

Meta-analysis of Genome-Wide Linkage Studies of Atopic Dermatitis

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Background: Atopic dermatitis (AD) is considered as a hereditary skin disease. Recently, a latest form of genetic research, genome-wide linkage study, has been carried out to detect its specific susceptibility genes. Since the results of these studies have been inconsistent, with linkage confirmed in different regions, we performed this meta-analysis.

Objective: To assess heritability and identify chromosomal regions showing evidence of AD.

Methods: A comprehensive and systematic search was conducted with PubMed databases. We combined results from three non-overlapping genome-wide linkage studies of AD, using the heterogeneity-based genome scan meta-analysis (HEGESMA) method, a rank-based analysis that assesses the strongest evidence for linkage within bins of a 30-centimorgan width on autosomes and the X chromosome.

Results: The following ten bins had a *p* value of less than .05 in both weighted and unweighted analyses, suggesting that these bins contain AD-linked loci: 3.2, 3.4, 3.5, 4.5, 5.1, 5.2, 6.3, 9.2, 9.4, and 22.1. HEGESMA produced significant genome-wide evidence for linkage on 3p14.1-q12.3, with high average rank and low between-study heterogeneity; 5pter-p15.1 represents new significant loci not reported previously.

Conclusion: HEGESMA confirms the region encoding CD80, CD86, and interleukins on chromosomes 3 and 5. The filaggrin gene (*FLG*) may be the susceptibility factor.

ATOPIC DERMATITIS (AD) is a hereditary, pruritic, inflammatory, and chronic skin disease that occurs most commonly in early childhood and may continue or recur later. Clinically, it is characterized by an intensely pruritic rash with a typical morphology, and validated scoring systems are available to gauge disease severity.¹

It is generally believed that the major reasons for the increasing incidence of AD (as with many multifactorial and polygenic diseases) must be found in our environment because the genetic pool has changed minimally during a relatively limited period. An epidemiologic study of AD in a wide variety of populations has been performed and indicates a lower prevalence among those living in lower and more tropical latitudes, rural areas, and less industrialized regions.² However, molecular genetics is believed to offer a better key to understanding the pathogenesis of AD and will eventually provide a platform from which our insight into the environmental risk factors can further

expand. Moreover, recent data from various studies support a genetic contribution to AD. Family and twin studies estimate genetic predisposition to AD to be considerable.^{3,4} One study showed that 90% of its patients with AD had one or both parents affected with AD; in 1991, another study showed that when both parents have AD, their children have up to a 75% risk of developing the disease.^{5,6} Twin studies also support the role of a strong genetic contribution, with a concordance rate of 0.86 in monozygotic twins and 0.21 in dizygotic twins.⁷ In a Norwegian twin study, the heritability of AD was estimated at 0.72.⁸ To identify susceptibility genes for AD, the candidate gene association study approach can be applied. So far, genetic investigations have been limited to association studies with few candidate genes.^{9,10} However, AD is a multifactorial and polygenic disease, and its mode of inheritance does not follow typical mendelian laws. Therefore, nonparametric methods, such as genome-wide linkage or association (across the entire genome, polymorphic deoxyribonucleic acid [DNA] markers positioned at specific intervals along each chromosome are screened for linkage to the disease of interest), have been used to localize susceptibility genes. Previous genome-wide scans for genes predisposing to atopy have focused on asthma and high immunoglobulin E (IgE) levels as phenotypes.¹¹⁻¹⁴ Recently, genetic research has focused on an AD

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phenotype, but these studies have thus far produced inconsistent results, in part because the linkage signals were rather low.¹⁵ To determine the specific susceptibility genes, we summarized the existing evidence on regions linked to AD by applying heterogeneity-based genome scan meta-analysis (HEGESMA) to available genome scan data.

