Association of polymorphisms in Th1, Th2 cytokine genes with hayfever and atopy in a subsample of EPIC-Heidelberg

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Summary

Background Hayfever is determined by an interaction of environmental and genetic factors and biologically characterized by an imbalanced T helper cell 1 (Th1) and Th2 immune response and elevated IgE levels against inhalant allergens. Indications exist that polymorphisms in cytokine genes involved in the regulation of the Th1/Th2 balance may contribute to the allergic phenotype.

Objective We investigate whether polymorphisms in genes directly or indirectly involved in Th1, Th2 immune response are associated with hayfever and elevated IgE levels against inhalant allergens.

Methods From a subsample of the European Prospective Investigation into Cancer and Nutrition-Heidelberg, 322 subjects with hayfever and 322 age- and sex-matched non-allergic controls were selected and genotypes determined for 15 polymorphisms in 13 genes. Plasma IgE against inhalant allergens was measured via CAPSX1 (Phadiatop) test. We computed odds ratios (ORs) by logistic regression, tests on group differences of IgE-levels in dependence upon genotype and tests for trend by means of non-parametric methods.

Results We found decreased OR for hayfever (OR = 0.60, 95%CI = 0.4–0.9) and sensitization to inhalant antigens (OR = 0.5, 95%CI = 0.4–0.8) in heterozygotes of the IL-6 −174 G/C polymorphism. Homozygotes of the G-allele in IL-2 −330 T/G were at increased risk of hayfever (OR = 2.6, 95%CI = 1.3–5.2). Moreover, we found indications for differences in IgE levels against inhalant allergens in dependence upon genotypes of polymorphisms in IL-4R, IL-6, IL-13, and IL-18.

Conclusion Our data suggest an association of genetic variants in IL-6 and IL-2 with hayfever, confirm a role of polymorphisms in IL-4R, IL-13, and IL-18 for the elevated IgE phenotype, and add IL-6 to the list of candidate genes.

Keywords allergies, cytokines, EPIC, epidemiology, hayfever, IgE, polymorphisms, Th1/Th2

Introduction

Seasonal allergic rhinitis (hayfever) is an atopic disease that affects an increasing portion of the western population and accounts for considerable morbidity and loss of quality of life. While susceptibility to disease appears to be determined by an interaction between environmental and genetic factors no simple pattern of inheritance has been shown [1]. Recent linkage studies and genome screens have identified a series of candidate genes possibly involved in the etiology of atopic diseases. These include a considerable number of cytokines [2]. Moreover, cytokines play a crucial role in the widely used immunological model that explains the increasing prevalence of atopic diseases by an altered balance between Th1 and Th2 immune responses triggered by lifestyle-determined changes in environment [3].

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Most genetic variation between individuals is believed to be due to single nucleotide polymorphisms (SNPs). Inter-individual variations in SNP profile may consequently contribute to the variability in immune responses, including those to environmental allergens, seen in human populations.

Recently identified cytokine polymorphisms, some of them with indications of functional relevance, have been associated with allergies, asthma, or elevated IgE levels (see section Discussion). The general observation, however, that many SNP-disease associations reported in the literature are not reproducible, calls into question the underlying methodology [4, 5] and argues for a careful interpretation of the results.

Based on data and blood specimens from a large cohort study on nutrition and cancer the present paper pursues the following goals: (a) to reproduce reported SNP-disease associations, (b) to explore potential polymorphism-disease associations for SNPs which have not yet been related to hayfever, and (c) to compare results of associations based on either self-reported hayfever or the results of an *in vitro* screening test on common inhalant allergens conducted with stored blood specimens.

