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Family based association analysis of the *IL2* and *IL15* genes in allergic disorders

Ulla Christensen[1], Annette Haagerup[1,2], Helle G Binderup[1], Jørgen Vestbo[3,4], Torben A Kruse[5] and Anders D Børglum*,[1]

[1]*Institute of Human Genetics, The Bartholin Building, University of Aarhus, Aarhus C, Denmark;* [2]*Department of Paediatrics, Aarhus University Hospital, Skejby, Denmark;* [3]*Institute of Preventive Medicine, Kommunehospitalet, Copenhagen, Denmark;* [4]*North West Lung Centre, Wythenshawe Hospital, Manchester, UK;* [5]*Department of Clinical Biochemistry and Genetics, Odense University Hospital, University of Southern Denmark, Odense, Denmark*

Allergic diseases affect an increasing number of individuals and are a major global health problem. A substantial genetic contribution in the aetiology of allergic diseases is well documented. We have previously reported linkage of allergic diseases and atopy to the region harbouring the *IL2* gene (4q27). *IL15* is located approximately 20 Mb distal to *IL2*. The two genes encode cytokines that are structurally and functionally related, both inducing T-cell activation and proliferation. We screened the two genes for sequence variation and applied the seven single-nucleotide polymorphisms (SNPs) identified in a family based association study of two Danish samples comprising a total of 235 families with allergic diseases. None of the *IL15* SNPs showed significant association and the haplotype analysis yielded inconsistent results in the two samples. In contrast, the two *IL2* SNPs showed association both separately and in haplotypes with several atopic phenotypes, most significantly with IgE-mediated allergy. (single SNP *P*-value 0.0005 for positive skin prick test, haplotype *P*-value 0.019 for positive RAST test). To our knowledge, this is the first study reporting association between *IL2* and IgE-mediated allergy, asthma and atopic eczema. The SNP (rs2069762) that showed the most consistent results is located in the promoter and has previously been shown to influence the level of *IL2* expression. We suggest that the observed overtransmission of the T allele of this SNP may convey increased susceptibility to allergic disease by skewing the Th1/Th2 balance towards Th2.
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Introduction

Allergic disease is affecting an increasing number of individuals. Worldwide, asthma affects 155 million people. Even a larger number of individuals suffer from allergic rhinitis (hay fever).[1] Atopic dermatitis (AD) at present

Correspondence: Professor AD Børglum, Institute of Human Genetics, The Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark. Tel: +45 8942 1672; Fax: +45 8612 3173;
E-mail: anders@humgen.au.dk

affects 21% of Danish children.[2] Due to the high incidence, the personal morbidity and the social costs, the allergic diseases are a major global health problem.

The pathogenesis of these disorders is multifactorial and poly- or oligogenic. Family, twin, and adoption studies have demonstrated the genetic component of the aetiology to be considerable.[3–8] Twin studies of asthma and rhinitis have estimated the heritability to 60–73%. For AD, 0concordance rates in monozygotic twins are reported to be 0.86, compared to 0.21 in dizygotic twins.[9]

Several genome scans have pointed out candidate regions for allergic disease. We have previously reported

linkage of allergic rhinitis to chromosome 4q24–27 in a genome scan of Danish sib-pair families (LOD score 3.26).[10] In a recent fine-mapping study, we found evidence of linkage of additional atopy phenotypes to the same region, including increased serum levels of specific IgE (positive RAST test) and asthma.[11]

The *IL2* gene is located at 4q27 and consists of four exons. IL2 is important for the proliferation of activated T lymphocytes and thereby for the activation of the phagocytes to destroy uptaken material (Th1-activation) and for the B-lymphocytes to produce antibodies (Th2-activation). Other important functions are the central role of IL2 in enhancing AICD (activation-induced cell death) of T lymphocytes, and thereby the elimination of self-reactive cells,[12] and the termination of lymphocyte response by inducing the production of suppressive T cells.[13] Due to this central position in the regulation of the immune response, *IL2* is an obvious candidate gene for allergic disease.

