

# Association between a new polymorphism in the activation-induced cytidine deaminase gene and atopic asthma and the regulation of total serum IgE levels

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**Background:** Activation-induced cytidine deaminase (AICDA) is a recently identified RNA-editing deaminase that plays an important role in class-switching. Defects in AICDA result in a hyper-IgM phenotype and lack of IgG, IgA, and IgE in both human beings and mice.

**Objective:** The aim of this study was to determine whether the AICDA gene is related to regulation of total serum IgE and development of atopic asthma.

**Methods:** We screened for polymorphisms in the 5'-flanking and coding regions of the AICDA gene in subjects with atopic asthma and analyzed the effect of these polymorphisms on the development of atopic asthma and on total serum IgE levels in Japanese asthmatic families.

**Results:** We identified 3 novel polymorphisms (5923A/G, 7888C/T, and 8578A/C) and 1 rare variant (Arg25Cys) in the AICDA gene. Transmission disequilibrium testing showed that the 7888C allele was transmitted preferentially to asthma-affected children ( $P = .007$ ). Mean log [total serum IgE] levels of parents with the 7888C/7888C, 7888C/7888T, and 7888T/7888T genotypes were 2.12, 1.99, and 1.77, respectively, and a significant association was observed between the genotypes ( $P = .02$ ). In RT-PCR experiments, we found 2 novel splice variants of AICDA, one lacking all of exon 4 (variant 1; 367 base pairs) and the other lacking the first 30 base pairs of exon 4 (variant 2; 453 base pairs). These variants were not associated with the 7888C/T polymorphism.

**Conclusion:** The 7888C/T polymorphism might be associated with the pathogenesis of atopic asthma and the regulation of total serum IgE levels. (*J Allergy Clin Immunol* 2001;108:382-6.)

**Key words:** Atopy, asthma, AICDA, total serum IgE, TDT, splice variants

## Abbreviations used

AICDA: Activation-induced cytidine deaminase  
HIGM2: Autosomal recessive form of hyper-IgM syndrome  
RT: Reverse transcriptase  
TDT: Transmission disequilibrium test

Atopic diseases such as asthma and atopic eczema are a major cause of morbidity in developed countries and have been increasing in frequency.<sup>1,2</sup> Atopy, which is characterized by increased levels of IgE against common environmental allergens, is considered the strongest predisposing factor for asthma. More than 90% of children with asthma develop specific IgE against house dust mite, and dust mite allergy is associated strongly with asthma.<sup>3,4</sup>

Total serum IgE levels are regulated by both genetic and environmental factors. Associations between total serum IgE levels and polymorphisms in candidate genes, such as IL-4 (C-590T), CD14 (C-159T),  $\beta$ 2-adrenergic receptor (Arg16Gly and Glu27Gln), and FCER1B have been reported, but the results have not been conclusive.<sup>5,6</sup>

Class-switching to IgE requires 2 signals. The first signal is delivered by cytokines IL-4 and IL-13, which target the Cepsilon gene for switch recombination. The second signal is due to the interaction of the B cell surface antigen CD40 with its ligand (CD40L), which is expressed on activated T cells; this activates deletional switch recombination.<sup>7</sup> Several other genes are also involved in this process.<sup>8</sup>

Activation-induced cytidine deaminase (AICDA) is a member of the cytidine deaminase family that encodes a 198-amino acid protein and is mapped to human chromosome 12p13.<sup>9</sup> AICDA is an RNA-editing deaminase that creates new functional products from mRNAs by base substitution. A recent knockout mouse study showed that AICDA deficiency leads to a complete defect in class-switching, resulting in a hyper-IgM phenotype and lack of IgG, IgA, and IgE.<sup>10</sup> Mutations of AICDA have been found in patients with an autosomal recessive form of hyper-IgM syndrome (HIGM2).<sup>11</sup> Some of these mutations yield truncated AICDA protein by generation of stop codons or deletions. Other mis-

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sense mutations have been localized within the putative cytidine deaminase catalytic region of the protein.<sup>9,11</sup>

Although the mechanism of class-switching to IgE is not fully understood, AICDA appears to be a good candidate gene for the regulation of IgE and asthma. In this study, we screened for polymorphisms in the 5'-flanking and coding regions of the AICDA gene in subjects with atopic asthma and controls. We found 4 novel nuclear variants in the AICDA gene and conducted an association study in Japanese families identified through asthmatic children.

