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ORIGINAL INVESTIGATION

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Association studies of 33 single nucleotide polymorphisms (SNPs) in 29 candidate genes for bronchial asthma: positive association a T924C polymorphism in the thromboxane A2 receptor gene

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Abstract Although intensive studies have attempted to elucidate the genetic background of bronchial asthma (BA), one of the most common of the chronic inflammatory diseases in human populations, genetic factors associated with its pathogenesis are still not well understood. We surveyed 29 possible candidate genes for this disease for single nucleotide polymorphisms (SNPs), the most frequent type of genetic variation, in genomic DNAs from Japanese BA patients. We identified 33 SNPs, only four of which had been reported previously, among 14 of those genes. We also performed association studies using 585 BA patients and 343 normal controls for these SNPs. Of the 33 SNPs tested, 32 revealed no positive association with BA, but a T924C polymorphism in the thromboxane A2 receptor gene showed significant association ($\chi 2$= 4.71, P=0.030), especially with respect to adult patients ($\chi 2$=6.20, P=0.013). Our results suggest that variants of the TBXA2R gene or some nearby gene(s) may play an important role in the pathogenesis of adult BA.

Introduction

Bronchial asthma (BA), one of the most common of all chronic inflammatory diseases in human populations, is considered to result from a combination of detrimental factors, both environmental and genetic. Several genome-wide linkage analyses and association studies suggested the involvement of IgE receptor Fc epsilon R1 beta (Hijazi et al. 1998), beta 2 adrenoreceptor (Hopes et al. 1998), or inteleukin 4 (Noguchi et al. 1998). However, contradictory reports followed almost immediately (Ishizawa et al. 1999; Deichmann et al. 1999; Noguchi et al. 1999). Although it is uncertain whether these discrepancies reflect ethnic differences or simply false-positive results, it is obvious that the genetic factors associated with BA are not well understood. One approach to addressing the genetic factors associated with BA is to undertake extensive surveys of candidate genes to look for variations, and to perform association studies by using any polymorphisms that are found. Genetic variations may involve insertion, deletion or base substitutions, but we have chosen to examine single-nucleotide polymorphisms (SNPs) that reflect one-base substitution, because they are the most abundant type of genetic variation in the human genome.

BA is characterized by constriction of the airways, invasion of inflammatory cells into the respiratory tract, and prolongation of the life span of mast cells (O'Byrne et al. 1997). Some inflammatory pathways appear to be directly or indirectly over-stimulated in BA patients, with the life-span of mast cells being prolonged by unknown factors that interfere with an apoptotic signaling pathway. Hence, we have selected, as candidates for screening, genes that may be related to inflammation or apoptosis, such as those encoding proteins related to cell-cell interactions (cytokines and their receptors), and those involved in the arachidonic acid cascade. These products are known to have various biological activities, and some have been shown to induce inflammation. Although some inhibitors of the arachidonic acid cascade are in use for treating BA patients (O'Byrne et al. 1997), the effects of these drugs vary among individuals (Drazen et al. 1999a, 1999b). The differences in response probably reflect subtle variations among genes encoding the proteins involved in this pathway. We have also chosen to survey genes belonging to tumor necrosis factor (TNF) and TNF-receptor families, because these proteins are expressed in inflammatory

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cells and are suspected of influencing the severity of inflammation (Walczak et al. 1997; Ashkenazi and Dixit 1998).

Here, we report the identification of 33 single nucleotide polymorphisms (SNPs), of which only four have been previously reported, among 29 candidate genes surveyed, and describe the results of association studies between each of these SNPs and BA. We document a positive association between adult BA and a previously reported T924C SNP (Cagill et al. 1999) in the thromboxane A2 receptor gene (TBXA2R).

Materials and methods

Samples

Peripheral blood samples were obtained from 585 Japanese individuals with BA and from 343 control individuals. The samples were collected at the Osaka Prefectural Habikino Hospital and the Miyatake Asthma Clinic. We used the International Consensus Report on the Diagnosis and Management of Asthma for the diagnosis of BA. DNA and RNA were prepared from each sample according to standard protocols.

