in the promoter region of the *CCL11* gene were associated with serum IgE levels in AD patients, the two and 67 G > A SNPs were not shown to be associated with susceptibility to AD.[67] This apparent specificity of this effect might be related to the diminutive size of the genotyped sample of this single study,[67] the low magnitude of the underlying genetic effect, and the power advantage associated with the use of quantitative traits. Consequently, these data require replication, as well as further study in a more substantial cohort of subjects. An orally available antagonist of the eotaxin 1 receptor (*YM-344031*) does, however, lend some support to the genetic findings because it has recently been shown to inhibit both immediate and late-phase antigen-induced cutaneous inflammation in a mouse model of allergy.[68] Significant linkage disequilibrium was observed between positions -426 and -384, and also between -384 and 67.[69]

The *CCL17* (*TARC*) maps on chromosome at 16q13. It covers 11.30 kb on the direct strand. The serum TARC levels of patients with AD were significantly elevated and correlated with disease activity, and immunoreactive TARC levels were detected in epidermal keratinocytes (KCs), dermal infiltrating cells, and endothelial cells in acute and chronic lesional skin.[70] These observations strongly suggest that KCs can be a source of TARC in the lesional skin of patients with AD and that KCs producing TARC may be involved in the pathogenesis of AD. Although some SNPs of *TARC* are candidates as a genetic factor in AD, no association between AD and the -431C > T SNP of the *TARC* gene was observed in a Japanese population.[71] The results of the study, however, should be considered very preliminary due to the small number of AD patients.

CCR3 maps on chromosome 3p21.3. It covers 143.70 kb on the direct strand. As stated earlier, CCR3 is a receptor for various chemokines which are important in the pathogenesis of AD. Therefore, the biological activities of *CCR3* suggest that polymorphisms of *CCR3* may impart an increased risk for AD. Several SNPs in *CCR3* have been reported, including silent mutations (51T > C[72,73] and 240C > T[73]) and missense mutations (652T > A,[74] 824 G > A,[72,73] 971T > C,[72] 1052T > C[73]). There was no significant difference in genotype frequencies of 51T > C SNP of the *CCR3* gene between AD patients and controls.[75]

CCR4 maps on chromosome 3p24. It covers 3.36 kb on the direct strand. There was no significant association with the 1014C > T SNP of the *CCR4* gene.[76]

CMA1 maps on chromosome 14q11.2. It covers 2.91 kb on the reverse strand. There have been several reports of a significant association between a *CMA1* promoter polymorphism (-1903A > G) and atopic eczema in Japanese adults and schoolchildren.[77] These results could not be confirmed in another Japanese study with 100 patients and 69 patient-parents-trios[78] and an Italian study with 70 patients.[79] There was also no association between *CMA1*-1903A > G genotype and AD risk in Japanese but there was a significant association between the *CMA1* genotype and AD patients with a serum IgE concentration of < 500 IU/mL.[80] Recently, a family-based association study in Caucasians revealed a significant association of this polymorphism with total IgE levels in patients with self-reported AD.[81] As in the study of Mao *et al.*[77] a significant association between the *CMA1*-1903A > G polymorphism and AD was observed by Weidinger *et al.*[82] It may be speculated whether this DNA variant alters the expression of chymase. Recently, it has been shown that CMA1 is increased in chronic atopic eczema skin lesions,[83] and a potential role of chymase in the promotion of skin barrier defects and cutaneous neovascularization has been suggested.[84] Preliminary studies in animal models have indicated a therapeutic potential of chymase inhibitors in AD.[85,86]

Ma *et al.*[77] suggested that variants of *CMA1* may have been one source of genetic risk for AD in a Japanese population while other Japanese studies[78,80] failed to confirm this association. Functional studies and analyses of other loci are needed to clarify the consequences of the -1903A > G polymorphism in the *CMA1* gene and might determine whether chymase will qualify as a target for therapeutic interventions in AD.

CYTOKINES

Cytokines have been functionally divided into two subgroups: Th1 cytokines, mainly interleukin (IL) 2, IL12, interferon (IFN)γ, and tumor necrosis factor (TNF)α, which activate the cellular machinery of the immune system; and Th2 (IL4, IL5, IL6, IL10 and IL13) cytokines, which activate the humoral machinery.[87-90] Unusual deviation of the balance between Th1 and Th2 results in many immune diseases such as allergy and autoimmune diseases. Excessive Th1 immune response has been implicated in autoimmune diseases such as rheumatoid arthritis and multiple sclerosis, liver injury and graft versus host disease while unusual deviation in Th2 immunity causes allergic diseases and systemic lupus erythematosus.

For example, IL2, mainly secreted by Th1 cells, is an autocrine stimulator of Th1 cell differentiation and proliferation resulting in a T cell shift towards the Th1 phenotype. IL12 is a proinflammatory interleukin mainly produced by macrophages, B cells, and DCs. It promotes Th1 cell function while suppressing Th2 cell function. IFNγ can down-regulate allergic airway inflammation and mucus production,[91] further supporting the critical importance of Th2 cells in allergy and asthma. Of note, airway hyperresponsiveness (AHR) is induced by the transfer of IL-4-deficient Th2 cells, with a concomitant marked reduction of eosinophilia. This suggests that Th2 cells can induce AHR

even via IL4-independent mechanisms, but not by transfer of Th1 cells. In contrast, Th1 cells induced neutrophilic inflammation without AHR.[92] In another murine model of acute eosinophilic airway inflammation, however, Th1 cells were required in addition to Th2 cells for endogenous eosinophil recruitment, suggesting that Th2 cells may need Th1-derived signals for effective recruitment to airways.[93] Finally, engineering of Th2 cells to produce latent transforming growth factor (TGF) β1 reverted allergen-induced AHR and inflammation, which supports the concept that TGFβ-producing T cells play an important regulatory role in asthma,[94] and also that airway fibrosis and remodelling may be the final consequence of chronic or repeated TGF-β production. TNFα is expressed on the cell membrane and is then hydrolyzed to release the soluble form, which forms homotrimers. TNFβ (LT-α) has no cell membrane attachment domain but can form either membrane-anchored heterotrimers with LT-β or soluble homotrimers.

It is of note that Th2 cytokines can account directly or indirectly for the great majority of pathophysiological manifestations of allergic patients. IL4 is potentially pro-inflammatory and pro-atherogenic and induces circulating eosinophils to roll on and adhere to endothelial cells.[95] These eosinophils can then be attracted to target tissues by both IL-5 and chemokines. IL10 is a prototype anti-inflammatory interleukin produced mainly by activated T cells, B cells, and macrophages. It was described in mice as a Th2 cytokine that selectively inhibits IFNγ and granulocyte-macrophage colony-stimulating factor 2 (GM-CSF or CSF2) production by the Th1 cells. IL-13 is responsible for mucus hypersecretion by mucus cells, and induces metaplasia of mucus cells.[96] IL4 and IL13 stimulate fibroblast growth and chemotaxis, as well as the synthesis of extracellular matrix proteins.[97,98] However, subepithelial fibrosis in asthma also results from the activity of TGFβ, produced by T cells, eosinophils and fibroblasts, as well as of IL-6 produced by several cell types, including Th2 cells themselves.[99] Taken together, these findings indicate that Th2 cytokines, either directly or indirectly, can account for the hallmarks of allergic inflammation.

