

Original article

TLR-related pathway analysis: novel gene–gene interactions in the development of asthma and atopy

Background: The toll-like receptor (TLR)-related pathway is important in host defence and may be crucial in the development of asthma and atopy. Numerous studies have shown associations of TLR-related pathway genes with asthma and atopy phenotypes. So far it has not been investigated whether gene–gene interactions in this pathway contribute to atopy and asthma development.

Methods: One hundred and sixty-nine haplotype tagging single nucleotide polymorphisms (SNPs) of 29 genes (i.e. membrane and intracellular receptors, TLR4 or lipopolysaccharide-binding/facilitating proteins, adaptors, interleukin-1 receptor associated kinases, kinases, chaperone molecules, transcription factors and inhibitors) were analysed for single- and multilocus associations with atopy [total and specific immunoglobulin E (IgE) at 1–2 and 6–8 years] and asthma (6–8 years). A total of 3062 Dutch children from the birth cohorts PIAMA, PREVASC and KOALA (Allergenic study) were investigated. Chi-squared test, logistic regression and the data mining approach multifactor dimensionality reduction method (MDR) were used in analysis.

Results: Several genes in the TLR-related pathway were associated with atopy and/or asthma [e.g. *IL1RL1*, *BPI*, *NOD1*, *NOD2* and *MAP3K7IP1*]. Multiple, single associations were found with the phenotypes under study. MDR analysis showed novel, significant gene–gene interactions in association with atopy and asthma phenotypes (e.g. *IL1RL1* and *TLR4* with sIgE to indoor allergens and *IRAK1*, *NOD1* and *MAP3K7IP1* with asthma). Interestingly, gene–gene interactions were identified with SNPs that did not have an effect on their own.

Conclusion: Our unbiased approach provided suggestive evidence for interaction between several TLR-related pathway genes important in atopy and/or asthma development and pointed to novel genes.

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The hygiene hypothesis has been developed in response to data suggesting that the increase in allergic diseases such as asthma is caused by reduced exposure to microbial stimuli (1). Indeed, several studies have provided suggestive evidence that high microbial exposure (e.g. living on a farm) protects against the development of asthma and atopy (reviewed in 2).

Microbial exposure acts via the gatekeepers of host immunity, the toll-like receptors (TLRs), as the latter can detect microbial components or pathogen-associated molecular patterns (PAMPs). TLRs are expressed by antigen presenting cells such as macrophages and dendritic cells and by T-regulatory (T-reg) and epithelial cells. A well-known bacterial cell wall product is

lipopolysaccharide (LPS) that is commonly found in dust from many domestic and occupational sources. When LPS binds to TLR4, this results in an increase of T helper 1 (Th1) cell activating cytokines and activation of T-reg cells (3, 4).

As TLR4 activation and LPS exposure are possible protective factors of asthma and allergy, it is of interest to assess whether polymorphisms in this TLR-related pathway are associated with asthma and allergy. Indeed, asthma and atopic phenotypes have been associated with several single nucleotide polymorphisms (SNPs) in genes located in this TLR-related pathway, e.g. *TLR4* (5–8) itself, *CD14* (reviewed in 9, 10), interleukin (IL)-1 receptor associated kinase 3 (*IRAK3*) (11), *IL1RL1* (12, 13), nucleotide-binding oligomerization domain (*NOD1*) (14–16) and *NOD2* (17, 18). So far interactions between these genes have not been described. These gene–gene interactions can be investigated using multifactor dimensionality reduction (MDR), which is designed to translate high-dimensional genetic data into a single dimension (19) and furthermore selects SNPs in an unbiased way. Thus, it is possible to analyse genes in a biologically plausible pathway relevant for the development of asthma and atopy, yet without knowledge beforehand which genes will be important.

We hypothesized that next to *TLR4* and above mentioned genes, other genes in the TLR-related pathway are also important in atopy and asthma development and that these genes interact. We investigated this hypothesis in 3062 children of the Allergenic study (composed of three Dutch birth cohorts PIAMA, PREVASC and KOALA) (20).

Subjects and methods

Study populations

Allergenic consists of three similarly ascertained prospective Dutch birth cohorts, i.e. PIAMA, PREVASC and KOALA (20). Local medical ethics committees of participating institutes approved all studies. Written informed consent was obtained from all parents.

IgE measurements

Total serum IgE levels were determined at 1, 4 and 8 years in PIAMA; 1, 2 and 6 years in PREVASC and 1 and 2 years in KOALA and measured using radioimmunoassay (Sanquin Research, Amsterdam, The Netherlands) as described previously (20). Total IgE and sIgE measurements for egg, milk and indoor allergens (cat, dog and housedust mite) were clustered at 1–2 and 6–8 years. Total IgE was analysed in tertiles (see online repository).

Asthma definition

Asthma was investigated at 6 years in PREVASC and 8 years in PIAMA, defined as at least one episode of wheeze or dyspnoea in the last year and/or the use of inhaled steroids.

SNP selection and genotyping

Haplotype tagging SNPs were selected from the HapMap database (21) or from the Innate Immunity web site (22), depending on the largest number of SNPs with a minor allele frequency >0.1 available in each database. Additionally, the biomedical literature was screened for SNPs with known functional impact or association with asthma or atopy as described previously (20). Genomic DNA was extracted from buccal swabs or blood using standard methods (23) and amplified using REPLI-g UltraFast technology (Qiagen™, Hilden, Germany). Genotyping was performed by Competitive Allele-Specific PCR using KASPar™ genotyping chemistry, performed under contract by K-Biosciences (Herts, UK) with extensive quality control as described previously (20).

Statistical methods

All SNPs were analysed for Hardy–Weinberg equilibrium (HWE) in the cohort overall, using chi-squared statistics ($P > 0.01$). We used chi-squared tests to analyse whether genotypes were associated with elevated serum IgE levels (1–2 and 6–8 years), with sIgE for milk and egg (1–2 years), sIgE for indoor allergens (6–8 years) and asthma (6–8 years), using a co-dominant model. For SNPs with a $P < 0.10$, Akaike Information Criterion was evaluated to determine the best fitting genetic model (additive, dominant or recessive). Odds ratios (ORs) and 95% confidence intervals (CI) were calculated using logistic regression analysis. *IRAK1* is located on the X-chromosome and therefore analysed separately for boys and girls. Calculations were performed using SPSS 14.0 (SPSS Benelux BV, Gorinchem, the Netherlands) statistical software. The single SNP analysis was corrected for multiple testing using false discovery rate (FDR) ($P \leq 0.05$ is significant).

Gene–gene and gene–environment interactions were analysed using MDR (version 1.0.0), as described and used by us previously (24, 25) (online repository). Logistic regression analyses were performed to confirm significant two SNP interaction results from MDR analyses, if the interaction term was significant (in a multiplicative model) the best fitting genetic model, e.g. dominant or recessive was analysed. The significance of the average prediction error was calculated using permutation tests: a value of $P \leq 0.05$ was considered significant.

Results

Study population

Characteristics of the 3062 genotyped children are shown in Table 1. Children of non-Caucasian descent were excluded (5.7%).