Screening of SNPs

Twenty-nine candidate genes were screened for SNPs by direct sequencing of genomic DNAs or cDNAs derived from 5–10 Japanese patients with BA. Genomic or mRNA sequences of candidate genes were obtained from the GenBank DNA database (http:www.ncbi.nlm.nih.gov/). In the case of there being no data on the sequences of those genes, we determined the DNA sequences. Genomic DNAs served as the templates for sequencing, except in the case of genes that had been published only as mRNA sequences. DNA sequences were determined using the BigDye Terminator RR Mix (Perkin Elmer) with ABI 377 sequencers.

Hybridization with allele-specific oligonucleotides

SNPs were genotyped by allele-specific oligonucleotide (ASO) hybridizations in which 50 ng each genomic DNA was amplified by the polymerase chain reaction (PCR); 2 μl each PCR product was spotted onto a nylon membrane (Biodyne, Washington, USA) and fixed according to standard protocols. The DNA sequences of the PCR primers used to amplify the 14 genes that contained SNPs are listed in Table 1. Allele-specific probes (^{32}P-labeled 13–15 mers) were hybridized to the PCR products on the membranes and washed according to standard protocols. The DNA sequences of the probes and reaction conditions are also listed in Table 1.

Analysis by PCR-restriction fragment length polymorphism

PCR/restriction fragment length polymorphism (PCR-RFLP) analysis was performed to genotype SNPs whenever a cleavage site for an endonuclease was created or destroyed by the base substitution. Such genomic DNAs (50 ng each) were amplified by PCR, and each PCR product was digested with the appropriate enzyme (Table 1) according to the manufacturer's protocol.

Statistical analysis

Data were tested by categories with the Pearson $\chi 2$ test. We compared individuals who carried two major alleles with others at each locus examined

Results

We surveyed 29 candidate genes by direct sequencing of genomic DNAs from a small number of Japanese patients with BA. The candidate group consisted of cytokines and their receptors, TNF and TNF-receptor families, and factors involved in the arachidonic-acid cascade (Table 2). By screening a total of 36,017 bp nucleotides, we identified 33 SNPs, 29 of them being novel, among 14 of the candidate genes. The genomic distributions of these SNPs were 1/737 bp in non-coding regions and 1/1517 bp in coding regions; thus, single-base substitutions were 2.1 times more frequent in non-coding DNA in the regions examined.

The characteristics of the SNPs and their allelic frequencies are summarized in Table 3. The polymorphisms consisted of 23 transitions (70%) and 10 transversions (30%). Among the 33 SNPs, eight were located in 5' flanking regions, two in 5' untranslated regions (UTRs), 15 in coding regions, and eight in 3'UTRs. Of the 15 coding SNPs, seven were non-synonymous substitutions, and five of those resulted in non-conservative amino acid changes.

We then searched for association of these SNPs with BA by using 585 patients and 343 normal controls. Although no association was observed for 32 of the 33 SNPs analyzed, we found a significant association of the T924C SNP ($\chi 2$=4.71, P=0.030; Table 4) in the TBXA2R gene with the disease. In the T924C SNP, the frequency of individuals having the major alleles on both chromosomes was higher in the BA population (425/584, 73%) than in normal controls (204/310, 66%). When we separated BA patients into two groups by age, the association between the T924C SNP and BA became even more significant in the adult BA group (Table 4).

Discussion

We surveyed 29 genes for SNPs that might be associated with BA and identified 33 polymorphisms among 14 of those genes; 29 of the SNPs were novel. By screening different genes by different methods in mixtures of multiple ethnic groups, other researchers have detected one SNP per 350 bases on average, with the same SNP frequency in coding and non-coding regions (Cargill et al. 1999). The incidence of SNPs in our study was much lower, probably because we examined no more than 20 chromosomes in order to detect them. SNPs reflecting nucleotide transitions were observed more frequently than transversions; this tendency corresponded to reports from others (Fitch 1967; Vogel 1972; Vogel and Kopun 1977).

