

Recent Developments in Genetic Study of Allergic Disease in the Japanese Population

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ABSTRACT

Allergy is a common immune disorder characterized by raised IgE levels, which leads to clinical disorders such as asthma, rhinitis and eczema. Our understanding of the pathogenesis of allergic disease is largely dependent on our current understanding of the related pathophysiology and the available technology. Recent advances in molecular biology techniques have allowed a rapid and accurate identification of polymorphisms in various genes that may be important for determining the susceptibility to allergic disorders. In this review, we present some developments in genetic studies of allergic disease with particular focus on asthma and atopy in the Japanese population.

KEY WORDS

allergic disease, asthma, atopy, Japanese, linkage, polymorphism

INTRODUCTION

Atopic and allergic diseases such as asthma, rhinitis, and eczema were rare a few decades ago but, today, constitute an increasingly severe public health problem especially in developed countries.^{1,2} The rising prevalence of allergic disease over the past decades suggests that environmental and lifestyle factors are important. However, atopy clustering in families suggest a strong genetic component to allergic disease.^{3,4} Significant progress has been made in the study of atopy genetics during the last decade since the Oxford group, in 1989, first reported a linkage between generalized IgE responsiveness and a DNA polymorphism on chromosome 11q13 in British families.⁵

Atopy is a key predisposing factor in the development of bronchial asthma between the ages of 3 and 20 years.^{6,7} The majority of asthmatics are also atopic, with manifestation of allergic diathesis including clinical allergy to aeroallergens and foods, or subclinical allergy manifested by skin test reactivity to allergen or elevated serum IgE. Bronchial hyperresponsive-

ness (BHR), an exaggerated bronchospastic response to specific and non-specific substances, represents the physiological hallmark of asthma induced by Th2 cytokines such as interleukin (IL)-4, -5, -9, -10 and -13. Twin studies have revealed that heritability estimates of asthma vary between 36% and 79%.⁸⁻¹⁰ Importantly, there is evidence that genetic liability for asthma, BHR and allergic traits are regulated through distinct loci, although there is likely some shared overlap as well.¹¹ To date, several multiple genome-wide linkage studies for asthma and allergy have been performed, and have identified no fewer than 20 distinct chromosomal regions with linkage to asthma or related traits in different populations.¹²⁻²¹ The known chromosomes 5q, 11q, 12q, and 16p are major candidates that may contribute to the development of asthma and allergy. Recently, Yokouchi *et al.*²¹ conducted a genome screen in Japanese children with hay fever (orchard grass pollinosis), and found linkage of chromosomes 3p, 5q, 12p and 12q with this seasonal allergic rhinitis. Interestingly, all of these regions have been previously reported to harbor candidate genes for asthma and atopy.

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FCER1B GENE

The first genetic region reported to show linkage to atopy was chromosome 11q.⁵ A significant portion of atopic asthmatic families in Caucasian and Japanese populations may be linked to chromosome 11q13 through the maternal line.^{22,23} The β -subunit of the high-affinity IgE receptor (*FCER1B*) gene has been mapped to chromosome 11q13 and is a candidate gene for atopy²⁴⁻²⁷ because of its important role in initiating type I hypersensitivity reactions by mast cells and basophils.

The *FCER1B* gene is composed of seven exons and six introns, spanning approximately 11 kb.²⁸ At least eight variants of this gene have been identified that include three coding variants (Ile181Leu, Val183Leu and Glu237Gly).²⁹⁻³¹ The minor allele of Glu237Gly has been found at a low frequency (approximately 5%) in a number of populations. However, the Gly237 allele occurs at a relatively high frequency in the Japanese population (approximately 20%) and has been found to be associated with atopic asthma (particularly in childhood asthma), allergic rhinitis (due to house dust mite and/or Japanese cedar pollen), as well as with high serum total and specific IgE levels.^{31,32}

IL4 AND IL13 GENES

IL-4 and IL-13 are type 2 cytokines and play a central role in IgE-dependent inflammatory reactions.^{6,7} IL-4 plays a central role in B cells switching to IgE antibody production, and in the maturation of helper T-cells towards the Th2 phenotype. IL-4 and IL-13 operate through the IL-4 receptor (IL-4R) or IL-13R, respectively. Both receptors share the IL-4R α chain, and promote STAT6 (signal transducer and activator of transcription 6) activation. Mice deficient in *IL4*, *IL13*, *IL4r* or *Stat6* lack IgE synthesis and Th2-type reactions,⁷ therefore, genetic variants of IL-4/IL-13 signalling might be crucial for the development of atopic disorders.

