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INVESTIGATIVE REPORT

The Wiskott-Aldrich Syndrome Gene as a Candidate Gene for Atopic Dermatitis

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Atopic dermatitis is a multifactorial disorder probably caused by environmental factors in combination with susceptibility genes. The clinical similarity between atopic dermatitis and the eczema manifestation in patients with Wiskott-Aldrich syndrome made the previously identified *WAS* gene in chromosome sub-band Xp11.23 an interesting candidate gene for atopic dermatitis. We studied linkage and association to the *WAS* gene region using four polymorphic microsatellite markers in 406 Swedish families with at least two siblings affected with atopic dermatitis (in total 1514 individuals). In the analyses, we studied two qualitative traits: *atopic dermatitis* and *elevated allergen-specific serum IgE antibodies*, and one quantitative trait, a *severity score of atopic dermatitis*. We found that the marker *MAOB* gave positive linkage with a maximum lod score of 1.68 ($p < 0.05$) to the *severity score of atopic dermatitis*. Association could not be seen to *atopic dermatitis* nor to *elevated allergen-specific serum IgE antibodies* in this region using the transmission disequilibrium test. Our results indicate that either the *WAS* gene or another gene in the area contributes to the severity of atopic dermatitis. *Key words: association; genetic linkage; IgE levels; transmission disequilibrium test.*

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Wiskott-Aldrich syndrome (WAS) [MIM 301000] is a genetically determined disorder clinically characterized by thrombocytopenia, immunodeficiency, severe eczema and susceptibility to lymphoid malignancies (1). Other features seen are asthma, food allergy and urticaria (1–3). Raised total IgE levels and low IgM levels in serum are also common (2). The number of peripheral lymphocytes is often decreased and the thrombocytes are reduced in number and/or efficiency. A typical finding is the reduced expression of the membrane glycoprotein CD43 present on lymphocytes and platelets (1). However, there is a significant variation in the clinical and immunological presentation among patients with WAS (4).

WAS occurs in about four in every million live births and is typically transmitted as an X-linked recessive trait. An autosomal-dominant variant has also been proposed (1) as girls affected with WAS have been described (5, 6). The gene in chromosome sub-band Xp11.23 responsible for WAS was identified in 1994 (7). The gene spans about 9 kb of genomic DNA and has 11 exons and encodes a 502-aminoacid proline-rich protein called WASp (2). WASp is expressed in cells of the hematopoietic cell lineage.

The eczema in WAS usually appears during the first month of life, and most commonly affects the scalp, face, flexures and diaper area. It is essentially indistinguishable from atopic dermatitis except for the frequent presence of purpura and excessive bleeding from the excoriations in WAS eczema (3). This could be explained by the defective thrombocytes in WAS patients, a feature not associated with atopic dermatitis.

Atopic dermatitis is a multifactorial disorder most likely caused by environmental factors in combination with susceptibility genes. Genetic heterogeneity probably exists. In the search for genetic explanations for atopic dermatitis, the clinical similarity between atopic dermatitis and the eczema manifestation in WAS made the *WAS* gene an interesting candidate gene. We studied linkage and association to the *WAS* gene in 406 families with at least two siblings affected with atopic dermatitis (in total 1514 individuals) using four microsatellite markers.

MATERIAL AND METHODS

Recruitment and clinical examination of patients

A large sample group consisting of 406 families with at least two children affected with atopic dermatitis was analysed, in total 1514 individuals containing 572 affected full-sib pairs and 30 affected half-sib pairs. The male to female sex ratio among the siblings was 1:1.5. Families used in the present study were a subset of a sample group described previously (8). Pedigrees are available on request. The same dermatologist diagnosed atopic dermatitis in all siblings applying the UK Working Party's Diagnostic Criteria (9–11). An arbitrary score for the severity of the atopic dermatitis among the siblings was obtained using the classification given in Table I. Parents were included regardless of their atopic status and answered a questionnaire regarding atopic manifestations. The Karolinska Hospital Ethics Committee approved the study.

Table I. *Severity scoring of atopic dermatitis (AD)*

Factor	Score
Age at onset < 2 years	1
Hospitalization for AD	1
No. of sites* manifesting AD at examination	
0	0
1–3	1
> 3	2
Raised total and/or allergen-specific serum IgE	1
Maximum score	5

*The presence of AD at one or both sites in bilateral structures was considered as being at one site.

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Quantification of IgE antibodies

In all affected siblings we measured: The total serum IgE concentration using the Pharmacia CAP System, IgE FEIA (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The cut-off was 2 kU/l. IgE antibodies to a mixture of inhalant allergens, Phadiatop® (Pharmacia CAP System Phadiatop® FEIA). The Phadiatop was reported as either positive or negative. IgE antibodies to a mixture of relevant food allergens (fx5) (Pharmacia CAP System RAST® FEIA). In the analysis, we used raised allergen-specific IgE antibodies to either Phadiatop and/or food allergens as a positive result.

Genotyping

Genomic DNA was extracted from blood using standard protocols. Four polymorphic microsatellite markers flanking the *WAS* gene were used: *DXS6810, MAOB, DXS1199* and *DXS7132* (Table II). The map distances are based on the Marshfield map (http://research.marshfieldclinic.org/genetics/) and the Genome Database Comprehensive Map (http://www.gdb.org/). PCR conditions are available on request. PCR reactions for each marker were performed separately in 96 well plates, with products being pooled prior to gel electrophoresis on an ABI377 (Applied Biosystems). The forward primers were fluorescently labelled and the resulting genotype data were analysed by use of GENESCAN 2.1 and GENOTYPER 2.0 software from ABI (Applied Biosystems). Allele numbers were assigned for each marker based on the size of the amplified fragments. All genotyped markers were checked for Mendelian incompatibilities using PedCheck 1.0 (12) or zGenStat1.126 (Henric Zazzi© 2000). Incompatibilities were either resolved unambiguously or individuals were discarded from linkage analyses. The expected number of homozygotes was calculated based on the estimated allele frequencies in women, and the observed number of homozygotes was compared with the number expected using the Pearson chi squared test in the zGenStat 1.126 software. None of the markers showed significant deviation from the expected homozygosity frequency. Heterozygosity and position for the markers are listed in Table II.