It is generally agreed that synonymous substitutions should be more frequent than non-synonymous ones, because non-synonymous SNPs could influence the function of gene products and would probably be eliminated by selective pressure during evolution. However, seven of the 15 SNPs found within coding regions in our study were non-synonymous. Given the relatively small number of

Table 1 PCR primers and methods for genotyping SNPs present in 14 candidate genes for bronchial asthma

Gene name	SNP	PCR primers for genotyping			Methods for genotyping				
		Forward	Reverse	Annealing temperature (°C)	ASO hybridization			PCR RFLP	Direct sequencing
					ASO probe	Hybridization (°C)	Wash (°C)		
IL1B	T(-578)C	5'-AGACAGGGAGGGCTATTGG-3'	5'-CTCGAAGAGGTTTGGTATC-3'	55	5'-GAAAGCCATAAAAAC-3'	55	38		
					5'-GTTTTTATAGCTTTC-3'	55	38		
IL8RB	C238T	5'-TATGCCCTGGTATTCCTGCT-3'	5'-CAAGGTCAGGGCAAAGAGTA-3'	57	5'-CGGAGC(A/G)GCCGAC-3'	35	40		
IL10	T(-854)C	5'-ATCCAAGACAACACTACTAA-3'	5'-TAAATATCCTCAAAGTTCC-3'	53	5'-GATGTAA(T/C)ATCTCTG-3'	37	36–42		
	A(-627)C	5'-ATCCAAGACAACACTACTAA-3'	5'-TAAATATCCTCAAAGTTCC-3'	53	5'-GGAGAAT(A/C)CTAATGA-3'	37	36–42		
CCR3	T(-520)G	5'-TGGATAGAGACTAAAGATCTAG-3'	5'-AAGTTAGCATGACCCGGCAT-3'	55				BsmA I	
	C(-174)T	5'-TGCCGTAAGAGACAGTAGTAA-3'	5'-GGATGTGGTACCAAAGGTCT-3'	55	5'-AATCCTT(C/T)TCCTGG-3'	37	36		
CCR5	G2076T	5'-AGAGCTGGTTGGGAAGACAT-3'	5'-GACTGTGTTCAAGCTCTGCA-3'	57	5'-TTTGG(G/T)TTGGAAGT-3'	35	44		
	G2918T	5'-CAGTGCACACAAGTGTAGGT-3'	5'-CACCGTTCATATTCAGAGGC-3'	57	5'-CTGTTCT(T/G)TCTCAT-3'	37	36–42		
CMA1	G(-1903)A	5'-AATGTGAGCAGATAGTGCAGT-3'	5'-TCCTTGTACCACTTTCTTCAC-3'	57	5'-CAGGTG(G/A)AGCAAA-3'	37	36–44		
	C(-1777)T	5'-AATGTGAGCAGATAGTGCAGT-3'	5'-TCCTTGTACCACTTTCTTCAC-3'	57	5'-GTTTAAG(C/T)CTCTGA-3'	37	42–46		
TNFSF10	C825T	5'-CGACAAACAAATGGTCCAATAT-3'	5'-ATCCTGAAAACTGAATAGTCAC-3'	57					seq
	A1140G	5'-TAGTTGGCTAACTGACCTGG-3'	5'-TCTCTTGATCTCGTGATCTAC-3'	57	5'-CCCAAGA(A/G)AATGAAA-3'	35	36–38		