## MATERIALS AND METHODS

### Subjects

Proband was children with asthma who visited the Pediatric Allergy Clinic of the University Hospital of Tsukuba. A full verbal and written explanation of the study was given to all family members interviewed, and 144 families (476 members) gave informed consent and participated in this study. The mean age of the probands and their siblings was 10.6 years (range, 3 to 29 years); the mean age of the parents was 40 years (range, 26 to 72 years). Each family member was questioned regarding allergic symptoms and underwent a physical examination performed by a participating pediatrician. Asthma was diagnosed in subjects according to the criteria of the US National Institutes of Health, with minor modifications.<sup>12</sup> Patients had to satisfy the following pair of criteria: (1) 2 or more episodes of wheezing and shortness of breath during the past year and (2) reversibility of the wheezing and dyspnea, either spontaneously or by bronchodilator treatment. Because of the low age of the asthmatic members of the families, methacholine challenge was not done; however, the diagnoses of asthma in this population were confirmed clinically by participating physicians or pediatricians who had treated the children for more than 2 years. Because wheezing is often associated with viral respiratory infection in young children, we excluded those younger than 3 years old.<sup>13</sup> The young adult patients included in this study had had chronic asthma since childhood. Total serum IgE levels and IgE levels specific for *Der-matophagoides farinae* were determined by means of the Pharmacia CAP System (Uppsala, Sweden). To achieve homogeneity of the asthmatic patients, we selected families having more than 2 asthmatic children with *D farinae* RAST scores greater than 3. The families examined in this study are in part the same ones that participated in our genome-wide study.<sup>14</sup>

We selected controls who met all of the following criteria: (1) no symptoms or history of allergic diseases, (2) no detectable dust mite-specific IgE antibody, and (3) total serum IgE levels below the general population mean for their ages.

This study was approved by the Committee of Ethics of the University of Tsukuba.

### Molecular method

DNA was extracted from peripheral blood leukocytes collected in EDTA. The 5 exons and the 5'-flanking region of the AICDA gene were amplified from the genomic DNAs of 16 asthmatic subjects and 16 controls. Asthmatics for the mutation study were selected randomly from the asthmatic probands described above.

The regions of all exons and exon-intron junctions and the 5'-flanking region of 748 base pairs (bp) from the translation initiation site were amplified by PCR. Primers for exons were designed according to the published sequence for the AICDA gene (GenBank accession numbers AB040430, AC013443). Sequencing was performed with the Big Dye Terminator kit (Applied Biosystems, Foster City, Calif) on an ABI PRISM 3100 DNA Sequencer (Applied Biosystems).

We identified one rare missense mutation (Arg25Cys) in exon 2, one silent mutation in exon 4 (7888C>T), one 3'-UTR mutation (8578A>C), and one intron mutation (5923A>G). To genotype 5923A/G, 7888C/T, and 8578A/C polymorphisms, the region of interest was amplified by PCR with primer pairs 5'-TTAGGGTGC-CCAGCATTAC-3' and 5'-AGCATCTTCTTTGGTTTAGC-3' for 5923A/G, with 5'-CTTCCCAGGCTTTGAAAGTT-3' and 5'-TGAATGGCTCAGAGACAAGG-3' for 7888C/T, and with 5'-TTTAGGATGCTCCCAGGTC-3' and 5'-ACAAAGCCATC-CACTCAGT-3' for 8578A/C. The PCR product sizes for the 5923A/G, 7888C/T, and 8578A/C polymorphisms were 485 bp, 196 bp, and 433 bp, respectively. The 5923A/G and 7888C/T mutations were identified by the loss of *AluI* and the gain of *NlaIII* restriction sites, respectively. Digested PCR fragments were subjected to agarose gel electrophoresis and visualized by means of ethidium bromide staining and ultraviolet transillumination. The 8578A/C polymorphism was determined by direct sequencing, as described above.