The *IL15* gene is located at 4q31. IL15 is functionally and structurally related to IL2. They share the receptor subunits IL2Rβ and γ_c and both cytokines induce T cell activation and proliferation. The number of CD8 + memory T cells is decreased by IL2 and increased by IL15; thus, the number of these cells in animals is controlled by the balance between IL15 and IL2.[14] This balance also controls self-tolerance, as the IL2-induced AICD is inhibited by IL15. There are essential differences between the cells producing the cytokines: whereas IL2 is produced only by activated Th1 cells, *IL15* is transcribed in a variety of cell types including epithelial cells and monocytes, but not primary T cells.[15]

In order to examine the involvement of *IL2* and *IL15* in allergic disease, we screened a number of individuals for variations in the genes. Subsequently, we performed an association analysis of the identified single-nucleotide polymorphisms (SNPs) in two Danish family samples including 235 families with a minimum of two sibs suffering from atopic disease.

Materials and methods

Subjects

Two Danish family samples were studied: samples A and B (Table 1). In sample A, 135 nuclear families with asthmatic sib pairs were enrolled primarily from patient's records in four clinical centres in Eastern, Western and Central part of Denmark. Inclusion criteria were recurrent cough, wheezing and dyspnoea, and a positive methacholine challenge using the methods described by Yan *et al*.[16] Both affected siblings were aged 15–45 years. A questionnaire was filled in for every family member. Skin-prick tests using 10 common aeroallergens and lung function measurements including FEV1 and FVC were performed on all subjects. Blood samples were analysed for allergen-specific IgE (CAP phadiatop; Pharmacia Upjohn, Copenhagen, Denmark); positive test was defined as IgE ≥ 0.35 kU/l for one or more of the allergens. The rhinitis and AD (eczema) phenotypes were self-reported in the questionnaires. Details about sample A have been described previously.[17]

Sample B included a total of 424 individuals from 100 Danish atopic families enrolled in the ITA project. Recruitment, examination and clinical information are described previously.[10,11] In short, patients were recruited through four paediatric and one adult outpatient allergy clinic in Western Denmark. Inclusion criteria were a minimum of two atopic siblings with doctor diagnosed asthma, hay fever or AD and reported effect of appropriate medication. Mean age among the offspring was 10.8 years. As in sample A, male/female ratio was equal to random distribution. All subjects were clinically examined and questionnaire tested by the same doctor. Atopic disease was diagnosed by this doctor according to standard criteria in consensus with a second doctor evaluating the questionnaires only. Blood was drawn for DNA extraction and serum measurement of specific IgE to 11 common allergens (CAP RAST FEIA, Pharmacia Upjohn); positive test defined as above.

Sequencing and genotyping

Genomic DNA was isolated from blood samples according to standard procedures. The *IL2* and *IL15* genes were

Table 1 Structure of the two family samples analysed

| | Number of families with affected children | | | |
	Asthma	Rhinitis	Atopic dermatitis	Positive RAST test
Sample A (135 families)				
Families with one affected child	0	47	51	35
Families with two affected children	135	74	68	73
Total	135	121	119	108
Sample B (100 families)				
Families with one affected child	31	36	33	36
Families with two affected children	45	35	34	40
Families with three affected children	12	8	6	7
Total	88	79	73	83

screened for sequence variation in the coding sequences, intron/exon boundaries, and 1405 bp (*IL2*) and 485 bp (*IL15*) of the promoter regions by direct sequencing using Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems). After ethanol precipitation, the resolved products were analysed on an ABI-310 sequenator from Applied Biosystems. Both strands were analysed.

