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Genetic Association Analysis using Microsatellite Markers in Atopic Dermatitis

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Atopic dermatitis (AD) is presumed to be influenced by genetic and environmental factors. In this study, 54 patients with AD were examined for disease association by the use of 12 microsatellite markers. Several significant associations were recognized in the alleles on chromosome 5, 7 and 11. AD genes were mapped near the FC ε RI β gene (around D11S1314 locus) on chromosome 11, the IL4 gene cluster on chromosome 5 and the TCR γ gene on chromosome 7. This distribution in close proximity to candidate loci for AD is very similar to that of atopic genes, therefore implying that an atopic trait is genetically responsible for the development of AD.

Key words : Atopic dermatitis, Microsatellite marker, Disease gene, Chromosome 11

INTRODUCTION

Atopic dermatitis (AD) is a common skin diseases prevalent throughout the world, and is likely to results from multifactorial inheritance, with interaction between genetic and environmental factors. An atopic trait, which has been defined as a positive skin test response and/or elevated total serum IgE and/or elevated specific IgE, is likely to be involved in the development of AD, but it still remains to be established to what extent atopy plays a decisive role in the pathogenesis of AD. In fact, not all parameters of atopy are present in every individual manifesting AD. Numerous genetic studies on atopy and atopic respiratory disease have been made mainly using the linkage approach. For example, Cookson *et al.* [1-4] reported that atopy was linked to a marker 11q13, on chromosome 11. Especially, Leu181, a common variant of the Fc ε RI β gene was shown to be maternally inherited with a strong association with atopy [5, 6]. More recently, Marsh *et al.* [7, 8] and Meyers *et al.* [9] presented evidence of linkage of a locus for total serum IgE levels to chromosome 5q31. On the other hand, linkage study of atopy underlying atopic dermatitis showed nonlinkage of atopy to chromosome 11q13 [10]. Others showed evidence of linkage of atopy to chromosome 1q21, 16q [11], 3q21 [12], 17q71 [11, 13], 14q11.2 [14]. Typing studies on human leukocyte antigens (HLA) and in AD patients were also reported by several investigators [15-17], but HLA association with AD remains controversial. These discrepancies have not yet been resolved. The overall agreement of these studies is the polygenic nature of atopy and AD. In this regard, we have been interested in physically mapping candidate genes predisposing to the development of AD.

AD is a phenotypically heterogeneous disorder difficult to diagnose, as it is variable in both place and time, and lacks a specific test

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which could serve as a reference standard. Some patients with AD like other atopic disorders produce high serum levels of IgE antibodies as a defense against common antigens such as mites, house dust, pollens, and fungi, but others show low or no serum IgE levels. Accurate diagnosis of phenotype is crucial to the validity of genetic studies. Therefore, in this report, in an attempt to localize candidate genes influencing AD on human chromosomes, we performed association analysis between AD patients selected by severe criteria for this diagnosis irrespective of showing high or low serum IgE levels or microsatellites polymorphisms. These satellites (Table 1) were selected mainly accordingly to the criteria of genetic markers in close linkage with candidate loci proposed for atopy disorders so far [7, 18–24].

MATERIALS AND METHODS

Patients

Fifty-four Japanese patients (30 males and 24 females) with atopic dermatitis (AD), and 100 unrelated, and sex- and age-matched Japanese healthy controls, were enrolled in this study. The controls were aged between 20 to 26 years (mean age, 23 years). These patients were diagnosed according to the criteria proposed by the Japanese Dermatological Association [25]. The total IgE levels of the patients varied from 5 to 31,100 IU/ml (mean level, 4952.5 ± 5976.9 IU/ml). Their ages at disease onset ranged from 16 to 43 years (mean age, 24 years). Peripheral blood was collected after informed consent was obtained for genetic screening from every subject.

Methods

1. PCR primers and allele typing

PCR conditions and allele determination for HUMTH01 loci have been previously described [26]. PCR amplification for the remaining microsatellites of dinucleotide repeat units (D5S2057, D7S2507, D11S4083, D11S902, D11S904, D11S935, D11S905, D11S987, D11S1314, D11S937, and D11S1358) was carried out according to the user's manual (ABI PRISMTM Linkage Mapping Set, Perkin Elmer, USA). The primer sets for markers D11S902, D11S904, D11S905, D11S935, D11S987, D11S1314, D11S937, and D11S1358 were obtained from ABI PRISMTM Linkage Mapping Set-Panel 13 (Perkin Elmer, USA). The others (D5S2057, D7S2507, and D11S4083) were selected from the linkage map generated by Genethon [27]. Each forward primer was labeled at the 5' end with a fluorescent dye, 6-carboxyfluorescein (6-FAM), hexachlorinated analogue (HEX), or tetrachlorinated analogue (TET).

Samples were automatically analyzed on an ABI 373 sequencer using the Genescan software 672, followed by electrophoresis on a 6 % denaturing PAG (acrylamide/bisacrylamide 19 : 1, containing 8.3 M urea) in 1 × TBE buffer. Single strand DNA size markers GS350 or GS-500 (Applied Biosystems) were used for determination of allele type.