### Expression of AICDA

RT-PCR was performed to determine whether any polymorphisms within the AICDA gene cause splice variants. Peripheral blood lymphocytes from 3 asthmatic families were purified with Ficoll-Paque TM gradient (Amersham Pharmacia Biotech AB, Uppsala, Sweden). RNA from lymphocytes was extracted with TRIzol (Life Technologies, Rockville, Md) according to the manufacturer's instructions. Complementary DNA was generated from RNA by the addition of random primers (Life Technologies) and SuperScript reverse transcriptase (RT) in vendor-supplied RT buffer (Life Technologies) at 42°C for 1 hour. Complementary DNA derived from the reactions was subjected to PCR with primers 5'-CGTAGT-GAAGAGGCGTGACA-3' and 5'-GCGTCTCGTAAGTCAT-CAACC-3' to amplify the region between exon 2 and exon 5 of AICDA. The expected product size was 483 bp.

### Statistics

Deviation from Hardy-Weinberg expectations was examined by  $\chi^2$  testing. Linkage disequilibrium and an allelic transmission disequilibrium test (TDT) were assessed with the SIB-PAIR program.<sup>15</sup> Quantitative TDTs for total serum IgE levels were performed with the QTDT program.<sup>16</sup> A proband was selected from each family for TDT analysis, and 144 trios were analyzed for TDT. An association between total serum IgE levels and genotypes was analyzed by ANOVA and the Wilcoxon test. A *P* value less than .05 was considered statistically significant.

## RESULTS

We identified one missense mutation (Arg25Cys) in exon 2, one silent mutation in exon 4 (7888C>T), one 3'UTR mutation (8578A>C), and one intron mutation (5923A>G). Of these 4 mutations, the Arg25Cys mutation was rare, appearing in only 1 of 100 subjects (Fig 1).

Significant linkage disequilibrium was observed neither between the 5923A/G and 7888C/T polymorphisms ( $n = 476$ ,  $D = 0.0142$ ,  $D' = 0.2075$ ,  $P = .07$ ) nor between the 7888C/T and 8578A/C polymorphisms ( $n = 476$ ,  $D = 0.0044$ ,  $D' = 0.1020$ ,  $P = .49$ ).

The frequencies of the parental genotypes for the 5923A/G, 7888C/T, and 8578A/C polymorphisms did not deviate from expected values on the basis of Hardy-Weinberg equilibrium ( $P > .1$ ). TDT analyses revealed that the 7888C allele was transmitted preferentially to asthmatic children ( $P = .007$ ; Table I).

