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Loci on murine chromosomes 7 and 13 that modify the phenotype of the NOA mouse, an animal model of atopic dermatitis

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Abstract The NOA (Naruto Research Institute Otsuka Atrichia) mouse is an animal model of allergic or atopic dermatitis, a condition characterized by ulcerative skin lesions with accumulation of mast cells and increased serum IgE. We reported earlier that a major gene responsible for dermatitis in the NOA mouse lay in the middle of chromosome 14, and that the incidence of disease clearly differed according to parental strain; the mode of inheritance was autosomal recessive with incomplete penetrance. In the study reported here, we searched for genes that might modify the NOA phenotype, and we identified two candidate loci that appeared to contain genes capable of modifying atopic or allergic dermatitis, one in the middle of chromosome 7 (χ^2 = 14.66; P = 0.00013 for D7Mit62) and the other in the telomeric region of chromosome 13 (χ^2 = 15.352; P = 0.000089 for D13Mit147). These loci correspond to regions of synteny in human chromosomes where linkages to asthma, atopy, or related phenotypes, such as serum IgE levels, have been documented.

Key words Atopic dermatitis · Animal model · Modifier gene · Backcross · Linkage

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Introduction

Atopic dermatitis, a condition characterized by spotty ulcerative eruptions, itching, and recurrent remission and progression, is one of the most common allergic diseases in human populations worldwide (Coleman et al. 1997). Because atopic dermatitis is a multifactorial disease and because its susceptibility and severity are under the influence of environmental factors and nutritional conditions, genetic investigations of the human disease are complicated (Folster-Holst et al. 1998; Forrest et al. 1999). Suitable animal models are extremely powerful resources for extending the reach of genetic analysis. The phenotype of the NOA (Naruto Research Institute Otsuka Atrichia) mouse, an animal model of allergic or atopic dermatitis, is characterized by ulcerative skin lesions with the accumulation of mast cells, high levels of serum IgE, and scratching behavior (Kondo et al. 1997), a pattern very similar to the clinical features of atopic dermatitis in humans.

Previously, using 559 backcrossed N2 offspring from five parental strains, we determined that a major gene responsible for dermatitis of the NOA mouse was present on murine chromosome 14 (Natori et al. 1999). The incidence of NOA backcross progeny derived from mice with Th-2 responses was about three times higher than that of Th-1 response mice (BALB/cByJ, 38.8%; DBA/2J, 31.4%; C3H, 12.2%; C57BL6/J, 10.6%). These findings pointed to the existence of at least one gene capable of modifying dermatitis, and we considered that nonaffected N2 mice, whose genotypes for markers at the major locus on chromosome 14 indicated homozygosity for the dermatitis-causing gene, might be influenced dominantly by suppressor alleles inherited from normal parental strains or recessively by NOA alleles predisposing to the phenotype. Therefore, we searched the genomes of N2 mice with homozygous alleles at the candidate locus on chromosome 14 for markers where mice with dermatitis revealed homozygosity and nonaffected mice were heterozygous. These efforts identified two candidate loci, one in the middle of chromosome 7, and the other near the telomere of chromosome 13.

Materials and methods

Animals

NOA mice, a strain developed at the Naruto Research Institute Otsuka Pharmaceutical Factory (Tokushima, Japan), were bred at Clea Japan (Shizuoka, Japan). We crossed male NOA mice with female DBA/2J mice and backcrossed female F1 progeny with male NOA mice.

N2 offspring were examined for the absence or presence of ulcerative skin lesions. We finally obtained 367 N2 offspring derived from DBA/2J; from among them we selected 173 N2 progeny whose genotypes were homozygous for the markers D14Mit39 and D14Mit160.

Linkage study for dermatitis-modifying loci

We isolated genomic DNAs from the tail tips of the N2 population by standard techniques (Siracusa et al. 1989) and genotyped each animal, using microsatellite markers. Primer sequences for amplifying each marker locus were obtained through the database at the Mouse Genome Informatics (MGI) website (distributed via World Wide Web; URL: http://mgd.niai.affrc.go.jp/strtools.html). Polymerase chain reaction (PCR) amplifications were performed as described by Dietrich and associates (1992). PCR conditions for most primers involved a denaturation step at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C–57°C for 30 s, 72°C for 30 s, and extension at 72°C for 7 min. PCR products were electrophoresed on 4% agarose or 6% polyacrylamide gels, and stained with ethidium bromide.

Statistical analysis

The numbers of affected or nonaffected mice, and whether each was a recombinant, were calculated for each marker locus. The resulting segregation ratios were analyzed by the χ^2 test.

Results

By whole-genome scanning, we analyzed backcross NOA mice whose genotypes indicated homozygosity for the markers D14Mit39 and D14Mit160. Using 173 animals, we searched for microsatellite loci for which affected and nonaffected mice showed significant differences of genotypes. Among the 94 markers that were informative, we found a significantly higher number of homozygotes in affected mice, and of heterozygotes in unaffected mice, at D7Mit62 and D7Mit321 (P values, 0.00013 and 0.00092, respectively; see Table 1). We also found significant differences at the D13Mit147 locus ($P = 0.000089$).