	C1201A	5'-TAGTTGGCTAACTGACCTGG-3'	5'-TCTCTTGATCTCGTGATCTAC-3'	57	5'-AAGAAAG(C/A)GCAACAA-3'	35	42		
	G1437A	5'-GCACCACTAAAAGATCGCAG-3'	5'-TGAAACCAAGTCTCGCTCTG-3'	57	5'-CTGAAAGT(A/G)CAAAAAT-3'	35	36–42		
	G1500A	5'-GCACCACTAAAAGATCGCAG-3'	5'-TGAAACCAAGTCTCGCTCTG-3'	57	5'-TGAGGCA(A/G)GAGAAGAAT-3'	35	42–44		
	C1507T	5'-GCACCACTAAAAGATCGCAG-3'	5'-TGAAACCAAGTCTCGCTCTG-3'	57	5'-GAGAAT(C/T)GTTTGAAC-3'	35	44		
TNFRSF10A	C(-397)A	5'-GGCAGGCTGAATCACTCGC-3'	5'-TGACCTCAGCCTTTCTGTGA-3'	56				Msp I	
TNFRSF10B	C(-1562)T	5'-ATCCACTGGGTGACGACACCT-3'	5'- AGTTGTTCTTTCCTCCCGGT-3'	58				Hha I	
	T95C	5'-AAAGGCACGGCCCAGGAC-3'	5'-ACCGCGGCGACAACGAG-3'	57	5'-CTGGGCCCCGGGT-3'	42	42		
					5'-ACCCGGAGCCCAGG-3'	42	44		
	C200T	5'-AGTCTGCTCTGATCACCCAA-3'	5'-GGCTGGACCTCTTTTGTTGT-3'	57	5'-CAGAGAG(C/T)GGCC-3'	37	38		
CYP4F3	C806A	5'-AAGCCCAGTGAATATATTGCC-3'	5'-CTTGCTCAGCAGGAGTACAT-3'	55				Aat II	seq
	G1043A	5'-TGAAGATGGGAAGAAGTTGTC-3'	5'-CATTCAATCTCTTTAGGCTCAC-3'	55				Msp I	seq
	A1073G	5'-TGAGCACATCAGCCTCATGA-3'	5'-GCAGCAGCGAGAGACGGCA-3'	55					seq
LTC4S	A(-444)C	5'-TACAACGACTAAGGCTGGCA-3'	5'-GCTGTGTGTGAAGGCGAGC-3'	55					seq
EPHX2	G847A	5'-AAGGCCAGCCCCAGTGAGG-3'	5'-GTAGAGCTGGTAATCAAATACT-3'	55					seq
	G1272A	5'-GCATGCTGGTGTGGTACATG-3'	5'-CAGAATCCAGCCACTTAATGA-3'	55					seq
	A1590C	5'-GCATGCTGGTGTGGTACATG-3'	5'-CAGAATCCAGCCACTTAATGA-3'	55					seq
	A1701G	5'-TGCCCACGCTCAGCAGGTG-3'	5'-CCTTCAGAACAATGCTGCCA-3'	60	5'-TGTGCC(A/G)TCCTTC-3'	37	36		
	T1759C	5'-TGCCCACGCTCAGCAGGTG-3'	5'-CCTTCAGAACAATGCTGCCA-3'	60	5'-ACATCT(T/C)GCATGGA-3'	37	36		
CYSLT1	T927C	5'-CTCTCCTATATTTCTTTTCTGG-3'	5'-CTATACTTTACATATTTCTTCTCC-3'	55	5'-TCTACATT(T/C)AGAAAGC-3'	37	44–46		
TBXA2R	G179T	5'-TGGTGACTGATCCCTCAGG-3'	5'-TCGAAGAGCGCGGCGTGCT-3'	55				Hha I	
	C795T	5'-CTTTGCAGGTCTTCAT-3'	5'-CCTCTTCCAATGTCTGCATG-3'	55	5'-TCTTCAT(T/C)GCCCAG-3'	37	42		
	T924C	5'-CTTTGCAGGTCTTCAT-3'	5'-CCTCTTCCAATGTCTGCATG-3'	55				Rsa I	

