

Short communication

No linkage and association of atopy to chromosome 16 including the interleukin-4 receptor gene

Background: Several susceptibility genes for atopy have been suggested in recent years. Few have been investigated as intensively as the interleukin-4-receptor α (IL4R α) gene on chromosome 16. The results remain in dispute. Therefore, in a robust design, we tested for association of type I allergy to the IL4R variations I50V and Q576R, and investigated chromosome 16 for atopy candidate regions in general.

Methods: We identified 100 Danish allergy sib-pair families. Five conservative phenotypes for type I allergy were defined and evaluated. The IL4R variations were genotyped in trios and evaluated by the transmission disequilibrium test (TDT). Multipoint linkage analysis and exclusion mapping were conducted with sib-pairs analyzed for 17 microsatellite markers.

Results: No evidence for association or linkage to the IL4R polymorphisms was found (P values: 0.12–0.90). Linkage analysis did not support linkage of any of the phenotypes to chromosome 16. Major parts of chromosome 16 were excluded as candidate regions harboring oligogenes for type I allergy.

Conclusions: We found chromosome 16 unlikely to harbor strong candidate genes for type I allergy. The role of the IL4R α gene in the inheritance of atopy was insignificant in the Danish population. The use of conservative allergy phenotypes in the search for genes predisposing to atopic disease was discussed.

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The interleukin-4 receptor gene (NCBI Unigene no. X52425) located on chromosome 16p12 is one of the few identified atopy candidate genes. Eight intragenic SNPs, single nucleotide polymorphisms causing amino-acid substitutions in the mature protein, have been identified (1–4). A strong association between atopy and the R576 allele of the interleukin-4 receptor α (IL4R α) subunit's R576Q variation has been reported (2). The phenotypes found to associate were hyper-IgE syndrome, severe atopic dermatitis, and atopy. The last was defined as an elevated total serum IgE level (≥ 95 IU per ml) and/or a positive radioallergosorbent test (RAST) in response to at least one of three specified allergens (RAST score of $\geq 3+$). The study was conducted in a limited sample size holding three, seven, and 20 persons with hyper-IgE syndrome, severe atopic dermatitis, and atopy, respectively. These results were supported by evidence of association of R576 to the prevalence and clinical severity of asthma (5), but not by reports showing no association of the R576 allele to the hyper-IgE syndrome (6) and to atopy and asthma (7). The I50V variant's I50 allele was shown to associate to asthma and upregulate receptor

response to IL4 and hence increase IgE production *in vitro* (8). These findings could not be reconfirmed *in vivo* (9), and a call for linkage and association studies in different populations was made. Evidence of association between various IL4R α gene alleles/ haplotypes and asthma and atopy was found in ethnically diverse populations (4). This study suggested that variation outside the coding region of the gene influences susceptibility. Specific IgE measurements showed linkage to the marker D16S401 in a German population when evaluated with respect to maternal inheritance (10). This result could not be reproduced in a Japanese population with the marker D16S403 (7).

Thus, results on the impact of the IL4R α gene as an atopy candidate gene are diverse, and more data are needed to elucidate the topic. We here present a study of 100 Danish atopy families, whom we tested for association of six different atopy phenotypes to the IL4R α variants I50V and Q576R by transmission disequilibrium test (TDT) analysis in trios. Furthermore, we tested for linkage of five different atopy phenotypes to a map of chromosome 16 markers by affected sib-pair analysis.

Material and methods

Patient material

One hundred Danish atopy families were enrolled in the Danish allergy project ITA (the Inheritance of Type I Allergy). Nuclear families were recruited through four pediatric clinics and one adult outpatient allergy clinic in Aalborg, Viborg, Herning, and Aarhus, all cities in western Denmark. A family was selected for the study if at least two full siblings had doctor-diagnosed symptoms of atopy, i.e., asthma, rhinitis, or atopic dermatitis, and reported effect of appropriate medication. Participation of both the biologic parents was necessary to qualify the family for the project. The total of 424 individuals, 200 parents and 224 children, were all clinically examined and questionnaire tested by one doctor. Each person had blood drawn for DNA analysis and for serum measurements of total IgE and specific IgE, by RAST, to 11 common allergens. The mean age among the offspring was 10.8 years, and the male/female sex ratio was 1.2, which is equal to random distribution ($P=0.35$). The ITA project was carried out according to the Helsinki Declaration and approved by the scientific ethics committee. All participants and/or their parents gave informed consent.

