1282G > A*), and 0.214 (*+168602C > T*) (n = 1,439). This information, including frequencies, heterozygosity, and Hardy-Weinberg equilibrium, is available on our website (http://www.snp-genetics.com/reference/Supplementary_information_to_RUNX1.doc). Six haplotypes of *RUNX1* were constructed, and haplotypes that had frequencies of less than 5% were excluded from the statistical analysis to avoid redundant statistical tests (Figure 1B). Linkage disequilibrium coefficients (|*D*'|) and r^2 values among polymorphisms were also calculated, and haplotypes that were equivalent with single polymorphisms or had frequencies of less than 5% were excluded from further statistical analysis.

Allele frequencies of polymorphisms and common haplotypes were compared between the patients

and the normal controls using logistic regression models. The genetic polymorphisms of *RUNX1* showed no association with asthma development (Table 2). However, two polymorphisms (-2084G > C and -1282 C > T) in the regulatory region, which were in tight linkage (|D'| = 0.965 and r^2 = 0.896), showed marginal associations with the IgE levels among asthmatics (0.03 and 0.03 in recessive models, respectively; Table 3).

Previous research has focused on genetic studies of RUNX1 binding sites of interacting candidate genes, probably because of the functional features of RUNX1 as a transcription factor. Based on interest in this area, RUNX1 has been investigated for its functional relationship with various inflammatory and autoimmune diseases, such as leukemia, SLE, psoriasis, and RA (Miyoshi *et al.,* 1995; Prokunina *et al.,* 2002; Helms *et al.,* 2003; Tokuhiro *et al.,* 2003). The genetic associations of *RUNX1* polymorphisms with the above diseases have not been well defined. Nevertheless, it is clear that the RUNX1 gene plays an important role in immune responses. We found that polymorphisms in promoter regions of the RUNX1 gene were associated with the production of total IgE. In addition, based on an *in silico* study using the TRANSFAC database, core and matrix similarities of the MAZ (MYC-associated zinc finger protein)-binding site to the sequence surrounding the minor allele *-2084C* (CCCTCCCT, where the fifth nucleotide is polymorphic) were predicted to be 0.95 and 0.90. The major *-2084G* allele (CCCTCGCT) lost both similarities (Heinmeyer *et al.*).

The effects of *RUNX1* polymorphisms on the production of IgE were not dramatic. When Bonferroni corrections were strictly adopted, associated *P* values were not significant (the threshold of significance was 0.007; 7 polymorphisms and 1 phenotype were analyzed). However, there was a chance of type 1 error due to multiple comparisons. Considering that the comparisons were not totally independent of each other due to tight LDs among SNPs/haplotypes, the significance of associations with IgE production among asthmatic patients is noteworthy. Further biological and/or functional evidence of polymorphisms in the regulatory region of the RUNX1 gene is needed to confirm our suggestive associations of *RUNX1* polymorphisms with asthma.

In summary, we identified 19 sequence variants of *RUNX1* and examined the association with risk of asthma and IgE production among asthmatic patients. Genetic analyses of *RUNX1* in asthmatic patients revealed putative associations of genetic polymorphisms with the production of IgE.

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