Materials and Methods

Subjects

The present study was performed as a case–control study embedded in the Heidelberg part of the European Prospective Investigation into Cancer and Nutrition (EPIC). The EPIC-Heidelberg cohort comprises 25 544 participants from Heidelberg city and neighbouring communities, with an age range of 35–65 years at recruitment in women and 40–65 years in men. The study participants are almost completely (>95%) of Caucasian origin. At recruitment, detailed information on participants was obtained by means of questionnaires, face-to-face interviews, and anthropometric measurements. Furthermore, all participants were asked for a 24 mL blood sample, which was obtained from 95.8% of the cohort and was subsequently aliquoted and stored in liquid nitrogen. The informed consent signed by the participants included an agreement with an analysis of non-cancer diseases. The study was approved by the responsible local ethics committee [6].

Additional personnel and technical capacity allowed for the storage of aliquots of blood (stored at $-80\,^{\circ}C$) remaining after the above-mentioned Europe-wide aliquotation system, for a subgroup of subjects ($n = 4000$) participating between March 1998 and the end of recruitment (October 1998). Within this subgroup, we excluded individuals with self-reported diabetes, rheumatoid arthritis, tumours, inflammatory bowel diseases, myocardial infarction and stroke. From the eligible group, all subjects reporting physician-diagnosed hayfever ($n = 322$) were selected (wording of the question: 'Have you or have you ever had physician diagnosed hayfever'). Among the hayfever cases, 44 individuals also reported asthma and 108 reported atopic dermatitis. Controls ($n = 322$) without hayfever, excema, asthma or other allergies (as reported in the same way) were identified from the same EPIC subgroup and matched by sex and 5-year age group. Due to analytical problems with the buffy coat of 4 samples the final sample size consisted of 318 cases (165 male, 153 female) and 322 controls (168 male, 154 female).

Genotyping

Genomic DNA from peripheral blood mononuclear cells (PBMCs) was extracted using the QiaAmp Blood kit, in accordance with the provider's instructions (Qiagen, Hilden, Germany). PCR amplifications were carried out as described by Nieters et al. [7]. Primers and genotyping methods were taken from the cited publications for IL-2 −330 T/G [8], IL-4 −589 C/T [9], IL-4R Q576R [10], IL-5 −746 T/C [11], IL-6 −174 G/C [12], IL-10 −1082 G/A and −819 C/T [13], IL-12 p40 1188 A/C [14], IL-13 R130Q [11], IL-18 −137 G/C and −607 C/A [15], TNF −307 G/A [13], IFN-γR2 R64Q [16], IFN-γ CA-repeat [17], CD14 −159 C/T [18].

IgE-measurements

To assess the prevalence of atopic sensitization due to inhalation allergens an *in vitro* screening test, CAPSX1 (Pharmacia Upjohn, Uppsala, Sweden) was applied to a group of 614 individuals for whom plasma samples were available. This ELISA-based test, also known as Phadiatop test, detects the presence of specific serum or plasma IgE against common allergens [timothy, rye, birch, mugwort pollen, house dust mite (*Dermatophagoides pteronyssinus*), cat and dog epithelia as well as *Cladiosporum herbarum* mould]. Measurements could not be performed for 26 individuals, due to lack of plasma samples. All plasma samples were analysed in a blinded manner according to the manufacturer's recommendations at the Federal State Health Office Baden-Wuerttemberg and kept at $-80\,^{\circ}C$ until examination. The results are expressed in seven CAP classes (0: <0.35 kU/L; 1: 0.35–0.70 kU/L; 2: 0.70–3.50 kU/L; 3: 3.5 − 17.50 kU/L; 4: 17.70–50.00 kU/L; 5: 50.00–100.00 kU/L; 6: >100.00 kU/L) with class 1 or higher (>0.35 kU/L) considered as evidence of allergic sensitization. The mean values for the signal intensities of the corresponding CAP classes are 1: 202; 2: 363; 3: 1734; 4: 7441; 5: 15 745; 6: 21 482. Ranking of signal intensities of the CAPSX1 test was performed to compare the different genotypes according to their relative IgE levels against inhalant allergens.

Statistical methods

Hardy–Weinberg equilibrium. Departure from Hardy–Weinberg equilibrium was tested by using a χ^2-test [19].