Genotyping was performed by multiplex single base extension (SBE) technology using the SNaP-shot Kit, according to the manufacturer's recommendations (Applied Biosystems). Standard PCR conditions were used for both sequencing and genotyping. Primer sequences are shown in Table 2. Two investigators performed scoring of the genotypes independently; in cases of disagreement the

results were reviewed and re-analysed. Genotypes were tested for Hardy–Weinberg equilibrium and Mendelian inheritance. Furthermore, around 150 genotypes were analysed twice: 70 genotypes analysed by both direct sequencing and SBE, and around 80 genotypes analysed twice by SBE. No inconsistencies were identified, indicating a genotyping error rate of less than 0.67%.

Statistical analysis

Pairwise linkage disequilibrium (LD) was calculated using Simwalk2[18] and the GOLD software package (http://www.sph.umich.edu/csg/abecasis/GOLD).[19]

The family based association analysis used the FBAT test allowing for analysis of single SNPs and haplotypes

Table 2 Primers used for PCR amplification, sequencing and genotyping

Gene	Region	Type[a]	Position UCSC May 04 assembly[b]	Sequence
IL2	Promoter	F		AGTGGTTTTTGGAGTCAGTA
	Promoter	R		TATACTTCTAATTTAACCTGG
	Promoter	F		CTTTACAAGGACTCTCTTTGA
	Promoter	R		GAGGTACTGTTTAACGCTATT
	Promoter	F		TGTACATAGACATTAAGAGAC
	Promoter	R		AGCCCACACTTAGGTGATAGC
	Promoter	SNP	123735586	CACATGTTCAGTGTAGTTTTA
	Promoter	F		CTTTTGTATCCCCACCCCCT
	Promoter	R		TGCAAGACTTAGTGCAATGC
	Exon 1+2	F		GTTCCCTATCACTCTTTAAT
	Exon 1+2	R		TTTCATATTACTTTGAATTTTATT
	Exon 1b	SNP	123735076	AAAATCATCTGTAAATCCAG
	Exon 1a	SNP	123735089	GCTACAACTGGAGCATTTAC
	Exon 3	F		ATTAATTTCTGATTCTGACCTC
	Exon 3	R		TGGTACTTTTCCCCCTACTT
	Exon 4	F		TATAAGGTAAACTACTCTGTA
	Exon 4	R		TACATTCAACAATAAATATAAAAT
IL15	Promoter	F		ATGGCATGCAAATCACTCAGC
	Promoter	R		CACCAGCCACCGGTCTCCT
	Promoter	SNP	142914864	AACATTTCCCCTAGTTGGAC
	UTR	F		TTAGGTGTCTTTCATTCTTAAC
	UTR	R		ACACTTCCTGGTGCTTCAG
	UTR	SNP	142928055	TTTCACCCATAGTCCCAAGTAC
	UTR	F		TCACATTTTCTCAAGGAATGG
	UTR	R		GGAATCTGGCCTGCTTCC
	UTR	SNP	142932872	ACTTCCATCAGGTGTTGTTC
	Exon 3	F		TTAAAAGGTAGGTGTTCAATAAG
	Exon 3	R		ACTCTGAGATTCAGAGGACC
	UTR	SNP	142998069	CACTAGTAAGAAAAAGAAAG
	UTR	SNP	142998123	GATTGTATTGTAGGAGGCAT
	Exon 4	F		TTTAGACCTAGAAATATGTTGAC
	Exon 4	R		TCCAAGGACAATCATCATGAC
	Exon 5	F		CTCTTTTGCTTATAGTATTCATC
	Exon 5	R		TGGTTTCCTTAAGACAGACAG
	Exon 6	F		ATGTTTTCAGTAGGTTGTTCTC
	Exon 6	R		ACCATATATTATAGAAACACACC
	Exon 7	F		GTAAAATTTCTTAAGGATATTGTG
	Exon 7	R		AATGACTTCTCCAAATCAGGC
	Exon 8	F		ATGGTTCAAACGATATGATATTC
	Exon 8	R		TATGTCTAAGCAGCAGAGTGA

[a]F: forward, R: reverse, SNP: primer immediately adjacent to the SNP used in the SNaP-shot reaction (Applied Biosystems) (see Materials and methods).
[b]URL: http://genome.ucsc.edu.