IL4 and *IL13* genes are localized within 25 kb on the proximal portion of chromosome 5q31, to which genome searches have identified a strong linkage with asthma and atopy.^{13,14} A dinucleotide repeat in the third intron of *IL4* is linked to total serum IgE levels but not to asthma in Caucasian populations,³³ whereas linkage to both high IgE levels and asthma has been found in a Japanese population.³⁴ These findings support the candidacy of *IL4* as an atopy locus on 5q31. To date, five putative variants have been reported in the *IL4* promoter region - four of them are very rare.^{35,36} A variant in the *IL4* promoter, -590 C/T, has been related to higher IgE levels in the Japanese population.³⁴

Deichmann *et al.*³⁷ performed a systematic search for variations in the *IL4RA* gene and found 13 polymorphic variants in the coding region, including

seven variants that resulted in amino acid substitution. The prevalence of Ile50 has been demonstrated to be higher than Val50 in individuals with atopic asthma, especially during childhood in the Japanese population.³⁸ Recently, Nakamura *et al.*³⁹ reported significant changes in the prevalence of Ile50Val and Glu375Ala variants of *IL4RA* in healthy subjects with a normal IgE value compared with those in healthy subjects with a higher allergen-specific IgE value in the Japanese population. Furthermore, it was suggested that Ile50Val and Glu375Ala seem to be related to Japanese cedar pollen sensitization, and subjects with Ile50 or Glu375 might develop Japanese cedar pollinosis (JCPsis) with increased exposure to cedar pollen.

IL-13 operates through IL-13R, a heterodimer of IL-4R α and IL-13R α 1 chains.^{6,7,40} IL-13 is crucial for allergen-induced BHR in experimental animals and may be relevant to human asthma.^{41,42} Significantly higher IL-13 levels have been found in asthmatic patients with and without atopy.⁴³ A novel variant of human *IL13*, Arg110 Gln, on chromosome 5q31 was found to be associated with asthma rather than IgE levels in case-control populations from Britain and Japan; the variant also predicted asthma and higher serum IL-13 levels in a general Japanese paediatric population.^{44,45} Moreover, Immunohistochemistry demonstrated that both subunits of IL-13R are prominently expressed in bronchial epithelium and smooth muscle from asthmatic subjects, and detailed molecular modelling analyses indicated that residue 110 of *IL13*, the site of the charge-modifying variants Arg and Gln, is an important constituent in the internal constitution of the ligand and crucial in ligand-receptor interaction.⁴⁴ These results suggest that the *IL13* variant Arg110Gln might be involved in asthma pathogenesis. However, recent association studies by Miyazawa *et al.*⁴⁶ and our group⁴⁷ showed that this potentially functional variant did not contribute significantly to JCPsis susceptibility and cedar pollen-specific IgE concentration in the Japanese populations. Further studies will be necessary to assess the relationship between other variants in *IL13* and susceptibility to JCPsis.

IL12 AND IL18 GENES

IL-12 is expressed by activated macrophages that serve as an essential inducer of Th1 cells development. IL-12 has been found to be important for sustaining a sufficient number of memory/effector Th1 cells which mediate long-term protection to an intracellular pathogen, while IL-18 can induce the interferon (IFN)- γ production of T cells. The combination of IL-12 and IL-18 has been shown to inhibit IL-4 dependent IgE and IgG1 production, and enhance IgG2a production of B cells. In addition, administration of IL-18 together with IL-12 is important in sustaining Th1 responses and monokine production.

Several polymorphisms of the *IL12B* gene (encoding IL-12 p40) have recently been reported to be associated with asthma-severity phenotype in ethnically diverse children.^{48,49} Although in a genome-wide screen, strong evidence for linkage of atopic asthma in childhood with marker D5S1352 (located close to *IL12B*) was found,⁵⁰ however a lack of association between the *IL12B* polymorphisms and susceptibility to atopic asthma or allergic rhinitis in the Japanese families was also reported,⁵¹ suggesting that polymorphisms in *IL12B* are not likely to be associated with the development of atopy-related phenotypes in this study population. The influences of racial and ethnic-specific differences in environmental and/or genetic risk factors on the development of complex common disease may account for the conflicting findings.