Odds ratio (OR). For the determination of an association between genotype and phenotype (disease) and the quantification of the relative risk, we computed ORs, *P*-values and the corresponding 95% confidence limits using the SAS procedure LOGISTIC with the respective most frequent allele ('wild type') as reference category. For the IFN-γ CA repeat polymorphism, 12/12 CA repeats where taken as reference category.

Test on group differences. For the test on group differences we used non-parametric tests, the Wilcoxon rank-sum test and the Kruskal–Wallis test. The computation was conducted using the SAS procedure NPAR1WAY.

Adjustment for multiple comparisons: The numerous *P*-values arising from OR and test of difference calculations for the set of genes and polymorphisms under consideration have been adjusted for multiple testing by controlling the false discovery rate (FDR) [20] using the SAS procedure MULTTEST with option FDR.

Test on trend. We calculated the Jonckheere–Terpstra test, which is a non-parametric test on ordered differences between classes. It can be computed using an option of the SAS procedure FREQ.

Population attributable risk. We computed the risk according to the formula $PAR = p \times (OR - 1)/(p \times (OR - 1) + 1)$ (p = prevalence of exposure) [21].

Results

Association between investigated polymorphisms and self-reported hayfever

Table 1 shows the genotype frequencies in individuals with self-reported physician diagnosed hayfever and controls. Given the definition of the more frequent allele as the 'wild type', the frequency of the homozygote mutants ranges between 0.94% (TNF −308 G/A) and 23% (IL-10 −1082 G/A). The allelic distributions were in Hardy–Weinberg

Table 1. Genotype frequencies of selected candidate genes for hayfever in subjects with self-reported hayfever and controls in EPIC-Heidelberg, and medians of signals of CAPSX1 tests for IgEs against inhalant antigens

		Cases ($n = 318$)			Controls ($n = 322$)		
Gene polymorphic site	Genotype	Number	Median* CAPSX1 signals	*P*-value for test for group differences	Number	Median* CAPSX1 signals	*P*-value for test for group differences
IL-2 –330 T/G	TT	151	3495		171	56	
	TG	139	3029	0.3256	138	58	0.3699
	GG	28	2327		13	78	
IL-4 –589 C/T	CC	225	3168		242	58	
	CT	86	2851	0.1485	71	54	0.5912
	TT	7	10 868		9	108	
IL-4R Q576R A/G†	AA	199	3313		210	59	
	AG	99	2996	0.3423	91	56	0.1991
	GG	17	2365		19	52	
IL-5 –746 T/C†	TT	167	3271		164	60	
	TC	122	2944	0.6102	130	53	0.3157
	CC	29	4361		27	51	
IL-6 –174 G/C†	GG	123	3650		95	63	
	GC	131	2592	0.0026‡	165	58	0.0361§
	CC	64	5028		59	53	
IL-10 –1082 G/A	GG	70	2862		78	58	
	GA	143	3029	0.6906	140	56	0.8059
	AA	105	3783		104	59	
IL-10 − 819 C/T	CC	193	3611		187	56	
	CT	106	2828	0.4801	105	58	0.4204
	TT	19	4440		30	60	
IL-12 p40 +1188 3′UTR A/C†	AA	197	3362		201	60	
	AC	101	2943	0.8367	104	55	0.0952
	CC	19	2981		16	75	
IL-13 Arg130Gln A/G†	GG	194	2982		207	57	
	GA	104	3252	0.3326	97	57	0.5970
	AA	20	3816		17	61	
IL-18 –137 G/C†	GG	157	3362		172	55	
	GC	138	3001	0.7717	130	62	0.0433¶
	CC	23	4004		19	58	
IL-18 –607 C/A	CC	101	3354		116	53	
	CA	161	2981	0.1686	157	60	0.0246 ‖
	AA	54	4482		48	69	
TNF − 307 G/A	GG	230	3245		230	57	
	GA	85	3255	0.2115	89	60	0.8988
	AA	3	592		3	52	
IFN-γR2 R64Q G/A	GG	239	3133		245	56	
	GA	71	4155	0.9494	68	60	0.5287
	AA	8	2077		9	63	
IFN-γ†	1 (12/12 CA)	65	3650		63	60	
	2 (12/13 CA)	128	2915		125	59	
	3 (12/14 CA)	15	4985		23	57	
	4 (12/15 CA)	8	3390	0.9583	12	50	0.6791
	5 (13/13 CA)	69	3568		67	53	
	6 (13/14 CA)	15	3970		16	62	
	7 (13/15 CA)	13	4921		11	53	
	8 (13/16 CA)	0			2	46	
	9 (14/15 CA)	2	2637		3	58	
CD14 − 159 C/T	TT	71	3067		61	62	
	TC	159	2963	0.4628	173	58	0.5252
	CC	88	3839		88	57	