IL1RN maps on chromosome 2q14.2. It covers 34.70 kb on the direct strand. The polymorphism in intron 2 of the *IL1RN* gene is caused by a variable copy number of an 86-bp sequence. The 4-repeat (*IL1RN*1*) and 2-repeat (*IL1RN*2*) alleles are most common, while the other alleles occur at a combined frequency of less than 5%. No association was found between the variable number of tandem repeat polymorphisms in intron 2 of the *IL1RN* gene and AD.[100]

IL1RL1 (*ST2*) maps on chromosome 2q12. It covers 40.54 kb on the direct strand. A significant association between AD and the -26999G > A SNP of the ST2 gene was found in a Japanese population (OR = 1.86, 95% CI = 1.42–2.45).[101] On the other hand, 2992C > T, 5283G > A, 5860C > A, 11147C > T, 744C > A and -27639A > G SNPs were not associated with AD risk.[101]

IL1A and *IL1B* map on chromosome 2q14. The former covers 11.48 kb on the reverse strand and the latter covers 7.16 kb on the reverse strand. The -899T > C SNP of the *IL1A* gene was not associated with AD risk.[102] No association was found between either the -511C > T, 3953T > C, 3953T > C, -1418T > C or the 315T > C SNPs of the *IL1B* gene and AD.[100,102,103]

IL4 maps on chromosome 5q31.1. It covers 9.01 kb on the direct strand. The T allele of -590T > C SNP was associated with an increased risk of AD in a Japanese population.[104] In Caucasians the T allele of IL4 -589C > T SNP was significantly associated with the development of AD at 24 months of age.[105] No association between SNPs (-590T > C and 33T > C) of *IL4* and AD was found in a Chinese population.[106]

IL4R maps on chromosome 16p11.2–12.1. It covers 50.86 kb on the direct strand. Many SNPs (-3112C > T, -1803T > C, -327C > A, -326A > C and -186G > A) or haplotypes (α) of the *IL4R* gene are associated with AD.[107] Seven SNPs (223C > G > T > A, 1199C > A, 1291C > T, 1307T > C, 1727G > A, 2356C > T) and a silent 1242T > G have been demonstrated to have functional significance. Caucasian children with the rare homozygous 1727G > A polymorphism had a higher prevalence of flexural eczema in the first 6 months compared with the heterozygote and the wild type homozygote genotypes combined.[108] It has been demonstrated that the 1727G > A SNP was significantly associated with AD in another Japanese population.[109] No association between SNPs (1199C > A, 1242T > G, 1507C > T and 1727G > A) of *IL4R* and AD was found in a Chinese population, however.[106]

IL5 maps on chromosome 5q31.1. It covers 2.08 kb on the reverse strand. The -703C > T SNP of *IL5* was not significantly associated with AD in Japanese.[110]

IL6 maps on chromosome 7p21. It covers 6.12 kb on the direct strand. No association was found between the -174C > G SNP of the *IL6* gene and AD.[100] Similarly, the -174C > G and -922A > G SNPs were not linked to AD.[102]

IL10 maps on chromosome 1q31–q32. It covers 4.89 kb on the reverse strand. The -1082A > G, -819T > C and -592A > C SNPs of the *IL10* gene did not contribute to the development of AD.[106] No association was found between AD and the -1082A > G SNP of the *IL10* gene.[100] Also, -571C > A, -854C > T and -1117G > A SNPs of the *IL10* gene were not associated with AD.[102]

IL12B maps on chromosome 5q31.1–q33.1. It covers 15.69 kb on the reverse strand. The AA genotype of *IL12B* 1188A > C SNP was associated with decreased risk of AD in a Japanese population (OR = 0.44, 95% CI = 0.20–0.95).[111] The 4237 G > A, 4496A > G and 4510G > A SNPs of the *IL12B* gene did not

contribute to the development of AD.[106]

IL12RB1 maps on chromosome 19p13.1. It covers 39.94 kb on the reverse strand. Among eight SNPs (-111A > T, -2C > T, 4443C > T, 5970 G > C, 17183T > C, 17369C > T, 25748T > C and 27637A > T), the TT genotype of the -111A > T SNP (OR = 2.39, 95% CI = 1.41–4.04) and the TT genotype of the -2C > T SNP (OR = 2.55, 95% CI = 1.43–4.57) were significantly associated with an increased risk of AD in a Japanese population.[112]

IL13 maps on chromosome 5q31. It covers 4.85 k on the direct strand. No association between the -1111C > T SNP of *IL13* and AD was found in a Chinese population.[106] The statistically significant association between the -1024C > T SNP of the *IL13* gene and AD was confirmed.[113] In the Japanese population there was no significant association between two SNPs of 704A > C and 1103C > T while the Arg allele of Arg144Gln SNP was significantly associated with an increased risk of AD.[114] A significant association was noted for the A allele of the Arg144Gln SNP and AD (OR = 1.77, 95% CI = 1.06–2.96).[115] In Caucasians, haplotypes consisting of *IL13* Arg144Gln with AD (P = 0.006) were associated with AD.[105] None of the three SNPs (-1111C > T, 1293C > T, and Arg144Gln) were associated with AD during the first year.[102]

IL18 maps on chromosome 11q22.2–q22.3. It covers 21.61 kb on the reverse strand. Among five SNPs (-132A > G, -133C > G, -137G > C, -113T > G and 127C > T), the C allele of the -137G > C SNP was associated with an increased risk of AD (OR = 4.28, 95% CI = 1.24–14.77).[116]

TGFB1 maps on chromosome 19q13.2. It covers 52.34 kb on the reverse strand. The C allele (a low TGFβ1 producer allele) of the TGFβ1 915 G > C SNP was associated with an increased risk of AD (OR = 4.8, 95% CI = 2.4–9.7) while there was no statistical significant difference in the frequencies of the 869T > C genotypes.[117] No association between AD and the -590C > T SNP was observed.[102]

TNFα and TNFβ share a common receptor on tumor cells whose expression is upregulated by gamma-interferon.[118] *TNF* maps on chromosome 6p21.3. No significant association was found between -308 G > A SNP of the *TNFα* gene and AD.[103] Neither -1031T > C, -863C > A, -857C > T, -308G > A nor -238G > A SNPs of the *TNFα* gene was associated with AD in a Chinese population.[106] No association was found between AD and -238G > A and -308G > A SNPs of the *TNFβ* gene in a German population.[100] Also, the two SNPs were not linked to AD risk in Americans.[102]

GM-CSF maps on chromosome 5q31.1. It covers 2.38 kb on the direct strand. The A allele of the -677A > C SNP in the promoter region of the *GM-CSF* gene was associated with an increased risk of AD in the United Kingdom (OR = 2.3, 95% CI = 1.4–3.6).[103] Although -1916T > C SNP was significantly associated with an increased risk of AD (OR = 1.9, 95% CI = 1.2–3.1), there was a strong linkage disequilibrium existed between the -677A > C and -1916T > C SNPs.[103] The 3606T > C and 3928C > T SNPs of the *GM-CSF* gene was not associated with susceptibility to AD in Japanese.[119] There was strong linkage disequilibrium between the two polymorphisms.