SNP selection and genotyping

Twenty-nine TLR-related pathway genes were selected (Fig. 1), i.e. membrane and intracellular receptors (*TLR4*, *CD14*, *NOD1*, *NOD2* and *ITGB2*), TLR4 or LPS-binding/facilitating proteins (*LBP*, *BPI*, *PI3* and *HSPA1A*), adaptors (*MYD88*, *TIRAP*, *TICAM1*, *TICAM2* and *TRAF6*), *IRAKs*, kinases [*MAP3K7*, mitogen-activated protein kinase kinase kinase 7 interacting protein 1 (*MAP3K7IP1*) and *MAP3K7IP2*], chaperone molecules (*MD2*, *CDC37* and *HSPAA1*), transcription

Table 1. Characteristics of participating children in the allergenic birth cohort

Characteristics	PIAMA	PREVASC	KOALA	P-value
Participants in genetic study, number (% from total cohort)	1037 (25.0)	374 (49.8)	1,651 (58.1)	–
Ethnicity (% Dutch origin)	95.1	95.7	95.2	–
Boys (%)	51.2	49.2	50.6	0.80
Family history (%)				
Atopy mother	66.5	51.4	33.3	<0.001
Asthma mother	16.7	31.4	8.8	<0.001
Atopy father	31.6	47.7	36.4	<0.001
Asthma father	7.4	21.9	10.0	<0.001
Phenotypes				
Total IgE 1–2 years lowest tertile vs highest tertile (<i>n</i>)	136/132	115/112	290/259	0.80
Total IgE 6–8 years lowest tertile vs highest tertile (<i>n</i>)	236/236	72/71	–	0.94
sIgE to egg 1–2 years <0.35 µg/ml vs ≥0.35 µg/ml (<i>n</i>)	317/21	130/25	449/48	<0.01
sIgE to milk 1–2 years <0.35 µg/ml vs ≥0.35 µg/ml (<i>n</i>)	248/90	–	404/118	0.20
sIgE to indoor allergens 6–8 years <0.35 µg/ml vs ≥0.35 µg/ml (<i>n</i>)	521/192	100/32	–	0.30
Asthma 6–8 years no vs yes (<i>n</i>)	763/158	163/44	–	0.16

Bold: significant difference between the cohorts, not tested.

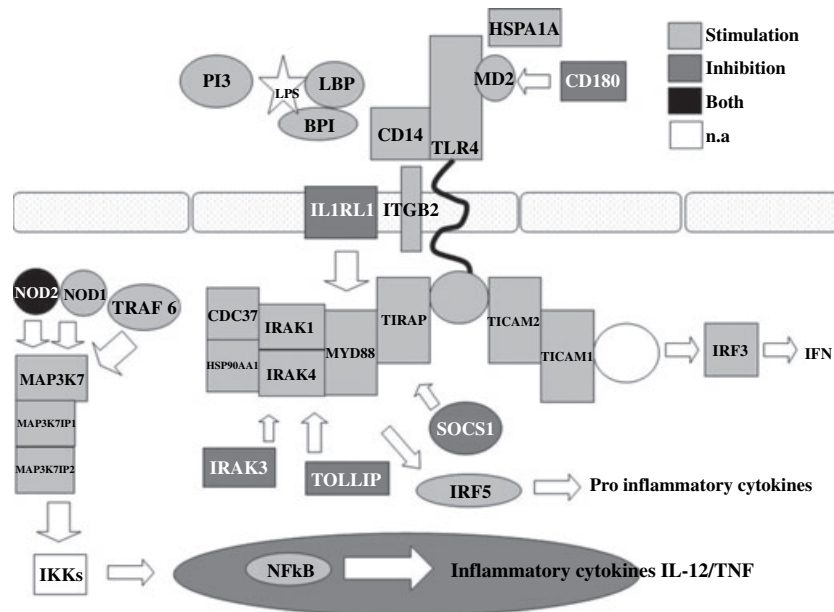


Figure 1. Schematic figure of the proteins involved in the TLR related pathway analyzed in this study. na: not analysed.

factors (*IRFs*) and inhibitors (*SOCS1*, *IL1RL1*, *TOLLIP* and *CD180*). Table 1 of online repository presents the function and location of the genes. The 29 genes were tagged by 169 SNPs (Table 2 of online repository) 10 SNPs deviated from HWE ($P < 0.01$) and were excluded from further analysis.

Single SNP analysis

Total IgE. Single nucleotide polymorphisms associated with total IgE at 1–2 years and total IgE at 6–8 years are shown in Table 3 of online repository.

The minor allele of an SNP in *IRAK4* associated with a reduced risk for high total IgE at both 1–2 and 6–8 years rs4251520, recessive model, ORs and (95% CI) 0.2 (0.0–

0.9) and 0.1 (0.0–0.9) respectively. Single nucleotide polymorphisms located in *NOD2*, *ITGB2*, *IL1RL1* and *TOLLIP* associated with total IgE at 1–2 and 6–8 years, but with different SNPs within the genes.

Specific IgE. Single nucleotide polymorphisms associated with sIgE to egg at 1–2 years, milk at 1–2 years and indoor allergens at 6–8 years are shown in Table 3 of online repository.

The minor allele of SNP rs1927911 in *TLR4* associated with increased sIgE to both egg and milk, ORs (95% CI) being 2.6 (1.3–5.3) and 1.8 (1.0–3.2), respectively, in a recessive model. Single nucleotide polymorphisms located in *NOD1* were associated with both sIgE to egg and milk at 1–2 years, but with different SNPs within the gene. Differ-

ent SNPs located in *TOLLIP* associated with sIgE to egg at 1–2 years and sIgE to indoor allergens at 6–8 years and different SNPs located in *ITGB2* and *TIRAP* associated with sIgE to milk at 1–2 years and sIgE to indoor allergens at 6–8 years. Finally, multiple SNPs located in *BPI* associated with sIgE to egg and milk at 1–2 years and sIgE to indoor allergens at 6–8 years. The minor allele of SNP rs1341023 in *BPI* was associated with increased sIgE to both egg and milk, ORs (95% CI) 2.1 (1.3–3.5) and 1.8 (1.3–2.6), respectively, in a recessive model. The minor allele of SNP rs6024876 in *BPI* was associated with increased sIgE to milk at 1–2 years and indoor allergens at 6–8 years, ORs (95% CI) being 1.4 (1.0–2.0) and 1.5 (1.1–2.0), respectively, in a dominant model.

Asthma. Table 3 of online repository shows SNPs associated with asthma. The strongest associations were found with SNPs located in *MAP3K7IP1* [e.g. rs6001585, OR (95% CI) 0.5 (0.3–0.7), dominant model].

FDR analysis

None of the above described associations remained significant after correction for multiple testing ($P \leq 0.05$).

MDR analyses (2 and 3 SNP interaction models)

Table 2 shows results of MDR for 2 and 3 SNP interaction models.

Total IgE

High total IgE at 1–2 years

2 SNP interaction. SNPs in *IL1RL1* and *BPI* were selected as the best model ($P = 0.02$). This was confirmed using logistic regression. Using a co-dominant model for rs17027006 (*IL1RL1*) and rs5741800 (*BPI*), both heterozygotes had a decreased risk for high total IgE at 1–2 years (P -value interaction term 0.03). **3 SNP interaction;** SNPs located in *MD2*, *ITGB2* and *BPI* were selected as the best model, ($P = 0.01$).

High total IgE at 6–8 years

2 SNP interaction. SNPs located in *IL1RL1* and *NOD1* were selected as best model ($P = 0.09$). This interaction was not significant in logistic regression analysis. **3 SNP interaction;** SNPs located in *MD2*, *MAP3K7IP2* and *BPI* were selected as the best model ($P = 0.001$).