Table 2 Distribution of 33 SNPs among 29 genes in Japanese BA patients. Nucleotides are numbered from the adenine of the translation initiation codon ATG. Nucleotides upstream of this adenine are signified by „–"

Gene name	Surveyed range	Non-coding (bp)	Coding(bp)	Total(bp)	Numbers of non-coding SNP(1SNP/bp)	Numbers of coding SNP (1SNP/bp)	Numbers of total SNP (1SNP/bp)
IL1B	(–87) – (–15)	73	0	73	1 (1/73)	0	1 (1/73)
IL8RB	21–484	0	464	464	0	1 (1/464)	1 (1/464)
IL10	(–1144) – (–607)	538	0	538	2 (1/269)	0	2 (1/269)
CCR3	(–630) – 1043	630	1043	1673	2 (1/315)	0	2 (1/837)
CCR5	401–3126	2013	713	2726	2 (1/1007)	0	2 (1/1363)
CMA1	(–1995) – (–1475)	521	0	521	2 (1/261)	0	2 (1/261)
TNFSF10	(–13) – 1573	740	846	1586	5 (1/148)	1 (1/846)	6 (1/264)
TNFRSF10A	(–461) – 1381	461	1381	1842	1 (1/461)	0	1 (1/1842)
TNFRSF10B	(–1844) – 1234	1844	1234	3078	1 (1/1844)	2 (1/617)	3 (1/1026)
CYP4F3	595–1572	9	969	978	0	3 (1/323)	3 (1/326)
LTC4S	(–972) – 462	983	451	1434	1 (1/983)	0	1 (1/1434)
EPHX2	14–2077	371	1693	2064	1 (1/371)	4 (1/423)	5 (1/413)
CYSLT1	22–990	0	969	969	0	1 (1/969)	1 (1/969)
TBXA2R	(–1050) – 1308	1315	1043	2358	0	3 (1/348)	3 (1/786)
IL4R	2129–2561	83	350	433	0	0	0
IL13RA1	(–23)–1334	76	1281	1357	0	0	0
SCYA5	(–2)–276	54	224	278	0	0	0
CMKR1	78–1320	232	1011	1243	0	0	0
CXCR4	42–1037	0	996	996	0	0	0
CCRD6	28–861	0	834	834	0	0	0
CCR7	(–17)–877	16	878	894	0	0	0
CCR10	19–1134	0	1116	1116	0	0	0
TNFRSF10C	(–114)–830	194	750	944	0	0	0
LTA4H	44–1868	2	1823	1825	0	0	0
PTGS2	(–87)–(–965)	879	0	879	0	0	0
ALOX5AP	All exons	696	483	1179	0	0	0
PGER4	29–1064	56	980	1036	0	0	0
cPLA2	All exons	1408	147	1555	0	0	0
PTGFR	(–59)–1085	64	1080	1144	0	0	0
Total (Average)		13258 bp	22759 bp	36017 bp	18 (1/737)	15 (1/1517)	33 (1/1091)

SNPs detected in more than 36,000 nucleotides, two reasons for obtaining this high incidence of non-synonymous SNPs occur to us. (1) The amino acid substitution may not influence the structure or polarity of the gene product and consequently have no effect on its function. However, as five of the seven non-synonymous SNPs found in our study probably do affect protein structure, function is almost certain to be affected. (2) Non-synonymous SNPs may confer some advantages under certain environmental conditions and expand their ratios accordingly.

Although in our association analyses, 32 of the 33 SNPs revealed no association with BA, we found a significant association between the T924C SNP in the gene and the disease. The T924C SNP is especially associated with adult BA patients. Others have suggested that molecular mechanisms of BA differ between adults and children, and our results support this idea.

Thromboxane A2, a constrictor of vascular and respiratory smooth muscles, has been implicated as a mediator of several diseases, including BA. Hence, any alteration of quality or quantity of TBXA2R, a receptor of thromboxane A2, is likely to have some effect on the respiratory constriction stimulated by inflammation and may influence susceptibility to BA. However, since the associated polymorphism is a synonymous substitution, it is unlikely to influence the function of the receptor, although it could possibly affect the efficiency of its transcription or translation. Cryptic splice sites can be generated by non-synonymous base substitutions (Richard and Beckman 1995; Siffert et al. 1998), but we can exclude this possibility here, because our RT-PCR experiments have revealed no alternatively spliced transcripts. Although the other two SNPs in the TBXA2R gene failed to show significant associations with BA; the G179T SNP has shown an association with a dominantly inherited bleeding disorder in Japanese patients (Hirata et al. 1994). As this gene has two separate transcriptional starting sites and two promoter regions (Nusing et al. 1993), we screened a total of 1473 bp of its two promoter regions for SNPs in 20 chromosomes but found no genetic variations. The possibility remains that variations exist in some genomic elements (i.e., enhancer, silencer) tightly linked to this polymorphism, or that some nearby gene(s) can influence the phenotype of BA.