STAT6 maps on chromosome12q13. It covers 16.79 kb on the reverse strand. There was no association between AD risk and the 2964 G > A SNP of the *STAT6* gene while the 13/15-GT repeat allele heterozygosity of the dinucleotide repeat in exon 1 (13-, 14-, 15- and 16-GT repeat alleles) was significantly associated with allergic disease including AD in Japanese.[120] However, the short tandem repeat in exon 1 was not associated with AD risk.[106]

IFNγ maps on chromosome 12q14. It covers 16.25 kb on the reverse strand. In a Chinese population, there was no association between short tandem repeats at the first intron of *IFNγ* gene and AD.[106]

ANTIGEN PRESENTATION MOLECULES

The human major histocompatibility complex (MHC, also called the human leukocyte antigen (HLA) complex) class I molecules are expressed on all human cells except erythrocytes and trophoblasts. HLA molecules are peptide-binding proteins on the surfaces of antigen-presenting cells. The complex of an HLA molecule and bound antigenic peptide forms a specific target for T cell recognition. HLA-A and HLA-B belong to the HLA class I heavy chain paralogues. This class I molecule is a heterodimer consisting of a heavy chain and a light chain. The heavy chain is anchored in the membrane. Class I molecules play a central role in the immune system by presenting peptides derived from the endoplasmic reticulum lumen. The class I molecules generally present antigens to $CD8^+$ T cells, and class II molecules present antigens to $CD4^+$ T cells. They are expressed in nearly all cells. On the other hand, HLA-DM belongs to the HLA class II beta chain paralogues. This class II molecule is a heterodimer consisting of an alpha (DMA) and a beta (DMB) chain, both anchored in the membrane. It is located in intracellular vesicles. HLA-DM plays a central role in the peptide loading of HLA class II molecules by helping to release the class II-associated invariant chain peptide molecule from the peptide binding site. Class II molecules are expressed in antigen presenting cells such as B lymphocytes, dendritic cells and macrophages. Both HLA class I and class II antigens contribute to the pathogenesis of AD.[121] In antigen presentation to cytotoxic T cells with HLA class I molecules, the antigen-processing pathway is controlled by the products of genes that are mapped within the HLA class I region, including low-molecular-weight polypeptide (LMP) and transporters associated with transporter for antigen presentation (TAP). TAP delivers cytosol-derived

peptides into the endoplasmic reticulum, where they bind to nascent HLA class I molecules. TAP is composed of two subunits, TAP1 and TAP2; deficiency of either subunit inhibits TAP function, reducing the supply and repertoire of peptides available for binding to HLA class I molecules. Many diseases associated with HLA have been investigated for the influence of TAP.[122-127] LMP products also have an important role in antigen presentation by class I HLA molecules. LMP subunit 2 (LMP2) and LMP7 are proteasome components, which enhance the proteolytic production of certain peptides.[128,129]

The *HLA* group of genes resides on chromosome 6p21.3. Eleven HLA-A (1, 2, 3, 11, 24, 26, 29, 30, 31, 33 and 66) and 27 HLA-B (7, 8, 13, 14, 16, 27, 35, 37, 38, 39, 46, 48, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 67, 71 and 75) alleles are frequently observed in Koreans. Among these, only the A24 allele of HLA was significantly associated with AD.[130] No HLA-DMA (Val140Ile, Gly155Ala, Ile179Thr, 184Arg-His-Cys) and HLA-DMB (144Ala-Glu-Val) alleles were associated with an increased risk of AD.[131]

LMP2 (*PSMB9*) and *LMP7* (*PSMB8*) map on chromosome 6p21.3. *LMP2* and *LMP7* genes encode two subunits of the proteasome, a cytoplasmic catalytic complex involved in the generation of antigenic peptides that are loaded on class I molecules within the endoplasmic reticulum.[132] LMP2/7 subunits may directly affect peptide cleavage specificity.[128,129] One dimorphic site within *LMP2*, Arg60His, and three dimorphic sites within *LMP7*, 3911 G > T, 3912C > T, 4069C > T, have been identified. SNPs of *LMP2* and *LMP7* genes were not significantly different for AD patients and controls.[130]

TAP1 and *TAP2* map on chromosome 6p21.3. *TAP* genes encode a heterodimer involved in the translocation of intracellular peptides across the endoplasmic reticulum membrane where they bind to class I molecules. Genes encoding the two *TAP* subunits, *TAP1* and *TAP2*, are located within the MHC class II region between the DPB1 and DQB1 loci[133,134] and variations in rat *TAP* genes have been reported to be associated with differences in the spectrum of MHC class I-binding peptides.[135] *TAP1* and *TAP2* genes are located at 6p21.3. Two dimorphic sites within *TAP1*, Val333Ile and Gly637Asp, and four dimorphic sites within TAP2, Ile379Val, Thr565Ala, Ala665Thr and Gln687Stop, have been widely investigated.[134] The 333Val and 637Gly alleles of the *TAP1* gene were significantly associated with an increased risk of AD in Tunisians[136] while allelic frequencies of the *TAP1* gene polymorphisms were similar in AD patients and controls.[130] The 565Ala and 665Thr alleles of the *TAP2* gene may be associated with increased risk of AD in a Korean population.[130]

OTHER MOLECULES

Cytotoxic T-lymphocyte-associated antigen-4 (CTLA4, also known as CD152) is a member of the Ig gene superfamily along with its homologue, CD28, a B7 binding protein.[137,138] CTLA4 is an inhibitory molecule that downregulates T-cell activation. Thus, ligation of CTLA4 on the T-cell surface initiates a cascade of biochemical events that attenuate an ongoing immune response.[139] Allergic diseases are characterized by a defective peripheral T cell tolerance to allergens, suggestive of possible CTLA4 dysfunction.[140,141]

Kallikrein (KLK, stratum corneum chymotryptic enzyme (SCCE)) localizes to the extracellular space of the stratum corneum and is specific for keratinizing cells undergoing desquamation.[142-145] KLK (SCCE) is secreted as an inactive zymogen that is activated by cleavage of an N-terminal peptide. Physiological activators of zymogens remain unknown but *in vitro* studies indicate that some kallikreins can undergo autoactivation while others may be activated by other kallikreins or endoproteases. Apart from its tissue localization, KLK (SCCE) has several properties and characteristics, including the pH and inhibitor profile of catalytic activity, matching the basic prerequisites for a crucial involvement in desquamation under *in vivo* conditions.[143,146,147] Transgenic mice overexpressing human KLK (SCCE) develop changes in their skin similar to those seen in chronic atopic dermatitis.[148] The overexpression of KLK (SCCE) initially may lead to a premature breakdown of the epidermal barrier. This would allow the penetration of irritants and allergens, triggering an inflammatory response, and subsequently a reactive hyperplasia. Therefore KLK (SCCE) is considered to be important in the pathogenesis of AD.

SLC9AR1 (solute carrier family 9, isoform 3 regulatory factor 1) is found in keratinocytes within the granular layer of the epidermis in normal skin and in T-cells and has been implicated in diverse aspects of epithelial membrane biology and immune synapse formation in cells. The downregulation of SLC9A3R1 after T-cell activation is consistent with its role as a negative regulator. SLC9A3R1 could have a similar role in the normal epidermis by modulating the keratinocyte response to a similar immune signal. NAT9 is also found in keratinocytes and T-cells and may play a role in glycosylation. Loss of RUNX1 binding has been shown to be associated with susceptibility to autoimmune diseases such as systemic lupus erythematosus.[149] RUNX1 is involved in CD4 silencing.[150] CD4 repression attributed to a RUNX1 mutation was found in only 18–30% of mature $CD8^+$ T lymphocytes.[150] SNPs lying between *SLC9A3R1* and *NAT9* result in the loss of a RUNX 1 binding site.[151] The loss of RUNX1 sites in alleles associated with autoimmune diseases suggest an important role for RUNX1 in tolerance. It may also suggest defective regulation of SLC9A3R1 or NAT9 by RUNX1 as a susceptibility factor for AD.

