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Genetic polymorphisms of Th2 interleukins, history of asthma or eczema and childhood acute lymphoid leukaemia: findings from the ESCALE study (SFCE)

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Abreviations
ALL Acute lymphoid leukaemia
OR Odds ratio
SNPs Single nucleotide polymorphisms
IL Interleukins

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Abstract (239 words)

Background: Previous studies on the putative role of allergy in the aetiology of childhood leukaemia have reported contradictory results. The present study aimed to analyse the relation between a medical history of asthma or eczema and childhood acute lymphoid leukaemia (ALL) in light of potential candidate gene-environment interactions.

Methods: Analyses were based on a subset of 434 cases of ALL and 442 controls successfully genotyped and of European ancestry children enrolled in a French population-based case-control study conducted in 2003-2004. Information about medical history was obtained during a standardized interview with the mothers. Candidate polymorphisms in genes of the Th2 cytokines IL4, IL10, IL13 and IL4-receptor, were genotyped or imputed.

Results: None of the variant alleles were directly associated with childhood acute lymphoid leukaemia. A medical history of asthma or eczema was reported more often in the control group (OR=0.7 [0.5-1.0]). This association was mostly seen in the group of children not carrying the *IL13*-rs20541 variant allele (Interaction Odds Ratio IOR 1.9, p-interaction=0.07) and in those carrying the *IL10* triple variant haplotype (IOR 0.5, p-interaction=0.04). No interaction was observed with the candidate polymorphisms in *IL4* and *IL4R*.

Conclusion: This study provides a new insight into the relationship between allergic symptoms and childhood acute lymphoid leukaemia, by suggesting this inverse association could be limited to children carrying certain genetic polymorphisms. If confirmed, these results could help better understand the biological mechanisms involved in the development of childhood acute lymphoid leukaemia.

1. **Introduction**

Acute lymphoid leukaemia (ALL) accounts for approximately 80% of all childhood acute leukaemias and a quarter of all childhood neoplasms in developed countries. Little risk factors have been identified to date, i.e. high-level ionizing radiation, certain chemotherapeutic agents, Down's syndrome and a few rare genetic disorders. It has long been hypothesized that a lack of immune stimulation through exposure to infectious agents in infancy and early childhood could be related to the development of ALL[1], although the epidemiological evidence is still contradictory[2]. To some extent, this parallels the hygiene hypothesis, which postulates that a lack of early immune stimulation could be associated with the rising incidence of allergies in developed countries. From these, one could expect atopy and allergy to be positively associated with childhood leukaemia. However, most questionnaire-based epidemiological studies reported an inverse association between atopy or allergy and childhood leukaemia, as reviewed by Linabery[3 , 4].

In the last few years, in an attempt to rely on unbiased sources of information, several publications have studied the association between atopy or allergy and childhood leukemia relying on objective sources of information, such as medical records[5, 6], health claim data[7], and IgE levels at recruitment[8]. Some of those studies[5, 8] corroborated the inverse association between allergy and childhood leukaemia found in the interview-based studies, whereas two other studies did not find this association[6, 7]. Recently, a Mexican study reported a positive association borderline of significance between bronchial asthma and ALL in children with Down syndrome[9].

Despite the increasing biological knowledge, the mechanisms leading to allergy still need to be unraveled. A role of Th2 cytokines has long been suspected in the etiology of allergy, supported by the key regulatory effects of the interleukins produced by T-lymphocytes undergoing a Th2 differentiation, such as interleukins (IL) IL4, IL10 and IL13. Additionally, some studies have reported associations between asthma and polymorphisms in the genes coding for IL13, IL4, IL10 and IL4R[10-13]. Polymorphisms in *IL13* and *IL4R* were also associated with allergic rhinitis, wheeze atopic dermatitis in an international study[14].

The role of interleukins in childhood leukaemia has been little investigated. In a Californian study, the levels of IL10 at birth were significantly lower in ALL cases than in controls[15]. In the same study, a genomic analysis showed a slight association between ALL and *IL10* tag-SNPs (Single Nucleotide Polymorphisms)[16]. Recently, two polymorphisms in *IL10* were reported to be associated with childhood leukaemia in Taiwan[17].

The aim of the present study was to provide a new insight in the relationship between allergic disorders and childhood ALL by taking into account genetic polymorphisms of several Th2 cytokines,

namely interleukins IL4, IL10, IL13 and IL4-receptor, suspected to be involved in the development of allergy.