Specific IgE

sIgE to egg at 1–2 years

2 SNP interaction. SNPs located in *IL1RL1* and *MAP3K7IP1* were selected as the best model ($P = 0.04$).

Table 2. Results of MDR analysis evaluating 29 TLR-related signalling genes

SNP*	Best model		Mean CV† consistency	Mean prediction error (%)	P-value‡
	Gene(s)	SNP(s)			
Total IgE 1–2 years					
2	<i>IL1RL1</i>	rs17027006	3.6	42.8	0.02
	<i>BPI</i>	rs5741800			
3	<i>MD2</i>	rs11786591	2.6	42.2	0.01
	<i>ITGB2</i>	rs2235133			
	<i>BPI</i>	rs5741800			
Total IgE 6–8 years					
2	<i>IL1RL1</i>	rs10208293	2.2	45.4	0.09
	<i>NOD1</i>	rs2075818			
3	<i>MD2</i>	rs11786591	2.0	37.7	0.001
	<i>MAP3K7IP2</i>	rs562824			
	<i>BPI</i>	rs5741798			
sIgE egg 1–2 years					
2	<i>IL1RL1</i>	rs1921622	2	42.8	0.04
	<i>MAP3K7IP1</i>	rs6001587			
3	<i>IL1RL1</i>	rs11685480	3.4	37.6	0.02
	<i>BPI</i>	rs4358188			
	<i>ITGB2</i>	rs4607021			
sIgE milk 1–2 years					
2	<i>MAP3K7IP2</i>	rs562428	3.6	41.4	0.01
	<i>BPI</i>	rs1341023			
3	No best model present				
sIgE indoor allergens 6–8 years					
2	<i>IL1RL1</i>	rs10208293	2.4	41.5	0.03
	<i>TLR4</i>	rs4986790			
3	<i>BPI</i>	rs1341023	1.4	40.5	0.005
	<i>ITGB2</i>	rs235331			
	<i>TIRAP</i>	rs8177376			
Asthma 6–8 years					
2	<i>SOC1</i>	rs4780355	2.8	41.1	0.008
	<i>MAP3K7IP1</i>	rs6001585			
3	<i>IRAK1</i>	rs1059701	1.6	39.8	0.002
	<i>NOD1</i>	rs2907749			
	<i>MAP3K7IP1</i>	rs6001585			

MDR, multifactor dimensionality reduction; TLR, toll-like receptor; SNP, single nucleotide polymorphism; sIgE, specific immunoglobulin E.

*Number of SNPs in model.

†CV = cross-validation, after exclusion of SNPs with $D^* > 0.8$.

‡Significance of prediction error (empirical P -value based on 1000 permutations).

and confirmed using logistic regression ($P = 0.01$). Carriers of both the G allele of rs1921622 (*IL1RL1*) and CC genotype of rs6001587 (*MAP3K7IP1*) had a higher risk of sIgE to egg. **3 SNP interaction;** SNPs located in *IL1RL1*, *BPI* and *ITGB2* were selected as the best model ($P = 0.02$).

Specific IgE to milk at 1–2 years

2 SNP interaction. SNPs located in *MAP3K7IP2* and *BPI* were selected as the best model ($P = 0.01$), but not confirmed using logistic regression analysis. **3 SNP interaction;** 3 SNP combinations did not provide a best model, as all five imputed data sets showed a different model.

Specific IgE to indoor allergens at 6–8 years

2 SNP interaction. SNPs located in *IL1RL1* and *TLR4* were selected as the best model ($P = 0.03$) and confirmed using logistic regression. Carrying the combination of the AA genotype of the functional SNP rs4986790 (*TLR4*) and A allele of rs10208293 (*IL1RL1*) in a dominant model decreased the risk (P -value interaction term 0.008; Fig. 2). **3 SNP interaction;** SNPs located in *BPI*, *ITGB2* and *TIRAP* were selected as the best model ($P = 0.005$).

Asthma

2 SNP interaction. SNPs located in *SOCS1* and *MAP3K7IP1* were selected as the best model ($P = 0.008$), which was not confirmed by logistic regression. **3 SNP interaction:** SNPs located in *IRAK1*, *NOD1* and *MAP3K7IP1* were selected as the best model ($P = 0.002$).

Overall conclusions regarding results of the analysis

Several genes important in the development of atopy and/or asthma were identified (e.g. *IL1RL1*, *BPI*, *NOD1*, *NOD2* and *MAP3K7IP1*). Multiple single SNPs were found to be associated with high total IgE at 1–2 and 6–8 years, and the presence of sIgE to egg, milk or indoor allergens and asthma, findings that did not remain significant after correction for multiple testing.

Multifactor dimensionality reduction analysis showed several 2 and 3 SNP interactions that were significant after permutation testing. Moreover, SNPs without an

effect when analysed as single SNP appeared to contribute significantly to atopy and asthma development in combination with other SNPs (see Fig. 3A,B for IgE at 1–2 years and IgE at 6–8 years; e.g. *MD2*, *BPI* and *MAP3K7IP2*).

Discussion

This investigation of the prospective Dutch Allergenic study identified novel gene-gene interactions of TLR-related pathway genes in association with the development of asthma and atopy, findings that were corroborated by their significance after permutation testing. We confirmed some previously found single SNP associations and additionally describe several novel single SNP associations with TLR-related pathway genes, although these single SNP analyses did not remain significant after correction for multiple testing. Interestingly, significant gene-gene interactions occurred even with SNPs not showing an effect on their own in single SNP analyses.

In the last two decades, multiple asthma and atopy genes have been found with generally moderate effects (26). It has become clear that interaction among SNPs in candidate genes may enhance the risk to develop atopic diseases (27–29). To our knowledge, we are the first to describe gene-gene interaction of TLR-related pathway genes in the development of asthma and atopy in an unbiased approach. We show that some gene-gene combinations increase the risk, whereas others reduce the risk for atopy or asthma development.

Several interesting genes have come forward. First, SNPs in *IL1RL1* were associated with total IgE levels in both age groups, and the SNPs showed up in the best MDR model of sIgE to egg (1–2 years) and indoor allergens (1–2 years) as well. Current results support our previous findings in two independent adult populations that strong associations exist between *IL1RL1* SNPs and atopy and asthma phenotypes (12). *IL1RL1* is the receptor for IL-33 which is located on mast cells, Th2 cells, regulatory T cells and macrophages and is also present in serum in a soluble form (30–33). There is increasing evidence that this gene is important in atopic diseases like eczema and asthma (12, 13). Studies have shown that *IL1RL1* exerts its role via TLR pathways and various forms of *IL1RL1* can either stimulate or inhibit Th2 responses (34–37). Functional *in vivo* studies show that administration of IL-33 stimulates Th2 cell production of IL-13, IL-4 and IL-5. Furthermore, intraperitoneal administration of IL-33 increases blood eosinophil numbers and IgE levels in addition to eosinophilic inflammation of the airways in mice (37). In this study, we provide supportive evidence that *IL1RL1* is important in the development of atopic phenotypes, and that gene-gene interaction with other SNPs in the TLR-related pathway contributes to this effect.