Serine protease inhibitor, Kazal-type, 5 (SPINK5) is

thought to be cleaved by furin to yield at least 14 independently working serine protease inhibitory domains.[152,153] Since SPINK5 and many tissue KLKs colocalize in the skin (in lamellar bodies of the uppermost epidermis and the pilosebaceous units of normal human skin tissue),[154] it has been hypothesized that these proteins may be part of a proteolytic enzyme-inhibitor system that controls skin desquamation and shedding.[152,154]

CTLA4 maps on chromosome 2q33. It covers 5.55 kbon the direct strand. *CTLA4* is linked to an increased incidence of autoimmune diseases.[155] A SNP at position 49 in exon 1 (49A > G) of *CTLA4* exerts differential functional effects on CTLA4 driven downregulation of T-cell activation.[156-158] The 49A > G SNP leads to a threonine to alanine change in the lead peptide. The 49A > G SNP of *CTLA4* gene was not correlated with AD, however.[159]

KLK7 gene maps on chromosome 19q13.33. It covers 7.57 kb on the reverse strand. Among all serine proteases within the human genome, the tissue *KLK* (*SCCE*) cluster is the largest. This cluster includes fifteen genes tandemly located on chromosome 19q13.4. KLKs generally share 30–50% sequence identity at the nucleotide and amino-acid levels.[160] The *KLK5* and *KLK7* genes were originally identified from a keratinocyte library and their products were first named human stratum corneum tryptic enzyme (hK5)[161] and human stratum corneum chymotryptic enzyme (hK7).[162] These KLKs seem to catalyze the degradation of intercellular structures in the most cornified layer of the skin and contribute to the normal cell shedding process at the skin surface.[163,164] *KLK5* and *KLK7* have been implicated in skin and brain diseases.[160,165,166] Recent studies have also revealed alterations of their expression in hormone-dependent cancers.[167-169] A 4-bp insertion was identified in the 3' untranslated region of the *KLK7* (*SCCE*) gene. The common allele was AACC, and the rare allele was AACCAACC. A significant genetic association was found between the rare AACCAACC variant of the *KLK7* (*SCCE*) gene and AD.[170]

Both *SLC9A3R1* and *NAT9* map on chromosome 17q25.1. The RUNX1 gene maps on chromosome 21q22.3. A SNP is located between the SLC9A3R1 and NAT9 genes in chromosome segment 17q25. This SNP occurs within a consensus binding site motif for RUNX1, a factor required for both differentiation and proliferation of haematopoietic cells. There was no significant allelic association between the RUNX1 polymorphism (rs734232) and AD in a small Japanese adult population.[171]

The serine protease inhibitor Kazal type 5 gene (*SPINK5*) expresses the 15 domain serine protease inhibitor lymphoepithelial Kazal-type-related inhibitor, named LEKTI.[172] *SPINK5* is located on chromosome 5q32 within a genomic region that has previously been linked to AD by a genome-wide linkage study and, consequently, SPINK5 has been implicated as a putative susceptibility gene for common, nonsyndromic AD.[173] Subsequently, Walley *et al.* reported a significant association of a nonsynonymous SNP located in exon 14 of SPINK5, consisting of a G-to-A transition (1258G > A) that leads to a Glu420Lys substitution in the encoded protein. In individuals affected by AD, a significant maternal over-transmission of the risk allele to their children was demonstrated.[173] Fölster-Holst *et al.*[174] studied 8 SNPs in different regions of the *SPINK5* gene, including 4 nonsynonymous SNPs leading to an amino acid change (Asp106Asn, Asn368Ser (1103A > G) and Asp386Asn (1156G > A), and Glu420Lys (1258G > A), Gly463Gly, Val553Val, Leu756Leu and Gly804Gly). None of the SNPs were associated with an increased risk of AD. Kato *et al.* examined associations between 8 SNPs (IV12-26C > T, IVS12-10A > G, IVS14 + 19 G > A, IVS 13-50 G > A, 1103A > G, 1156 G > A, 1188T > C, 1258G > A) of the *SPINK5* gene and AD in a Japanese population and found a positive association of 7 *SPINK5* SNPs (except for 1156G > A) with AD.[175] Nishio *et al.*[176] also reported a significant association between *SPINK5* 1258G > A and AD risk. No statistically significant association between *SPINK5* 1258G > A genotypes and AD was observed in a large German population.[177]

DRUG-METABOLIZING ENZYMES

The picture of drug-metabolizing enzymes is complicated, because AD is polygenic in nature, and susceptibility is also influenced markedly by environmental factors. In addition, evidence is emerging that certain metabolic polymorphisms may influence the pathogenesis of allergy. The metabolism of xenobiotics includes oxidation, reduction, and hydrolysis (phase I) and conjugation (phase II) reactions.[178,179] Glutathione S-transferase (GST) enzymes belong to the phase II detoxification system and are responsible for biotransformation and degradation of certain electrophilic compounds. Oxidative stress, with the formation of reactive oxygen species (ROS), is a key component of inflammation.[180] Members of the GST supergene family are critical for protecting cells from ROS because they can utilize a wide variety of products of oxidative stress as substrates and also influence the synthesis of eicosanoid-like mediators via modulation of ROS levels.[181,182]

N-acetyltransferase (NAT) is one of the conjugation enzymes and transfers acetate from acetyl coenzyme A to the functional groups of primary arylamine and hydrazine to form acetamides and hydrazides, in xenobiotics, containing arylamine and hydrazine groups[178,179,183] NAT enzymes, NAT1 and NAT2, are involved in the metabolism of these carcinogens via O- and N- acetylation.[184] Therefore, NAT2 and NAT1 are involved in the detoxification and bioactivation of carcinogens.[185,186] Individuals in a population are

either rapid or slow acetylators, depending on their ability to acetylate certain NAT substrates. Generally, acetylation is bimodally distributed in different populations. An association between NAT2 slow acetylation and allergic diseases and extrinsic asthma in patients with atopic characteristic has been reported.[187,188]

Certain genes within the *GSTM*, *GSTT* and *GSTP* subfamilies (*GSTM1*, *GSTT1* and *GSTP1*) are polymorphic in humans and the levels of individual enzymes expressed can be influenced by induction and genetic polymorphism. The *GSTM1*, *GSTT1* and *GSTP1* genes are located on chromosomes 1p13.3, 22q11.23 and 11q13, respectively. Lack of GSTM1 and GSTT1 activity is caused by the homozygous deletion of these intact genes (the null genotype). The non-null genotype is the wild type or heterozygote. The 1404A > G (Ile105Val) and 2294C > T (Ala114 Val) SNPs of the *GSTP1* gene confers lower levels of enzyme activity toward a variety of carcinogens and anticancer agents. As compared with the combined *GSTM1* non-null genotype (*GSTT1* non-null genotype and *GSTP1* AG), the combined *GSTM1* null genotype (*GSTT1* null genotype and *GSTP1* AA) was associated with a significantly increased risk of AD (OR = 9.43, 95% CI = 1.06–438.6).[189]

NAT2 gene maps on chromosome 8p23.1–p21.3. It covers 9.97 kb on the direct strand. N-acetylation is an important genetic polymorphic pathway in the biotransformation of one or more single-based mutations in the *NAT2* gene known to cause low expression levels of functional *NAT2* enzyme.[190,191] Individuals who carry two slow *NAT2* SNPs are slow acetylators, whereas those who are homozygous or heterozygous for wild-type *NAT2* alleles are rapid acetylators.[192,193] The presence of 481C > T, 590 G > A and 857G > A SNPs would lead to slow acetylation.[194] 481C > T (synonymous mutation) and 590G > A SNPs were not related to an increased risk of AD.[195] Moreover, 481C > T, 590 G > A and 857G > A, were not associated with an increased risk of AD.[196]

DISCUSSION AND CONCLUSION

The most important problems facing AD research are identifying "at-risk" individuals and implementing clinical surveillance, prevention practices, and follow-up care. The immune system plays an important role in AD. Although the increased/decreased risk associated with individual immune system SNPs may be small compared to that conferred by high-penetrance cancer genes, their public health implications may be large because of their high frequency in the general population. It is thus essential that epidemiological investigations of immune system polymorphisms are adequately designed. Unfortunately a fairly large number of studies are limited by their sample size and subsequently suffer from lack of power to detect effects that may truly exist. Also, given the borderline significance of previously reported associations and multiple comparisons, it is possible that one or more findings are false-positives.[197] Large and combined analyses may be preferred to minimize the likelihood of both false-positive and false-negative results. In addition, controls should be chosen in such a way that, if they were cases, they would be included in the case group; when controls are matched to cases, it is essential to account for matching in the analysis. When appropriate, confounding factors should be controlled for, with particular consideration of race and ethnicity.