2. <u>Material and methods</u>

2.1. Study

Study design and sampling have been extensively described elsewhere[18, 19]. Briefly, the ESCALE study is a population-based case-control study conducted in France in 2003-2004. Eligible children were residing in mainland France, had not been adopted, and had a living French-speaker biological mother without a serious psychiatric disorder.

Children diagnosed with leukaemia before the age of 15, between 01/01/2003 and 31/12/2004, were detected and recruited using the detection system of the French national childhood haematological malignancies registry. Children who were deceased or under palliative care were ineligible. Out of the 714 eligible children diagnosed with acute lymphoid leukaemia, 648 were enrolled in the study (participation rate 91%).

Controls were randomly selected from the general population using random digit dialing contemporaneously with the recruitment of the cases. Quotas were applied to obtain a control group comparable to the whole case group (leukaemias, lymphomas, CNS tumours and neuroblastomas) in terms of gender and age (0-1, 2, 3, 4, 5-6, 7-8, 9-11, 12-14 years) and to the general population regarding the number of children living in the household. The participation rate of the controls was 71%, so that 1,681 controls were enrolled out of the 2,361 eligible children.

2.2. Information on medical history

Information about socio-demographic characteristics and suspected risk factors, such as maternal exposures during pregnancy, perinatal characteristics, personal and familial medical history was obtained over the telephone. The children biological mothers were interviewed using a standardized questionnaire identical for cases and controls. Before the interview, mothers were asked to have a copy of the child health record on hand, so they could get back to the records if needed. A large proportion of mothers followed this recommendation (97.2% for ALL cases and 98.2% for controls). Mothers were specifically asked whether the participating child had developed asthma or eczema, and, if so, the age at onset. Moreover, a specific item regarded regular intakes of bronchodilators, corticoids, or antihistamines/anti-allergic drugs.

Reported medical history of asthma and eczema were analysed as independent variables. More specific variables were also analysed, considering a latency period of one year by ignoring the asthma

or eczema diagnoses that occurred in the year prior to the age at diagnosis (for cases) or recruitment for controls).For asthma, we also considered the reported treatments with antihistamines, corticoids or bronchodilators.

2.3. Biological specimens

Biological specimens were requested from each participant. They consisted in blood samples taken from cases during care, prior to initiation of chemotherapy, and saliva taken at home using swab brushes for the controls. Biological samples were obtained from 619 ALL cases (96%) and 810 controls (48%).

After DNA extraction, a sufficient DNA quantity was available for genotyping of 513 ALL cases and 570 controls. Cases were genotyped on an Illumina 370k Quad platform (283,027 single nucleotide polymorphisms [SNPs]), and a custom iSelect platform (4,868 SNPs) was used for the controls. The comparability of the two genotyping methods was assessed and confirmed by re-genotyping 96 randomly-selected cases on the platform used for the controls (100% agreement). Fulfilled quality checks, including a call-rate >97% for the cases and 95% for the controls, and a 46, XX or 46, XY formula were considered mandatory criteria for subsequent genetic analyses, leading to the exclusion of 42 ALL cases (including 6 children with Down syndrome) and 109 controls.

A principal component analysis (PCA) using information from 96,609 SNPs was performed in the case group in order to determine the Caucasian origin from the CEPH (Human Polymorphism Study Center). Because of the platform used for genotyping the controls, such a PCA could not be performed for the control group. However, in the case group, having at least two European-born grandparents, a proxy variable derived from the questionnaire, showed to be very predictive of the CEPH classification (sensitivity 98.2%, specificity 94.3%)[20]. Assuming that this proxy variable was as good a predictor in the control group, it was used to restrict both the case and control samples on European ancestry, in order to limit the potential for population stratification bias.

Finally, 434 cases with ALL and 442 controls, all genotyped children of European ancestry ($\geq$ 2 grandparents born in Europe), were available for the present analysis.

2.4. Genotyping of candidates SNPs

Polymorphisms in Th2 cytokines genes (*IL4*, *IL10*, *IL13* and *IL4R*) were identified through a literature search and selected *a priori* as a sample of SNPs having been reported to be associated with allergy, asthma[10-13], or with IgE level[14]. Additional selection criteria were the effect of the genotype on the protein function or production level, and its availability after genotyping. Those polymorphisms selected for analysis were the haplotype rs2070874-rs2243250 in *IL4*, rs20541 in *IL13*, rs1801275 in *IL4R* and the haplotype rs1800896-rs1800871-rs1800872 in *IL10*.

