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Mutation and association analysis of the interferon regulatory factor 2 gene (*IRF2*) with atopic dermatitis

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Abstract Interferon regulatory factor 2 (IRF-2) is a member of a family of transcriptional factors involved in the modulation of cellular responses to interferons (IFNs) and viral infection as well as in the regulation of cell growth and transformation. *Irf2* knockout mice show T helper 1 (Th1) cell development defect and spontaneous development of an inflammatory skin disease. To determine if there are any mutations in *IRF2* associated with development of atopic dermatitis (AD), we screened for mutations in the 5' flanking and coding regions of *IRF2* in AD patients and control subjects by single-strand conformational polymorphism (SSCP) analysis. We found three mutations in the promoter region ([−829C>T, −830C>T], −684C>T, and −467G>A), one silent mutation in exon 9 (921G>A), and a 10-bp deletion in the 3' untranslated region (1739[ATCCC]8>6). Among them, the −467G allele and the haplotype of the −467G, 921A, and 1739(ATCCC)8 alleles were transmitted preferentially to AD-affected children ($P = 0.02$ and $P = 0.007$, respectively). Our data suggest that IRF-2 plays some role in the development of AD in the Japanese population.

Key words Atopic dermatitis · *IRF2* · Polymorphism · Japanese population · Transmission disequilibrium test

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Introduction

Atopic dermatitis (AD) features an intensely pruritic rash with a characteristic morphology and distribution. It affects 10%–20% of children in developed countries and shows strong familial aggregation (Larsen et al. 1986). Eighty percent of AD cases show elevated total serum IgE levels (Juhlin et al. 1969), and atopic mechanisms dominate current understanding of the pathogenesis of this disease (Bos 2000).

Interferon regulatory factors (IRFs) comprise a family of transcriptional factors that includes IRF-1, IRF-2, ICSBP, p48, IRF-3, IRF-4, IRF-7, and vIRFs. They are involved in modulation of cellular responses to interferons (IFNs) and to viral infection and in the regulation of cell growth and transformation. IRF-2 was originally described as an antagonist of IRF-1-mediated transcriptional regulation of IFN-inducible genes (Harada et al. 1989). IRF-1 deficiency results in increased production of T helper (Th) 2-related cytokines and a compensatory decrease in the expression of naive cell- and Th1-related cytokines (McElligott et al. 1997). *Irf2* knockout mice show a defect in Th1 cell development, impairment of natural killer cells (Lohoff et al. 2000), and spontaneous development of an inflammatory skin disease involving CD8+ T cells (Hida et al. 2000). These observations suggest that *IRF2* might be involved in the pathogenesis of AD.

We screened for mutations in the 5' flanking and coding regions of *IRF2* on chromosome 4q35 in AD patients. We found five novel variants in *IRF2* and conducted a transmission disequilibrium test (TDT) in families identified through patients with AD.

Subjects and methods

Subjects

Probands of the families studied were patients with AD who visited the Department of Dermatology or Pediatric

Allergy Clinic of the University Hospital of Tsukuba, Japan. A full verbal and written explanation of the study was given to all family members interviewed, and 49 families (180 members, including children with atopic asthma) gave informed consent and participated in the study. The mean age of the probands and their siblings was 13 years (range, 1 to 36 years), and the mean age of the parents was 42 years (range, 28 to 72 years). Each family member was questioned regarding allergic symptoms, and each underwent physical examination by a participating dermatologist. AD was diagnosed in subjects according to the criteria of Hanifin and Rajka (1980).

Screening for mutations

Genomic DNA was isolated from peripheral blood leukocytes collected in ethylenediaminetetraacetate by the standard phenol/chloroform extraction method. The nine exons and the promoter region of *IRF2* were amplified from genomic DNA of 24 subjects with AD and 24 control subjects. Subjects with AD were selected randomly for the mutation study from among the AD probands described above.

All exons and exon-intron junctions and the 5' flanking region 1655bp upstream of the translation initiation site were amplified by polymerase chain reaction (PCR). Primers for exons were designed according to the published sequence of *IRF2* (GenBank Accession Numbers L24442, NM_002199) (Cha and Deisseroth 1994; Harada et al. 1989). Single-strand conformational polymorphism (SSCP) analysis was done for all PCR products (each exon and the promoter region for each individual). PCR products with variant SSCP patterns were subjected to sequence analysis.

Genotyping

To genotype the polymorphisms, each region of interest was amplified by PCR with a primer pair as shown in Table 1. The [−829C/T, −830C/T] (the first nucleotide of NM_002199 was denoted +1), −684C/T, −467G/A, and 921G/A mutations were identified by the loss of *Fnu4HI*, gain of *MseI*, gain of *NlaIII*, and loss of *Fnu4HI* restric-

tion sites, respectively. Digested PCR fragments and PCR products for the 1739(ATCCC)6–8 polymorphism were subjected to agarose gel electrophoresis and visualized by ethidium bromide staining and ultraviolet transillumination.

Statistical analysis

Deviation from Hardy-Weinberg expectancy was examined by χ -squared test. An allelic transmission disequilibrium test (TDT) was performed via the GENEHUNTER version 2 program (<http://www-genome.wi.mit.edu/ftp/distribution/software/genehunter/>). *P* values less than 0.05 were considered significant.