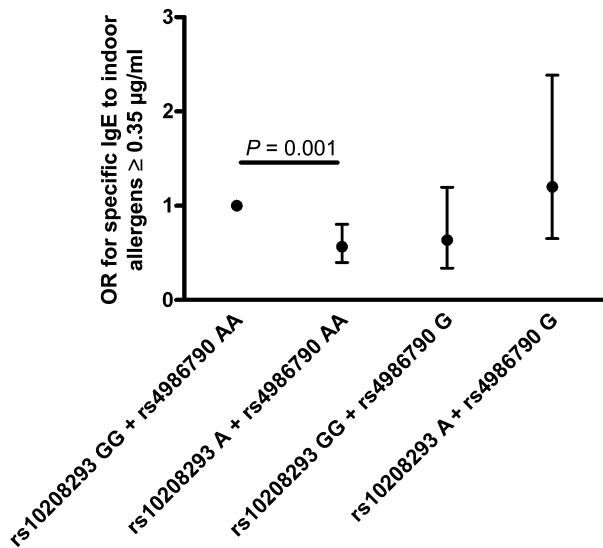


Figure 2. Association of genes in the TLR related pathway and specific IgE to indoor allergens: *IL1RL1* and *TLR4* Best 2 SNP model for sIgE for indoor allergens ≥ 0.35 $\mu\text{g/ml}$. OR (odds ratio) and 95% confidence interval are shown, rs10208293 GG and rs4986790 AA is reference category.

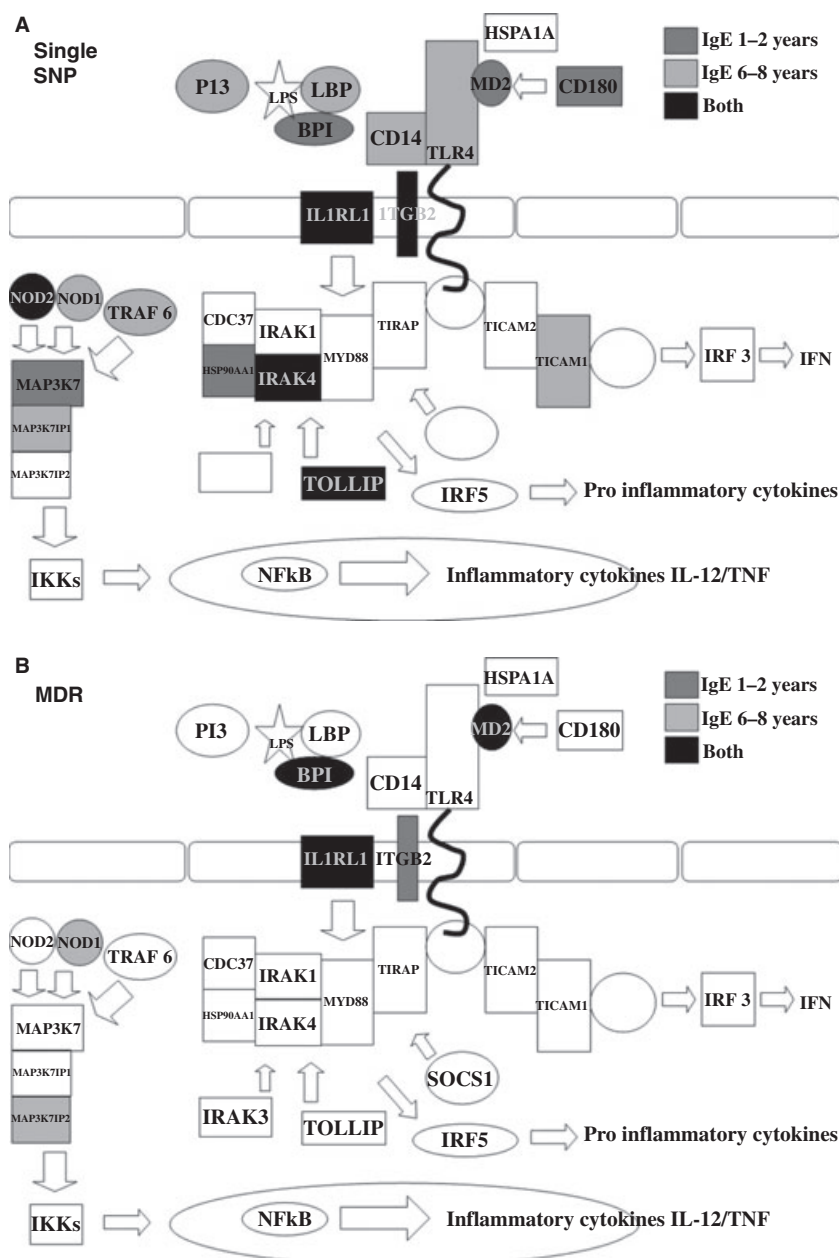


Figure 3. Comparison of single SNP (A) and MDR analysis (B) for total IgE at age 1–2 and 6–8 years and at both age groups. *MD2*, *BPI* and *MAP3K7IP2* did not come forward in the single SNP analysis for total IgE at age 6–8. However, MDR analysis shows an important effect for these genes with respect to the development of total IgE. Light Grey: Genes associated with total IgE at age 1–2 years; Dark Grey: Genes associated with total IgE at age 6–8 years; Black: Genes associated with both IgE at age 1–2 and IgE at age 6–8 years.

A second gene of interest is *BPI*, as SNPs in this gene are associated with total IgE and sIgE at 1–2 years, and *BPI* SNPs are selected using MDR with all atopic phenotypes under study. *BPI* is a polypeptide with antimicrobial (especially Gram-negative bacterial) capacities by its properties to damage Gram-negative bacterial membranes. *BPI* competes with LBP for binding to LPS and consequently inhibits LPS–LBP binding to the TLR4–CD14 complex (38). This inhibition of LPS signalling was

confirmed clinically in that *BPI* administration decreases mortality of children with severe meningococcal disease (39). An SNP located in *BPI* has been associated previously with Crohn's disease (40), and we are the first to report its association with atopic phenotypes.

The *NOD1* and *NOD2* both function as intracellular PAMP receptors (41). Single nucleotide polymorphisms located in *NOD1* and *NOD2* associated with autoimmune diseases like Crohn's disease and rheumatoid arthritis

(42–44). The previous studies have shown that carriers of the major alleles of *NOD1* SNPs (rs2907748 CC and rs2907749 AA, respectively) were at risk for developing high IgE levels (OR 3.07 and 1.63), in addition to preferential transmission of the rs2907749 A allele to asthma affected children (15). We show that the minor alleles of these SNPs protect towards the development of sIgE to egg (single SNP analysis) and that rs2907749 plays a role in asthma development (MDR analysis), thereby replicating the previous results. The association with different phenotypes (sIgE to egg vs total IgE) and the additional association of rs2075818 in our population may be due to the difference in populations and/or unknown environmental exposures. Single nucleotide polymorphisms in *NOD2* have been associated with asthma and atopic phenotypes in two earlier studies (17, 18). We also found association with some of these *NOD2* SNPs (e.g. rs313550 and rs5743266), however, with different alleles and different atopic phenotypes which is classified as loose replication (45).

Some different genes in the TLR-related pathway became apparent when investigating asthma and atopy. Mitogen-activated protein kinase kinase kinase 7 interacting protein 1 (*MAP3K7IP1*) also known as TAB1 is a kinase which forms a complex with *MAP3K7IP2* and *MAP3K7* (also known as TAB2 and TAK1, respectively) and regulates the transcription factor NF κ B after TRAF6 activation (46). In this way, *MAP3K7IP1* plays a role in TLR signalling and SNPs located in *MAP3K7IP1* might alter its signalling function. This gene is also important in the inhibition of transforming growth factor (TGF)- β -induced gene expression (47). As TGF- β contributes to airway remodelling in asthma (48), it seems plausible that SNPs in *MAP3K7IP1* may influence TGF- β inhibition. We are the first to point out the importance of *MAP3K7IP1* in the development of asthma; further genetic and functional studies have to confirm our results.