The polymorphisms that could not be genotyped on the cases' platform, namely IL4-rs2070874 and rs2243250, IL10-rs1800871 and rs1800872, or that showed evidence of genotyping errors in the controls (departure from Hardy-Weinberg), e.g. IL10-rs1800896 and IL4R-rs1801275, were imputed

using the Impute V2 software[21]. Information for imputation was obtained from all SNPs genotyped in our participants in the 100,000 bp in the vicinity of the gene, and using the CEU samples from the *HapMap* and *1000 Genomes* projects (respectively, *HapMap* 43 release 28 CEU, phases 1, 2 and 3 [http://hapmap.ncbi.nlm.nih.gov], *1000 Genomes* phase pilot 1-low coverage [http://www.1000genomes.org]) as reference panels. Inferred genotypes were further considered for analyses when the post-imputation probability reached at least 90%. However, sensitivity analyses were conducted using a 0.80 cutoff. Additionally, the quality of imputation was assessed for each SNP, by the *Info* and *Certainty* criteria provided by Impute V2 software.

The genotypes were analysed separately, and then assuming a dominant model of expression (carrying at least one variant allele *vs.* none). Since some SNPs in *IL4* and *IL10* (IL4-rs2070874 and rs2243250; IL10-rs1800871 and rs1800872) have been shown to be in linkage disequilibrium, the haplotypes were deduced from the genotypes. The haplotypes for *IL4* rs2070874-rs2243250 were CC (reference haplotype) and TT (double variant haplotype), and those for *IL10* rs1800896-rs1800871-rs1800872 were CGG (reference haplotype), TGG and the triple variant TAT haplotype.

2.5. Statistical analyses

For each SNP, the Hardy-Weinberg equilibrium test was systematically performed in the group of 442 controls of European ancestry, and there was no evidence of a departure from the equilibrium. Unconditional logistic regression models were used to compare cases and controls with regards to their history of asthma or eczema, and genotypes. All analyses presented hereafter were adjusted for a gender*age variable, birth order, maternal highest diploma, and professional category of the household[20]. Interactions were assessed using likelihood ratio tests.

3. Results

3.1. Study sample

The table 1 shows a brief description of the subsample of genotyped children of European ancestry. The distribution of gender was not different in cases and controls, but controls tended to be globally older than ALL cases. Cases were more likely than controls to be first-born children, and not to have been breastfed. The parental socio-professional characteristics were differently distributed, with higher educational levels and higher professional category in the control group. Common precursor-B cell leukaemia was the most common subtype (n=355), with the group of genotyped cases also including less common subtypes, such as pro-B (n=10), mature B-cell (n=19), T-cell (n=40) and other types of lymphoid leukaemia (n=40).

3.2. Association between asthma or eczema and childhood ALL

In this subsample of 434 ALL cases and 442 controls genotyped and of European origin, asthma and eczema were both inversely associated with the occurrence of childhood ALL (respective OR=0.6 [0.4-1.1] and 0.8 [0.5-1.1]) (table 2), as observed in the whole ESCALE study[18]. These associations were also observed when considering a latency of 1 year, and when changing the definition of asthmatic children to those treated with anti-allergic, bronchodilators or corticoid drugs. Having a history of either asthma or eczema was significantly associated with childhood ALL with the same order of magnitude (OR=0.7 [0.5-1.0]). Associations were unchanged when histories of asthma and eczema were mutually adjusted for.

3.3. Association between candidate polymorphisms and childhood ALL

Table 3 shows the associations between the candidate polymorphisms in *IL13*, *IL10*, *IL4* and *IL4R* genes and childhood ALL. None of the genotypes of the candidate SNPs analysed separately were associated with childhood ALL. The *IL4* double variant haplotype (TT haplotype) was little associated with childhood ALL (OR=1.3 [0.9-1.8], p=0.14), but the *IL10* TAT triple variant haplotype was not associated with ALL.

3.4. Gene-gene and gene-environment interactions

The analyses suggested a gene-gene interaction between *IL4* TT haplotype and *IL13*-rs20541 (IOR 1.8, p=0.10), between *IL13*-rs20541 and *IL10* TAT haplotype (IOR 0.6, p=0.10), and between *IL4* TT haplotype and *IL4R*-rs1801275 (IOR 1.8, p=0.07), , despite lack of statistical significance (table 4).

The analyses of interaction between a reported medical history and the candidate polymorphisms are shown in table 5. The results suggested there might be an interaction between *IL13*-rs20541 and a medical history of asthma or eczema. Indeed, the inverse association between childhood ALL and asthma/eczema was only seen in the group of children carrying no variant allele (OR=0.5 [0.4-0.8] *vs.* OR=1.1 [0.6-1.9] in the carrier group [not shown], interaction test p=0.07). There was also some evidence of an interaction with the *IL10-TAT* haplotype, with the association between a history of asthma or eczema and childhood ALL observed in those carrying the triple variant haplotype (IOR=0.5 [0.2-1.0], p interaction 0.04). There was no evidence of an interaction between asthma/eczema and *IL4* or *IL4R* polymorphisms.