Continued advances in SNP maps and in high-throughput genotyping methods will facilitate the analysis of multiple polymorphisms within genes and the analysis of multiple genes within pathways. The effects of polymorphisms are best represented by their haplotypes. Data from multiple polymorphisms within a gene can be combined to create haplotypes, the set of multiple alleles on a single chromosome. A few studies reviewed here reported haplotype associations, although several studies analyzed multiple polymorphisms within a gene, sometimes with inconsistent results. Haplotype analysis can increase the power to detect disease associations because of higher heterozygosity and tighter linkage disequilibrium with disease-causing mutations. In addition, haplotype analysis offers the advantage of not assuming that any of the genotyped polymorphisms is functional; rather, it allows for the possibility of an ungenotyped functional variant to be in linkage disequilibrium with the genotyped polymorphisms.[198] An analysis of data from multiple genes within the same pathway can provide more comprehensive insight into the studied associations. Such an analysis may shed light on the complexities of the many pathways involved in the immune system and AD development, providing hypotheses for future functional studies. Because of concerns over inflated type I error rates in pathway-wide or genome-wide association studies, methods of statistical analysis seeking to obviate this problem are under development.[199] The ability to include haplotype information and data from multiple genes, and to model their interactions, will provide more powerful and more comprehensive assessments of the immune system.

Although the summary risk for developing AD in individuals of each genotype may not be large, AD is such a common disease that even a small increase in risk can translate to a large number of AD cases. Therefore, polymorphisms, even those not strongly associated with AD, should be considered as potentially important public health issues. In addition, it is important to keep in mind that a susceptibility factor in one population may not be a factor in another. There are differences in the prevalence of immune system polymorphisms across populations. In a population where the prevalence of an "at-risk" genotype

in a given polymorphism is very low, the "at-risk" allele or "at-risk" genotype may be too infrequent to assess its associated risk. At a population level, the attributable risk must be small simply because it is an infrequent allele. Finally, the major burden of AD in the population probably results from the complex interaction between many genetic and environmental factors over time. Many harmful substances in the environment first require metabolic activation by Phase I enzymes to their ultimate forms and then the activated forms are detoxified by Phase II enzymes.[200,201] Thus, genetically determined susceptibility to AD may depend on the balance between drug-metabolizing enzyme activity and immune capacity. Further investigations of the combined effects of polymorphisms between immune response genes and drug-metabolizing genes may also help to clarify the influence of genetic variation in the AD development. Consortia and international collaborative studies, which may be a way to maximize study efficacy and overcome the limitations of individual studies, are needed to help further illuminate the complex landscape of AD risk and genetic variations.

The characterization of the genetic factors involved in this common, chronic disorder may provide important clues to its relationship to other diseases, such as asthma and allergic rhinitis, and is ultimately hoped to lead to more effective interventional strategies.

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ELSEVIER

Ambient Formaldehyde Levels and Allergic Disorders Among Japanese Pregnant Women: Baseline Data From the Osaka Maternal and Child Health Study

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PURPOSE: The effects of formaldehyde (FA) exposure on allergic disorders are not clearly understood. This cross-sectional study examined the relationship between FA exposure and the prevalence of allergic disorders in Japan.

METOHDS: Subjects were 998 pregnant women. Participants were considered to have asthma, atopic eczema, or allergic rhinitis (including cedar pollinosis) if they had received any medical treatment for any of these allergic disorders during the previous 12 months. Passive air sampling tubes were worn for 24 hours and analyzed for FA.

RESULTS: When FA levels were categorized into four groups, there was a tendency for a positive exposure-response relationship between FA levels and the prevalence of atopic eczema, although the adjusted odds ratio for highest vs. lowest FA categories did not reach statistical significance. When FA levels were categorized into two groups to assess the effects of exposure to high levels of FA on allergic disorders, FA levels of 47 ppb or more were independently associated with an increased prevalence of atopic eczema (adjusted odds ratio = 2.25; 95% confidence interval, 1.01–5.01). The positive association was more pronounced in women with a negative familial allergic history than in those with a positive familial allergic history. No clear association was found between FA levels and the prevalence of asthma or allergic rhinitis.

CONCLUSIONS: FA exposure may be associated with an increased prevalence of atopic eczema in Japanese pregnant women.

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KEY WORDS: Asthma, Cross-sectional Studies, Eczema, Japan, Pregnant Women, Rhinitis.

INTRODUCTION

A worldwide increase in allergic disorders in both children (1–3) and adults (4, 5) has been observed during the past few decades. A number of causes have been proposed for this increase. They include fewer infections during early childhood (6), exposure to microbial compounds in the environment and underlying innate and adaptive immune responses (7), exposure to cigarette smoke (8) and other outdoor and indoor irritants and allergens (9), and damp housing and the presence of molds in the home (10). However, the etiology of allergic disorders is still not sufficiently well understood.

Because people spend much of their time indoors, exposure to organic chemicals that arise from synthetic materials and furnishings, such as formaldehyde (FA), is inevitable. Several epidemiological studies examined the association between FA exposure and respiratory symptoms and allergic disorders (11–18). A cross-sectional study of Swedish adults showed that FA at home was significantly associated with an increased prevalence of nocturnal breathlessness (11). In another Swedish cross-sectional study, the prevalence of asthma increased among adults with domestic exposure to newly painted indoor surfaces that was related to a significant increase in FA concentrations (12). In contrast, FA

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Selected Abbreviations and Acronyms

FA = formaldehyde
OMCHS = Osaka Maternal and Child Health Study
OR = odds ratio
95% CI = 95% confidence interval

at home was not related to respiratory symptoms or diseases in a U.S. cross-sectional study (13). A cross-sectional study in Swedish school personnel found no relationship between FA in the classroom air and nasal symptoms (14). A case–control study in Australian children showed that FA exposure was positively associated with asthma (15). In a cross-sectional study in Swedish secondary schoolchildren, schools with greater concentrations of FA had more pupils with current asthma (16). A marginally significant positive association was observed among Australian children between FA exposure and the prevalence of atopy, but not asthma or respiratory symptoms (17). There was no relationship between FA and persistent wheezing illness in a case-control study of U.K. children (18).

To our knowledge, no epidemiologic study has assessed the association between FA and atopic eczema all over the world, and there has been no epidemiological information regarding the relationship between FA exposure and allergic disorders in Japan. Using a cross-sectional design, we analyzed baseline data from the Osaka Maternal and Child Health Study (OMCHS) to investigate the association between FA exposure and the prevalence of asthma, atopic eczema, and allergic rhinitis in Japanese pregnant women.