Finally, an intriguing interaction became apparent between rs10208293 (*IL1RL1*) and rs4986790 (*TLR4*) with respect to sIgE to indoor allergens. The functionality of SNP rs10208293 (*IL1RL1*) is unknown with respect to increased or decreased expression of *IL1RL1*. Carrying of the G allele of the functional *TLR4* SNPs rs4986790 has been associated with hyporesponsiveness and reduced Th1 cytokine production in response to inhaled endotoxin (49). We show that carrying the AA genotype of rs4986790 combined with the minor allele of rs10208293 indeed decreases the risk to develop high sIgE to indoor allergens. Biologically, this can be achieved by enhanced TLR4 signalling and subsequently Th1 cell activation in individuals with these SNP combinations.

This study has some limitations, first no single SNP analysis was significant after multiple testing corrections, and one can argue that our observations were found by chance. However, most MDR results were statistically significant after 1000 permutations, convincing us that a

type 1 error did not likely occur. We wish to emphasize that this study is an unbiased, data mining approach and other studies have to replicate our findings. Furthermore, we pooled three different birth cohorts (PIAMA, PREVASC and KOALA) and reported previously (20) that the three cohorts were recruited and phenotyped in a similar way and only Caucasian children were entered in the analysis. Moreover, we previously published that the cohorts showed similar results when analysed separately for genes with a larger effect (20). Nevertheless, we cannot fully rule out population stratification. The genes selected for analysis were based on literature available till October 2005, we acknowledge that more genes are located in these pathways.

In conclusion, we identified several genes important in the development of atopy and/or asthma (e.g. *IL1RL1*, *BPI*, *NOD1*, *NOD2* and *MAP3K7IP1*). This is the first report on gene–gene interactions of TLR-related pathway genes, providing supportive evidence that multiple genes in this pathway contribute to asthma and atopy development. Of interest for the field of genetic research is the observation that several SNPs without an effect on their own come forward significantly in gene–gene interactions. Future studies have to replicate our findings and investigate whether environmental factors act on different SNPs, whereas functional studies have to investigate the exact biological mechanism of these gene–gene interactions.

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Web resources

HapMap database URL: <http://www.hapmap.org>; data release 19, phase II, October 2005.

Innate Immunity web site. Innate Immunity PGA. NHLBI Program in Genomic Applications URL: <http://www.innateimmunity.net/data/homology>; October 2005.

K-Biosciences, Cambridge, UK URL: <http://www.kbiosciences.co.uk>

Competing interests

There are no competing interests.

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TLR related pathway analysis: novel gene-gene interactions in the development of asthma and atopy

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Multifactor Dimensionality Reduction (MDR)

MDR is a method that reduces dimensionality of multifactor information and aims to improve the identification of multiple factors associated with disease, i.e. MDR reduces multifactor information into one dimension, i.e. high versus low risk. MDR reports the performance of (combination of) SNPs that represent the best predictor model for each number of (e.g. 2 or 3) combinations. The dataset is divided into a trainingset (9/10 of the data) and a testing set (1/10 of the data). The best predictor model is the model with the lowest prediction error in the training set. The final prediction error is calculated using the testing set. This procedure is repeated 10 times in the dataset. Cross-validation consistency is the number of time the combination is selected as best predictor model in the 10 times the data is analysed. Missing data were imputed for missing genotypes by multiple imputations using the Multivariate Imputation by Chained Equations (Mice) procedure that runs under the statistical program R version 2.4.1. After 100 imputations, convergence was achieved resulting in 5 imputed datasets. The model with the highest cross validation consistency and lowest prediction error in the 5 imputed datasets was selected as “best model”. To examine the significance of testing accuracies, we determined 1000 MDR analyses for each test after permutation (200 of each imputation file). The null-hypothesis was rejected when the one-sided p-value, as estimated by 1000 permutations in Monte Carlo simulation, was 5%. In that case the “best model” predicts the status of cases and controls better than chance without the need to correct for multiple testing.

The haplotype tagging SNPs and gender were included in the MDR analysis. Best models were computed for 2 and 3 SNPs. To exclude the possibility that low cross validation consistency was caused by SNPs that were highly correlated with SNPs present in the best

model, MDR was performed a second time and SNPs that showed strong LD ($D' > 0.8$) with SNPs in the best model were excluded from analyses.

Total IgE tertiles

Cases and controls were defined as children with a serum IgE level in the highest, respectively the lowest tertile, as estimated at each age in boys and girls separately. We clustered measurements from ages 1 and 2 years, and 6 and 8 years. In the subgroup of children with IgE measurements available at both 1 and 2 years, the highest and lowest tertiles were determined from the mean of the two measurements.

Specific IgE measurements

Positive specific IgE was defined as 1) specific IgE > 0.35 $\mu\text{g/ml}$ for milk at age 1 and 2 years (PIAMA at age 1, Koala at age 1 or 2 years), 2) specific IgE > 0.35 $\mu\text{g/ml}$ for egg at age 1 and 2 years (PIAMA at age 1, PREVASC and KOALA at age 1 or 2 years), 3) specific IgE > 0.35 $\mu\text{g/ml}$ for house dust mite, dog or cat (indoor allergens) at age 6 and 8 (PREVASC at age 6, PIAMA at age 8 years).

Table 1. Gene nomenclature, location and function of all 29 TLR related pathway genes.

HUGO gene nomenclature	Full name	Location	Function in TLR related pathway	
			Receptors	
TLR4	Toll like receptor 4	9q32		Transmembrane receptor that binds PAMPs (LPS a.o.). Activation causes release of antimicrobial peptides, inflammatory cytokines, chemokines, and costimulatory molecules. ¹
NOD2	Nucleotide binding oligomerisation domain 2	16q21		Intracellular PAMP receptor, can crosstalk with TLR pathway ²
NOD1	Nucleotide binding oligomerisation domain 1	7p15		Intracellular PAMP receptor, can crosstalk with TLR pathway ²
CD14		5q31		Surface membrane receptor binds LPS-LBP complex and facilitates its binding to TLR4. ³
ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	21q22		LPS binding protein, synergises with CD14 ⁴
			TLR4 or LPS binding/facilitating proteins	
LBP	Lipopolysaccharide Binding Protein	20q11		Extracellular protein binds LPS to facilitate binding to CD14-TLR4-LY96 complex. ⁵
MD2	myeloid differentiation protein-2	8q21		Binds to TLR4 and allows the forming of a stable receptor complex, also known as LY96 ⁶
BPI	bactericidal/permeability-increasing protein	20q11		BPI competes with LBP for binding to LPS and promotes CD14-independent delivery to dendritic cells. ⁷
PI3	Peptidase Inhibitor 3	20q12		LPS detoxification by binding to LPS or activation of innate immune responses. ⁸
HSPA1A	Heat shock 70 kda protein 1A	6p21		Up regulates TLR4. ⁹
			Adaptors¹⁰	
TIRAP	Toll-interleukin 1 receptor (TIR) domain containing adaptor protein	11q24		intracellular adaptor molecule
MYD88	myeloid differentiation primary response gene 88	3p22		intracellular adaptor protein
TRAF6	TNF receptor-associated factor 6	11p12		intracellular adaptor protein