*IgE measurements performed for 308 cases and 306 controls. †Missing values for IL-4R (three cases, two controls); IL-5 (one control); IL-6, IFN-γ (three controls); IL-12, IL-13, IL-18 –137 (one case, one control), IL-18 –607 (one control, two cases). ‡*P*-value under controlling the FDR: 0.039. §¶ ‖ *P*-value under controlling the FDR: 0.217.

equilibrium for all of the investigated variants except for both IL-10 polymorphisms (data not shown).

In Table 2 the association of individual genotypes of the investigated polymorphisms and hayfever was explored. Homozygosity of the IL-2 polymorphism correlated with an increased risk of self-reported hayfever (OR = 2.6, 95% CI = 1.3–5.2). A decreased risk of self-reported hayfever was seen in heterozygotes of the IL-6 promoter polymorphism (OR = 0.6, 95%CI = 0.4–0.9). After adjustments for multiple testing both estimates, however, lack statistical significance (P-value = 0.152 in both instances).

Association between investigated polymorphisms and case definition on the basis of IgE measurements

Subsequently, we used the CAPSX1-test results for the definition of the atopy case/control status (cases CAPSX1 class $\geqslant 1$, controls CAPSX1 class = 0) and examined (a) how close the test-based atopy status matched the questionnaire-based case/control status, and (b) whether atopy as defined by the CAPSX1 test was associated with the genotype status under consideration.

We found that 78% of the questionnaire-based controls were also test-based negative and 86% of the questionnaire-based cases were test-based positive.

When we explored the association of this case definition with the genotype status, a negative correlation with the IL-6 − 174 G/C polymorphism was found. Heterozygotes for the IL-6 promoter polymorphism were at 50% reduced risk for elevated IgE levels against inhalant antigens (95%CI = 0.4–0.8). After controlling for the FDR, the P-value for association remained statistically significant ($P = 0.045$). Among the other variants, the IL-4R heterozygotes showed a negative association, whereas the AA-homozygotes of the IL-18 − 607 polymorphism and of the IL-13 Arg130Gln polymorphism were at increased risk of elevated IgE levels against inhalant antigens. Significance disappeared under controlling the FDR. A positive Phadiatop test did not correlate with variants in IL-2, IL-4, IL-5, IL-10, IL-12, TNF, IFN-γR2 and CD14.

Association between investigated polymorphisms and levels of inhalant IgE

In an exploratory analysis, correlation of gene variants with medians of the CAPSX1-test signal intensities as indicators of IgE levels against inhalant allergens revealed significant group differences for several genotypes.

Table 1 shows, for cases and controls separately, the median values of the test results by genotype, and the P-values of the Kruskal–Wallis test on group differences. Statistically significant group differences of IgE levels depending on the genotype were evident in cases and controls for IL-6 − 174 G/C (cases $P = 0.0026$; controls: $P = 0.0361$), and in controls only for IL-18 − 137 G/C ($P = 0.0433$) and IL-18 − 607 C/A ($P = 0.0246$).