(HBAT).[20,21] Primarily, the data were analysed using an additive model, which does not assume any specific mode of inheritance and is in general the most sensitive test.[21] The additive model is equivalent to the classic transmission disequilibrium test (TDT).[22] In addition, we also analysed the data assuming dominant and recessive modes of inheritance. The basic null-hypothesis tested by the FBAT program is 'no linkage and no association', and the first *P*-values (before the slashes) in Table 5 and 6 relate to this null-hypothesis. However, in the presence of linkage and when there is more than one affected child per pedigree, which is the case in most of our data, the FBAT program offers the possibility of testing the null-hypothesis 'linkage but no association' by use of the empirical variance for the test statistic. The second *P*-values (after the slashes) in Table 5 and 6 are calculated using the empirical variance, thus testing for association in the presence of linkage.

P-values <0.05 are referred to as significant. For each gene, none of the tests performed both regarding phenotypes, models and SNPs/haplotypes are independent, and no correction for multiple testing was performed.

In silico analysis
The impact of an SNP on potential binding sites for transcription factors was assessed using the program Matinspector (URL) (www.genomatix.de).[23,24] This program utilizes a library of matrix descriptions for transcription factor binding sites to locate matches in sequences and assigns a quality rating of matches. Thus, the influence of an SNP on the binding of possibly important transcription factors can be investigated by loading the program with the wild type and the variant sequence, respectively. The possible effect of an SNP on splicing was investigated using the programs ESEfinder release 2.0 (http://rulai.cshl.edu/tools/ESE)[25] and NNSPLICE version 0.9 (www.fruitfly.org/seq_tools)[26] and comparing the results from analyses of the allelic sequences. ESEfinder identifies putative exon splicing enhancers responsive to the human SR proteins SF2/ASF,

SC35, SRp40 and SRp55, and NNSPLICE predicts potential splice sites based on neural network. Both programs assign quality ratings for the predictions.

Results
In order to identify potentially functional sequence variations in the *IL2* and *IL15* genes, we analysed 10 unrelated atopic patients from families that showed evidence of linkage to the region (4q27) in a recent linkage study (sib-pairs sharing both haplotypes identical by descent)[11] and two healthy controls. Two SNPs were identified in *IL2* and five in *IL15* (Table 3).

These seven SNPs were subsequently applied in a family based association analysis of two Danish samples comprising 135 and 100 atopic families, respectively. In addition, an apparently nonsynonymous SNP (rs3087209, Table 3) from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP) was also tested in the two samples. However, this putative SNP showed no variation in the entire data set and is most likely not a true SNP, at least not in the Danish population.

The two *IL2* SNPs, rs2069762 and rs2069763, were found to be in weak LD with each other with Lewontin's standardized disequilibrium coefficient $D' = 0.334$ ($P < 0.0001$). The five IL5 SNPs showed a moderate degree of pair wise LD as shown in Table 4.

All variants were found to be in Hardy–Weinberg equilibrium

Table 4 Pairwise linkage disequilibrium of *IL15* SNPs measured as Lewontin's standardized disequilibrium coefficient D'^a

	Rs1519551	Rs2322262	Rs1493012	Rs2254514
Rs3806798	0.777	0.781	0.701	0.793
Rs1519551		0.584	0.601	0.513
Rs2322262			0.889	0.901
Rs1493012				0.889

[a]All D' values were statistically significant with *P*-values less than 0.001.