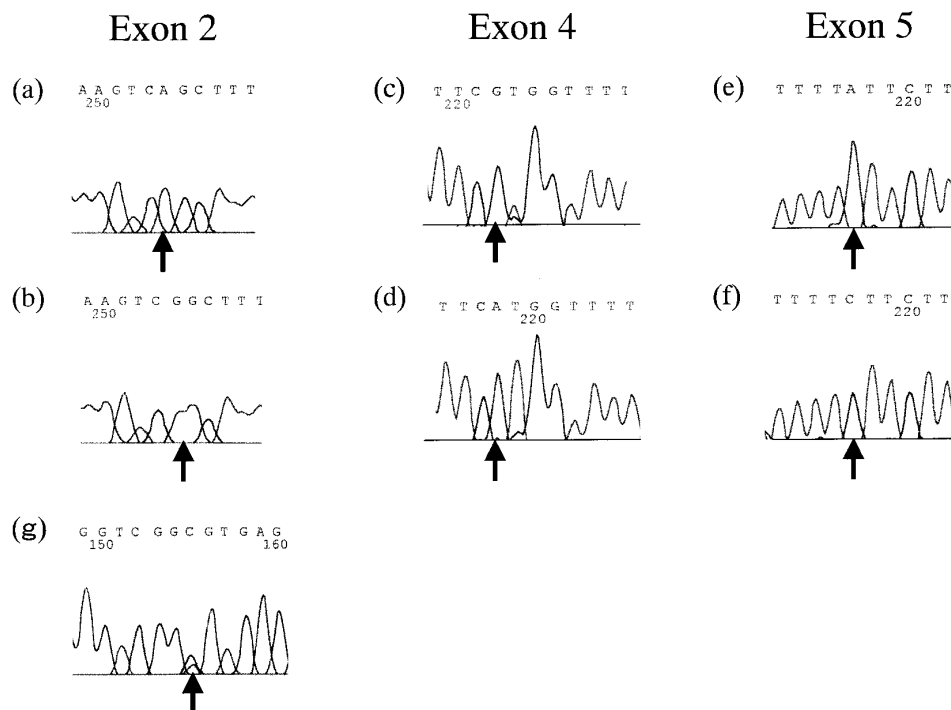


**FIG 1.** Panels show sequences of wild-type and mutant alleles for polymorphisms. **A** and **B**, 5923A/G; **C** and **D**, 7888C/T; **E** and **F**, 8578A/C; **G**, a rare mutation, Arg25Cys. Arrows indicate the polymorphic sites.

**TABLE I.** TDTs for the 5923A/G, 7888C/T, and 8578A/C polymorphisms (n = 432; 144 trios)

| Polymorphism | Allele | No. transmitted | No. not transmitted | P value |
|--------------|--------|-----------------|---------------------|---------|
| 5923A/G      | A      | 22              | 16                  | .42     |
| 788C/T       | C      | 56              | 30                  | .007    |
| 8578A/C      | A      | 21              | 15                  | .41     |

Mean log [total serum IgE] levels of parents with 7888C/7888C, 7888C/7888T, and 7888T/7888T genotypes were 2.12, 1.99, and 1.77, respectively, and a significant association was observed between the groups ( $P = .025$  for ANOVA and  $P = .020$  for the Wilcoxon test). The mean log [total serum IgE] levels of the probands with these genotypes were 2.85, 2.83, and 2.63, respectively, and no significant association was observed ( $P > .1$ ). In quantitative TDT analysis, no associations were observed between the log [total IgE] level and either allele of the 7888C/T polymorphism ( $P > 0.1$ ).

In RT-PCR experiments, we found 2 novel splice variants of the AICDA gene (Fig 2). One lacked all of exon 4 (variant 1; 367 bp), and the other lacked the first 30 bp of exon 4 (variant 2; 453 bp). Among 10 subjects, 7 had variant 1 and 6 had variant 2. These variants were not associated with the 7888C/T polymorphism. In family 3, variant 1 and variant 2 appeared in the child but neither parent had either variant, suggesting that these variants are probably not caused by genetic polymorphisms.

## DISCUSSION

We have identified 3 polymorphisms and 1 rare variant in the AICDA gene. AICDA is a novel member of the RNA-editing deaminase family. Some genes, such as apolipoprotein B, are edited by the RNA-editing deaminases, resulting in the production of novel proteins with different functions.<sup>17,18</sup> Recent studies have shown that AICDA deficiency prevents immunoglobulin class-switch recombination, prevents immunoglobulin somatic hypermutations, and causes lymph node hyperplasia because of the presence of a giant germinal center. Rare variants in human AICDA gene have been identified and shown to cause HIGM2. Most of the variants identified in patients with HIGM2 are deletions or stop codons, which result in truncated AICDA proteins or missense mutations localized within the putative cytidine deaminase catalytic region of the protein.<sup>9,11</sup> The mechanism of IgE class-switching is not fully understood; however, it is suggested that AICDA is important in the processing of pre-RNA(s) encoding proteins involved in the mechanisms of switch recombination or that AICDA mediates the construction of ribozymes with nuclease activity.<sup>19</sup>

We identified a novel missense mutation, Arg25Cys, in the coding region of exon 2; however, this mutation is located outside of the putative cytidine deaminase catalytic region. The total serum IgG, IgA, IgM, and IgE levels of the subject with the Arg/Cys genotype were 516 mg/dL, 74 mg/dL, 17 mg/dL, and <0.34 IU/mL, respectively, indicating that this missense variant does not profoundly influence AICDA protein function, at least not in heterozygotes.