HEGESMA can perform both genome scan meta-analysis and heterogeneity testing across genome searches.^{16–18} HEGESMA is currently being used for a number of ongoing meta-analyses of genome scans, including those of osteoporosis (bone mineral density and fracture risk), Parkinson disease, autism, schizophrenia and bipolar disorder, pre-eclampsia, and myocardial infarction.^{19–23}

Materials and Methods

Eligible Genome Scans

We considered all AD genome-wide scans published before December 2008 by searching the PubMed database with the following terms, restrictive to human studies and English publications: genome wide search, genome wide scan, genome wide screen, genome wide association, genome wide linkage, genomic search, genomic scan, genomic screen, susceptibility loci, and atopic dermatitis. Manual searches of reference lists were also carried out. We identified four genome-wide studies that fulfilled the following inclusion criteria: (1) DNA markers as microsatellites were typed throughout the genome; (2) the required linkage score data were published on the Web,

provided by the authors, or extractable from published graphs; and (3) there was no sample overlapping. Atrial scans and candidate region studies were excluded. However, one study was excluded because linkage statistics were not sufficient to perform HEGESMA, which required the results of at least 20 bins in the genome-wide scan and attempts to contact the authors were unsuccessful.²⁴ Therefore, a total of three genome-wide scans are included in the present meta-analysis^{25–27} (Fig 1).

The search strategy was approved by the authors. The literature search and the article retrieval and evaluation were performed by Ming Liao and Xiaoli Huang.

Data Extraction

The following information was extracted from each eligible study: first author, journal, year of publication, country of recruitment, ethnicity of study population, number of families, number of affected cases, number of markers, genotyping, linkage statistic, type of statistical analysis, and software used for linkage analysis and determining linkage site (Table 1). In an article describing one genome-wide scan performed by Bradley and colleagues, one of the figures indicated three kinds of logarithm of the odds (LOD) values of the multipoint linkage analysis for three qualitative phenotypes: AD, AD combined with elevated allergen-specific serum IgE levels, and extreme AD.²⁶ As there were overlapping cases in this study, we extracted the LOD value in the largest studies from the figure. The data on linkage test statistics of the remaining three genome-wide scans were extracted from published graphs.²⁶

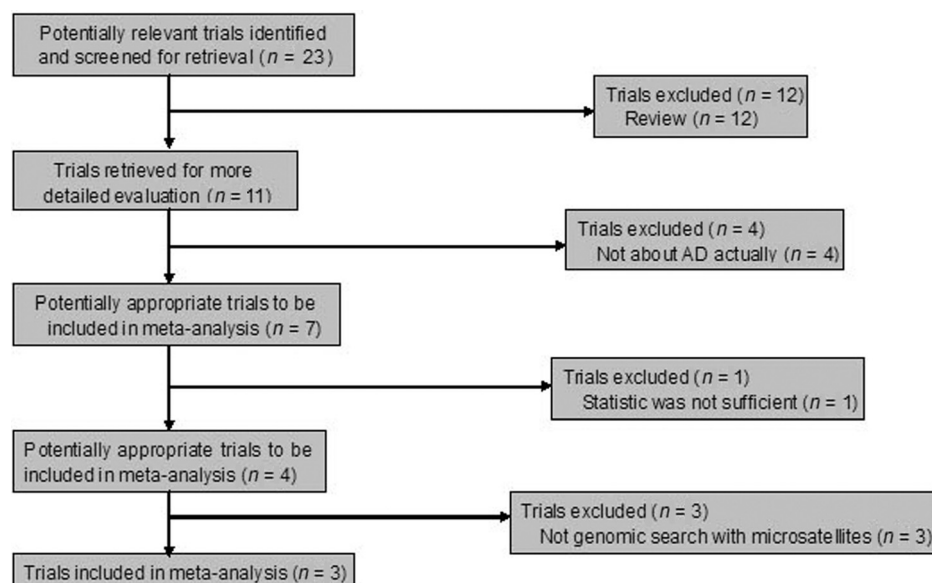


Figure 1. Outline of the search-flow diagram. AD = atopic dermatitis.

Table 1. Summary of Genome-Wide Studies

Year	First Author	Population	Phenotype	Microsatellites	Pedigrees	Statistic	Software	Type of Analysis	Linkage Regions
2000	Lee	German, Italian, Swedish, Dutch	AD	380	199	<i>p</i> Value	PedCheck	Multipoint linkage	3q21
2002	Bradley	Swedish	AD; AD and specific IgE; AD and severity score	367	109	LOD score	Allegro and MAPMAKER/SIBS	Multipoint linkage	3p24-22, 18q21, 3q14, 13q14, 15q14-15, 17q21
2004	Haagerup	Danish	AD and specific IgE	446	23	LOD score	MAPMAKER/SIBS	Multipoint linkage	3p26-3p24, 4p15-4p14, 18q11-18q12

AD = atopic dermatitis; IgE = immunoglobulin E; LOD = logarithm of the odds.