METHODS

Study Population

The OMCHS is an ongoing prospective cohort study that was initiated to investigate preventive and risk factors for maternal and child health problems such as allergic disorders and postpartum depression. The background and general procedure of the OMCHS have been described previously (19). In brief, the subjects, who were pregnant women at enrollment, were asked to complete a baseline survey that was followed by several postnatal surveys. Originally, eligible subjects were restricted to pregnant women in Neyagawa City, which is one of the 43 municipalities in Osaka Prefecture. The Prefecture has a total population of approximately 8.8 million people. Between November 2001 and March 2003, there were 3,639 eligible subjects in Neyagawa City, and 627 pregnant women (17.2%) chose to participate in this study. Because of the low participation rate in Neyagawa City, the opportunity to enroll was extended to eligible subjects who lived in other municipalities in Osaka Prefecture. Eight pregnant women who did not live in Neyagawa City but who learned of the study at an obstetric clinic before August 2002 participated in this study. In addition, 77 participants who heard the accounts of the OMCHS from public health nurses in six other municipalities from August 2002 to March 2003 were enrolled into the study. From October 2002 to March 2003, 290 participants were recruited from a university hospital and 3 obstetric hospitals in three other municipalities; these women were recommended for participation in the OMCHS by an obstetrician. Finally, a total of 1002 participants gave their fully informed consent in writing and completed the baseline survey. Missing data on FA exposure caused the exclusion of four subjects. There were 998 participants left for analysis. The OMCHS was approved by the ethics committees of the Osaka City University School of Medicine and the Osaka Prefectural Institute of Public Health.

Measurements

After enrollment, each participant completed a set of two self-administered questionnaires. One involved demographic and health indication data, and the other diet history data. The participants were requested to wear a passive diffusion sampling tube to measure FA and nitrogen dioxide and to collect two dust samples of the bedclothes and flooring for the detection of mite antigen. Participants mailed these materials to the data management center. Research technicians reviewed the questionnaire, and missing or illogical data were completed by telephone interview. Data regarding diet and concentrations of nitrogen dioxide were not used in this study because our other papers will report or have reported the associations of these variables with allergic disorders (20–22).

One of the self-administered questionnaires inquired about age; gestation; parity; indoor domestic pets; family income; education; personal history of asthma, atopic eczema, and allergic rhinitis; family history of asthma, atopic eczema, and allergic rhinitis; smoking habits; current passive smoking exposure; and the presence of mold in the kitchen. Current asthma, atopic eczema, and allergic rhinitis (including Japanese cedar pollinosis) were considered present if subjects received any medical treatment for any of these allergic disorders during the previous 12 months. Detailed data on the types of medications and the duration of their use were not collected. A family history of asthma, atopic eczema, and allergic rhinitis (including Japanese cedar pollinosis) was defined as present if a parent or sibling had exhibited any of these doctor-diagnosed allergic disorders.

The passive air sampling tubes contained silica gel impregnated with triethanolamine (Sibata Scientific Technology Co, Ltd, Tokyo, Japan). The sampling tubes were worn for 24 hours and analyzed for FA by a spectrophotometrical method that is highly correlated with results from liquid chromatography (23, 24). Concentrations of FA are

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reported in parts per billion (ppb) and represent the mean concentrations for the exposure periods.

Two dust samples were collected from a 1-m^2 area of the bedclothes and flooring for 1 minute using a vacuum cleaner fitted with a collection apparatus. Antigen levels of *Dermatophagoides farinae* mite from extracts of fine dust fractions were measured with a double-antibody sandwich enzyme-linked immunosorbent assay using a soluble antigen prepared from the whole mite bodies as a reference standard and expressed as antigen equivalent in micrograms per square meter of surface area (Mitey checker; Shinto Fine Co, Ltd, Osaka, Japan) (25, 26). The antigen levels were semi-quantitatively classified with scores of – (<2 μg/m^2), ± (5 μg/m^2), + (10–15 μg/m^2), and ++ (>35 μg/m^2). In the present study, we used only results from the bedclothes because the correlation between antigen levels from the bedclothes and flooring was almost collinear (Spearman correlation coefficient = 0.54; $p < 0.0001$).

Statistical Analysis

FA exposure was categorized into four groups. The cut-off points were at the 30th, 60th, and 90th percentile values on the basis of the distribution for all study subjects (<18, 18–27, 28–46, and ≥47 ppb) because the distribution was markedly skewed toward high values (skewness = 1.69, kurtosis = 4.75). Also, FA exposure was classified into two groups using a cut-off point at the 90th percentile to assess the effects of exposure to high levels of FA on allergic disorders. Covariates included in the multivariate models were age (<30 and ≥30 years); gestation (<18 and ≥18 weeks); parity (0 and ≥1); family history of asthma, atopic eczema, and allergic rhinitis; cigarette smoking (never, former, and current); current passive smoking at home and work; mold in the kitchen; indoor domestic pets; family income (Japanese yen <4,000,000, 4,000,000–5,999,999, and ≥6,000,000 / year); education (<13, 13–14, and ≥15 years); mite antigen level in house dust (–, ±, and + or ++); and season when data were collected (spring, summer, fall, and winter).

Logistic regression analysis was used to estimate odds ratios (ORs) and their 95% confidence intervals (CIs) for allergic disorders in relation to FA levels. To examine whether the prevalence increased with increases in levels of FA, the trend of association was evaluated using a logistic regression model assigning the median value in each exposure category as the representative score. A two-sided p value less than 0.05 was considered to indicate statistical significance. Also, an exposure-response relationship was regarded as marginally significant when the p value (two-sided) ranged from 0.05 to less than 0.10. We also conducted analyses stratified by a familial allergic history to assess possible effect modification by this variable. Because a familial allergic history is a convincing risk factor for allergic disorders, FA exposure might be expected to have a smaller effect in the development of allergic disorders among individuals with a positive familial allergic history. The homogeneity of odds ratios between pregnant women with a positive and negative familial allergic history was tested by including an interaction term of a familial allergic history × FA exposure into the model. All computations were performed using the SAS software package version 9.1 (SAS Institute, Inc, Cary, NC).

RESULTS

The prevalence of current asthma, atopic eczema, and allergic rhinitis was 2.1%, 5.7%, and 14.0%, respectively. About half of the participants were 30 years or older and enrolled by the 17th week of gestation (Table 1). High mite antigen

TABLE 1. Distribution of selected characteristics in 998 pregnant women, Osaka Maternal and Child Health Study, Japan, November 2001 to March 2003

Variable	n (%)
Age, years	
<30	471 (47.2)
≥30	527 (52.8)
Gestation, weeks	
<18	507 (50.8)
≥18	491 (49.2)
Parity ≥1	511 (51.2)
Family history of asthma	101 (10.1)
Family history of atopic eczema	138 (13.8)
Family history of allergic rhinitis	428 (42.9)
Cigarette smoking	
Never	694 (69.5)
Former	120 (12.0)
Current	184 (18.4)
Current passive smoking at home	493 (49.4)
Current passive smoking at work	120 (12.0)
Mold in kitchen	204 (20.4)
Indoor domestic pets (cats, dogs, birds, or hamster)	113 (11.3)
Mite antigen level in house dust[a]	
–	433 (43.4)
±	297 (29.8)
+ or ++	268 (26.9)
Family income (Japanese yen/year)	
<4,000,000	298 (29.9)
4,000,000–5,999,999	403 (40.4)
≥6,000,000	297 (29.8)
Education, years	
<13	322 (32.3)
13–14	410 (41.1)
≥15	266 (26.7)
Season when data were collected	
Spring	310 (31.1)
Summer	176 (17.6)
Fall	207 (20.7)
Winter	305 (30.6)

[a]Antigen levels were semiquantitatively classified with scores of – (<2 μg/m^2), ± (5 μg/m^2), + (10 to 15 μg/m^2), and ++ (>35 μg/m^2).

levels of 10 μg/m^2 or greater were measured in the houses of 27% of the participants. The median and maximum level of FA among all participants was 24 and 131 ppb, respectively. A total of 13 samples (1.3%) exceeded the current Japanese indoor guideline of 80 ppb. FA levels varied by season and the highest levels were found during the winter (Kruskal-Wallis test; $p < 0.0001$).