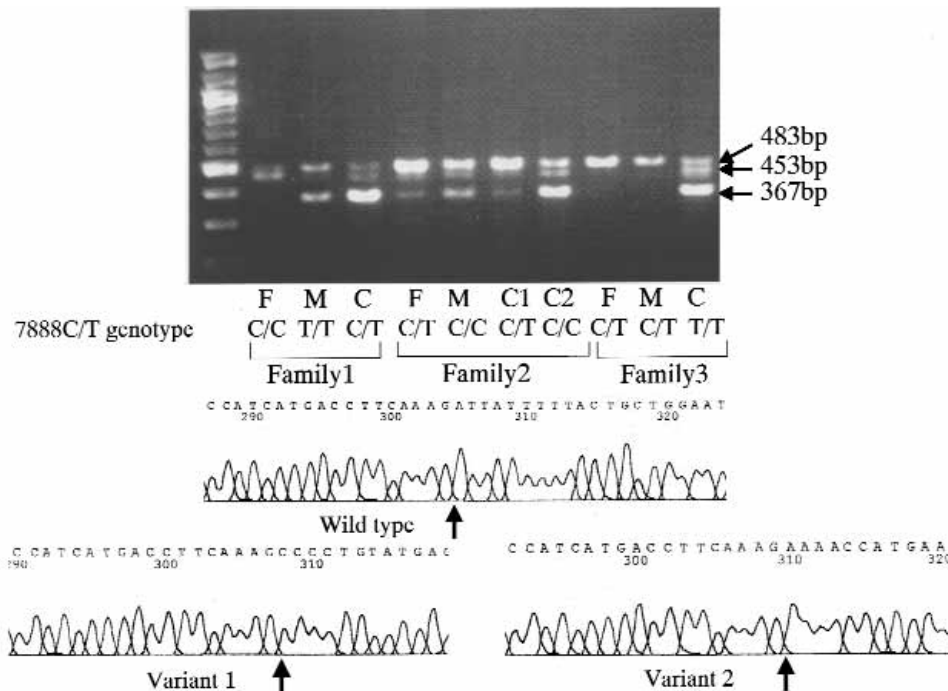


**FIG 2.** Top, RT-PCR results between exons 2 and 5 of AICDA. F, father; M, mother; C, child. Bottom, Sequence of wild-type and splice variants of AICDA. Arrows indicate exon 3 and exon 4 boundaries. Exon 5 starts after the end of exon 3 in variant 1; the first 30 bp of exon 4 are missing in variant 2.

We have shown that the 7888C allele is associated with development of atopic asthma and with total serum IgE levels. In our genome-wide search for atopic asthma in 47 families with affected sibling pairs (sib-pairs),<sup>14</sup> we genotyped the microsatellite marker D12S336, which is located at 13265 kb, according to the National Center for Biotechnology Information map viewer. This marker is approximately 4 Mb from the AICDA gene. The non-parametric linkage (NPL) score for this marker is 0.67 ( $P > .05$ ). With our genome-scan families, the expected NPL score for the 7888C/T polymorphism is 0.4 if it is assumed that the 7888C allele is transmitted 1.9 times more frequently to asthma-affected children.

Asthma is considered a complex disease in which many genes with small effects are involved. To detect genes in a complex trait, disequilibrium mapping is more powerful than linkage analysis by affected sib-pair methods.<sup>20</sup> Therefore, it is likely that failure to find linkage in this region is due to the low power of affected sib-pair methods and to the small sample size of our genome-wide families.