All of the genome-wide linkage studies included were performed in 331 Caucasian populations from Denmark, Sweden, Great Britain, Germany, Italy, and the Netherlands. This is the first meta-analysis of whole-genome linkage studies to identify susceptibility genes for AD.

Genome Scan Meta-analysis and Heterogeneity Testing

The genome scan meta-analysis starts by sorting the chromosomes into “bins” (loci) of similar length; each bin usually has a width of approximately 30 centimorgans (cM), giving 120 bins in total for the whole genome.^{17,18,28,29} For nomenclature purposes, “bin c.n” would be the *n*th bin on chromosome *c*. For each genome scan, the most significant result of the test statistic obtained within the bin is recorded. Test statistics may include LOD score, maximum logarithm of odds score, nonparametric linkage score, and *z* statistics, depending on the mode of analysis of the linkage data.³⁰ Then for each scan, the bins are ranked according to the significance of their results, and the ranks for each bin are summed across scans. The significance of the average rank of each bin is assessed empirically again at the distribution of average ranks. When a bin has a high average (or summed) rank, this is considered evidence for linkage. Equal test statistics for several bins within a study were assigned as tied ranks.

The extent of heterogeneity between studies for each bin can be estimated by the statistics *Q*, *Ha*, and *B*, which have been developed by the authors. The *Q* statistic is defined as the sum of the squared deviations of each study's bin rank from the mean of the ranks. In genome scan meta-analysis, the presence of low between-study heterogeneity for a specific with high ranks in all studies can be interpreted as further evidence for the importance of this bin. Conversely, significantly high between-study heterogeneity suggests that there may be major differences in genetic effect, design, population, or other factors across the combined studies. Besides the *Q* statistic, two other heterogeneity metrics (*B* and *Ha*) have been proposed, but inferences with these metrics were similar to the *Q* metric.^{17,18}

The statistical significances of the average rank and the *Q* metric were assessed by the Monte Carlo method. In a run of the Monte Carlo method, the ranks of each study are randomly permuted and the simulated average rank and *Q* metric are calculated; then the procedure for 50,000 runs is repeated and a null distribution for the average rank (and for the *Q* metric) is constructed. A Monte Carlo

test was performed, generating null distributions separately for each bin, considering only the simulated distributions of the Q metric (Q adjusted) for bins with the neighboring simulated average rank (± 2) as the bin being considered each time.

In the present study, genome scan meta-analysis and heterogeneity testing were performed unweighted and weighted. The evaluation of the significance of average ranks and the significance of heterogeneity was performed with *HEGESMA* software (Department of Biomathematics, University of Thessaly).

Results

Figure 2 shows the weighted and unweighted average rank statistics for each 30 cM bin (vertical axis), plotted against the bin location within the chromosomes (horizontal axis). The bins with $p < .05$ in both weighted and unweighted analysis are located above the 5% boundary. Ten bins in chromosomes 3, 4, 5, 6, 9, and 22 are of great significance. The high average rank of chromosome is considered as evidence for linkage with AD.

The results of *HEGESMA* are shown in Table 2. Of the ten significant bins for which p is less than .05, bin 5.1 is most significant, with the lowest p value in either weighted or unweighted analysis (weighted, $p = .005$; unweighted, $p = .006$). This greatly suggests that the susceptibility loci exist in this chromosomal bin, and the low Q metric of bin 5.1 is further evidence of linkage.

Moreover, of the ten significant bins, bin 3.4 (with high average rank and low p value) is found to have the lowest Q value, indicating the lowest between-study heterogeneity. Besides the Q statistic, two other heterogeneity metrics

(B and H_a) are also similar to the Q metric (see Table 2), which can be interpreted as further support for the importance of this bin.

The results of the heterogeneity test by *HEGESMA* are shown in Table 3. The heterogeneity statistic of bin 3.4 indicates the lowest heterogeneity again (left-sided p value for Q, H_a , or B is the lowest), which suggests that there may be little difference in genetic effect, design, population, or other factors across the combined studies.

Discussion

Our findings with respect to 3p14.1-q12.3 on chromosome 3 are similar to the results of three other studies included.^{25–27} The 3p14.1-q12.3 region may include one or more AD susceptibility genes for our population as it is possible that the true disease susceptibility gene is located close to the actual linkage peak.