2. Statistical analysis

Gene frequencies and phenotype frequencies were estimated by direct counting. The significance of the distribution of alleles between patients with AD diseases and normal controls were tested by the chi-square (χ^2) method with continuity correction and Fisher's exact probability test (P value test). Comparison between two groups was made with 95 % confidence interval to estimate statistical significance.

RESULTS

1. Association analysis of AD and a microsatellite marker D5S2057 on chromosome 5

The D5S2057 locus corresponded to an AFM (Association Francaise contre les Myopathies) c003xe9 marker from the CEPH/CHLC databases [21]. D5S2057, showing polymorphism of dinucleotide repeat units (CA)n, was located in close proximity to the IL4 cytokine-gene cluster in chromosome 5q31.1. Eight alleles were found in the patients as compared to 10 alleles in normal controls. As listed in Table 2, within the patient group, allele 113 was observed at a significantly low phenotype frequency of 11 % compared to 25 % in the control group (R.R. = 0.38, $\chi^2 = 4.21$, P = 0.040).

2. Association analysis of AD and a microsatellite marker D7S2507 on chromosome 7

The D7S2507 locus is mapped in the vicinity of the TCR γ gene region (7p15). Eleven alleles were observed in this analysis. Among them, the phenotype frequency of allele 175 in the patient group was statistically

Table 1 Characteristics of microsatellite markers

Makers	Chromosome	Locus	Structure	No. of alleles	Heterozygosity
D5S2057	5	5q23-q31	CA	10	0.64
D7S2507	7	7p14-p13	CA	11	0.73
HUMTH01	11	11p15.5	AATG	7	0.76
D11S902	11	11p15	CA	12	0.78
D11S904	11	11p14	CA	7	0.72
D11S935	11	11p13	CA	9	0.68
D11S4083	11	11p12	CA	18	0.96
D11S905	11	11p12	CA	12	0.82
D11S987	11	11p11	CA	11	0.65
D11S1314	11	11q13	CA	10	0.83
D11S937	11	11q13	CA	14	0.72
D11S1358	11	11q14	CA	5	0.65

higher than that in the control group (R.R. = 3.81, χ^2 = 7.31, P = 0.007).

3. Association analysis of AD and 10 microsatellites markers on chromosome 11

Genetic polymorphisms of 10 microsatellites scattering on chromosome 11 were investigated (Fig. 1). Chromosome 11 is known to encompass a recombination distance region of approximately 100 cM (centiMorgans). Each index map covering a resolution of approximately cM is shown in Fig. 1. The HUMTH01 (Human Tyrosine Hydroxylase gene, intron1) locus is located in 11p15.5-p15, and shows a tetranucleotide repeat polymorphism 4bp (AATG)n [28]. Other STRs (short tandem repeat) have 2bp (CA) repeat units. It was noteworthy that the significance of the allelic frequency between the normal and patient groups was observed at 5 loci as listed in Table 2 (D11S904, D11S935, D11S4083, D11S1314, and D11S937). High value for statistical significance and relative risk (R.R.) was obtained in alleles 202 (R.R. = 2.29, χ^2 = 5.92, P = 0.015) on the D11S904 locus. Two alleles were also recognized with statistical significance on locii in the region of 11q13 ; allele 91 on the D11S1314 locus (R.R. = 6.13, χ^2 = 5.91, P = 0.022) and allele 157 on the D11S937 locus (R.R. = 3.40, χ^2 = 6.07, P = 0.014).

DISCUSSION

Many patients with AD are characterized by allergen-specific IgE antibodies against several environmental antigens, but some patients produce low or no IgE antibodies. It has remained uncertain whether an atopy treat is a prerequisite to develop AD. In this study we analyzed the disease susceptibility to atopic dermatitis (AD) with various serum levels of IgE using microsatellite markers which are localized around candidate loci predisposing to the atopic trait.

IL-4 is known to play a role in the induction of IgE by B cell. The IL4 gene has recently emerged as a major candidate for IgE responsiveness and atopy [6]. It was found that microsatellite markers around the IL-4 locus on chromosome 5q23-q31 are linked to genetic control of elevation of total, but not specific, serum IgE levels. The marker D5S2057 (5q23-q31) is close to the IL4 cytokine-gene cluster on chromosome 5q31.2-q33. The frequency of allele 113 out of 10 alleles found in this study was significantly decreased in the patient group (R.R. = 0.38, χ^2 = 4.41, P = 0.04).