Phenotype definitions

In the ITA project, we have chosen to evaluate five distinct phenotypes of type I (immediate type) allergy.

- 1) *atopy* defined by high levels of IgE directed against at least one of the tested allergens, i.e., RAST of $\geq 1+$
- 2) *atopic asthma* characterized by RAST of $\geq 1+$ and clinical symptoms of asthma (11) and effect of standard asthma medication
- 3) *allergic rhinitis* defined as RAST of $\geq 1+$ and traditional symptoms of rhinitis according to international guidelines (12)
- 4) *atopic dermatitis* characterized by RAST of $\geq 1+$ and positive diagnosis of atopic dermatitis by standard clinical criteria (13)
- 5) *elevated total IgE* on an atopic basis defined as RAST of $\geq 1+$ and total serum IgE measurement larger than 100 kU/l.

All the clinical diagnoses were determined by two doctors separately – first, one doctor did the interview and examination of the allergy families in the outpatient clinic; second, another doctor evaluated only the questionnaires. A sixth phenotype, defined as t-IgE of ≥ 95 and/or RAST of $\geq 3+$, was also tested for association to the IL4R α , because of the positive results on this specific definition of atopy earlier reported (2).

IgE measurements

The IgE measurements were done by using the Pharmacia CAP IgE FEIA and the Pharmacia CAP RAST FEIA from Pharmacia, Uppsala, Sweden. Specific IgE values, considered to be evaluated at ≥ 0.35 kUAl ($\geq$ class 1), were determined for 11 common allergens: grass, birch, mugwort, olive, *Parietaria*, cat, dog, horse, mite (*Dermatophagoides pteronyssinus* and *D. farinae*), and *Cladosporium herbarum*.

IL4R mutation analysis

For the purpose of association analysis by a trio approach, all 424 individuals were tested for the two IL4R α variations, I50V and R576Q. We developed a simple assay for testing the R576Q variation on gDNA by PCR amplification followed by RFLP analysis. The primers were 5'-GCCCCACCACTGGCTACC-3', sense; and 5'-ACCCAAGCCCAACCGCA-3', antisense. The annealing temperature was 58°C; the PCR fragment of 82 bp was digested with *MspI* for 2 h; and the resulting fragments of 82 bp

(wild-type) and 64 bp (mutant) were separated in a 1% NuSieve and 2% agarose gel. The I50V variation was likewise tested on gDNA as suggested by Mitsuyasu et al. (8).

Chromosome 16 marker analysis

For nonparametric linkage analysis, the families containing two full sibs with RAST of $\geq 1+$, 48 families in total, were selected. The two markers D16S401 and D16S403 were built into a set of highly polymorphic microsatellite markers (<http://www.marshmed.org/genetics/>) evenly scattered over the entire chromosome 16 with a mean density of 7.5 cM. Multiplex PCR with scan primers (DNA Technology, Aarhus, Denmark) was done, and the products were separated and genotyped on an ABI 310 Genetic Analyzer (Perkin Elmer, Foster City, CA, USA).

Statistical analysis

The TDT (14) was used to test for association to the two biallelic IL4R markers. The test reduces the risk of false positive results due to population stratification by using nuclear family data. We selected trios from the 100 nuclear families by the oldest child with the phenotype of interest. Only one trio was scored per family to secure an independent data set. The chi-square test with 1 d.f. was calculated for every phenotype.

Multipoint affected sib-pair linkage analysis was performed with the program MAPMAKER/SIBS (15). Maximum MLS analyses were carried out for all types of sib-pair combinations but presented as a LOD score curve based on "all independent pairs of affected/phenotyped sibs" (Fig. 1). A multipoint exclusion map was obtained for disease susceptibility models with λ_s of 1.2, 1.5, 2, 3, 5, and 10. The results are shown as an exclusion LOD score curve only for $\lambda_s=5$ (Fig. 2).

Results

We found no association or linkage of the six allergy phenotypes to the two IL4R polymorphisms I50V and R576Q (Table 1). The sixth "and/or-phenotype" was included to test the association previously reported (2). We found no maternal inheritance for either I50 or R576 for any of the phenotypes. For the two mutations I50 and R576, the allele frequencies in the founder population were 0.54 and 0.29, respectively.

Our multipoint linkage analysis showed no evidence of linkage to chromosome 16. The maximum likelihood IBD for the tested five phenotypes were all clearly below a minimum critical LOD of 2 for linkage of complex traits (16). In particular, no significant linkage was found to the markers D16S403 and D16S401 or the IL4R locus between markers D16S769 and D16S409 (Fig. 1).