The analysis of the combined data of cases and controls (data not shown) revealed the following associations:

For the IL-6 promoter polymorphism the P-value for genotype specific differences in signal intensities was $P = 0.0006$ (median of CASPSX1 signals was ‘980’ in wild-type homozygotes, ‘173’ in heterozygotes and ‘911’ in mutant homozygotes, respectively). After correction for FDR, statistical significance remained for the IL-6 polymorphism analysed in the whole study population ($P = 0.009$) and in hayfever patients ($P = 0.039$).

For the IL-4R polymorphism a tendency of higher medians with increasing number of A-alleles was apparent ($P_{\text{trend}} = 0.0302$). When the whole study population was explored, tests for trend were also significant for both IL-18 polymorphisms ($P = 0.041$ for IL-18 − 137 and $P = 0.013$ for IL-18 − 607). Median CAPSX1 signal intensities were ‘192’, ‘791’, ‘1355’ for the IL-18 − 137 genotypes (GG, GC, CC, respectively) and ‘160’, ‘345’, ‘1122’ for the CC-, CA-, AA-genotypes of the − 607 polymorphism. Compared with the combined Arg/Arg and Arg/Gln genotypes of the IL-13 − 330 A/G polymorphism, mutant (Gln) homozygotes had substantially increased median CAPSX1 signal intensities ($P = 0.046$). Adjustments for age and sex in the logistic regression model did not change the data markedly (data not shown).

Discussion

The most consistent findings of this study are the observed associations between the IL-6 − 174 G/C promoter polymorphism and hayfever as well as CAPSX1-test results. To our knowledge, it is the first time that allelic variants of the IL-6 gene have been associated with these phenotypes. IL-6 is a multi-functional cytokine involved in numerous processes shaping the immune response and acute phase reactions. In general, the involvement of IL-6 in atopic diseases is underlined by its role in immune responses after antigen presentation: in suppression of dendritic cell (DC) development [22] and in mast cell and DC-mediated response to allergens [23].

Increasing evidence suggests an important function of IL-6 for promoting the development of Th2 mediated diseases, like allergies [24, 25]. It has been demonstrated that IL-6 is able to induce initial IL-4 production in naïve T cells, thereby polarizing these cells into Th2 cells [26]. In addition, in human T cells, IL-6 was shown to have a short-term and long-term effect on IL-5 secretion, the main growth factor for eosinophils [24]. By inhibition of IFN-γ, IL-6 on the other hand, has a negative effect on Th1 responses [27, 28].

Levels of IL-6 have been found to be correlated with clinical allergy status: decreased levels correlated with decreased skin prick test results [29]; allergic patients showed higher IL-6 levels in nasal secretion than controls [30], and monocytes from newborns with a high risk to develop allergies produced significantly larger amounts of IL-6 than monocytes from newborns with a low risk to develop allergies [31].

The C-allele of the IL-6 − 174 G/C polymorphism was previously associated with both decreased transcription and lower plasma levels of IL-6 [32]. The fact that the lower median IgE levels as measured via CAPSX1 and also the negative association with hayfever were found among the IL-6 − 174 G/C heterozygotes compared with both groups of homozygotes, deserves special attention. Initially this appears

Table 2. Odds ratios, 95% confidence limits and *P*-values for associations between genotypes and (a) reported hayfever and (b) sensitisation to inhalant allergens* in a subgroup of EPIC-Heidelberg ($n = 640$)