3.5. Sensitivity analyses

Similar patterns of interaction were observed after taking into account a one-year latency, and lowering the imputation probability cutoff to 0.80.

All analyses repeated with a further adjustment for repeated infections in infancy, maternal pesticides use during pregnancy, paternal smoking before conception, familial history of cancer, and road traffic showed compatible estimates, although the association between a history of asthma or eczema and

childhood ALL was weaker (OR=0.8 [0.6-1.1]). Adjusting all models for attendance to full-time day care before the age of 1 year did not change the results. We also repeated the analyses adjusting for the number of grandparents born in Europe (2, 3 or 4) and the results did not change.

The associations between the various SNPs in *IL4* and *IL10* and asthma or eczema in the control group were compatible with the positive associations reported in previous literature, with OR estimates ranging between 1.2 and 1.4, although not reaching statistical significance (dominant model, data not shown). However, the association with *IL13*-rs20541 and *IL4R*-rs1801275 (OR=0.8 [0.5-1.2] and OR=0.9 [0.6-1.4]) did not replicate the associations found in previous studies.

All analyses were repeated in the 422 cases and 434 controls whose mother had a copy of the medical record at the interview, and showed similar results.

4. <u>Discussion</u>

4.1. *Main results*

Not only do our results support an inverse association between childhood ALL and a reported history of asthma or eczema, but they suggest that this association could depend on the children's genotypes for Th2 cytokine genes. Indeed, in this study, the inverse association was essentially observed in individuals not carrying the variant *IL13*-rs20541 allele, and in those carrying the *IL10* TAT haplotype.

4.2. *Strength and limits*

The registry-based methods used for the identification of the eligible cases and the high participation rate ensured that the recruitment of cases was nearly exhaustive. Only 41 leukaemia cases were not eligible for medical reasons, being dead or under palliative care, which is unlikely to have biased our results. Biological sampling was generally well accepted in this population-based study, and later decreases in numbers were mainly due to unsuccessful genotyping.

The controls were randomly selected from the general population with quotas on the sex and age distributions ensuring comparability with the cases. Moreover, the selection process made the control group representative of the general population in terms of region and number of children living in the household. In the control group, the age at recruitment, parental education level and professional category were related to the acceptance and success of the genotyping, the latter being probably due to the sampling method (saliva). These variables were considered potential confounders and all analyses were systematically adjusted for them.

It was not possible to run a PCA to determine whether controls were of Caucasian origin because the genotyping platform used for the controls had much fewer SNPs than the one used for the cases. In order to limit the possibility of a population stratification bias, we however restricted our study to children with a European ancestry as derived from the country of birth of their grandparents. In the ESCALE study, this variable was found to be a good proxy for the Caucasian origin in the case group[20]. The comparability of the different genotyping platforms used for cases and controls was assessed by re-genotyping 96 cases on the controls platform.

Because of the apparent discrepancy in published results between interview-based studies and two of the studies relying on medical and administrative data, misclassification bias is a particular concern when studying the relationship between a reported history of allergy and childhood ALL. In the ESCALE study, mothers were asked about the index child's medical history, and they were encouraged to use the child health record as a support for answering. Additional analyses showed robust results when restricted to children whose mother had the health record on hand during the interview. The questions about the age at onset and related treatments gave additional information to classify children on their medical history. The frequency of asthma in the ESCALE control group was very close to that observed in another French study[22] (respectively 11.1% and 12.7% of the 11-14 year-old children). Applying a one-year latency and restricting the definition of asthma to those under treatment did not change the magnitude of the association with childhood ALL, although leading to higher p-values. Because asthma and eczema can occur without a proper allergy and, conversely, allergy can manifest itself with other symptoms, the present results have a limited generalizability regarding allergy in childhood.

Candidate SNPs were selected for their potential association with allergy or symptoms, and their functional consequences. We used imputation to infer the genotypes for 4 SNPs in *IL4* and *IL10* that were not available in the case sample after genotyping, under strict quality criteria (Info and Certainty, post-imputation probability). To be considered for analysis, an individual genotype had to be imputed with a very high probability. Still, the sensitivity analyses showed that the results were robust to a lower imputation probability cutoff.