Exclusion LOD scores at $\lambda_s=5$ were below the stringent critical value $\text{LOD}=-2$ (17) for D16S403, D16S401, and D16S769–D16S409 for four, five, and three phenotypes, respectively (Fig. 2). For every phenotype, parts of chromosome 16 could be excluded – atopy (89%), atopic asthma (26.5%), allergic rhinitis (36.5%), atopic dermatitis (57%), and elevated t-IgE (95%).

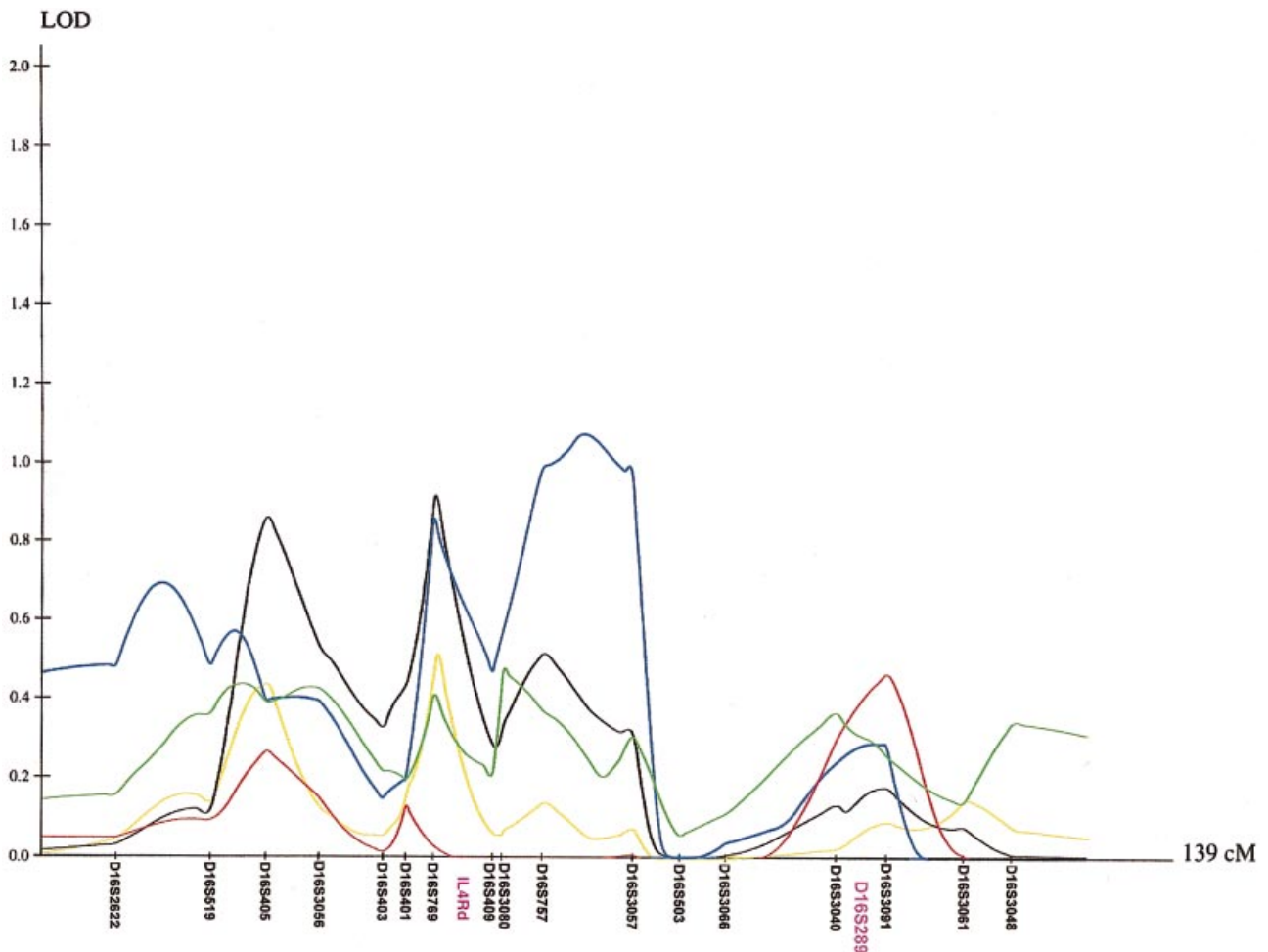


Figure 1. Chromosome 16 maximum likelihood IBD curves for five distinct atopy phenotypes: atopy (black), atopic asthma (blue), allergic rhinitis (green), atopic dermatitis (red), and elevated total IgE (yellow). Chromosome 16 marker map used is listed on x-axis, and two known potential atopy loci on chromosome 16, IL4R α gene and D16S289, are shown (pink).

