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Identification of novel polymorphisms in the *Adam33* gene

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Abstract *Adam33* is a member of a family of genes that encode membrane-anchored proteins with a disintegrin and a metalloprotease domain and that are primarily expressed in lung fibroblasts and bronchial smooth muscle. The human *Adam33* gene is located on chromosome 20p13, a region that has been linked to asthma and bronchial hyperresponsiveness. Recently, the polymorphisms in *Adam33* have been found to be associated with asthma. In this study, we performed polymorphism scanning of the entire genomic region, including the promoter region of *Adam33*, by direct sequencing. We identified 16 novel polymorphisms in the *Adam33* gene. Among these novel polymorphisms, three polymorphisms (–2154G → A, –753T → A, and –330C → T) were found to be in the promoter region and one polymorphism (13491 G → A) was located in 3' untranslated region of the *Adam33* gene.

Keywords Adam33 · Polymorphism · Promoter · Asthma · Metalloprotease

Introduction

The membrane-anchored proteins of the *Adam* gene family possess a unique domain structure, having a disintegrin and a metalloprotease domain (Stone et al. 1999; Wolfsberg et al. 1995a). Adam proteins also contain a signal sequence, prodomain, catalytic domain, cysteine-rich domain, epidermal-growth-factor-like domain, and a cytoplasmic domain (Black and White 1998; Primakoff and Myles 2000).

These domains of the *Adam* gene family have been suggested to be involved in adhesive interactions, cell fusion, and proteolysis and in numerous biological processes, including myoblast fusion, neurogenesis, sperm maturation, and protein-ectodomain shedding of cytokines and other cell surface proteins (Howard et al. 2000; Shi et al. 2000; Wolfsberg et al. 1995b). Over 30 *Adam* genes have been identified in various species. Many of the *Adam* genes show tissue-specific expression in testes and are thought to be involved in spermatogenesis and fertilization (Cerretti et al. 1999; van Huijsduijnen 1998; Wolfsberg and White 1996; Zhu et al. 1999). In addition, Kuzbanian (*Adam10*) has been shown to play an important role in neurogenesis and neural network formation (Hattori et al. 2000; Pan and Rubin 1997). Adam proteins have also been implicated in several disease processes, including Alzheimer's disease (Moss et al. 2001; Yamamoto et al. 1999).

Recently, the *Adam33* gene has been identified and characterized in mouse and human (Gunn et al. 2002; Yoshinaka et al. 2002). It maps to human chromosome 20p13, encodes an open reading frame of 2442 bp consisting of 22 exons, and is expressed in lung fibroblasts, heart, and bronchial smooth muscle, but not in bronchial epithelial cells (Van Eerdewegh et al. 2002; Yoshinaka et al. 2002). Van Eerdewegh et al. (2002) have reported that the *Adam33* gene is associated with asthma and bronchial hyperresponsiveness. In this study, we have scanned the entire

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Table 1 Primer sequences used in this study