The power conditions to detect an interaction with IOR=2 between a reported history of asthma or eczema (frequency≈20%) and the various candidate polymorphisms (frequency ranging from 25% to 40%) in the subsample of 434 ALL cases and 442 controls were moderate, between 47% and 53%. Our study might have suffered from under-power to detect interactions with small effect. However, our analyses highlighted two interactions between a reported history of asthma or eczema and both *IL10* TAT haplotype and *IL13*-rs20541 alleles. Due to the number of tests performed, caution is required with regards to the interpretation of the p-values and these findings need replication.

4.3. Comparison with previous literature

Showing a negative association between ALL and history of asthma or eczema in the ESCALE study is in line with the results of most of the interview-based studies reporting on the association between allergy and childhood leukaemia[23-26]. A concern has been raised about the consistency of this inverse association, since two studies relying on objective data did not replicate this finding[6, 7]. On the other hand, in a study using medical diagnoses from the children's medical record[5], an inverse association was also found between childhood ALL and the definite diagnoses of allergy, eczema and hay fever. A meta-analysis by Linabery et al[3] replicated the overall inverse association between atopy/allergy and childhood ALL, and underlined the lack of robustness of this association when taking into account a latency period. In the ESCALE study, the available data allowed taking into account a one-year latency and, although limited by numbers, those analyses showed an inverse association of the same order of magnitude.

The genotypic analyses showed a non-significantly raised OR related to the *IL4* haplotype, but not with *IL10* promoter haplotype that is related to a decrease in IL10 synthesis. In the NCCLS, Chang et al[16] had found a slight association between three tag-SNPs in *IL10* gene and childhood ALL that was not robust when taking into account multiple testing. In the same Californian study[15], the analysis of blood samples taken at birth showed that cases featured lower IL10 levels than controls, whereas the levels of other Th1 and Th2 cytokines, including IL4 and IL13, were not different. Our results further suggest that the suspected negative association between childhood ALL and a medical history of asthma or eczema could be limited to children carrying certain genetic polymorphisms, such as the *IL10* promoter TAT haplotype or the IL13-rs20541 GG genotype. Unlike with *IL10*, the interaction with *IL13* could partly explain the discrepancies observed with the US[6] and Taiwanese[7] studies. Indeed, genotyping from the 1000 Genomes project (phase 1) showed a lower proportion of IL13-rs20541 GG genotype in people with Asian (42.7%, ASN sample) or Mexican ancestry (19.7%, MXL sample) than in those with European ancestry (60.0%, CEU sample).

The inverse occurrence of allergy and childhood ALL suggested by epidemiological studies remains largely unclear. There is a growing body of evidence for a protective effect of a highly-active immune system against leukaemia, with studies having found an inverse association between childhood acute lymphoblastic leukaemia and allergy and early immune stimulations indicators such as daycare in infancy[2, 27] and breastfeeding[2, 28]. Immune responses are normally inhibited by the regulatory T cells (Treg), thus avoiding allergy and autoimmune diseases. As explained by the "reduced immune suppression" hypothesis, a lack of Treg stimulation could lead to inappropriately overactive Th1 and Th2 responses[29], which, one could hypothesize, could promote both allergy and the expansion of preleukaemic cells. Interestingly, this hypothesis aligns with the deficit in IL10, regulatory cytokine also produced by Treg cells, reported at birth in ALL cases by Chang and colleagues[15].

In conclusion, this study suggests that the association between allergic phenotypes and childhood ALL could be modified by genetic polymorphisms of Th2 cytokines such as IL13 and IL10. While these results warrant further replication, they provide further evidence for a reduced risk of ALL among children developing allergic symptoms, and suggest that this association could be related to immune suppression mechanisms involving IL10.

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Table 1: Characteristics of the sample of genotyped cases and controls of European ancestry

	Controls N	Controls %	ALL cases N	ALL cases %
Total	442		434	
Sex and age				
Boys				
0 - 2 yrs	47	*10.6*	62	*14.3*
3 - 4 yrs	27	*6.1*	64	*14.7*
5 - 8 yrs	56	*12.7*	51	*11.8*
9 - 14 yrs	97	*21.9*	56	*12.9*
Girls				
0 - 2 yrs	59	*13.3*	58	*13.4*
3 - 4 yrs	34	*7.7*	52	*12*
5 - 8 yrs	45	*10.2*	63	*14.5*
9 - 14 yrs	77	*17.4*	28	*6.5*
Birth order				
1	181	*41.0*	223	*51.4*
2	175	*39.6*	137	*31.6*
3+	86	*19.5*	74	*17.1*
Breastfeeding (any duration)				
No	191	*43.2*	225	*51.8*
Yes	251	*56.8*	209	*48.2*
Maternal educational level				
No diploma	31	*7.0*	44	*10.1*
Lower secondary diploma	102	*23.1*	140	*32.3*
High school diploma	74	*16.7*	79	*18.2*
University degree	235	*53.2*	171	*39.4*
Paternal educational level				
No diploma	29	*6.6*	52	*12*
Lower secondary diploma	159	*36.0*	186	*42.9*
High school diploma	55	*12.4*	66	*15.2*
University degree	196	*44.3*	126	*29*
missing	3	*0.7*	4	*0.9*
Parental professional category				
Intellectual/scientific jobs, managers, Intermediate professions	248	*56.1*	149	*34.3*
Administrative and sales workers	98	*22.2*	149	*34.3*
Service workers	40	*9.0*	57	*13.1*
Factory and agricultural workers, unemployed	56	*12.7*	79	*18.2*