Discussion

Because the effort to sequence the human genome is near completion, many research efforts have been shifting to focus on the analysis of complex human diseases. Atopic dermatitis is one of the most common of such conditions, with an overall prevalence as high as 20%–30% in Western countries (Coleman et al. 1997), where 40%–50% of young

Table 1. Association of dermatitis with microsatellite loci on chromosomes 7 and 13 among N2 murine offspring whose genotypes at D14Mit39 and D14Mit160 were homozygous

Chromosome 7		Dermatitis (+)		Dermatitis (−)			
Marker	cM	Homo	Hetero	Homo	Hetero	χ^2	P
D7Mit117	11.0	44	33	42	54	3.066	0.08
D7Mit199	27.8	46	31	40	56	5.583	0.018
D7Mit62	42.6	53	24	38	58	14.660	0.00013
D7Mit321	48.5	50	27	38	58	10.988	0.00092
D7Mit40	53.0	50	27	41	55	8.467	0.0036
D7Mit101	60.0	47	30	43	53	4.519	0.034
Chromosome 13		Dermatitis (+)		Dermatitis (−)			
Marker	cM	Homo	Hetero	Homo	Hetero	χ^2	P
D13Mit3	9.0	43	34	49	47	0.396	0.53
D13Mit117	19.0	46	31	48	48	1.634	0.201
D13Mit91	30.0	48	29	42	54	5.915	0.015
D13Mit290	40.4	48	29	34	62	12.420	0.00043
D13Mit147	51.0	47	30	30	66	15.352	0.000089
D13Mit213	59.0	45	32	33	63	9.996	0.0016
D13Mit151	71.0	44	33	32	64	9.834	0.0017

Homo, Homozygous; hetero, heterozygous

adults show positive skin-prick tests to house dust or grass pollen (Cline and Burrows 1989; Holford-Strevens et al. 1984). However, because multiple genetic and environmental factors influence the onset and severity of the condition, studies using human clinical materials are extremely complicated and difficult. Appropriate murine models showing pathological features similar to those in human diseases have already contributed much to the investigations of other multifactorial diseases in humans, such as essential hypertension and diabetes mellitus (Jacob et al. 1991; Todd et al. 1991). Animal models offer controlled exposure, limited and consistent genetic variation, and unlimited sizes of sib-pairs. It seems likely that animal models hold considerable potential for elucidating the genetics of atopic disease as well.

New therapies for allergic diseases are being developed by improving existing classes of drugs or by discovering new classes of drugs. Interferon-gamma (IFN-γ) is considered to be a candidate for suppressing allergic conditions, as it is a cytokine that downregulates Th2-cell function; in placebo-controlled trials it has reduced clinical severity associated with atopic dermatitis and decreased the number of circulating eosinophils (Schneider et al. 1998; Stevens et al. 1998). However, the identification of suppressor genes should provide another useful approach to the development of novel therapies for atopic dermatitis.

In this study, we searched for genes that could modify the gene responsible for the NOA phenotype in our murine model. We identified two candidate loci, one in the middle of chromosome 7, and the other in the telomeric region of chromosome 13. Interleukin 16 (IL-16), a natural ligand for the CD4 receptor that acts as a chemoattractant for CD4+ T cells and as a modulator of T-cell activation, is located in the vicinity of D7Mit62 on chromosome 7 (Center et al. 1996; Cruikshank et al. 1994). IL-16 plays a crucial role in recruiting CD4+ cells to sites of inflammation during the acute phase of atopic dermatitis (Laberge et al. 1998). As for the other locus, the murine gene encoding PIK3R1 (phosphoinositide-3-kinase regulatory subunit, polypeptide 1 [p85 α]) is located in the vicinity of D13Mit147. The phosphoinositide-3 kinases constitute a family of enzymes that phosphorylate the 3'hydroxy group on phosphatidylinositol to generate membrane-associated lipid mediators, which act as second messengers to recruit and activate a series of intracellular kinases (Carpenter and Cantley 1996). The crosslinking of IgE-bound high affinity Fc receptor for IgE (FcεRI) with polyvalent antigen leads to Ca^{2+}-dependent degranulation of mast cells and basophils, thereby initiating the allergic response. PI3K regulates the signaling events that lie downstream of the FcεRI-mediated activation of tyrosine kinases (Barker et al. 1999).

Each of the candidate modifier loci we have identified in the mouse is syntenic to human chromosomal regions 11q13 and 5q13 (Stephenson and Lueders 1998; Williams et al. 1998). These regions represent so-called consensus areas of linkage to asthma, atopy, or related phenotypes such as serum IgE level (Cox et al. 1998; CSGA 1997; Daniels et al. 1996; Dizier et al. 1999; Ober et al. 1998). The identification of genes underlying shared human and mouse linkages may provide new insights into the etiology of allergic or atopic dermatitis.

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