Our linkage region on chromosome 3 was located near 3q21, which was previously reported as a region linked to AD.¹⁴ Genes encoding two type I membrane proteins of the immunoglobulin superfamily, CD80 and CD86 antigens, have been mapped to this region. Both CD80 and CD86 interact with CD28 to provide co-stimulatory signals for T-cell activation and have been implicated in the activation of the Th2 subset of CD4⁺ T helper lymphocytes that are thought to have a pivotal role in mediating allergic inflammation.^{31,32} In this region, CD80/CD86 are interesting candidate genes because they are involved in co-stimulatory signals in T-cell activation.³³ Specifically, the memory T cells that encounter their specific antigen will generate an allergen response that then activates leukocytes, leading to the production of several cytokines and to atopic skin inflammation.³⁴

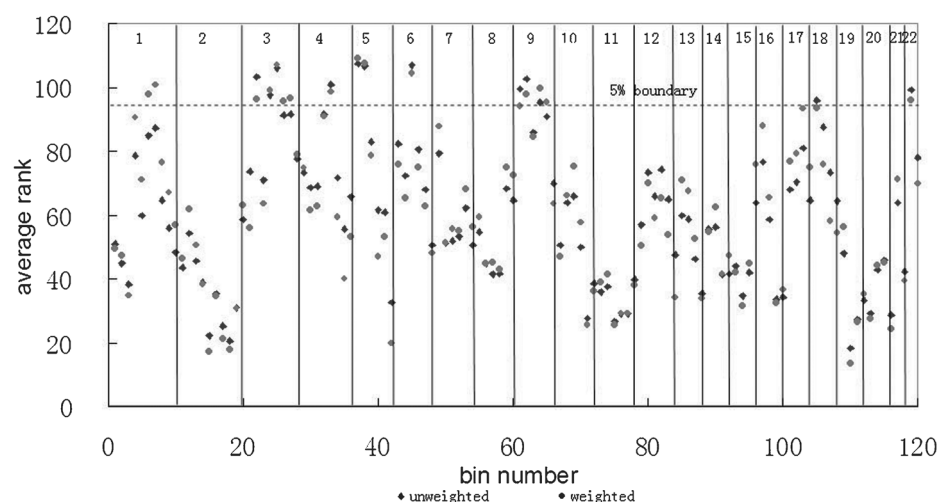


Figure 2. Weighted and unweighted average ranks for three atopic dermatitis genome scans with 120 bins.

Table 2. Results of HEGESMA

Chromosome	Bin	Interval (cm)	Linkage Region	Weighted					Unweighted				
				Observed	Observed	Observed	Observed	Observed	Observed	Observed	Observed	Observed	Observed
				Q	Ha	B	p Value for Mean	Q	Ha	B	p Value for Mean	Q	Ha
3	3.2	32.36–63.12	3p25.3-p22.1	641.902	24.049	12.226	.047	1568.667	64.667	98.000	.014	48.667	11.333
	3.4	88.6–117.76	3p14.1-q12.3	11.828	3.236	1.325	.032	48.667	11.333	18.000	.031	434.000	32.000
	3.5	117.76–146.6	3q12.3-q22.1	173.897	12.440	6.859	.008	266.000	26.000	58.000	.009	128.667	16.667
4	4.5	109.02–134.74	4q24-q28.3	112.743	10.111	5.396	.035	194.667	22.667	36.000	.007	312.000	28.000
5	5.1	0–31.78	5pter-p15.1	32.500	4.413	2.839	.005	554.667	37.333	64.000	.015	392.667	28.667
	5.2	31.78–64.14	5p15.1-q11.2	63.472	7.370	3.946	.007	428.667	31.333	58.000	.023		
6	6.3	65.14–99.01	6p21.1-q15	132.494	10.948	5.876	.015						
9	9.2	27.32–53.6	9p22.3-p21.1	206.812	13.059	7.094	.039						
	9.4	84.9–109.85	9q21.32-q31.1	123.609	9.156	5.303	.029						
22	22.1	0–33.76	22pter-q12.3	106.062	8.432	4.989	.048						

cM = centimorgans.