<i>TICAM1</i>	Toll like receptor adaptor molecule 1	19p13		MYD88-independent intracellular adaptor molecule
<i>TICAM2</i>	Toll like receptor adaptor molecule 2	5q23		Intracellular MYD88-independent adaptor molecule
			IL-1 receptor Associated kinases¹¹	
<i>IRAK1</i>	Interleukin-1 receptor-associated kinase 1	Xq28		Transmits intracellular TLR4 signalling
<i>IRAK3</i>	Interleukin-1 receptor-associated kinase 3	12q14		Intracellular regulatory protein, inhibits TLR4 signalling by inhibiting IRAK4 and IRAK1
<i>IRAK4</i>	Interleukin-1 receptor-associated kinase 4	12q12		Transmits intracellular TLR4 signalling by activating IRAK1
			Kinases¹²	
<i>MAP3K7</i>	mitogen-activated protein kinase kinase kinase 7	6q16		TAK1 is activated by TRAF6 and mediates IKK and MAPK pathways
<i>MAP3K71P1</i>	mitogen-activated protein kinase kinase kinase 7 interacting protein 1	22q13		Downstream signalling protein forms a kinase complex with TRAF6, MAP3K7 and MAP3K7IP2, required for the activation of NFkB
<i>MAP3K71P2</i>	mitogen-activated protein kinase kinase kinase 7 interacting protein 2	6q25		Downstream signalling protein forms a kinase complex with TRAF6, MAP3K7 and MAP3K7IP1, required for the activation of NFkB
			Transcription factors¹³	
<i>IRF3</i>	interferon regulatory factor 3	19q13		TLR4-MYD88 independent pathway transcription factor, activates transcription of target genes like type 1 interferons
<i>IRF5</i>	interferon regulatory factor 5	7q32		TLR4 MYD88 dependent pathway transcription factor, induces expression of pro inflammatory cytokines
			Chaperone molecules¹⁴	
<i>CDC37</i>	cell division cycle 37 homolog	19p13		Intracellular chaperone of IRAK1
<i>HSP90AA1</i>	heat shock protein 90kDa alpha (cytosolic), class A member 1	14q32		Intracellular chaperone of IRAK1
			Inhibitors¹	
<i>CD180</i>		5q12		Inhibits binding of LPS to TLR4-LY96 complex
<i>IL1RL1</i>	Interleukin 1 receptor like 1	2q12		Membrane and soluble receptor, inhibits TLR4 signalling via inhibition of MYD88 and TIRAP
<i>SOCS1</i>	suppressor of cytokine signalling 1	16p13		Intracellular protein, inhibits TLR4 signalling by inhibiting TIRAP

<i>TOLLIP</i>	Toll interacting protein	11p15		Intracellular protein, inhibits TLR4 signalling by inhibiting IRAK ¹⁵
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Table 2. Selected haplotype tagging SNPs of all 29 TLR related pathway genes.

SNP	Gene	Minor Allele frequency	HW	Call rate (%)
rs1341023	<i>BPI</i>	45.7		97.0
rs2154374	<i>BPI</i>	43.5		97.1
rs4358188	<i>BPI</i>	49.9		97.2
rs5741798	<i>BPI</i>	17.2		98.4
rs5741800	<i>BPI</i>	45.9		95.2
rs5741806	<i>BPI</i>	36.7		96.9
rs5743507	<i>BPI</i>	12.1		98.6
rs6024718	<i>BPI</i>	18.9		98.0
rs6024876	<i>BPI</i>	26.3		96.9
rs6069757	<i>BPI</i>	43.7		98.0
rs6099053	<i>BPI</i>	11.3	<0.01	98.5
rs6127776	<i>BPI</i>	31.5		98.7
rs1077861	<i>CARD15</i>	31.2		97.7
rs2067085	<i>CARD15</i>	38.8		97.4
rs3135500	<i>CARD15</i>	39.3		96.7
rs5743266	<i>CARD15</i>	32.2		98.1
rs5743289	<i>CARD15</i>	20.1		98.1
rs5743291	<i>CARD15</i>	9.1		98.7
rs2066847	<i>CARD15</i>	0.0		99.1
rs17221417	<i>CARD15</i>	32.5		96.6
rs2066845	<i>CARD15</i>	02.1		98.7
rs2066844	<i>CARD15</i>	05.5		97.5
rs2075818	<i>CARD4</i>	23.5		97.7
rs2075822	<i>CARD4</i>	21.6		97.1
rs2529440	<i>CARD4</i>	45.2	<0.01	96.8
rs2907748	<i>CARD4</i>	25.2		97.4
rs2907749	<i>CARD4</i>	28.9		98.0
rs6949758	<i>CARD4</i>	24.9		98.1
rs7789045	<i>CARD4</i>	45.1		98.2
rs10267377	<i>CARD4</i>	27.3	<0.01	86.2
rs1558066	<i>CARD4</i>	17.8	<0.01	98.1
rs932272	<i>CARD4</i>	39.2		95.2
rs2563298	<i>CD14</i>	27.3		98.4
rs2569190	<i>CD14</i>	47.9		97.6
rs2569191	<i>CD14</i>	48.2		98.3
rs2915863	<i>CD14</i>	40.5		94.3
rs5744455	<i>CD14</i>	23.1		96.7
rs3756561	<i>CD180</i>	6.3		98.2
rs5744463	<i>CD180</i>	10.4		98.6
rs5744464	<i>CD180</i>	07.8		97.8
rs3733912	<i>CD180</i>	22.6		98.1
rs11879191	<i>CDC37</i>	20.3		97.5
rs12982179	<i>CDC37</i>	30.1		97.1
rs1190592	<i>HSP90AA1</i>	20.6		97.9
rs11621560	<i>HSP90AA1</i>	35.2		97.3
rs1008438	<i>HSPA1A</i>	40.7		95.7
rs1043618	<i>HSPA1A</i>	39.2		97.3