		Reported hayfever			Sensitisation to inhalant allergens		
Gene polymorphic site	Genotype	Odds ratio	95% confidence limit	*P*-value	Odds ratio	95% confidence limit	*P*-value
IL-2 –330 T/G	TT	1	–	–	1	–	–
	TG	1.1	0.8–1.6	0.5489	0.9	0.6–1.2	0.4283
	GG	2.6	1.3–5.2	0.0101†	1.7	0.8–3.5	0.1641
IL-4 –589 C/T	CC	1	–	–	1	–	–
	CT	1.2	0.8–1.8	0.3268	0.8	0.5–1.2	0.3239
	TT	0.7	0.3–2.2	0.5858	0.8	0.2–2.6	0.6870
IL-4R Q576R A/G	AA	1	–	–	1	–	–
	AG	1.2	0.8–1.7	0.4316	0.7	0.5–1.0	0.0420‡
	GG	1.0	0.5–2.0	0.9258	0.8	0.4–1.6	0.4564
IL-5 –746 T/C	TT	1	–	–	1	–	–
	TC	0.9	0.7–1.3	0.6981	0.8	0.5–1.1	0.1837
	CC	1.1	0.6–2.0	0.8241	0.9	0.5–1.7	0.7436
IL-6 –174 G/C	GG	1	–	–	1	–	–
	GC	0.6	0.4–0.9	0.0089†	0.5	0.4–0.8	0.0015§
	CC	0.8	0.5–1.3	0.4329	0.7	0.5–1.2	0.1806
IL-10 –1082 G/A	GG	1	–	–	1	–	–
	GA	0.9	0.6–1.3	0.4740	0.8	0.5–1.2	0.1808
	AA	0.7	0.5–1.2	0.2119	0.6	0.4–1.0	0.0533
IL-10 –819 C/T	CC	1	–	–	1	–	–
	CT	0.9	0.6–1.3	0.4148	0.8	0.5–1.1	0.1763
	TT	0.6	0.3–1.1	0.0869	0.7	0.3–1.4	0.3023
IL-12 p40 +1188 3′UTR A/C	AA	1	–	–	1	–	–
	AC	1.0	0.7–1.5	0.8302	0.9	0.7–1.3	0.6925
	CC	1.3	0.6–2.7	0.4547	2.0	0.9–4.4	0.0792
IL-13 Arg130Gln A/G	GG	1	–	–	1	–	–
	GA	1.1	0.8–1.6	0.5580	1.1	0.8–1.6	0.6240
	AA	1.3	0.6–2.8	0.5005	2.3	1.0–5.3	0.0496‡
IL-18 –137 G/C	GG	1	–	–	1	–	–
	GC	1.0	0.6–1.6	0.9433	1.2	0.8–1.9	0.4383
	CC	1.2	0.5–2.8	0.6564	0.8	0.3–1.9	0.5433
IL-18 –607 C/A	CC	1	–	–	1	–	–
	CA	1.1	0.7–1.7	0.7836	1.1	0.7–1.8	0.6971
	AA	1.2	0.6–2.3	0.6366	2.3	1.2–4.7	0.0161¶
TNF −307 G/A	GG	1	–	–	1	–	–
	GA	0.9	0.6–1.3	0.6672	1.1	0.8–1.7	0.4048
	AA	1.2	0.2–6.5	0.8310	0.4	0.1–2.4	0.3047
IFN-γR2 R64Q G/A	GG	1	–	–	1	–	–
	GA	1.0	0.7–1.6	0.8365	1.1	0.7 – 1.6	0.5848
	AA	1.0	0.4–2.7	0.9865	0.6	0.2 – 1.6	0.7246
IFN-γ	(12/12 CA)	1.0	–	–	1		–
	(12/x CA)	1.0	0.6–1.5	0.8328	0.6	0.7–1.8	0.6423
	(Without 12 repeats)	1.0	0.6–1.5	0.8196	0.6	0.6–1.5	0.2771
CD14 –159 C/T	TT	1	–	–	1	–	–
	TC	0.8	0.5–1.2	0.1951	1.0	0.6–1.5	0.8558
	CC	0.8	0.5–1.4	0.4712	1.0	0.6–1.7	0.9641

*As measured by CAPSX1 test, cases are defined by CAPSX1 class $\geqslant 1$ and controls by CAPSX1 class = 0. †*P*-value under controlling the FDR: 0.152. ‡*P*-value under controlling the FDR: 0.320. §*P*-value under controlling the FDR: 0.045. ¶*P*-value under controlling the FDR: 0.242.