The association between the log [total IgE] level and 7888C/T was significant in adults but not in children. This might be due to an age effect,<sup>21</sup> because the variability of total serum IgE is most marked in children and teenagers and is less obvious in adults. Moreover, all of the children in this study were mite-sensitive asthmatics, who might be prone to show changes in total serum IgE levels as a result of the exposure to allergens.<sup>22</sup>

Because the 7888C/T polymorphism is synonymous, we tried to evaluate the relation between AICDA mRNA

and the polymorphism. The RT-PCR experiment with sample families detected novel AICDA splice variants lacking all or part of exon 4. These variants, however, were not associated with the 7888C/T polymorphism. Exon 4 is not in the putative cytidine deaminase catalytic region. Revy et al<sup>11</sup> showed that subjects with homozygous missense mutation or a stop codon in exon 4 developed HIGM2. Therefore, it is unlikely that this splice variant has enzymatic activity.

Although the possible functional consequence is unknown, our data suggest that the 7888C/T polymorphism or its associated polymorphisms of AICDA contribute to the development of asthma and the regulation of total IgE levels.

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#### REFERENCES

1. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 1998;351:1225-32.
2. Gergen PJ, Weiss KB. The increasing problem of asthma in the United States. *Am Rev Respir Dis* 1992;146:823-4.
3. Shibasaki M, Tajima K, Morikawa A, Mitsuhashi M, Sumazaki R, Tokuyama K. Relation between frequency of asthma and IgE antibody levels against *Dermatophagoides farinae* and total serum IgE levels in schoolchildren. *J Allergy Clin Immunol* 1988;82:86-94.
4. Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin Exp Allergy* 1989;19:419-24.

5. Nanavaty U, Goldstein AD, Levine SJ. Polymorphisms in candidate asthma genes. *Am J Med Sci* 2001;321:11-6.
6. Sandford AJ, Pare PD. The genetics of asthma. The important questions. *Am J Respir Crit Care Med* 2000;161(3 Pt 2):S202-S206.
7. Bacharier LB, Geha RS. Molecular mechanisms of IgE regulation. *J Allergy Clin Immunol* 2000;105(2 Pt 2):S547-S558.
8. Nagumo H, Agematsu K, Shinozaki K, et al. CD27/CD70 interaction augments IgE secretion by promoting the differentiation of memory B cells into plasma cells. *J Immunol* 1998;161:6496-502.
9. Muto T, Muramatsu M, Taniwaki M, Kinoshita K, Honjo T. Isolation, tissue distribution, and chromosomal localization of the human activation-induced cytidine deaminase (AID) gene. *Genomics* 2000;68:85-8.
10. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 2000;102:553-63.
11. Revy P, Muto T, Levy Y, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). *Cell* 2000;102:565-75.
12. National Heart, Lung, and Blood Institute NIH. Guidelines for the diagnosis and management of asthma. Washington DC: US Government Printing Office; 1997.
13. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;332:133-8.
14. Yokouchi Y, Nukaga Y, Shibasaki M, et al. Significant evidence for linkage of mite-sensitive childhood asthma to chromosome 5q31-q33 near the interleukin 12 B locus by a genome-wide search in Japanese families. *Genomics* 2000;66:152-60.
15. Duffy DL. SIB-PAIR v.0.93. Queensland Institute of Medical Research, Brisbane. Available at <http://www.qimr.edu.au/davidd.html>. 1996. Accessed June 2000.
16. Abecasis GR, Cardon LR, Cookson WO. A general test of association for quantitative traits in nuclear families. *Am J Hum Genet* 2000;66:279-92.
17. Mehta A, Kinter MT, Sherman NE, Driscoll DM. Molecular cloning of apobec-1 complementation factor, a novel RNA-binding protein involved in the editing of apolipoprotein B mRNA. *Mol Cell Biol* 2000;20:1846-54.
18. Teng B, Burant CF, Davidson NO. Molecular cloning of an apolipoprotein B messenger RNA editing protein. *Science* 1993;260:1816-9.
19. Oettgen HC. Regulation of the IgE isotype switch: new insights on cytokine signals and the functions of epsilon germline transcripts. *Curr Opin Immunol* 2000;12:618-23.
20. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996;273:1516-7.
21. Barbee RA, Halonen M, Lebowitz M, Burrows B. Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity. *J Allergy Clin Immunol* 1981;68:106-11.
22. Graves PE, Kabesch M, Halonen M, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000;105:506-13.

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