There was a tendency for a positive exposure-response relationship between FA levels and the prevalence of atopic eczema, although the crude OR for highest vs. lowest FA categories did not reach statistical significance (Table 2). Adjustments for age; gestation; parity; family history of asthma, atopic eczema, and allergic rhinitis; cigarette smoking; current passive smoking at home and work; mold in the kitchen; indoor domestic pets; family income; education; mite antigen level in house dust; and season did not appreciably change these results. When FA levels were categorized into two groups using a cut-off point at the 90th percentile, FA levels of 47 ppb or more were independently associated with an increased prevalence of atopic eczema in the multivariate model (adjusted OR, 2.25; 95% CI, 1.01–5.01). The same result was observed if levels of FA were treated as a continuous variable. For every 10-unit (ppb) increase in FA exposure, there was an increase of 16% in the prevalence of atopic eczema (adjusted OR, 1.16; 95% CI, 0.99–1.35). No clear association was found between FA levels and the prevalence of asthma or allergic rhinitis.

When subjects were divided according to familial allergic history in at least one parent or sibling, an increased prevalence of atopic eczema in relation to FA levels was more pronounced in those with a negative than with a positive familial allergic history, after multivariate adjustment (Table 3). This association was marginally significant only among women with a negative familial allergic history. The multivariate OR of atopic eczema for comparison of a positive with a negative familial allergic history was 1.78 (95% CI, 1.01–3.22). No significant interaction was found in the association of FA levels with the prevalence of atopic eczema between pregnant women with a positive and negative familial allergic history ($p = 0.69$, 0.44, and 0.30 for homogeneity of OR for the second, third, and fourth categories, respectively).

DISCUSSION

In the present cross-sectional study of Japanese pregnant women, we found that FA exposure was positively related to the prevalence of atopic eczema, especially in women with a negative familial allergic history. However, no clear association was shown between FA and the prevalence of current asthma or allergic rhinitis.

No epidemiologic studies, to our knowledge, have examined whether there is an association between FA levels and

TABLE 2. Crude and adjusted ORs and 95% CIs for current allergic disorders in relation to formaldehyde exposure levels, Osaka Maternal and Child Health Study, Japan, November 2001 to March 2003

		Crude		Adjusted[a]	
Formaldehyde levels (ppb)	Prevalence (%)	OR (95% CI)[b]	OR (95% CI)[c]	OR (95% CI)[b]	OR (95% CI)[c]
Asthma					
<18	7/298 (2.4)	1.00		1.00	
18–27	6/299 (2.0)	0.85 (0.28–2.56)	1.00	0.80 (0.23–2.84)	1.00
28–46	5/301 (1.7)	0.70 (0.22–2.24)		0.72 (0.19–2.77)	
≥47	3/100 (3.0)	1.29 (0.33–5.07)	1.51 (0.44–5.23)	2.15 (0.41–11.28)	2.65 (0.63–11.11)
		(p for trend = 0.87)		(p for trend = 0.47)	
Atopic eczema					
<18	15/298 (5.0)	1.00		1.00	
18–27	15/299 (5.0)	1.00 (0.48–2.08)	1.00	1.03 (0.47–2.29)	1.00
28–46	17/301 (5.7)	1.13 (0.55–2.31)		1.11 (0.50–2.42)	
≥47	10/100 (10.0)	2.10 (0.91–4.83)	2.01 (0.98–4.12)	2.36 (0.92–6.09)	2.25 (1.01–5.01)
		(p for trend = 0.08)		(p for trend = 0.08)	
Allergic rhinitis					
<18	45/298 (15.1)	1.00		1.00	
18–27	41/299 (13.7)	0.89 (0.57–1.41)	1.00	1.06 (0.65–1.73)	1.00
28–46	37/301 (12.3)	0.79 (0.49–1.26)		0.85 (0.51–1.40)	
≥47	17/100 (17.0)	1.15 (0.63–2.12)	1.29 (0.74–2.25)	1.17 (0.60–2.28)	1.22 (0.68–2.20)
		(p for trend = 0.91)		(p for trend = 0.91)	

[a]Based on multiple logistic regression controlling for age; gestation; parity; family history of asthma, atopic eczema, and allergic rhinitis; cigarette smoking; current passive smoking at home and work; mold in the kitchen; indoor domestic pets; mite antigen level in house dust; family income; education; and season when data were collected.
[b]Formaldehyde levels were categorized into 4 groups using cut-off points at the 30th, 60th, and 90th percentile values.
[c]Formaldehyde levels were categorized into 2 groups using a cut-off point at the 90th percentile value.

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TABLE 3. Adjusted ORs and 95% CIs for current atopic eczema in relation to formaldehyde exposure levels by familial allergic history, Osaka Maternal and Child Health Study, Japan, November 2001 to March 2003[a]

	Negative familial allergic history (n = 488)			Positive familial allergic history (n = 510)		
Formaldehyde levels (ppb)	Prevalence (%)	OR (95% CI)[b]	OR (95% CI)[c]	Prevalence (%)	OR (95% CI)[b]	OR (95% CI)[c]
<18	4/141 (2.8)	1.00		11/157 (7.0)	1.00	
18–27	5/156 (3.2)	1.37 (0.33–5.79)	1.00	10/143 (7.0)	0.80 (0.30–2.12)	1.00
28–46	7/147 (4.8)	1.88 (0.49–7.23)		10/154 (6.5)	0.92 (0.35–2.45)	
≥47	4/44 (9.1)	4.21 (0.90–19.85)	2.96 (0.87–10.12)	6/56 (10.7)	1.45 (0.42–4.93)	1.63 (0.58–4.57)
		(*p* for trend = 0.06)			(*p* for trend = 0.50)	

[a]Based on multiple logistic regression controlling for age; gestation; parity; cigarette smoking; current passive smoking at home and work; mold in the kitchen; indoor domestic pets; mite antigen level in house dust; family income; education; and season when data were collected.
[b]Formaldehyde levels were categorized into four groups using cut-off points at the 30th, 60th, and 90th percentile values.
[c]Formaldehyde levels were categorized into two groups using a cut-off point at the 90th percentile value.

atopic eczema. There are potential reasons why such an association may exist. In a climate chamber study, FA exposure induced a significant increase of transepidermal water loss in patients with atopic eczema but not in control subjects (27). The irritant properties of FA may impair the epidermal barrier function (27). In our study, approximately 50% of the participants who had atopic eczema at the time of data collection had been treated with medications before 12 years of age. Therefore, exposure to FA may exacerbate atopic eczema symptoms in adults. Alternatively, the observed association might be attributed to unrecognized environmental factors associated with FA.