Table 3 Single-nucleotide polymorphisms (SNPs) applied in the family based association study

Gene	SNP	Rs number	Position UCSC May 04 assembly[a]	Position in gene[b]	Alleles (frequencies in parentheses)[c]
IL2	SNP1	Rs2069762	123735586	Promoter	T (0.717), G (0.283)
	SNP2	Rs2069763	123735076	Exon 1, synonymous	G (0.622), T (0.372)
	SNP3	Rs3087209	123735089	Exon 1 nonsynonymous	T (1.000), G (0.000)
IL15	SNP1	Rs3806798	142914864	Promoter	A (0.902), T (0.098)
	SNP2	Rs1519551	142928055	Intron 1	A (0.577), G (0.423)
	SNP3	Rs2322262	142932872	Intron 1	T (0.736), C (0.264)
	SNP4	Rs1493012	142998069	Intron 2	A (0.567), G (0.433)
	SNP5	Rs2254514	142998123	Exon 3, UTR	C (0.720), T (0.280)

[a]URL: http://genome.ucsc.edu/
[b]UTR: untranslated region.
[c]Frequencies in two family samples combined. No significant difference in allele frequencies was observed between the two samples (results not shown).

Table 5 Association analysis[a] of two *IL2* SNPs in two Danish family samples, separately and combined, employing additive and dominant modes of inheritance

	Asthma		Positive RAST test		Positive skin test		Rhinitis		Atopic dermatitis	
	Additive	Dominant	Additive	Dominant	Additive	Dominant	Additive	Dominant	Additive	Dominant
Sample A										
Rs 2069762	**0.018/0.022** (90/50)	0.059/**0.047** (83/31)	**0.018/0.030** (72/51)	**0.018/0.010** (30/27)	0.077/0.087 (63/43)	0.098/0.072 (25/23)	**0.012/0.014** (80/54)	**0.013/0.022** (74/61)	**0.027/0.030** (78/55)	0.050/0.070 (71/60)
Rs 2069763	0.890/0.890 (84/49)	0232/0.227 (43/37)	0.810/0.800 (70/42)	0.055/**0.030** (36/32)	0.483/0.394 (60/34)	**0.007/0.0005** (29/26)	0.564/0.547 (75/51)	0.314/0.267 (38/36)	0.931/0.925 (72/50)	0.290/0.205 (35/33)
Sample B										
Rs 2069762	0.758/0.770 (54/46)	0.539/0.563 (50/44)	0.918/0.922 (53/43)	0.115/0.077 (10/10)	Np	Np	1.00/1.00 (51/42)	0.115/0.077 (11/10)	0.418/0.463 (44/37)	0.268/0.310 (42/36)
Rs 2069763	0.174/0.201 (50/35)	0.271/0.319 (27/32)	0.293/0.319 (52/40)	0.369/0.306 (42/37)	Np	Np	0.128/0.163 (49/39)	0.183/0.261 (25/34)	**0.030/0.018** (43/28)	0.059/**0.031** (34/28)
Samples A and B										
Rs 2069762	**0.031/0.038** (144/96)	0.060/0.082 (133/102)	0.070/0.091 (125/94)	0.140/0.169 (113/37)	Np	Np	**0.044/0.046** (131/96)	**0.017/0.024** (120/101)	**0.022/0.029** (102/92)	**0.026/0.042** (113/96)
Rs 2069763	0.498/0.508 (134/84)	0.269/0.256 (110/99)	0.633/0.631 (122/82)	0.162/0.136 (104/88)	Np	Np	0.621/0.624 (124/90)	0.570/0.549 (103/87)	0.205/0.167 (115/78)	0.102/0.083 (98/85)

[a]*P*-values less than 0.05 are highlighted in bold. *P*-values after the slash are calculated using the empirical variance of the test statistic (see Materials and methods). The number of informative families is shown in parentheses (), the number after the slash refers to the number of informative families when applying the empirical variance. NP = not performed.

IL2 association analysis

Table 5 presents the results of the association analysis of the two *IL2* SNPs analysed separately. The promoter variant (rs2069762) showed significant association with all three clinical phenotypes asthma, rhinitis, AD, and positive RAST test in sample A (with *P*-values between 0.01 and 0.05). The T allele was transmitted more frequent than what would be expected by chance; the G allele less frequent. In sample B, this SNP did not show any significant association. However, analysis of the two samples combined showed again significant association with the three clinical phenotypes (whereas positive RAST test did not reach significance level with *P*-values around 0.1). For this promoter SNP, the additive and dominant models yielded similar *P*-values, while no evidence of association was observed under a recessive model. Likewise, no big differences were observed when the empirical variance was used to account for sibling genotype correlation for families with more than one child.