The contribution of IL-18 to asthma has been studied in human beings and mice. In humans, the level of serum IL-18 was elevated during acute asthmatic exacerbations⁵² and secretion of IL-18 by peripheral blood mononuclear cells was increased in asthma and atopic dermatitis.⁵³ Yoshizawa *et al.*⁵⁴ reported that significantly higher serum IL-18 levels were found in patients with atopic dermatitis than in control subjects, and that serum IL-18 levels were correlated with disease severity and with the number of eosinophils in peripheral blood. However, reduced expression of IL-18 mRNA in atopic dermatitis has also been described.⁵⁵ An important role of IL-18 has also been found in rheumatoid arthritis, which is a typical Th1-mediated disease.⁵⁶

The human *IL18* gene is located on chromosome 11q22.2–22.3, and it is composed of six exons and five introns spanning about 19.5 kb. Higa *et al.*⁵⁷ examined whether or not polymorphisms occurred in the coding region of the IL-18 gene and, if so, whether there was a relation between polymorphism and asthma. They detected one new polymorphism in the coding region of the IL-18 gene linked with substitution A to C at position 105, but this polymorphism was silent. The increase of the frequency of the A allele was found to be associated with increased susceptibility to asthma. There was also an increase of serum IL-18 levels in patients with allergic asthma compared with control subjects, but no significant association was detected between the IL-18 levels and genotypes of 105A/C polymorphism in *IL18*.

TLR2 AND TLR4 GENES

Gene-environment interaction is likely to impact on the development of allergic disease.^{3,4} The mammalian host defense against environmental pathogens can be classified into two major forms of immune response: innate and acquired. Innate immune responses are triggered through the binding of bacterial lipopolysaccharide (LPS) and other bacterial components to receptors such as CD14 and Toll-like receptors (TLRs). Recent studies have revealed that

TLRs play an important role in innate immune responses and subsequent activation of acquired immune responses.⁵⁸ To date, 10 distinct human TLRs have been identified and shown to mediate specific cellular responses to pathogen-associated molecular patterns. Binding of LPS to CD14 initiates cytoplasmic signal transduction through the TLR4.⁵⁹

Common, cosegregating missense mutations (Asp299Gly and Thr399Ile) in the *TLR4* gene have been identified in humans and found to be associated with hyporesponsiveness to inhaled LPS.⁶⁰ A recent study in Swedish children showed that decreased LPS-induced IL-12 (p70) and IL-10 responses were associated with the Asp299Gly polymorphism of *TLR4* and independently with asthma, especially atopic asthma.⁶¹ In addition, this polymorphism was associated with a 4-fold higher prevalence of asthma in school-aged children. Also, genetic variation in the *TLR2* gene was suggested to be a major determinant of the susceptibility to asthma and allergies in children of European farmers.⁶² However, no evidence was found to support the argument that genetic variations in *TLR2* and *TLR4* contribute to the development of atopy-related phenotypes in a Japanese population.⁶³

The genes encoding TLRs show a high variability in human populations, but whether these genetic variations modify the interaction with microbial molecules and thereby modify the frequency of asthma and allergies is not yet known. This data is expected to be elucidated in future research.

ADAM33 GENE

Recent information regarding asthma genetics comes from the work of Van Eerdewegh and coworkers.⁶⁴ These investigators performed a genetic study by combining linkage and association approaches in Caucasian affected sib-pair families from the UK and the US, and identified *ADAM33* (a disintegrin and metalloprotease domain 33) as a major susceptibility gene for asthma linked to BHR. The association of *ADAM33* polymorphisms with asthma and related phenotypes has been subsequently replicated in several different populations.^{65–69}

Most recently, our group examined the association between *ADAM33* polymorphisms and susceptibility to JCPsis, a most common seasonal allergic rhinitis in Japan.⁷⁰ We conducted a case-control association study among a Japanese population, involving 95 adult individuals with JCPsis and 95 healthy controls. A total of 22 single-nucleotide polymorphisms (SNPs) in the *ADAM33* gene were genotyped using PCR-based molecular methods. Six SNPs of *ADAM33* gene, three in introns (7575G/A, 9073G/A and 12540 C/T) and three in the coding region (10918G/C, 12433T/C and 12462C/T), were strongly associated with JCPsis susceptibility and most of the SNPs were in linkage disequilibrium with each other. A higher