A more evident positive relationship between FA and atopic eczema was found in our subjects who had a negative familial allergic history than in those with a positive familial allergic history. A 4-year follow-up study in Swedish schoolchildren showed that among children without a history of atopy a new asthma diagnosis was more common at higher levels of FA in classroom air (28). The present findings are in partial agreement with this observation. Further studies are necessary to understand the interaction between FA and genetic factors in the manifestation of atopic eczema.

We found no significant association between FA and the prevalence of current asthma. The present findings are in agreement with previous observations in the United States showing no association between FA at home and respiratory symptoms or diseases in adults (13) and between exposure to FA vapor and bronchoconstriction among medical students (29). However, they are in disagreement with results of a previous study showing a positive association between FA exposure at home and asthma (12). It may be difficult to detect a clear positive association between FA and current asthma in our study population that had only a few asthmatics.

In our study, no significant association between FA and the prevalence of current allergic rhinitis was detected. As far as we know, only one epidemiologic study has investigated whether there was an association between FA and allergic rhinitis. A cross-sectional study in Sweden found no association between FA levels in classroom air and nasal symptoms in primary school personnel (14). Their results are compatible with our findings.

Our participants were relatively homogeneous because all were pregnant and we obtained extensive information on potential confounding factors. Although there were 3,639 eligible pregnant women in Neyagawa City, only 627 (17.2%) took part in this study. We do not know if a difference existed between participants and nonparticipants because data on personal characteristics, such as age, socioeconomic status, and history of allergic disorders, were not available for nonparticipants. Regarding the remaining 374 participants who were not residents of Neyagawa City, we could not calculate the participation rate because the exact number of eligible women was not available. Also, we could not compare participants with nonparticipants in the four collaborating hospitals and six municipalities. Because our subjects might not be representative of Japanese pregnant women in general, the present findings cannot be generalized. In fact, educational levels were greater in the present study population than in the general population. According to the 2000 population census of Japan, the proportions of women ages 30 to 34 years in Osaka Prefecture with years of education of <13, 13 to 14, ≥15, and unknown were 49.2%, 32.3%, 13.6%, and 4.9%, respectively (30). The corresponding figures for the present study were 32.3%, 41.1%, 26.7%, and 0.0%, respectively. No attempt was made to ascertain outcome status through reviews of medical records. Moreover, we did not use validated diagnostic criteria for allergic disorders, such as those reported in the International Study of Asthma and Allergies in Childhood. Because the definition of allergic disorders was based on self-reporting and medical treatment in the past 1 year, there was probably a loss of milder cases of allergic disorders. In particular, women who want to become pregnant or who are pregnant might tend to avoid drugs.

The main sources of emission of FA in homes are gas appliances, open fireplaces, tobacco products, furniture, woodchip boards and other building materials containing

FA (31). Asthma, atopic eczema, and allergic rhinitis sufferers might not be aware of the main sources of FA and the possible ill effects of FA exposure. In our study, furthermore, the exposure measurements were performed in parallel with completing the questionnaires, so the former might not be able to influence the reporting of allergic disorders. The consequence would have given rise to an underestimation of our findings because of nondifferential outcome misclassification.

Each participant wore a passive diffusion sampling tube on a usual day, and then the tube was analyzed by laboratory personnel blind to health status to minimize observer bias. Both measurement errors of FA and day-to-day variations in personal exposures would have most probably led to nondifferential exposure misclassification.

The interface between allergy/immunology and pregnancy should be discussed, which may have an influence on the association of interest. It has been suggested that pregnancy involves a shift to the Th2 side of the immune response (32) whereas the importance of the role of NK and interleukin (IL)-12, IL-15, and IL-18 tripods in successful or failed pregnancy in humans was suggested beyond the Th1/Th2 paradigm (33). Pregnancy does not appear to have a consistent effect on the frequency or severity of asthma (34). Rhinitis symptoms during pregnancy may be attributable to the hormonal changes in pregnancy. However, rhinitis solely ascribed to pregnancy may not be a distinct entity because most pregnant women do not have significant nasal symptoms (33). Symptoms of atopic eczema may worsen with pregnancy in some patients and appear to improve in others (35). Atopic eczema in pregnancy is not likely to be a distinct entity. Regarding the interpretation and generalization of our results, the interface between allergic disorders and pregnancy is likely to be a minor problem.

This is the first epidemiological study on the association between exposure to FA and allergic disorders in Japan. Because this was a cross–sectional study, we could not establish a cause and effect relationship for the associations under study. Further evaluations in prospective studies are needed to draw a conclusion regarding whether FA exposure increases the likelihood of atopic eczema.

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APPENDIX

Space limitations preclude the inclusion as authors of the following members of the Osaka Maternal and Child Health Study Group:

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ORIGINAL PAPER **Epidemiology of Allergic Disease**

Tuberculin reactivity and allergic disorders in schoolchildren, Okinawa, Japan

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Clinical and Experimental Allergy

Summary

Background Bacillus Calmette-Guérin (BCG) vaccination triggers a T-helper type 1 response. Whether BCG vaccination and positive tuberculin reactivity are preventive against allergic disorders remains controversial.

Objective The current cross-sectional study investigated the relationship of BCG vaccination and tuberculin reactivity with the prevalence of allergic disorders using data from the Ryukyus Child Health Study (RYUCHS).

Methods Subjects were 5717 schoolchildren aged 8–11 years in Okinawa, Japan. The RYUCHS collected information on symptoms of allergic disorders and potential confounding factors. The outcomes were based on diagnostic criteria from the International Study of Asthma and Allergies in Childhood. Data on BCG vaccination and tuberculin tests were obtained from school records. Allowance was made for grade, sex, sibship size, smoking in the household, paternal and maternal history of asthma, atopic eczema, and allergic rhinitis, and paternal and maternal educational level.

Results No measurable relationship was found between BCG vaccination in infants and the prevalence of allergic disorders. Among 5567 BCG-vaccinated children, positive tuberculin reactivity (induration ≥10 mm) in the first grade was independently associated with a decreased prevalence of wheeze, asthma, and atopic eczema: the multivariate odds ratios for wheeze, asthma, and atopic eczema were 0.80 (95% confidence interval [CI], 0.67–0.94), 0.78 (95% CI, 0.64–0.95), and 0.77 (95% CI, 0.62–0.95), respectively. The inverse associations were more pronounced in children with a negative parental allergic history than in those with a positive parental allergic history. There was no significant relationship between tuberculin reactivity and allergic rhinoconjunctivitis.

Conclusions The findings suggest that positive tuberculin reactivity may be inversely associated with the prevalence of wheeze, asthma, and atopic eczema, but not allergic rhinoconjunctivitis, especially among Japanese children without a parental allergic history.

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Keywords allergic rhinoconjunctivitis, asthma, atopic eczema, Japanese children, tuberculin reactivity, wheeze

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Introduction

The increase in allergic disorders during the past decades might be to some extent explained by a reduction in bacterial and viral infections and comprehensive vaccination programmes in Japan as well as in Western societies [1]. If an individual has predominantly T-helper type 2 (Th2) cells, the Th2 phenotype interacts with environmental allergens to develop allergic disorders [2]. Infections and immunizations may alter the balance between Th1 and Th2 phenotypes [2].

Bacillus Calmette-Guérin (BCG) vaccination triggers a Th1 response in both healthy adults [3] and newborns [4]. Shirakawa et al. [5] demonstrated a clear relationship among Japanese children between delayed hypersensitivity to tuberculin at the age of 12–13 years and a decreased prevalence of asthma, rhinitis, and eczema, lower levels of total serum IgE and Th2 cytokines, and higher levels of the