The role of the T cell receptor (TCR) in allergic reactions is not yet clear. However, specific IgE response might be induced after recognition of foreign antigens by the TCR. Moffatt *et al.* [21] analyzed linkage between specific IgE reactions, and the TCR α and β gene complexes on chromosomes 14 and 7 with the use of TCR α and β-linked microsatellite markers, respectively. They suggested that some gene (or genes) associated with TCR α -microsatellite alleles in the TCR

Table 2 Statistically significant alleles associated with atopic dermatitis

Markers	Significant alleles	Atopic dermatitis (n = 54)	Healthy controls (n = 100)	R.R.	χ^2	p value
D5S2057	113	11 %	25 %	0.38	4.21	0.040
D7S2507	175	91 %	72 %	3.81	7.31	0.007
D11S904	190	11 %	2 %	6.13	5.91	0.022 *
D11S904	202	57 %	37 %	2.29	5.92	0.015
D11S935	188	9 %	25 %	0.31	5.54	0.019
D11S4083	176	2 %	12 %	0.14	4.67	0.024 *
D11S4083	184	11 %	2 %	6.13	5.91	0.022 *
D11S4083	190	17 %	5 %	3.80	5.78	0.019 *
D11S1314	91	11 %	2 %	6.13	5.91	0.022 *
D11S937	157	20 %	7 %	3.40	6.07	0.014

*: obtained by Fisher's exact test
R.R.: relative risk

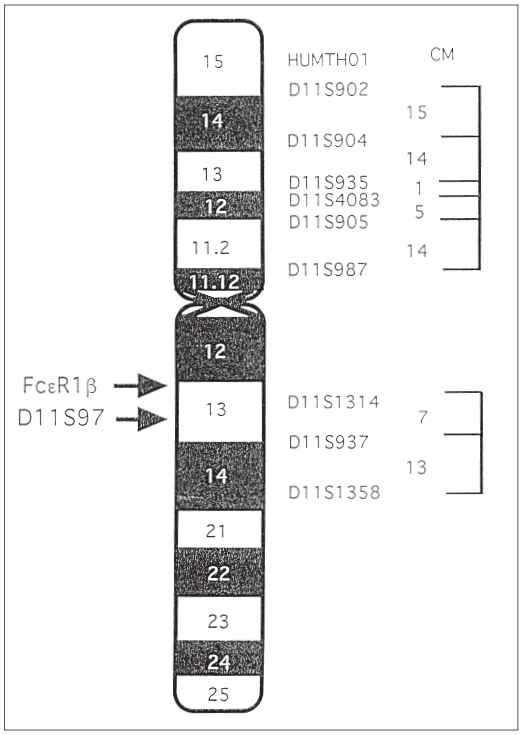

Fig. 1 Gene map of 10 microsatellite markers investigated in this study on chromosome 11. The sex-averaged length estimates (cM: centiMorgans) are from Genethone [27] and Litte *et al.* [33] and Fain *et al.* [34]. Mapping data are available from the GENELINK database (http://www.genlink.wustl.edu) and GenBank database (http://www.ncbi.nlm.nih.gov).

α region modified specific IgE responses [21]. γ / δ T cells, which are usually less abundant than $\alpha\beta$ T cells, only 1–10 % of total T cells, mainly localized in the skin, uterine, tongue, and intestinal epithelia [29, 30], raising the possibility that γ / δ T cells are somehow involved in the development of atopic dermatitis. The TCR γ gene resides on chromosome 7p15–p14. We carried out association analysis between the patient and control groups using the D7S2507 (7p14–p13) microsatellite which maps near the TCR γ gene. The phenotype frequency of allele 175 was significantly increased in the patient group (R.R. = 3.81, χ^2 = 7.31, P = 0.007).

Two potential candidate genes for atopy were identified in the region of 11q13 [1, 16, 31, 32]. They encode the lymphocyte surface marker CD20 and the β subunit of the high-affinity IgE receptor (Fc ε RI β) on mast cells and basophils. The high-affinity receptor for IgE (Fc ε RI) is composed of α, β and γ subunits. The role of Fc ε RI β is to control IgE-mediated mast-cell degranulation and the release of cytokines which enhance IgE production through the α chain. We investigated the susceptibility to AD using 10 microsatellite markers dispersed on chromosome 11 (Fig. 1). Among them, the loci of D11S1314 (11q13) and D11S937 (11q13) are the closest to the Fc ε RI β gene. There was statistical significance of the phenotype

frequencies between the patient and the normal groups for a total of 6 alleles on 5 different loci (D11S904; 11p14, D11S935; 11p13, D11S4083; 11p12, D11S1314; 11q13, D11S937; 11q13) as summarized in Table 2. Among them, 2 alleles (allele 91 on D11S1314 and allele 157 on D11S937) showed the most conspicuous differences in the phenotype frequencies. Especially, allele 91 on the D11S1314 locus was observed at the significantly high phenotype frequency of 11 % compared to 2 % in the random control group (R.R. = 6.13, $\chi^2 = 5.91$, P = 0.022).

Collectively, AD genes may be mapped near the Fc ε RI β gene on chromosome 11 (11q13), the IL4 gene cluster on chromosome 5 and the TCR γ gene on chromosome 7 in addition to 11p12, 11p13 and 11p14 on chromosomes 11. It must be noted that this distribution in close proximity to candidate loci for AD on human chromosomes is very similar to that of atopic genes in spite of the fact that the AD patients enrolled in this study were selected irrespective of serum IgE levels, implying that an atopy trait is genetically involved in the development of AD. Further studies to systematically survey muany more microsatellite markers in AD patients may be crucial to pinpoint AD candidate genes more precisely. Such studies are now under way in our laboratories.

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