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Table 2: Association between a reported history of asthma or eczema and childhood ALL

	Controls N=442		ALL N=434				
	n	%	n	%	OR[a]	95%CI	p
Reported asthma history							
No	399	90.3	410	94.5	1.0	Ref.	
Yes	42	9.5	24	5.5	0.6	[0.4-1.1]	ns
missing	1	0.2					
With a latency							
No	404	91.4	412	94.9	1.0	Ref.	
Yes	36	8.1	22	5.1	0.7	[0.4-1.3]	ns
missing	2	0.5					
With treatment[b]							
No	409	92.5	415	95.6	1.0	Ref.	
Yes	32	7.2	19	4.4	0.7	[0.3-1.2]	ns
missing	1	0.2					
Reported eczema history							
No	346	78.3	355	81.8	1.0	Ref.	
Yes	95	21.5	77	17.7	0.8	[0.5-1.1]	ns
missing	1	0.2	2	0.5			
With a latency							
No	363	82.1	359	82.7	1.0	Ref.	
Yes	77	17.4	72	16.6	0.9	[0.6-1.3]	ns
missing	2	0.5	3	0.7			
History of asthma and/or eczema							
None	319	72.2	339	78.1	1.0	Ref.	
Asthma without eczema	27	6.1	16	3.7	0.6	[0.3-1.3]	
Eczema without asthma	79	17.9	69	15.9	0.8	[0.5-1.1]	
Asthma and eczema	15	3.4	8	1.8	0.6	[0.2-1.4]	ns
Missing	2	0.5	2	0.5			
Neither asthma, nor eczema	319	72.2	339	78.1	1.0	Ref.	
Asthma or eczema	122	27.6	93	21.4	0.7	[0.5-1.0]	*
missing	1	0.2	2	0.5			

[a] Odds ratios (OR) 95% confidence intervals (95%CI) estimated using a logistic regression model adjusted for a sex*age variable, maternal educational level, household socioprofessional category and birth order.
[b] treatments with antihistamines, corticoids or bronchodilators
ns: p≥0.05, * p<0.05

Table 3: Associations between candidate polymorphisms in *IL13*, *IL4*, *IL10* and *IL4R* and childhood ALL