In silico analysis of this promoter variant using the program MatInspector revealed a potential binding site for heterodimers of TCF-11 (a transcription factor of the bzip type[27,28]) and MafG (a member of the Maf subfamily of the transcription factor AP-1[29]) when there was a T at the position of the polymorphism. Noteworthy, AP-1 is known to play a key role in the regulation of *IL2* expression.[30–32] Changing the T allele to a G eliminated this potential binding site reflecting a marked reduction in the predicted binding affinity of the complex.

The synonymous exon 1 SNP (rs2069763) showed highly significant association with the skin prick test under a dominant model (*P*-value 0.0005, using the empirical variance). The T allele was overtransmitted. This association could not be tested in sample B where the skin test was not performed. A significant association was also found with AD, but only in sample B (*P*-value 0.018, additive model, empirical variance).

In silico analyses indicated that this variant might affect splicing of the transcript in two ways. First, according to the ESEfinder program, the score for potential binding sites for the splicing factors SC35, SRp40 and SRp55 was altered when changing the G allele to T. A predicted binding site for SC35 was eliminated in the T allele sequence, a potential binding site for SRp55 existed only in the T allele sequence and the score for two potential SRp40 binding sites changed significantly when changing G to T.

Second, splice site analyses using the NNSPLICE program found that the score for a potential splice site was 0.86 for the T-allele and only 0.57 for the G-allele. Thus the G-allele seems to inactivate a potential pseudo-splice site that might lead to a better splicing of the normal transcript.

The two SNPs in *IL2* define the following four haplotypes mentioned with the promoter SNP first and estimated

Table 6 Association analysis[a] of haplotypes of two *IL2* SNPs in two Danish family samples, separately and combined, employing additive and dominant modes of inheritance

	Asthma		Positive RAST test		Positive skin test		Rhinitis		Atopic Dermatitis	
	Additive	Dominant	Additive	Dominant	Additive	Dominant	Additive	Dominant	Additive	Dominant
Sample A										
Haplotype H4 (GT) undertransmitted	**0.004/0.030**	**0.004/0.030**	**0.007/0.035**	**0.007/0.035**	**0.004/0.031**	**0.004/0.031**	**0.004/0.037**	**0.004/0.031**	0.011/0.054	0.011/0.054
Sample B										
Haplotype H1 (TT) overtransmitted	0.174/0.226	0.617/0.621	0.231/0.263	0.423/0.383	Np	Np	0.114/0.151	0.373/0.351	**0.025/0.022**	**0.049/0.039**
Haplotype H2 (TG) undertransmitted	0.135/0.172	0.129/0.162	**0.022/0.034**	0.082/0.098	Np	Np	0.051/0.054	0.081/0.092	0.982/0.982	0.982/0.982
Samples A and B										
Haplotype H1 (TT) overtransmitted	0.174/0.226	0.617/0.621	0.189/0.204	0.063/0.058	Np	Np	0.134/0.143	0.183/0.172	**0.033/0.03**	**0.022/0.021**
Haplotype H4 (GT) under-transmitted	0.135/0.173	0.129/0.163	**0.005/0.019**	**0.005/0.019**	Np	Np	**0.003/0.02**	**0.003/0.02**	0.024/0.072	0.024/0.072

[a]*P*-values less than 0.05 are highlighted in bold. *P*-values after the slash are calculated using the empirical variance of the test statistic (see Materials and methods). NP = not performed.

frequencies in parantheses H1: TT (0.378), H2: TG (0.342), H3: GG (0.269) and H4: GT (0.011). The results of the association analysis of these *IL2* haplotypes are presented in Table 6.