implausible and inconsistent with the expectation of an allelic 'dose-response' effect as indicated, for example, for the polymorphism in the IL-4R. However, a special phenotype in IL-6 heterozygotes has also been found in other studies. Heesen et al. [33] presented a significantly reduced IL-6 production in lipopolysaccharide stimulated blood cells of heterozygotes as compared with both groups of homozygotes. Also in the context of one of our own projects [7], measurements of IL-6 secretion after *in vitro* T cell stimulations of PBMCs revealed significantly lower levels of IL-6 in heterozygotes. The medians were 341.3 ng/mL in GG-homozygotes, 288.0 ng/mL (GC heterozygotes) and 385.2 ng/mL (CC homozygotes) with *P*-values <0.05. Underlying mechanisms of this 'molecular heterosis' effect are discussed in Comings and MacMurray [34]. Also Sandford and Paré [35] comment on the issue of heterozygous advantage or disadvantage in the

context of an asthma gene (IL-12B) for which equivalent data was provided [36].

With the available data, the public health relevance of the association between the IL-6 promoter polymorphism and hayfever can roughly be assessed. Converting the decreased OR of the mutant allele carriers (Table 2 left column) to the corresponding elevated OR for the wild-type homozygotes (OR = 1.5, CI = 1.1–2.1) and taking the frequency of the wild-type homozygote genotype (about 35% with a rough range of 30–40%) into account (combining all mutant allele carriers leads to a lower OR compared with considering only the heterozygotes and to a smaller proportion of subjects being 'exposed' to the risk-increasing gene variant and is thus a conservative quantification), the proportion of risk in the population which is attributable specifically to this polymorphism ranges between 2.1% and 30.6% with a point estimate of 14.6%, making it worthy of further consideration.

Additional findings indicate a potential association between the IL-2 promoter polymorphism and hayfever. Hoffmann et al. [37] correlated this variant with IL-2 protein levels in *in vitro* stimulation experiments of peripheral blood lymphocytes, where they reported an early and sustained enhancement of IL-2 production in mutant homozygotes. Because IL-2 is a Th1 cytokine, this should imply a reduced risk of hayfever in this group. However, in our study the risk is increased relative to the wild-type allele carriers. A more complex regulatory network and phenotypic differences between the *in vitro* system and the *in vivo* situation may account for the inverse association with hayfever seen here.

We found some indications of marginal statistical significance for a role of polymorphisms in the Th1 gene IL-18 and IgE-levels. Recently also Kruse et al. [38] reported an association of IL-18 variants with specific sensitization to common allergens and allergic rhinitis. They found the C-allele of the −137 G/C polymorphism positively correlated with specific and total serum IgE. Our data point in the same direction of increasing CAPSX1 signal intensities seen with increasing numbers of C-alleles. We, however, did not find an association with self-reported hayfever. The promoter polymorphism in IL-18 appears to have phenotypic relevance. Carriers of the G-allele of the −137 G/C polymorphism had significantly higher transcriptional activity of the IL-18 promoter than carriers of the C-allele [15]. These data are in line with the association we observed between GG-homozygosity and lower IgE levels as indicated by CAPSX1 signal intensities ($P = 0.049$, data not shown). Recently, Watanabe et al. [39] identified a deletion of amino acid 317 of the IL-18R gene associated with lower IFN-γ production after IL-18 stimulation and elevated IgE levels. Homozygosity of this mutant was more frequent in atopic children compared with normal controls [39]. This supports our data suggesting that differences in the IL-18 signal cascade due to variants in the corresponding genes is associated with modulated IgE levels and atopic diseases.