rs1420102	<i>IL1RL1</i>	49.1		95.4
rs1861246	<i>IL1RL1</i>	22.5		96.0
rs1921622	<i>IL1RL1</i>	45.0		96.0
rs6543115	<i>IL1RL1</i>	24.8		97.7
rs6543116	<i>IL1RL1</i>	25.0		96.7
rs6719130	<i>IL1RL1</i>	14.0		98.4
rs12712142	<i>IL1RL1</i>	40.7		97.2
rs12999364	<i>IL1RL1</i>	40.6		97.7
rs13424006	<i>IL1RL1</i>	36.5		96.6
rs17027006	<i>IL1RL1</i>	30.2		93.9
rs17696376	<i>IL1RL1</i>	9.1		96.6
rs1041973	<i>IL1RL1</i>	19.7		96.8
rs10208293	<i>IL1RL1</i>	26.7		98.1
rs11685480	<i>IL1RL1</i>	49.1		98.2
rs1059701	<i>IRAK1</i>	19.0		98.0
rs1059703	<i>IRAK1</i>	14.0		97.0
rs2239673	<i>IRAK1</i>	19.0		97.2
rs1168643	<i>IRAK3</i>	34.4		95.7
rs1732886	<i>IRAK3</i>	30.0		97.9
rs3782347	<i>IRAK3</i>	29.2		97.9
rs1152912	<i>IRAK3</i>	42.4		95.8
rs1168757	<i>IRAK3</i>	8.1		91.0
rs1461567	<i>IRAK4</i>	29.3	<0.01	97.0
rs4251513	<i>IRAK4</i>	49.4		97.2
rs4251520	<i>IRAK4</i>	11.8		98.3
rs2304207	<i>IRF3</i>	13.7		98.4
rs2304204	<i>IRF3</i>	24.1		97.0
rs7808907	<i>IRF5</i>	48.9		96.7
rs752637	<i>IRF5</i>	39.2		96.9
rs180318	<i>ITGB2</i>	40.1		95.9
rs235331	<i>ITGB2</i>	23.3		96.2
rs760456	<i>ITGB2</i>	31.7		95.7
rs760458	<i>ITGB2</i>	23.4		97.7
rs1474552	<i>ITGB2</i>	8.6		97.3
rs2235133	<i>ITGB2</i>	46.9		97.0
rs2838732	<i>ITGB2</i>	18.5		98.5
rs2838733	<i>ITGB2</i>	27.6		97.3
rs2838735	<i>ITGB2</i>	42.7		95.4
rs2838737	<i>ITGB2</i>	15.0		96.8
rs3746972	<i>ITGB2</i>	18.5		97.8
rs3788150	<i>ITGB2</i>	16.0		98.2
rs3788151	<i>ITGB2</i>	6.4		98.5
rs5030671	<i>ITGB2</i>	25.7		96.6
rs684	<i>ITGB2</i>	21.6		97.4
rs235325	<i>ITGB2</i>	42.2		96.1
rs4607021	<i>ITGB2</i>	39.7		97.4
rs235326	<i>ITGB2</i>	33.0		95.3
rs4818740	<i>ITGB2</i>	12.6		97.2
rs2329940	<i>ITGB2</i>	12.7		98.5
rs745144	<i>LBP</i>	43.2		97.6
rs1780616	<i>LBP</i>	33.4		96.6
rs2232596	<i>LBP</i>	49.8		96.8

rs5741812	<i>LBP</i>	12.6		97.5
rs6025083	<i>LBP</i>	45.1		96.9
rs11536945	<i>LBP</i>	24.3		98.6
rs11536949	<i>LBP</i>	7.1		99.0
rs11536988	<i>LBP</i>	4.5		98.6
rs7838114	<i>MD2</i>	19.4		97.6
rs10808798	<i>MD2</i>	47.5		97.6
rs17226566	<i>MD2</i>	23.4		97.6
rs11786591	<i>MD2</i>	33.0		96.5
rs16938758	<i>MD2</i>	18.5		97.1
rs157692	<i>MAP3K7</i>	31.2		95.9
rs1144159	<i>MAP3K7</i>	15.6		96.0
rs1145727	<i>MAP3K7</i>	29.4		96.7
rs13208824	<i>MAP3K7</i>	13.7		96.8
rs3799912	<i>MAP3K7</i>	10.0		97.7
rs1972280	<i>MAP3K7IP1</i>	26.5		96.3
rs5750824	<i>MAP3K7IP1</i>	18.0		97.6
rs6001585	<i>MAP3K7IP1</i>	19.8		97.9
rs9611174	<i>MAP3K7IP1</i>	15.1		96.3
rs6001587	<i>MAP3K7IP1</i>	31.1		97.2
rs237012	<i>MAP3K7IP2</i>	49.9		96.9
rs237033	<i>MAP3K7IP2</i>	11.3		97.7
rs480526	<i>MAP3K7IP2</i>	10.4		97.5
rs562428	<i>MAP3K7IP2</i>	37.1		97.2
rs2789490	<i>MAP3K7IP2</i>	11.5	<0.01	95.3
rs6932778	<i>MAP3K7IP2</i>	23.4		97.4
rs6853	<i>MYD88</i>	12.1		97.2
rs7744	<i>MYD88</i>	13.6		98.4
rs1983649	<i>PI3</i>	41.5		97.4
rs2664581	<i>PI3</i>	17.6		98.1
rs6032040	<i>PI3</i>	15.9		98.4
rs193779	<i>SOCS1</i>	21.8	<0.01	98.5
rs4780355	<i>SOCS1</i>	30.3		97.7
rs419939	<i>TICAM2</i>	29.2		95.6
rs2052834	<i>TICAM2</i>	36.9		96.8
rs2559990	<i>TICAM2</i>	49.7		97.5
rs698365	<i>TICAM2</i>	46.0		95.3
rs625413	<i>TIRAP</i>	22.0		97.4
rs1786704	<i>TIRAP</i>	19.6		98.0
rs1893352	<i>TIRAP</i>	16.3		97.7
rs8177375	<i>TIRAP</i>	12.2		96.8
rs8177376	<i>TIRAP</i>	23.9		97.9
rs4986790	<i>TLR4</i>	7.0		98.2
rs2770150	<i>TLR4</i>	26.9		96.8
rs6478317	<i>TLR4</i>	34.8		98.3
rs10759932	<i>TLR4</i>	12.8		96.4
rs11536878	<i>TLR4</i>	12.3		97.0
rs11536889	<i>TLR4</i>	13.8		97.7
rs4986791	<i>TLR4</i>	7.2		98.0
rs1927911	<i>TLR4</i>	25.4		92.6
rs10759931	<i>TLR4</i>	43.1	<0.01	97.0
rs3793966	<i>TOLLIP</i>	47.5		96.4

rs3793969	<i>TOLLIP</i>	46.1		97.4
rs4963060	<i>TOLLIP</i>	28.6		97.7
rs5743854	<i>TOLLIP</i>	12.3	<0.01	98.4
rs5743859	<i>TOLLIP</i>	22.6		97.5
rs5743890	<i>TOLLIP</i>	14.3		98.3
rs5743894	<i>TOLLIP</i>	16.4		98.5
rs5743902	<i>TOLLIP</i>	26.8		97.3
rs5743915	<i>TOLLIP</i>	43.9		97.7
rs5743944	<i>TOLLIP</i>	26.2		97.6
rs5743955	<i>TOLLIP</i>	4.0		98.0
rs5743973	<i>TOLLIP</i>	3.2	<0.01	93.7
rs5743987	<i>TOLLIP</i>	5.8		96.4
rs5744038	<i>TOLLIP</i>	6.1		98.7
rs5744034	<i>TOLLIP</i>	16.7		96.9
rs2303439	<i>TRAF6</i>	15.5		98.0
rs5030419	<i>TRAF6</i>	12.9		98.3
rs8120	<i>TRIF</i>	25.8		97.9
rs1046673	<i>TRIF</i>	15.3		98.1

HW: Hardy Weinberg Equilibrium; if <0.01 excluded from further analysis (n=10).
Call rate; percentage of DNA successfully genotyped.

Table 3

Single SNPs that are associated with increased serum IgE at age 1-2 or at age 6-8 years; with positive specific IgE for egg or milk at age 1-2, positive specific IgE for indoor allergens at age 6-8 or asthma at age 6-8 ($p < 0.05$).