Table 3 Allelic frequencies of the SNPs detected in the Japanese population (conserved amino acids are *underlined*)

Gene name	SNP (amino acid)	Region	Flanking sequence (5'flank-SNP-3'flank)	Number of genotyping	Homozygosity of major allele (%)	Heterozygosity (%)	Homozygosity of rare allele (%)	Number of alleles	Major allele (%)	Minor allele (%)
IL1B	T(-578)C	5'UTR	TTTTGAAAGC (T/C) ATAAAAACAG	160	58 (36)	78 (49)	24 (15)	320	194 (61)	126 (39)
IL8RB	C238T (R80C)	coding	CAGGGTCGGC (C/T) GCTCCGTCAC	48	47 (98)	1 (2)	0 (0)	96	95 (99)	1 (1)
IL10	T(-854)C	5'flank	GGTGATGTAA (T/C) ATCTCTGTGC	48	18 (38)	22 (46)	8 (17)	96	58 (60)	38 (40)
	A(-627)C	5'flank	CCCCGCCTGT (A/C) CTGTAGGAAG	48	18 (38)	19 (40)	11 (23)	96	55 (57)	41 (43)
CCR3	T(-520)G	5'flank	ATAATGAATG (T/G) CTCATCATTA	179	61 (34)	82 (46)	36 (20)	358	204 (57)	154 (43)
	C(-174)T	5'UTR	CTCAATCCTT (C/T) TCCTGGCACC	68	29 (43)	29 (43)	10 (15)	136	87 (64)	49 (36)
CCR5	G2076T	3'UTR	GTGGATTTGG (G/T) TTGGAAGTGA	48	11 (23)	26 (54)	11 (23)	96	48 (50)	48 (50)
	G2918T	3'UTR	TTTCTGTTCT (G/T) TCTCATATGA	48	11 (23)	27 (56)	10 (21)	96	49 (51)	47 (49)
CMA1	G(-1903)A	5'flank	CAGGCAGGTG (G/A) AGCAAAACTT	173	100 (58)	68 (39)	5 (3)	346	268 (77)	78 (23)
	C(-1777)T	5'flank	TTTGTTTAAG (C/T) CTCTGATTTT	218	61 (28)	120 (55)	37 (17)	436	242 (56)	194 (44)
TNFSF10	C825T (synonym)	coding	CCAGTTTTTT (C/T) GGGGCCTTTT	51	14 (27)	31 (61)	6 (12)	102	59 (58)	43 (42)
	A1053G	3'UTR	TATCCCAAGA (A/G) AATGAAATTG	44	17 (39)	21 (48)	6 (14)	88	55 (63)	33 (38)
	C1202A	3'UTR	TAGAAGAAAG (C/A) GCAACAATCC	48	17 (35)	25 (52)	6 (13)	96	59 (61)	37 (39)
	G1438A	3'UTR	TACTGAAAGT (G/A) CAAAAATTAG	48	17 (35)	24 (50)	7 (15)	96	58 (60)	38 (40)
	G1501A	3'UTR	GGCTGAGGCA (G/A) GAGAAT(C/T)GTT	48	16 (33)	23 (48)	9 (19)	96	55 (57)	41 (43)
	C1508T	3'UTR	GCA(A/G)GAGAAT (C/T) GTTTGAACCC	48	18 (38)	22 (46)	8 (17)	96	58 (60)	38 (40)
TNFRSF10A	C(-397)A	5'flank	TCACTTCGCC (C/A) GGTAGTGACG	179	77 (43)	80 (45)	22 (12)	358	234 (65)	124 (35)
TNFRSF10B	C(-1562)T	5'flank	CACCAATCAG (C/T) GCCCTGTCAA	80	75 (94)	5 (6)	0 (0)	160	155 (97)	5 (3)
	T95C (<u>L32P</u>)	coding	AGGCCTGGGC (T/C) CCGGGTCCCC	48	37 (77)	10 (21)	1 (2)	96	84 (88)	12 (13)
	C200T (<u>A67 V</u>)	coding	CAGCAGAGAG (C/T) GGCCCCACAA	456	261 (57)	165 (36)	30 (7)	912	687 (75)	225 (25)
CYP4F3	C806A (A269D)	coding	TTCACAGATG (C/A) CGTCATCCAG	132	95 (72)	35 (27)	2 (2)	264	225 (85)	39 (15)
	G1043A (synonym)	coding	CAAAGCACCC (G/A) GAATACCAGG	131	87 (66)	40 (31)	4 (3)	262	214 (82)	48 (18)
	A1073G (synonym)	coding	GGCAGGAGGT (A/G) CAAGAGCTTC	47	18 (38)	17 (36)	12 (25)	94	53 (56)	41 (44)
LTC4S	A(-444)C[a]	5'flank	GGATGGGGAC (A/C) GGGAACAGAT	63	49 (78)	14 (22)	0 (0)	126	112 (89)	14 (11)
EPHX2	G847A (R283E)	coding	GCAGGTTACC (G/A) GGTCCTAGCT	54	37 (69)	13 (24)	4 (7)	108	87 (81)	21 (19)
	G1272A (synonym)	coding	TCTGTGAAGC (G/A) GGAGGACTTT	54	29 (54)	21 (39)	4 (7)	108	79 (73)	29 (27)
	A1590C (synonym)	coding	TGGACAAGCC (A/C) ACCGAGGTGA	46	13 (28)	23 (50)	10 (22)	92	49 (53)	43 (47)