In sample A, evidence of under-transmission of the H4 haplotype was found in all phenotypes, most significantly for the asthma and rhinitis phenotypes and positive skin-prick test (*P*-values 0.004/0.03). In sample B, the same trend was observed for H4 without reaching significance (results not shown), but in the combined sample significant results were again observed, in particular for rhinitis and positive RAST test.

The H1 haplotype was found overtransmitted in sample B and in the combined sample for the AD phenotype with *P*-values around 0.02. For both H4 and H1, the additive and dominant model yielded very similar results. For the H4 haplotype, the use of the empirical variance had quite a dramatic effect on the *P*-values, which were increased by a factor of around 3, suggesting that some of the positive signal in the default test were due to linkage and not to association alone. The same effect was not seen for the H1 results.

An overall test analysing the total distribution of all haplotypes and thereby introducing increased degrees of freedom was not significant in any of the phenotypes.

IL15 association analysis

None of the five SNPs in *IL15* showed significant association with any of the atopic phenotypes when examined separately. The haplotype analysis examined haplotypes of varying length including two, three, four or all five SNPs in a sliding window fashion analysing neighbouring SNPs from one end of the gene to the other. The haplotypes mentioned below refer to the names (SNP 1–5) and order listed in Table 3.

Few significant results were obtained. In sample A for the AD phenotype, evidence of under-transmission was observed for a set of frequent haplotypes with the same core (estimated frequencies around 0.4) under a recessive model. The haplotypes were TAT, TATA, and TATAC of SNP 1–3, 1–4 and 1–5, respectively. All haplotypes showed similar results, most significantly for the short TAT haplotype yielding a *P*-value of 0.025 that increased to 0.038 using the empirical variance.

In sample B, a set of haplotypes showed association with the two phenotypes postive RAST and rhinitis with positive RAST with equal results under both the dominant and additive model. The haplotypes were all under-transmitted, had a common core, and similar frequencies of around 0.05, thus probably reflecting the same haplotype in all tests, namely: TACGT (SNP 1–5). Both 2-, 3-, 4-, and 5-point combinations of this haplotype yielded almost identical results, slightly more significantly for the ACGT haplotype showing a *P*-value of 0.005, and 0.010 using the empirical variance.

Neither of the haplotypes showing association in the two samples separately showed significant association in the combined sample. For positive RAST test, a single haplotype of SNP 2–4, ATG, with an estimated frequency of 0.033 was found under-transmitted with marginally significant *P*-values under both the additive and dominant model. The two models yielded identical *P*-values of 0.044 that were reduced to 0.036 using the empirical variance.

Discussion

We have examined the possible role of the *IL2* and *IL15* genes in the aetiology of atopy and allergic diseases in a family based association study of two Danish samples including a total of 235 families with allergic diseases. Both of the genes are obvious functional candidate genes, and *IL2* is also a positional candidate gene as we have previously reported evidence of linkage to the region harbouring *IL2* (4q27).[10,11] The *IL15* gene is located outside the maximal linkage signal approximately 20 Mb distal to *IL2*.

We found association between *IL2* and allergic diseases and IgE-mediated allergy (positive RAST test, positive skin prick test), both when testing the two *IL2* SNPs separately and in haplotypes. This association was observed in particular in sample A, while significant replication in sample B was seen only for positive RAST test and AD and with different SNPs/haplotypes.

The paucity of significant results in sample B might partly be explained by demographic and clinical differences between the two populations. The two groups have different age distributions: All the individuals in sample A are older than 15 years whereas in sample B the probands are mainly children. Other inclusion criteria and diagnostics are not completely identical either. Thus, even though the diagnoses are the same, the underlying risk alleles might not be identical or of equal importance in the two groups.