Gene	Alleles*	Rs number	Total IgE 1-2 yrs [#]	Total IgE 6-8 yrs [#]	Specific IgE Egg 1-2 yrs [#]	Specific IgE Milk 1-2 yrs [#]	Specific IgE Indoor Allergens 6- 8 yrs [#]	Asthma 6-8 yrs [#]
Receptors								
<i>TLR4</i>	T/C	rs2770150		2.0 (1.1-3.8) R				
<i>TLR4</i>	A/G	rs6478317			1.8 (1.0-3.2) R			
<i>TLR4</i>	C/T	rs1927911		0.7(0.5-0.0) D	2.6 (1.3-5.3) R	1.8 (1.0-3.2) R		
<i>NOD2</i>	G/A	rs3135500					0.7 (0.5-0.9) D	
<i>NOD2</i>	G/A	rs5743266					1.5 (1.1-2.0) D	
<i>NOD2</i>	G/A	rs5743291	0.7 (0.5-1.0) D					
<i>NOD2</i>	C/G	rs17221417		1.8 (1.3-2.4) D			1.4 (1.0-1.9) D	
<i>NOD1</i>	C/G	rs2075818		0.7 (0.5-0.9) D				
<i>NOD1</i>	C/T	rs2907748			0.6 (0.4-0.9) D			
<i>NOD1</i>	A/G	rs2907749			0.5 (0.4-0.9) D			
<i>NOD1</i>	C/T	rs932272				0.5 (0.3-0.9) R		
<i>CD14</i>	G/A	rs2569190		1.5 (1.0-2.3) R				
<i>ITGB2</i>	T/A	rs235331	0.8 (0.6-1.0) D					
<i>ITGB2</i>	G/T	rs760458					0.7 (0.5-1.0) D	0.1 (0.0-0.7) R
<i>ITGB2</i>	A/G	rs1474552		1.7 (1.1-2.7) D			1.6(1.1-2.4) D	
<i>ITGB2</i>	A/G	rs2235133	0.7 (0.5-0.9) R			0.7 (0.5-0.9) D		
<i>ITGB2</i>	C/T	rs3788151		1.6 (1.0-2.7) D			2.1 (1.4-3.2) D	
<i>ITGB2</i>	A/G	rs4607021				0.7 (0.5-1.0) D		
<i>ITGB2</i>	C/T	rs235326				0.5 (0.3-1.0) R		
TLR4 or LPS binding/ facilitating proteins								
<i>LBP</i>	C/T	rs745144		1.6 (1.1-2.3) D				
<i>LBP</i>	A/G	rs2232596						1.6 (1.1-2.3) D
<i>LBP</i>	A/T	rs5741812			2.0 (1.3-3.1) D			0.6 (0.4-0.9) D
<i>MD2</i>	G/A	rs7838114	0.4 (0.2-0.9) R					
<i>MD2</i>	T/C	rs10808798	0.7 (0.6-1.0) D					
<i>BPI</i>	T/C	rs1341023	1.4 (1.0-1.9) R		2.1 (1.3-3.5) R	1.8 (1.3-2.6) R		
<i>BPI</i>	G/A	rs2154374			1.9 (1.1-3.2) D			
<i>BPI</i>	C/G	rs5741798			0.6 (0.3-0.9) D			
<i>BPI</i>	A/G	rs5741800	0.7 (0.5-0.9) D			0.7 (0.5-1.0) D		
<i>BPI</i>	C/T	rs5741806						
<i>BPI</i>	G/C	rs5743507	1.4 (1.0-1.9) D			1.6 (1.1-2.3) D		
<i>BPI</i>	A/G	rs6024876	1.4 (1.1-1.7) D			1.4 (1.0-2.0) D	1.5 (1.1-2.0) D	
<i>PI3</i>	A/C	rs2664581		0.6 (0.4-0.9) D				

<i>PI3</i>	T/A	rs6032040		1.5 (1.1-2.2) D	1.9 (1.2-2.9) D			
<i>HSPA1A</i>	T/G	rs1008438				1.4 (1.0-2.0) D		
<i>HSPA1A</i>	G/C	rs1043618				1.4 (1.0-2.0) D		
<i>Adaptors</i>								
<i>TIRAP</i>	C/T	rs625413				0.7 (0.5-1.0) D		
<i>TIRAP</i>	T/C	rs1786704					2.7 (1.3-5.6) R	
<i>TIRAP</i>	A/G	rs1893352				1.5 (1.1-2.1) D		
<i>MYD88</i>	A/G	rs7744			1.6 (1.0-2.6) D			
<i>TRAF6</i>	C/T	rs2303439		0.7 (0.5-1.0) D				
<i>TRAF6</i>	C/G	rs5030419			1.7 (1.1-2.8) D			
<i>TICAM1</i>	C/T	rs1046673		0.7 (0.5-1.0) D				
<i>Associated kinases</i>								
<i>IRAK4</i>	T/C	rs4251520	0.2 (0.0-0.9) R	0.1 (0.0-0.9) R	1.6 (1.1-2.4) A			
<i>Kinases</i>								
<i>MAP3K7</i>	C/A	rs13208824	1.4 (1.0-1.9) D					
<i>MAP3K7</i>	A/G	rs3799912				4.0 (1.1-15.1) R		
<i>MAP3K7I P1</i>	A/T	rs1972280		0.7 (0.5-1.0) D				0.6 (0.4-0.8) D
<i>MAP3K7I P1</i>	A/G	rs5750824			1.6 (1.0-2.5) D			
<i>MAP3K7I P1</i>	A/C	rs6001585						0.5 (0.3-0.7) D
<i>MAP3K7I P1</i>	C/T	rs6001587			0.6 (0.4-1.0) D			0.6 (0.5-0.9) D
<i>MAP3K7I P2</i>	G/A	rs237033			4.4 (1.1-17.3) R			
<i>MAP3K7I P2</i>	T/C	rs562428				1.6 (1.2-2.3) D		0.7 (0.5-0.9) D
<i>MAP3K7I P2</i>	C/T	rs6932778						0.7 (0.5-0.9) D
<i>Chaperone molecules</i>								
<i>CDC37</i>	G/A	rs11879191						0.3 (0.1-1.0) R
<i>HSP90AA1</i>	A/C	rs11621560	0.7 (0.6-1.0) D					
<i>Inhibitors</i>								
<i>CD180</i>	C/T	rs3756561	1.5 (1.0-2.2) D					
<i>CD180</i>	G/C	rs5744463	0.7 (0.5-1.0) D				0.7 (0.4-1.0) D	
<i>IL1RL1</i>	G/A	rs1861246		2.5 (1.1-5.5) R				
<i>IL1RL1</i>	G/C	rs6543115		2.4 (1.2-4.9) R				
<i>IL1RL1</i>	G/A	rs6543116		2.6 (1.3-5.4) R				
<i>IL1RL1</i>	G/C	rs17027006	0.8 (0.6-1.0) D				1.8 (1.1-3.1) R	
<i>IL1RL1</i>	G/A	rs10208293					0.7 (0.5-0.9) D	
<i>SOCS1</i>	T/C	rs4780355						0.4 (0.2-0.8) R
<i>TOLLIP</i>	C/T	rs3793966	0.7 (0.6-1.0) D					
<i>TOLLIP</i>	A/T	rs3793969						0.6 (0.4-0.9) R
<i>TOLLIP</i>	A/G	rs5743890			2.9 (1.0-8.0) R			
<i>TOLLIP</i>	A/G	rs5743894					0.1 (0.0-0.8) R	0.6 (0.4-0.9) D

<i>TOLLIP</i>	C/T	rs5743915		1.4 (1.0-2.0) D				
<i>TOLLIP</i>	G/A	rs5743944		1.4 (1.0-1.9) D				
<i>TOLLIP</i>	T/C	rs5744034					0.2 (0.1-1.0) R	

*Wild type allele is first; Odds ratios are calculated using wild type as a reference category. #OR = odds ratio calculated by logistic regression (95% confidence interval) and model (respectively D= minor allele is dominant, R=minor allele is recessive, A=Additive model).

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