Discussion

Numerous studies report investigation of variants in the IL4-receptor and their possible role in allergic predisposition. The results remains conflicting, and the studies vary in design; i.e., there have been variations in sample sizes, internal vs external controls, population stratification, and phenotype inconsistency among them. Two genome-wide searches for asthma susceptibility loci have been published. One reports potential linkage of total IgE levels to chromosome 16 (D16S289) (18), and the other found no linkage of asthma to chromosome 16 in three ethnically diverse populations (19). A meta-analysis mapping oligogenes for atopy and asthma based on 156 papers published until the end of 1998 classified chromosome 16 as a strong candidate chromosome (20). The study suggests a proximal candidate region containing IL4R and a distal region with D16S289.

The goal of our study was both to scan chromosome 16 for type I allergy candidate loci, and to test the proposed association of allergy to the IL4 receptor in a

Danish Caucasian population. The Danish population is ethnically very homogeneous, and, traditionally, allergy patients are clinically very well characterized. We chose a robust study design using:

- 1) nonparametric multipoint linkage analysis of affected sib-pairs (15)
- 2) the TDT test on trio families, which is more robust to population stratification by means of internal controls (14), as a test of linkage disequilibrium (21)

The type I allergy phenotype definitions (nos. 1–5) were conservative in the sense that, in addition to the clinical diagnosis or biomarker tested, they all included specific sensitization to at least one allergen. By the exclusion LOD score analysis, major parts of chromosome 16 could be excluded, at $\lambda_s = 5$, as regions likely to harbor oligogenes for allergy. The proximal candidate region was excluded at D16S403 for all phenotypes except rhinitis, at D16S401 for all five phenotypes, and at the IL4R interval for three phenotypes, not counting