Primer name	Forward primer sequence (5' → 3')	Reverse primer sequence (5' → 3')
Adam33-1	AGTGGCTGTCTGGGGTTG	TTTCCCAGGCTGGTCTCA
Adam33-2	TGGCCAAGACCAGGAGTT	GGGAAAAGTCTCCGTGCTC
Adam33-3	GAAAATCAAAGTGCAGAAAAACG	CCGGAGATGTTAACTTTAAGCAA
Adam33-4	AGCCCAGATCATGCCATT	GCCCCAAGGATAGGAAG
Adam33-5	TTATGCAATTCTGCCCTCTGG	GGGCGGAGGAGGGAATC
Adam33-6	TCACAGCTATGGGCTGGAG	GAGTCCTAAGGGGTGGAGGA
Adam33-7	CTGCCTGGATTCTCGCTCT	CAGGGGTCCCATCACCTC
Adam33-8	CCCCTCTGAGCCCTATCC	CAGTCCTGCTGGGCTGAT
Adam33-9	CAGCCCAGCAGGACTGAG	CGGCGATCACAAGGAAGA
Adam33-10	TCCTTCCCCTCTGCACTG	CCCAGTGCCTTCTTCTGC
Adam33-11	GTCCCCAGAACCACCAAGT	GCCCTCTTAGTTTGACAGCAC
Adam33-12	GACCACCCCTGTGCTTC	CCTTTGGGGCTCTCCTCT
Adam33-13	TGGGGTCACTCTGCCTCT	TGTGGGAACACCCCACTT
Adam33-14	AGCCAGGCTGAGTGGACA	CGTGGAGGAGGTGAGAACA
Adam33-15	AGGGAGGATGCTGGTGTG	GCCAGCAGGGAGCTAACA
Adam33-16	AGGGAGGATGCTGGTGTG	TGCTCCCCTCCCTGTAGA
Adam33-17	GGGGCAGCAGTTTGTCTG	TTTCCCACCGATGGAT
Adam33-18	GAGGCCAAGGTGAGGTTG	GGGACAGGAGCAATGGTG
Adam33-19	TCCCTGGTGCTTCCCATA	TCTCCCAGAGCTCACCTCA
Adam33-20	CCTCTTGCCCCCTCTTGCT	TGAGCCCTGACCACCAAT
Adam33-21	GAAGAGACGGGAATTCACAGG	ACTGCAGGAAGGCCAGAGA
Adam33-22	TCGCCCTCAGCTTCTCAG	AAACGCAGCGGAGGAAGT
Adam33-23	CTCTACGGCCGCACTGAC	CGCGCAAAGTCACACAAC
Adam33-24	AGGGGTGGGGATCAGAGA	GAGAGGTCCATGCCGAGA
Adam33-25	AATTGGTGCCCTCAGGTTT	CTGCACAGGGCAGGAAGT
Adam33-26	GCCTGTTTCCAGGTGGTG	AGGTCACCCCCACTCCTC
Adam33-27	TGCCTTCCAGGAGCTTCA	CTCTGGGGCTGCTCTCTG
Adam33-28	GCCTGCTTCTGAGCCTA	TGTCAGACATGGCCACAGA
Adam33-29	CTCTGTGACCTGGGTGGTG	CAGCAGCCTGGCACTCTC
Adam33-30	GCGGACATCCAAGGTTCTT	GGGTCTGGGAGAAATGGTG
Adam33-31	CTTCCAGATGGGCAGCAC	TGCCCTGCAGTTCAAGT
Adam33-32	GCCAAGATCCTGCCTCTG	GGTGTAGCACTGGGATTGG

Adam33 gene sequence, including the promoter region, by direct sequencing in order to detect polymorphic sites in *Adam33*.

Materials and methods

DNA samples

Blood samples were obtained from 24 healthy Korean individuals. Genomic DNAs were extracted from leukocytes of peripheral blood by a standard phenol-chloroform method.

Polymerase chain reaction

Genomic regions of *Adam33* containing the promoter region were partially amplified and sequenced by using the 32 primer pairs shown in Table 1. The polymerase chain reaction (PCR) was carried out in a 25- μ l reaction volume containing 50 ng genomic DNA, 0.5 μ M primers, 0.2 mM dNTP, 1.5 mM MgCl₂, 10 mM TRIS-HCl (pH 8.3), and 1 U Ex *Taq* polymerase (TaKaRa). PCR was run for 30 cycles of denaturation at 95°C for 30 s, annealing at the melting temperature of each primer pair for 1 min, and extension at 72°C for 1 min on a GeneAmp PCR system 9700 thermocycler (Applied Biosystem).

Sequencing analysis

Both the sense and antisense strands of the PCR products were directly sequenced by using the same primers as those for the PCR amplification. PCR products purified by a PCR purification kit (Millipore, USA) were used as the template DNA for cycle sequencing. Sequence analysis was performed by BigDye Termini-

Fig. 1 Locations of each single-nucleotide polymorphism (SNP) in *Adam33* (black blocks coding exons). The positions of SNPs were calculated from the translation start site. The reference sequence is based on the human chromosome 20 genomic contig defined by the Human Genome Project. Size bar 1 kb