Table 1 Asthma and atopy candidate genes reported in literatures

Chromosome	Gene	Gene description *
1p32-p31	<i>VCAM1</i>	vascular cell adhesion molecule 1
1p31-p22	<i>CLCA1</i>	chloride channel, calcium activated, family member 1
1q21-q22	<i>DAP3</i>	death associated protein 3
1q22-q25	<i>SELP</i>	selectin P (granule membrane protein 140kDa, antigen CD62)
1q31-q32	<i>IL10</i>	interleukin 10
1q41-q44	<i>CHRM3</i>	cholinergic receptor, muscarinic 3
2q14	<i>IL1A</i>	interleukin 1, alpha
	<i>IL1B</i>	interleukin 1, beta
2q14.1	<i>DPP10</i>	dipeptidylpeptidase 10
2q14.2	<i>IL1RN</i>	interleukin 1 receptor antagonist
2q22.1	<i>HNMT</i>	histamine N-methyltransferase
2q33	<i>CTLA4</i>	cytotoxic T-lymphocyte-associated protein 4
3p26-p24	<i>IL5RA</i>	interleukin 5 receptor, alpha
3p21.3	<i>TLR9</i>	Toll-like receptor 9
	<i>CCR3</i>	chemokine (C-C motif) receptor 3
3p21	<i>CCR5</i>	chemokine (C-C motif) receptor 5
4p14	<i>TLR6</i>	Toll-like receptor 6
4q13-q21	<i>MUC7</i>	mucin 7, salivary
4q22.3	<i>PGDS</i>	prostaglandin D2 synthase, hematopoietic
4q31.22-q31.23	<i>EDNRA</i>	endothelin receptor type A
4q32	<i>TLR2</i>	Toll-like receptor 2
4q34.1-q35.1	<i>IRF2</i>	interferon regulatory factor 2
4q35	<i>TLR3</i>	Toll-like receptor 3
5q31-q32	<i>ADRB2</i>	adrenergic, beta-2-, receptor, surface
5q31	<i>IL13</i>	interleukin 13
5q31.1	<i>IL4</i>	interleukin 4
	<i>IL5</i>	interleukin 5 (colony-stimulating factor, eosinophil)
	<i>CSF2</i>	colony stimulating factor 2 (granulocyte-macrophage)
	<i>IRF1</i>	interferon regulatory factor 1
	<i>CD14</i>	CD14 antigen
5q31.1-q33.1	<i>IL12B</i>	interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)
5q32	<i>SPINK5</i>	serine protease inhibitor, Kazal type 5
	<i>UGRP1</i>	uteroglobin-related protein 1 (secretoglobin, family 3A, member 2; <i>SCGB3A2</i>)
5q33.2	<i>TIM1</i>	T cell immunoglobulin domain and mucin domain 1 (hepatitis A virus cellular receptor 1; <i>HAVCR1</i>)
5q33.3	<i>TIM3</i>	T cell immunoglobulin domain and mucin domain 3 (hepatitis A virus cellular receptor 2; <i>HAVCR2</i>)
5q35	<i>LTC4S</i>	leukotriene C4 synthase
6p24.1	<i>EDN1</i>	endothelin 1
6p21.3	<i>HLA</i>	human leukocyte antigen
	<i>TAP1</i>	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
	<i>LTA</i>	lymphotoxin alpha (TNF superfamily, member 1)
	<i>TNF</i>	tumor necrosis factor (TNF superfamily, member 2)
6p21.2-p12	<i>PAFAH</i>	platelet-activating factor acetylhydrolase, plasma (phospholipase A2, group VII; <i>PLA2G7</i>)
6q23-q24	<i>IFNGR1</i>	interferon gamma receptor 1
7p14.3	<i>GPRA</i>	G protein-coupled receptor for asthma susceptibility
7q11.23	<i>CCL24</i>	chemokine (C-C motif) ligand 24 (<i>eotaxin-2</i>)
	<i>CCL26</i>	chemokine (C-C motif) ligand 26 (<i>eotaxin-3</i>)
7q31.2	<i>CFTR</i>	cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)
7q36	<i>NOS3</i>	nitric oxide synthase 3 (endothelial cell)
8p22	<i>NAT2</i>	N-acetyltransferase 2 (arylamine N-acetyltransferase)
9q31	<i>IKAP</i>	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein
9q32-q33	<i>TLR4</i>	toll-like receptor 4
9q33-q34	<i>C5</i>	complement component 5
10q11.1	<i>CXCL12</i>	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1; <i>SDF1</i>)
10q11.2	<i>ALOX5</i>	arachidonate 5-lipoxygenase
11q12.3-q13.1	<i>CC16/CC10</i>	secretoglobin, family 1A, member 1 (uteroglobin); <i>SCGB1A1</i>
11q13	<i>FCER1B</i>	Fc fragment of IgE, high affinity I, receptor for; beta polypeptide
	<i>GSTP1</i>	glutathione S-transferase pi
11q22.2-q22.3	<i>IL18</i>	interleukin 18 (interferon-gamma-inducing factor)
12p13.31	<i>C3AR1</i>	complement component 3a receptor 1
12p13	<i>AICDA</i>	activation-induced cytidine deaminase
12q13	<i>STAT6</i>	signal transducer and activator of transcription 6, interleukin-4 induced
12q14	<i>IFNG</i>	interferon, gamma
12q24.2-q24.31	<i>NOS1</i>	nitric oxide synthase 1 (neuronal)
13q14.2	<i>PHF11</i>	PHD finger protein 11
14q11.2	<i>TCRA/D</i>	T cell receptor, alpha/delta
14q22.1	<i>PTGDR</i>	prostaglandin D2 receptor (DP)
14q32	<i>AACT</i>	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
16p12-q21	<i>CARD15</i>	caspase recruitment domain family, member 15
16p11.2-12.1	<i>IL4RA</i>	interleukin 4 receptor
17q11.2-q12	<i>NOS2A</i>	nitric oxide synthase 2A (inducible, hepatocytes)
	<i>CCL5</i>	chemokine (C-C motif) ligand 5 (regulated upon activation, normally T-expressed and secreted; <i>RANTES</i>)
17q11.2-q21.1	<i>CCL2</i>	chemokine (C-C motif) ligand 2 (monocyte chemoattractant protein 1; <i>MCP1</i>)
17q21.1-q21.2	<i>CCL11</i>	chemokine (C-C motif) ligand 11 (<i>eotaxin</i>)
17q23	<i>ACE</i>	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1
17q23.1	<i>EPO</i>	eosinophil peroxidase; <i>EPX</i>
19p13.3	<i>TBXA2R</i>	thromboxane A2 receptor
19p13.3-p13.2	<i>C3</i>	complement component 3
19q13.1	<i>TGFB1</i>	transforming growth factor, beta 1 (Camurati-Engelmann disease)
20p13	<i>ADAM33</i>	a disintegrin and metalloproteinase domain 33
21q22.11	<i>IFNGR2</i>	interferon gamma receptor 2 (interferon gamma transducer 1)
Xq24	<i>IL13RA1</i>	interleukin 13 receptor, alpha 1