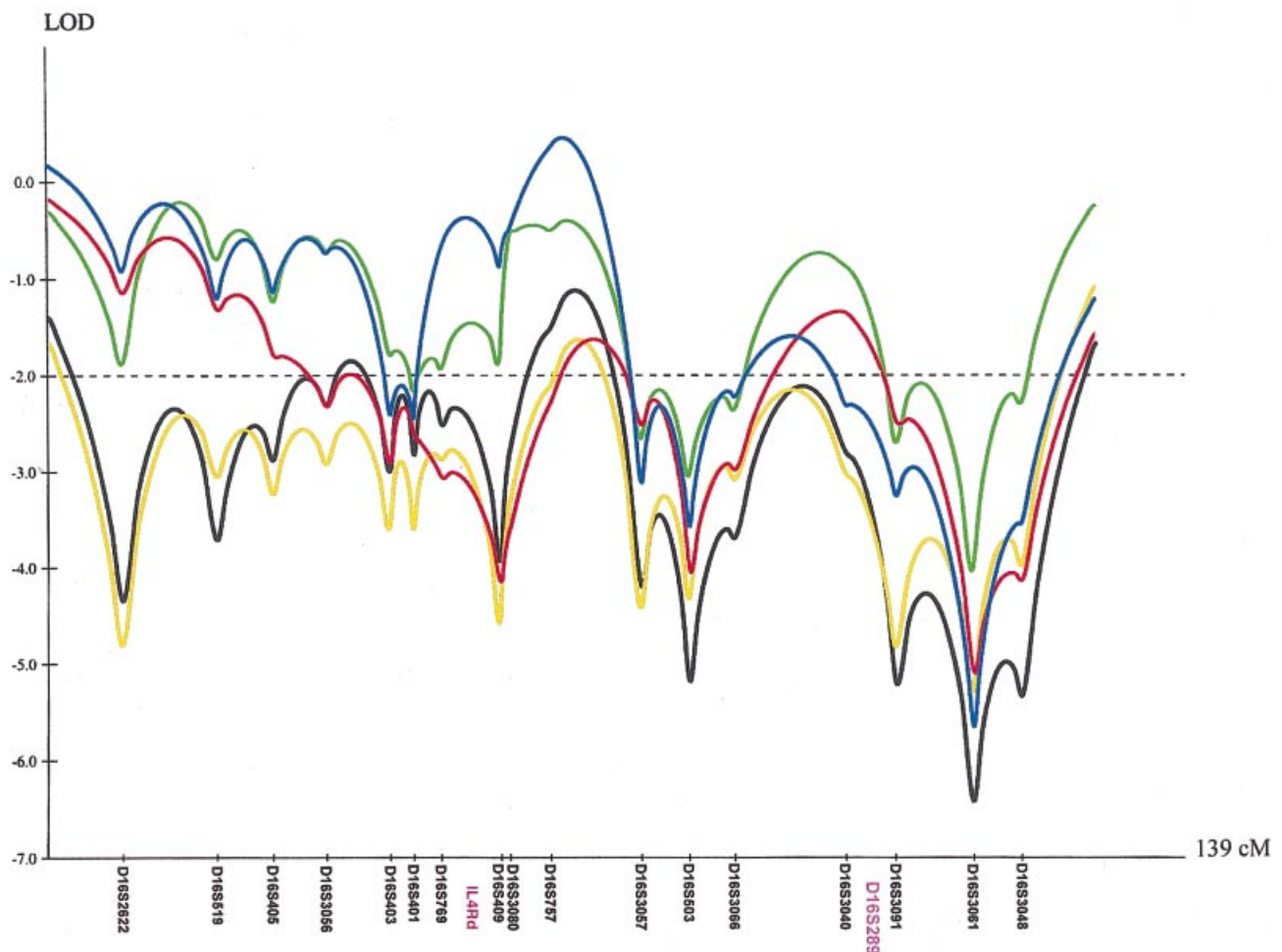


Figure 2. Chromosome 16 exclusion LOD scores at $\lambda_s=5$ for five distinct atopy phenotypes: atopy (black), atopic asthma (blue), allergic rhinitis (green), atopic dermatitis (red), and elevated total IgE (yellow). Chromosome 16 marker map used is listed on x-axis, and two known potential atopy loci on chromosome 16, IL4R α gene and D16S289, are shown (pink).

asthma and rhinitis. The distal candidate region appointed by D16S289 was in our map flanked by D16S3040 and D16S3091. Exclusion in this region was total with the exception of the phenotypes rhinitis and atopic dermatitis when testing close to locus 14. Under a less strict exclusion criteria, LOD = -1, the only phenotypes not excluded were asthma in the IL4R interval and rhinitis at the D16S3040 locus. In spite of this substantial exclusion at $\lambda_s=5$, these results also illustrate the general difficulty in large-scale strong

exclusion of regions harboring polygenes, i.e., $\lambda_s < 3$, for complex diseases. In many cases, this would require a larger number of sib-pairs than presented in our study (17).

In conclusion, our results showed no evidence of association or linkage by TDT to the tested IL4R polymorphisms. Nor did we find linkage by maximum likelihood IBD analysis to chromosome 16 of five allergy phenotypes.

In the ITA project, we have chosen to focus on clinical

Table 1. TDT for IL4R variations I50V and Q576R for six distinct allergy phenotypes. Data of heterozygous parents (I50/V50 and Q576/R576) to affected trio index patients are shown for heterozygous parents

Phenotype	Transmitted alleles I50V parents (mothers)			χ^2	P	Transmitted alleles Q576R parents (mothers)			χ^2	P
	I50	V50	Total			Q576	R576	Total		
1. Atopy	50	52	102	0.04	0.84	40	30	70	1.43	0.23
2. Atopic asthma	38	44	82	0.44	0.51	28	30	58	0.07	0.79
3. Allergic rhinitis	39	44	83	0.30	0.58	23	35	58	2.48	0.12
4. Atopic dermatitis	34	33	67	0.01	0.90	19	25	44	0.82	0.37
5. Elevated t-IgE and atopy	50	43	93	0.53	0.47	27	29	56	0.07	0.79
6. t-IgE ≥ 95 and/or RAST $\geq 3+$	53	56	109	0.08	0.77	34	39	73	0.34	0.56

symptoms, since localizing genes predisposing to clinical atopic disease seem to have the greatest clinical relevance. In addition to all the clinical diagnosis put independently by two doctors, an elevated specific IgE measurement to at least one tested allergen was included in all phenotypes as described. We believe that this narrower phenotype definition will decrease the degree of genetic heterogeneity and thereby increase the power of the study. For the association study reported, we used internal controls in order to reduce the risk of false positive results.

In future searches for genes predisposing to clinical atopic disease, we recommend more strict and conservative allergy phenotype definitions, as introduced in this study. A total genome scan based on these new

phenotype definitions might be helpful in confirming known atopy candidate regions and reveal loci still unknown.

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