Statistical analysis

Linkage analysis. In the analyses, we studied two qualitative traits: *atopic dermatitis* and *elevated allergen-specific serum IgE antibodies*, and one quantitative trait: the severity score of atopic dermatitis. Multipoint non-parametric sib pair linkage analyses were performed using MAPMAKER/SIBS (13), which tests linkage by the maximum likelihood ratio approach. In order to achieve a Z-score with known statistical properties, linkage analysis was performed separately on brother–brother, sister–sister and sister–brother pairs (14, 15). For families with more than two affected siblings, all independent pairs were used. Allele frequencies were estimated from all individuals in the material. Linkage analysis for the quantitative trait, the *severity score of atopic dermatitis*, was performed using MAPMAKER/SIBS (13), using the non-parametric command. MAPMAKER/SIBS (13) was also used for exclusion mapping for the qualitative traits, analysing brother–brother, brother–sister, sister–sister pairs both separately and together.

Association. A transmission/disequilibrium test (TDT) (16) was performed in order to evaluate the evidence for association between the marker locus and the two qualitative phenotypes – *atopic dermatitis* and *elevated allergen-specific serum IgE antibodies*. For this we used ASPEX software (17). P-values were corrected for the number of markers tested. In the TDT analysis only one affected child (the oldest) in each family was included.

Table II. *Position and heterozygosity of the markers*

Marker	Position*(cM)	Heterozygosity
DXS6810	42.75	0.63
MAOB	47.00	0.78
WAS	50.33	
DXS1199	51.50	0.68
DXS7132	52.50	0.76

*Position in centimorgan (cM) of markers from pter to qter.

RESULTS

We found linkage with a maximum lod score (MLS) of 1.68 ($p < 0.05$) in the single-point analyses to marker *MAOB* following the *severity score of atopic dermatitis*. We did not find any evidence of linkage to the other markers in either the single-point or multipoint analyses. When studying the phenotypes *atopic dermatitis* and *elevated allergen-specific serum IgE antibodies*, we could not show linkage to any of the markers and we could exclude the presence of a susceptibility gene with a major effect. However, we could not exclude a susceptibility gene with a minor effect, i.e. that would have increased the expected 50% sharing of alleles to 57% or less in the trait *atopic dermatitis* and to 67% or less in the trait *allergen-specific IgE antibodies*. In the TDT analysis, none of the markers showed any significant association to the traits of *atopic dermatitis* or *elevated allergen-specific serum IgE antibodies*.

DISCUSSION

The Wiskott-Aldrich syndrome (WAS) is a severe illness with manifestations in many different organs. One of the clinical manifestations in WAS is an atopic dermatitis-like eczema. In the search for genetic explanations for atopic dermatitis, the clinical similarity between atopic dermatitis and the eczema manifestation in WAS made the *WAS* gene a possible candidate gene. We believe that WAS is an interesting syndrome that might teach us more about the pathogenesis of atopic dermatitis.

It is known that the product from the *WAS* gene (WASp) acts in many different pathways affecting the cytoskeleton, cell proliferation and the immune system. A typical finding in WAS patients is the reduced expression of the membrane glycoprotein CD43 on most of their blood cells. The WAS protein (WASp) is known to interact with the expression of CD43 either directly or through a more complex mechanism (1). CD43 has also recently been claimed to be involved in self-tolerance selection of autoreactive T cells in the thymus (18). Interestingly, it has been proposed by Thestrup-Pedersen et al. that the T cells of atopic dermatitis patients are "dysmatured" due to a faulty selection in the thymus, and that they "home" to the skin because the ectodermal compartment resembles the epithelium in the thymus (19).

In the present study, we found linkage between the marker *MAOB* and the trait severity score of atopic dermatitis. Our linkage does not reach the genome-wide significance levels ($p < 2.2 \times 10^{-5}$) that have been suggested (20), and hence confirmation from an independent patient sample group is required. No association could be seen to this region in the qualitative traits, *atopic dermatitis* or *elevated allergen-specific serum IgE antibodies* using the transmission/disequilibrium test.

The marker *MAOB* is located approximately 3cM centromeric of the *WAS* gene, so the gene that contributes to the linkage in our study can be either the *WAS* gene or another gene in the area. One could speculate that it might be a gene with a similar function, since genes with similar function tend to cluster, as for example interleukin genes cluster on chromosome 5 and the HLA genes cluster on chromosome 6. The

findings could also be coincidental, having nothing to do with the *WAS* gene. The exclusion mapping tells us that this area does not include a gene with major effect contributing to the traits of *atopic dermatitis* and *elevated allergen-specific serum IgE antibodies*. Recently, there have been two genome scans in atopic dermatitis (21, 22). None of the groups reports linkage or association to the WAS region on the X chromosome.

In summary, our results indicate that either the *WAS* gene or another gene in the area may contribute to the severity of the atopic dermatitis. However, confirmation from an independent patient sample group is required. A better understanding of the WASp function and its interactions, especially with CD43, might illuminate the pathogenesis of atopic dermatitis.

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