	Controls N=442		ALL N=434			
	n	**%**	**n**	**%**	**OR[a]**	**95%CI**
IL13-rs20541						
GG	290	*65.6*	268	*61.8*	1.0	Ref.
AG	134	*30.3*	153	*35.3*	1.1	[0.8-1.5]
AA	18	*4.1*	13	*3.0*	0.7	[0.3-1.6]
AG+AA *vs GG*	152	*34.4*	166	*38.2*	1.0	[0.8-1.4]
IL4 - rs2070874						
CC	334	*75.6*	296	*68.2*	1.0	Ref.
CT	103	*23.3*	120	*27.6*	1.3	[0.9-1.8]
TT	5	*1.1*	6	*1.4*	1.4	[0.4-5.2]
missing			12	*2.8*		
CT+TT vs CC	108	*24.4*	126	*29.0*	1.3	[0.9-1.8]
IL4-rs2243250						
CC	324	*73.3*	292	*67.3*	1.0	Ref.
CT	112	*25.3*	119	*27.4*	1.1	[0.8-1.6]
TT	6	*1.4*	6	*1.4*	1.0	[0.3-3.7]
missing			17	*3.9*		
CT+TT vs CC	118	*26.7*	125	*28.8*	1.1	[0.8-1.5]
IL4 haplotype						
≥1 TT	108	*24.4*	125	*28.8*	1.3	[0.9-1.8]
IL10-rs1800896						
CC	98	*22.2*	88	*20.3*	1.0	Ref.
CT	211	*47.7*	215	*49.5*	1.1	[0.8-1.7]
TT	133	*30.1*	131	*30.2*	1.0	[0.7-1.5]
CT+TT vs CC	344	*77.8*	346	*79.7*	1.1	[0.8-1.5]
IL10-rs1800871						
GG	261	*59.0*	229	*52.8*	1.0	Ref.
AG	153	*34.6*	153	*35.3*	1.1	[0.8-1.5]
AA	28	*6.3*	20	*4.6*	0.9	[0.5-1.7]
missing			32	*7.4*		
AG+AA vs GG	181	*41.0*	173	*39.9*	1.1	[0.8-1.5]
IL10-rs1800872						
GG	261	*59.0*	229	*52.8*	1.0	Ref.
GT	153	*34.6*	170	*39.2*	1.2	[0.9-1.7]
TT	28	*6.3*	24	*5.5*	1.1	[0.6-2.1]
missing			11	*2.5*		
GT+TT vs GG	181	*41.0*	194	*44.7*	1.2	[0.9-1.6]
IL10 haplotypes						
≥1 TAT	181	*41.0*	173	*39.9*	1.1	[0.8-1.5]
≥1 TGG	226	*51.1*	198	*45.6*	0.9	[0.6-1.1]
IL4R-rs1801275						
AA	274	*62.0*	281	*64.7*	1.0	Ref.
AG	154	*34.8*	135	*31.1*	0.8	[0.6-1.2]
GG	14	*3.2*	18	*4.1*	1.3	[0.6-2.9]
AG+GG vs AA	168	*38.0*	153	*35.3*	0.9	[0.7-1.2]

[a] Odds ratios (OR) 95% confidence intervals (95%CI) estimated using a logistic regression model were adjusted for a sex*age variable, maternal educational level, household socioprofessional category and birth order.

Table 4: Gene*gene interaction analyses for the candidate polymorphisms in *IL13*, *IL4*, *IL10* and *IL4R*, in childhood ALL

| | Case-control models | | | | |
| | Without interaction | | With interaction | | |
	OR	95%CI	OR	95%CI	p int
***IL13-rs20541* x *IL4* TT haplotype**					
Variant *IL13* rs20541	1.0	[0.7-1.4]	0.8	[0.6-1.2]	
IL4 TT haplotype carrier	1.3	[0.9-1.8]	1.0	[0.6-1.6]	
Variant *IL13-rs20541* x *IL4* haplotype			1.8	[0.9-3.5]	*0.10*
***IL13-rs20541* x *IL10* TAT haplotype**					
Variant *IL13* rs20541	1.1	[0.8-1.4]	1.3	[0.9-2.0]	
IL10 TAT haplotype carrier	1.1	[0.8-1.5]	1.3	[0.9-1.9]	
Variant *IL13-rs20541* x *IL10* haplotype			0.6	[0.3-1.1]	*0.10*
***IL13-rs20541* x *IL4R*-rs1801275**					
Variant *IL13* rs20541	1.0	[0.8-1.4]	0.9	[0.6-1.4]	
Variant *IL4R* rs1801275	0.9	[0.7-1.2]	0.8	[0.5-1.2]	
Variant *IL13* x variant *IL4R*			1.3	[0.7-2.5]	*ns*
***IL4* TT haplotype x *IL10* TAT haplotype**					
IL4 TT haplotype carrier	1.3	[1.0-1.9]	1.5	[1.0-2.3]	
IL10 TAT haplotype carrier	1.1	[0.8-1.5]	1.2	[0.8-1.6]	
IL4 haplotype x *IL10* haplotype			0.8	[0.4-1.5]	*ns*
***IL4* TT haplotype x *IL4R*-rs1801275**					
IL4 TT haplotype carrier	1.3	[0.9-1.8]	1.0	[0.7-1.5]	
Variant *IL4R* rs1801275	0.9	[0.7-1.2]	0.8	[0.5-1.1]	
IL4 haplotype x variant *IL4R*			1.8	[0.9-3.6]	*0.07*
***IL10* TAT haplotype x *IL4R*-rs1801275**					
IL10 TAT haplotype carrier	1.1	[0.8-1.5]	1.2	[0.8-1.7]	
Variant *IL4R* rs1801275	0.9	[0.6-1.2]	0.9	[0.6-1.4]	
IL10 haplotype x variant *IL4R*			0.8	[0.4-1.5]	*ns*

Odds ratios (OR) and 95% confidence intervals (95%CI) estimated using a logistic regression model adjusted for a sex*age variable, maternal educational level, household socioprofessional category and birth order.