Table 3 (continued)

Gene name	SNP (amino acid)	Region	Flanking sequence (5'flank-SNP-3'flank)	Number of genotyping	Homozygosity of major allele (%)	Heterozygosity (%)	Homozygosity of rare allele (%)	Number of alleles	Major allele (%)	Minor allele (%)
	A1701G (synonym)	coding	CAGGTGTGCC (A/G) TCCTTCCACC	82	30 (37)	36 (44)	16 (20)	164	96 (59)	68 (41)
	T1759C	3'UTR	ACACACATCT (T/C) GCATGGATGG	79	29 (37)	34 (43)	16 (20)	158	92 (58)	66 (42)
CYSLT1	T927C (K309 N)	coding	TGTCTACATT (T/C) AGAAAGCATT	175	73 (42)	85 (49)	17 (10)	350	231 (66)	119 (34)
TBXA2R	G179T (R60L)[a]	coding	TCGCACACGC (G/T) CTCCTCCTTC	165	164 (99)	1 (1)	0 (0)	330	329 (99.7)	1 (0.3)
	C795T (synonym)[a]	coding	TGGTCTTCAT (C/T) GCCCAGACAG	302	105 (35)	154 (51)	43(14)	604	364 (60)	240 (40)
	T924C (synonym)[a]	coding	CCTGGGTGTA (T/C) ATCCTGTTCC	310	204 (66)	93 (30)	13 (4)	620	501 (81)	119 (19)

[a]SNPs reported previously

Table 4 Association of a T924C synonymous base substitution in the TBXA2R gene with BA

T924C	Number of genotyping	Homozygosity of major allele (%)	Heterozygosity (%)	Homozygosity of rare allele (%)	χ^2-value	*P*-value
Normal control	310	204 (66)	93 (30)	13 (4)		
BA	584	425 (73)	121 (21)	38 (7)	4.71	0.030
≥18 years old	260	196 (75)	47 (18)	17 (7)	6.20	0.013
<18 years old	324	229 (71)	74 (23)	21 (6)	1.74	0.188

We believe that the SNPs documented here will be useful for studies concerned with molecular mechanisms of various inflammatory diseases, as a step toward the ultimate goal of clarifying the genetic backgrounds of common diseases including BA.

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