For the *IL2* promoter SNP (rs2069762), the G allele was transmitted less often than expected by chance to the atopic individuals, while the T allele conversely was transmitted more often than expected. Several observations indicate that this variant (often referred to as the '−330' polymorphism) has a functional impact on the expression of *IL2*. Hoffmann *et al*[33] found that cultured peripheral blood lymphocytes from healthy individuals who were homozygous for the G allele produced over three times as much IL2 protein as individuals with T/T or G/T. In accordance with this observation, a recent study using a reporter-gene (luciferase) assay showed that the G allele induced twice as high gene expression as the T allele in transfected Jurkat cells.[34] However, the same study reported an apparently opposite effect in *in vivo* analyses, suggesting the existence of additional unknown polymorphisms affecting gene regulation.

Our *in silico* analysis of the promoter SNP revealed an impact on a potential binding site for the activation factor heterodimer of TCF-11 and the AP-1 protein MafG. Johnsen *et al*[35] proposed that the TCF11/MafG complex may contribute to the negative regulation of transactivating transcription. This corroborates that the T allele may induce a relative decrease in *IL2* expression and offers a possible molecular explanation that should be experimentally tested.

Since IL2 is a Th1 cytokine, a variant that diminishes the IL2 production will tend to skew the Th1/Th2-balance towards Th2, which is typical for atopic individuals. Thus, the results showing decreased IL2 expression for the T allele as opposed to the G allele correlate well with our results. That is, the observed overtransmission of the T allele may convey an increased susceptibility to atopy and allergic disease by a relative decrease in IL2 expression.

Our results for the *IL2* promoter SNP contradict the findings of a recent study, indicating that individuals homozygous for the G allele may have an increased risk of rhinitis.[36] However, in the present study, the haplotype results and the strong association observed between the synonymous exon 1 SNP (rs2069763) and positive skin prick test suggest that other *IL2* variants might be of functional importance. Thus, in different populations different functional variants may predominate and, due to LD between the variants, possibly give rise to conflicting results.

The synonymous exon SNP disclosed exceedingly significant association with the skin prick test. Unfortunately, this association could not be tested in sample B where prick test was not performed. The fact that this SNP did not show significant single marker association using other models than the dominant model except for the AD phenotype in sample B, and only showed moderately significant association with positive RAST test (which normally correlates well with skin prick test) in sample A indicates, though, that this result should be interpreted with some caution.

However, haplotype analyses including this variant revealed significant results for all phenotypes in both sample A and the combined sample (except for asthma in the combined sample), and the results of the *in silico* analysis indicated a possible impact of the variant on splicing mechanisms. Functional analyses are needed to clarify the latter.

The two *IL2* variants may actually influence the disease risk as indicated by the analyses mentioned above, but it should be kept in mind that the associations might also merely reflect LD to one or more causal variants in the surrounding region. Data provided by the HapMap project (www.hapmap.org) show extended LD in the region with a large, relatively well-defined haplotype block of around 580 kb. Besides *IL2* the block contains two genes: Testis nuclear RNA-binding protein (*Tenr*) and interleukin 21 (*IL21*). IL21 is a member of the family of immunomodulatory cytokines also including IL2 and IL15, and it would be interesting to examine this gene in future studies.

A recent case–control study reported association between haplotypes of five *IL15* SNPs and asthma in a German population; no significant association was observed with any of the SNPs separately.[37] In our study, different haplotypes showed association to different phenotypes in the two samples. Analysing the samples together a single three-SNP-haplotype showed evidence of under-transmission for positive RAST test. However, considering the number of tests performed and the lack of convergent results in the two samples, we conclude that *IL15* does not play a major role in the aetiology of asthma and related atopic disorders in the Danish population.

To our knowledge, we are the first to report association between *IL2* gene variants and IgE-mediated allergy (positive RAST test/skin prick test), asthma and AD (eczema). Regarding the association observed for the promoter SNP functional evidence support that the observed overtransmission of the T allele may convey increased disease susceptibility by a relative decrease in IL2 expression skewing the Th1/Th2 balance towards Th2. However, further studies are needed to shed light on this matter.

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