* <http://www.ncbi.nlm.nih.gov/LocusLink/>

frequency of the common alleles of these SNPs was noted for the subjects with JCPsis in comparison with healthy controls. Haplotype analysis using four SNPs (7575G/A, 9073G/A, 10918G/C and 12540C/T) showed that AACT haplotype was significantly associated with decreased risk of JCPsis. In addition, associations were found between *ADAM33* polymorphisms and various JCPsis-related phenotypes including clinical severity, eosinophil counts in nasal secretion and allergen-specific IgE levels in sera, but not total serum IgE levels.

ADAM33 belongs to a family of membrane-anchored metalloproteinases that also have fusogenic, adhesion and intracellular signaling properties. *ADAM33* might play a key role in predisposing to the reduced lung function characteristic of asthma, possibly by influencing airway wall remodeling.⁷¹ Our finding also raises the possibility that polymorphic variation in *ADAM33* might contribute to allergic rhinitis through influencing the course of epithelium inflammation, damage, repair, and consequent tissue remodeling in the nasal airways.⁷⁰ Although the gene function is at present unclear, these potential actions of *ADAM33* provide many possibilities for further research.

CONCLUDING REMARKS

The understanding of the genetics of asthma and allergy has evolved rapidly since the sequencing of the human genome and in this past year has led to the isolation of several novel asthma-susceptibility genes, such as *ADAM33*. Although these discoveries have shed new light on the pathophysiology of allergic disease, it is clear that no single genetic risk factor is responsible for the development of asthma and atopy. Cumulative information suggests that the development of the disease in an individual will depend upon the interaction of a number of genes together with various environmental factors. Recently, numerous studies have been carried out to examine the association between asthma or its related traits and genetic variations in candidate genes in ethnically diverse groups including the Japanese population (Table 1).⁷² The results from these studies have often shown discrepant outcomes in different populations. However, many of these studies are limited with respect to sample size, thereby severely hampering their interpretation due to the reduced statistical power. Also, allergy is a multifactorial disease affected by interacting genetic and environmental factors. However, it remains unknown how environmental and relevant variants interact. Future studies will continue to improve our understanding of the role of genetic variants, gene-gene interaction, and gene-environment interaction in the development of allergic diseases, and eventually reveal new approaches for modulating this system in ways that favorably affect disease mechanisms and clinical settings.

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