p int: p for interaction test; ns: p>0.10

Table 5: Interaction analyses between the candidate polymorphisms and a reported history of asthma or eczema, in childhood ALL

	Asthma			Eczema			Asthma or eczema		
	OR	95%CI	p int	OR	95%CI	p int	OR	95%CI	p int
IL13-rs20541									
Medical history vs no	0.5	[0.3-1.1]		0.6	[0.4-1.0]		0.6	[0.4-0.9]	
≥1 variant allele vs none	1.0	[0.7-1.4]		0.9	[0.7-1.3]		0.9	[0.6-1.2]	
Interaction history x allele	1.8	[0.5-6.0]	ns	1.7	[0.8-3.7]	ns	1.9	[0.9-3.8]	0.07
IL4-rs2070874									
Medical history vs no	0.6	[0.3-1.3]		0.7	[0.5-1.1]		0.7	[0.4-1.0]	
≥1 variant allele vs none	1.3	[0.9-1.9]		1.3	[0.9-1.9]		1.3	[0.9-1.9]	
Interaction history x allele	0.9	[0.3-2.9]	ns	0.9	[0.4-2.0]	ns	1.1	[0.5-2.2]	ns
IL4-rs2243250									
Medical history vs no	0.7	[0.3-1.3]		0.7	[0.5-1.2]		0.7	[0.4-1.0]	
≥1 variant allele vs none	1.2	[0.8-1.6]		1.1	[0.8-1.7]		1.1	[0.8-1.6]	
Interaction history x allele	0.8	[0.2-2.8]	ns	0.9	[0.4-2.0]	ns	1.1	[0.5-2.3]	ns
IL4 TT haplotype									
Medical history vs no	0.6	[0.3-1.3]		0.7	[0.5-1.2]		0.7	[0.4-1.0]	
≥1 variant allele vs none	1.3	[0.9-1.9]		1.3	[0.9-1.9]		1.3	[0.9-1.9]	
Interaction history x allele	0.9	[0.3-2.9]	ns	0.9	[0.4-2.0]	ns	1.0	[0.5-2.2]	ns
IL10-rs1800896									
Medical history vs no	0.9	[0.3-2.6]		0.9	[0.4-2.2]		0.8	[0.4-1.7]	
≥1 variant allele vs none	1.1	[0.8-1.6]		1.2	[0.8-1.7]		1.2	[0.8-1.7]	
Interaction history x allele	0.7	[0.2-2.4]	ns	0.8	[0.3-1.9]	ns	0.8	[0.4-2.0]	ns
IL10-rs1800871									
Medical history vs no	1.1	[0.5-2.3]		0.9	[0.6-1.5]		1.0	[0.6-1.5]	
≥1 variant allele vs none	1.2	[0.9-1.6]		1.2	[0.9-1.7]		1.3	[0.9-1.8]	
Interaction history x allele	0.3	[0.1-1.0]	0.04	0.6	[0.3-1.2]	ns	0.5	[0.2-1.0]	0.04
IL10-rs1800872									
Medical history vs no	1.1	[0.5-2.3]		0.9	[0.6-1.5]		1.0	[0.6-1.5]	
≥1 variant allele vs none	1.4	[1.0-1.8]		1.4	[1.0-1.9]		1.5	[1.0-2.0]	
Interaction history x allele	0.2	[0.1-0.8]	0.02	0.6	[0.3-1.3]	ns	0.5	[0.3-1.0]	0.06
IL10 TAT haplotype									
Medical history vs no	1.1	[0.5-2.3]		0.9	[0.6-1.5]		1.0	[0.6-1.5]	
≥1 variant allele vs none	1.2	[0.9-1.6]		1.2	[0.9-1.7]		1.3	[0.9-1.8]	
Interaction history x allele	0.3	[0.1-1.0]	0.04	0.6	[0.3-1.2]	ns	0.5	[0.2-1.0]	0.04
IL4R-rs1801275									
Medical history vs no	0.4	[0.2-0.8]		0.8	[0.5-1.3]		0.7	[0.4-1.0]	
≥1 variant allele vs none	0.8	[0.6-1.1]		0.9	[0.7-1.3]		0.9	[0.6-1.2]	
Interaction history x allele	3.7	[1.1-12.6]	0.04	0.8	[0.4-1.8]	ns	1.2	[0.6-2.4]	ns

Odds ratios (OR) and 95% confidence intervals (95%CI) estimated using a logistic regression model adjusted for a sex*age variable, maternal educational level, household socioprofessional category and birth order.
p int: p for interaction test; ns: p>0.10

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