Results and discussion

We identified three mutations in the promoter region ([−829C>T, −830C>T], −684C>T, and −467G>A), one silent mutation in exon 9 (921G>A, G248G), and a 1739(ATCCC)8>6 in the 3' untranslated region (UTR) (Fig. 1). Among these five polymorphisms, [−830C/T, −829C/T] and −684C/T were rare, appearing in only 1 and 6 of 92 subjects, respectively.

The 1739(ATCCC)6–8 polymorphism was in complete linkage disequilibrium with the 921G/A polymorphism ($n = 168$, $D = 0.0428$, $D' = 1.0$, $P < 0.0001$), but no significant linkage disequilibrium was observed between the −467G/A and 921G/A polymorphisms ($n = 160$, $D = 0.0147$, $D' = 0.176$, $P = 0.3$).

The genotype frequencies of the −467G/A and 921G/A and 1739(ATCCC)6–8 polymorphisms in the parents did not deviate from expected Hardy-Weinberg equilibrium frequencies ($P > 0.05$). According to TDT analysis, the −467G allele was transmitted preferentially to AD-affected children ($P = 0.02$, Table 2), but statistical significance was not reached after correction for multiple comparison ($P_c = 0.06$). Neither allele of the 921G/A or 1739(ATCCC)6–8 polymorphisms was transmitted preferentially to AD-affected children (Table 2). Because the TDT analysis included only informative families and was susceptible to chance factors, haplotype TDT was performed. The haplo-

Table 1. Primers for amplification of interferon regulatory factor 2 (*IRF2*) gene polymorphisms

Region	Polymorphism	Primer sequence (5' to 3')	Annealing temp.	Expected product size	Diagnostic restriction enzyme
Promoter	[−829C/T, −830C/T]	AAGGGTGACCAAGCCAGTGAG GCGCTCCGCTCCATTTAGGA	60°C	245bp	<i>Fnu4HI</i>
Promoter	−684C/T	GATGGGCCTCCTTTTCGTTCTA TGTGACACCGACCACCTCCAAG	55°C	180bp	<i>MseI</i>
Promoter	−467G/A	GGTAAGGACGCAAGTAATGC CAAGCAGCGGCTCGTGAGTC	59°C	316bp	<i>NlaIII</i>
Exon 9	921G/A	GGCTGGTCTCAAACTCCTGA ACCGGATTGCTCTCCTCTTT	56°C	316bp	<i>Fnu4HI</i>
3'UTR	1739(ATCCC)6–8	CGGACGAGATAATGTGAAC TTAAAGCAGTCTGAAAATGG	56°C	334bp	

Fig. 1. Genomic structure and locations of polymorphic sites in the human interferon regulatory factor 2 (*IRF2*) gene

Table 2. Transmission disequilibrium tests for the *IRF2* polymorphisms

	Allele/haplotype	(Frequency)	Transmitted	Not transmitted	χ -squared	<i>P</i>
Polymorphism	-467G/A					
	G allele	(0.86)	19	7	5.54	0.02
	A allele	(0.61)	27	33	0.6	0.44
Haplotype	921G/A					
	G allele	(0.93)	9	10	0.05	0.82
	A allele	(0.52)	14	21	1.4	0.24
Haplotype	1739(ATCCC)6-8					
	GG8 haplotype	(0.28)	23	8	7.36	0.007
	GA8 haplotype	(0.05)	6	6	0.08	0.78
	AG8 haplotype	(0.09)	5	7	0.33	0.56
	AA8 haplotype	(0.06)	1	7	4.5	0.03

type comprising the -467G, 921A, and 1739(ATCCC)8 alleles (haplotype GA8) was transmitted preferentially to AD-affected children ($P = 0.007$, $P_c = 0.03$).

In the promoter of *IRF2*, there are four Sp-1 sites, one NF-kappaB site, and one IRF-1 binding site (Cha and Deisseroth 1994; Harada et al. 1994). The -467G/A polymorphism is not located within these regions. However, TFSEARCH analysis predicted that the polymorphism may lie within the upstream stimulating factor (USF) binding region (<http://www.rwcp.or.jp/papia/>). Thus, it is possible that the -467G/A polymorphism affects transcriptional activity of *IRF2*. On the other hand, the haplotype GA, but not GG, was associated with AD, indicating that the -467G/A polymorphism alone is not likely to be associated with AD. Another possibility is that unidentified mutations that are in linkage disequilibrium with the GA8 haplotype or the haplotype GA8 itself may be associated with AD.

A mouse lacking IRF-2 shows spontaneous development of an inflammatory skin disease involving CD8+ T cells (Hida et al. 2000). Histopathological analysis of disease tissues in *IRF2* knockout mice revealed thickening of the epidermis due to proliferation of keratinocytes in the basal epidermis, similar to the histological changes observed in psoriasis (Hida et al. 2000). Recent genome-wide studies showed loci linked to AD on human chromosomes 3q21 (Lee et al. 2000), 1q21, 17q25, and 20p (Cookson et al. 2001), which are coincident with major psoriasis loci.

Although AD is distinct from psoriasis, both diseases are characterized by dry, scaly skin, disturbed epidermal differentiation, and inflammation that is responsive to T cell-specific antigens. The data suggest that AD is influenced by susceptibility genes common to psoriasis. Further studies are needed to clarify the role of the *IRF2* -467G/A polymorphism in the pathogenesis of AD.

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