Chemokines reportedly play fundamental roles in the regulation of several types of T cells and in the development, homeostasis, and function of the immune system, especially in leukocyte trafficking. During the multistep process of leukocyte trafficking, chemokine ligand-receptor interactions mediate the firm adhesion of leukocytes to the endothelium and initiate transendothelial migration from the blood vessel into perivascular pockets.³⁵ From perivascular spaces, matrix-bound sustained chemokine gradients direct skin-infiltrating leukocyte subsets to subepidermal or intraepidermal locations. In AD regions, chemokines recruit pathogenic leukocytes to skin in response to mechanical injury such as scratching.³⁶

The region on chromosome 5 was a new locus that has not been reported to be in association with AD. The chromosome 5q region harbors many candidate genes for AD, including genes for interleukin (IL)-4; IL-13; IL-5; IL-12 β ; and serine protease inhibitor, Kazal-type 5 (*SPINK5*).⁹ Other candidate genes include the high-affinity IgE receptor beta-chain gene (*FCER1B*), mast cell chymase gene (*CMA1*), and IL-4 receptor alpha-chain gene (*IL4RA*).⁹

Recently, the lost function of the skin barrier in the development of AD has been emphasized.³⁷ The filaggrin gene (*FLG*) is involved in aggregation of the keratin cytoskeleton, which causes the collapse of granular cells into flattened anuclear squames.¹⁰ The condensed cytoskeleton is cross-linked by transglutaminases during the formation of the cornified cell envelope (the outermost barrier layer of the skin), which prevents water loss and impedes the entry of allergens and infectious agents.¹⁰ Recently, two loss-of-function variants in the gene encoding filaggrin at 1q21 were shown to be strongly associated with AD.¹⁰ As genome-wide scans have revealed a conspicuous overlap of susceptibility loci for psoriasis and AD (both of which can be characterized by epidermal differentiation and inflammation), involving chromosomal regions 1q21, 3q21, 17q25, and 20p12, our linkage region 3p14.1-q12.3 is very close to one of them, 3q21. Therefore, we suspect that some specific genes encoding FLG may exist on chromosome 3, linked to the susceptibility of chronic cutaneous inflammation disturbing epidermal barrier function, as in AD.³⁸

In this present research, two genomic linkage studies for AD did not meet our inclusion criteria and were thus excluded. One study used microsatellites but single-nucleotide polymorphisms, and the other did not contain the statistics we required. However, these two genomic linkage studies both concluded that chromosome 1 is the

Table 3. Results of Heterogeneity Test by HEGESMA

Chromosome	Bin	Interval (cM)	Linkage Region	Weighted			Unweighted		
				Left-Sided p Value for Q	Left-Sided p Value for Ha	Left-Sided p Value for B	Left-Sided p Value for Q	Left-Sided p Value for Ha	Left-Sided p Value for B
3	3.2	32.36–63.12	3p25.3-p22.1	.522	.523	.766	.991	.992	.990
	3.4	88.6–117.76	3p14.1-q12.3	.024	.027	.017	.041	.048	.043
	3.5	117.76–146.6	3q12.3-q22.1	.772	.796	.860	.742	.736	.782
4	4.5	109.02–134.74	4q24-q28.3	.228	.259	.245	.294	.316	.308
5	5.1	0–31.78	5pter-p15.1	.263	.205	.286	.285	.260	.331
	5.2	31.78–64.14	5p15.1-q11.2	.390	.412	.414	.370	.412	.355
6	6.3	65.14–99.01	6p21.1-q15	.463	.499	.508	.645	.676	.671
9	9.2	27.32–53.6	9p22.3-p21.1	.339	.336	.330	.656	.681	.672
	9.4	84.9–109.85	9q21.32-q31.1	.273	.236	.263	.291	.255	.330
22	22.1	0–33.76	22pter-q12.3	.175	.149	.178	.415	.398	.461

cM = centimorgans.

linkage region. Thus, the present meta-analysis cannot deny that 1q21 may be one of the susceptibility factors whereas 3q21 has been associated with AD.

Conclusion

Due to the lack of supportive evidence, more genome-wide linkage studies of AD that use heterogeneity-based genome scan meta-analysis are needed. Interpretation of genome-wide meta-analyses needs to take into account a number of limitations. First, the technique requires a detailed presentation of linkage data, which we were unable to obtain in one identified study. Second, the summation that is performed involves the mixing of genetically distinct populations. In our analysis, the population was all Caucasian, with no contributions from other populations. Replication of findings is an important requirement in any genetic association study, and larger investigations in other populations are still required. Finally, the specific susceptibility genes remain to be found, and the results from candidate gene studies and genome-wide linkage studies require independent replication and identification of the functional basis of the association determined in the two identified studies.

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