Table 2 Summary of SNPs detected in the *Adam33* gene

Position ^a	Region	Amino acid	Frequency	Position ^a	Region	Amino acid	Frequency
–2225G → A ^b	Promoter		7/44[A]	7667 A → G	Intron 7		1/42[G]
–2154G → A	Promoter		14/48[A]	8227 G → A	Exon 9	Gly288Gly	6/48[A]
–1263C → G	Promoter		3/40[G]	8889 C → T	Intron 11		5/48[T]
–753T → A ^b	Promoter		1/44[A]	9511 G → T	Intron 13		2/48[T]
–330C → T ^b	Promoter		3/44[T]	10453 C → T	Intron 16		5/48[T]
381 A → G	Intron 1		20/32[G]	10474 C → T ^b	Intron 16		4/48[T]
419 C → T ^b	Intron 1		4/48[T]	10895 G → A	Exon 19	Val710Ile	1/46[A]
517 A → G ^b	Intron 1		13/34[G]	10918 G → C	Exon 19	Gly717Gly	10/44[C]
819 C → G	Intron 1		5/42[G]	11188 T → A	Intron 19		17/46[A]
1109 C → T	Intron 1		17/42[T]	11391 T → C	Intron 19		18/46[C]
1712 G → C	Intron 1		41/46[C]	11434 A → C	Intron 19		24/46[C]
1733 A → G ^b	Intron 1		6/40[G]	11495 T → C	Intron 19		24/46[C]
1870 G → A	Intron 1		10/48[A]	11966 G → A	Intron 19		7/48[A]
2062 G → A ^b	Intron 1		9/48[A]	12136 G → C ^b	Intron 19		17/40[C]
2272 C → T ^b	Intron 1		1/38[T]	12433 T → C	Exon 20	Met764Thr	1/42[C]
2991 T → C ^b	Intron 2		1/42[C]	12462 C → T	Exon 20	Pro774Ser	2/44[T]
3012 C → G	Intron 2		24/44[G]	12540 C → T	Intron 20		1/48[T]
3728 G → A	Intron 2		21/48[A]	12864 C → G	Intron 21		1/46[G]
4448 G → A	Intron 2		6/48[A]	12946 G → A	Intron 21		9/44[A]
4565 G → A ^b	Intron 2		12/48[A]	12954 C → T	Intron 21		7/42[T]
4855 T → A	Intron 2		2/48[A]	12988 C → A	Intron 21		9/44[A]
4856 C → T	Intron 2		2/48[T]	13204 C → T	3'UTR		31/44[T]
5065 G → C ^b	Intron 3		1/48[C]	13236 T → C	3'UTR		8/44[C]
5272 C → T ^b	Intron 3		1/46[T]	13491 G → A ^b	3'UTR		1/46[A]
6139 G → C ^b	Intron 3		1/48[C]	13506 C → G	3'UTR		33/48[G]
7575 G → A	Intron 7		12/48[A]				

^aCalculated from the translational start site

^bNovel SNPs identified in this study

nator cycle sequencing (Applied Biosystems) on an ABI 3700 Prism Automated DNA sequencer (Applied Biosystems) according to the manufacturer's instructions.

Results and discussion

To discover novel single-nucleotide polymorphisms (SNPs) in the *Adam33* gene, we performed scanning by direct sequencing on genomic DNA samples isolated from 24 Korean healthy individuals. We found a total of 51 SNPs in the *Adam33* gene, 16 SNPs of which were identified as novel polymorphic sites (Fig. 1, Table 2). Among the novel SNPs, three SNPs (–2154G → A, which is a G to A substitution at position –2154 counting from the translation start site, –753T → A, and –330C → T) were located in the promoter region, five SNPs (419C → T, 517A → G, 1733A → G, 2062G → A, and 2272C → T) were located in intron 1, two SNPs (2291T → C and 4565G → A) were located in the intron 2, three SNPs (5065G → C, 5272C → T, and 6139G → C) were located in intron 3, and 10474C → T, 12136G → C, and 13491G → A were located in intron 16, intron 19, and the 3' untranslated region (UTR), respectively. Among the polymorphic sites located in introns, polymorphic site 12136G → C was located in intron 19 and showed a high allele frequency (42.5%). Van Eerdewegh et al. (2002) showed that some of the SNPs having significant association with asthma were located in the introns and 3'UTR of the *Adam33* gene. Indeed, several SNPs located in intron 19, such as 12136G → C, were significantly associated with asthma

(Van Eerdewegh et al. 2002). The SNPs identified in this study should provide important information for case-control association studies. Linkage disequilibrium (LD) coefficients between each SNP (381A → G, 1712G → C, 11434A → C, and 13506C → G) pair including our two novel SNPs (517A → G and 12136G → C) were calculated. We observed no strong LD (data not shown).

In many genes, SNPs located within promoter regions have been described as affecting gene transcription and causing inter-individual variations in gene production. We detected three novel SNPs in the promoter region of the *Adam33* gene. Specifically, a G to A polymorphism at the position –2154 had a high allele frequency (29.2%). It would be interesting to analyze the allele frequency of these variances with large samples of patients with autoimmune and allergic diseases, since evidence for the involvement of *Adam33* in asthma and bronchial hyperresponsiveness has been reported (Van Eerdewegh et al. 2002).

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