The relevance of variants of the IL-4R and IL-13 genes in allergies and IgE regulation is well established. A number of studies and reviews on this topic have been published. For the explored polymorphisms in these genes the results of our study are in agreement with similar investigations performed by other authors. Significantly decreasing median levels of the CAPSX1 signals with increasing number of mutant G-alleles of the IL-4R Q576R polymorphism (Table 1, $P_{trend} = 0.03$) are in line with data first reported by Kruse et al. [40]. Moreover, increased median levels of CAPSX1 signals ($P = 0.046$, data not shown) and a positive association with atopy among the IL-13 mutant (Gln) homozygotes, support a role of this variant in elevated IgE phenotypes, as summarized by Vericelli [41].

The clinical outcome 'hayfever' was not associated with polymorphisms in (a) the IL-4 gene previously associated with atopic asthma by Noguchi et al. [42], (b) the IL-12 p40 gene previously associated with mite-sensitive childhood asthma [43], and (c) the IFN-γ gene previously associated with allergy [7]. Finally, we could not confirm an association of self-reported allergic symptoms including hayfever with the polymorphism in the CD14 gene as reported by Koppelman et al. [44]. When we correlated genetic variants with reported asthma, TNF and IFN-γR2 polymorphisms showed evidence for association. In line with Gao et al. [16], none of the asthmatics ($n = 43$) had the Gln/Gln genotype, whereas 3% of the controls ($n = 306$) presented this genotype. Similarly, as shown by Winchester et al. [45] and others, AA homozygotes of the TNF promoter polymorphism were at increased risk of asthma. Whereas 7% of the asthmatics were homozygous for the mutant variant, only 1% of the controls had this genotype (OR = 6.6, 95%CI = 1.05–41.3). These were the only suggestive results from the asthma related analysis and their agreement with previous results does at least not argue against the quality of the data.

The various tests calculated to obtain the just discussed results raise the issue of multiple comparisons. This problem is clearly crucial in the setting of a genome-wide scan. However, its importance in the present setting may be questionable for the following reasons: Being aware of the multiple tests problem, we decided not to carry out a 'blind' search for associations, but pursued the candidate gene approach with polymorphisms for which potential relevance for hayfever was assumed on the basis of previous reports or indirectly by earlier functional data. As long as no rule of good scientific practice exists to report the number of all comparisons which have been carried out with the data set under consideration or by the statistician in charge (see for this problem [46], p. 32), it is common practice to publish only highlights (i.e. 'significant' results) and to leave 'uninteresting' results out of consideration. This is a way to circumvent, but not to solve the problem of multiple comparisons. It reappears twice, in form of significant, but non-reproducible results, and in form of publication bias for the omitted findings. On the other hand, since the existing methods of adjustment for multiple tests are known to be too conservative, and science is interested in detecting associations with potential public health relevance, epidemiologists prefer not to adjust for multiple comparisons, as long as no definite confirmation of an issue is demanded. Instead, they put emphasis on the discussion of the plausibility of their results [21, 47]. In order to offer full transparency to the reader, we decided to present the results of all the comparisons, which we have carried out and to show both, the individual 'raw' *P*-values and the critical *P*-values adjusted for multiple comparisons. In the interpretation of our data we, however, prioritize the individual *P*-values and the discussion of the biological plausibility of the respective relationships.

Biased results due to population stratification cannot be excluded in this study setting and may in principle be the reason for the violation of the HWE for the IL-10 gene variants. However, since cases and controls are sampled from one source population consisting of individuals of largely Caucasian origin, we do not expect population stratification to be of major concern in this project [48].

In conclusion, we present for the first time indications for an association of the IL-6 −174 G/C polymorphism with elevated IgE levels against inhalant antigens. Furthermore our data are in agreement with previous studies, correlating some Th1, Th2 cytokine gene polymorphisms, such as in IL-4R, IL-13, and IL-18 with IgE phenotypes. Promoter polymorphisms in IL-6 and IL-2 appear to modulate the risk of hayfever in this population. Currently, we corroborate these findings with independent material, coming from a dedicated allergy project and include the consideration of haplotypes.

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