

ORIGINAL ARTICLE

A TLR2 polymorphism is associated with type 1 diabetes and allergic asthma

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Type 1 diabetes (T1D) and allergic asthma are immune-mediated diseases. Pattern recognition receptors are proteins expressed by cells in the immune system to identify microbial pathogens and endogenous ligands. Toll-like receptors (TLRs) and CD14 are members of this family and could represent a molecular link between microbial infections and immune-mediated diseases. Diverging hypotheses regarding whether there exists a common or inverse genetic etiology behind these immune-mediated diseases have been presented. We aimed to test whether there exist common or inverse associations between polymorphisms in the pattern recognition receptors TLR2, TLR4 and CD14 and T1D and allergic asthma. Eighteen single nucleotide polymorphisms (SNPs) were genotyped in TLR2 (2), TLR4 (12) and CD14 (4) in 700 T1D children, 357 nuclear families with T1D children and 796 children from the 'Environment and Childhood Asthma' study. Allele and haplotype frequencies were analyzed in relation to diseases and in addition transmission disequilibrium test analyses were performed in the family material. Both T1D and allergic asthma were significantly associated with the TLR2 rs3804100 T allele and further associated with the haplotype including this SNP, possibly representing a susceptibility locus common for the two diseases. Neither TLR4 nor CD14 were associated with T1D or allergic asthma.

Genes and Immunity advance online publication, 15 January 2009; doi:10.1038/gene.2008.100

Keywords: association study; allergic asthma; type 1 diabetes; Toll-like receptor; CD14

Introduction

Type 1 diabetes (T1D) is characterized by immunological destruction of pancreatic β -cells mainly through a T-helper 1 (Th1) driven process. Allergic asthma, moreover, is associated with a Th2-dominated inflammatory response in the bronchial airways.¹ However, in both conditions, the adaptive and the innate immune system are assumed to be involved in complex manners.

The factors that contribute to or trigger these immune processes are poorly understood. Pattern recognition receptors (PRRs) are proteins expressed by cells of the immune system. Toll-like receptors (TLRs) and CD14 are important PRR with a crucial role in the activation of the innate immune system and the first-line defense against foreign intruders such as different infectious microbes.² Certain host molecules may also act as ligands and activators of TLRs.³ Activation by ligands leads to

cytokine production necessary for pathogen eradication, as well as maturation of certain immune cells causing expression of cytokines that promotes the differentiation of Th1 in preference to Th2 cells.⁴ This supports evidence that exposure to high levels of certain microbial agents may decrease the risk of asthma.⁵

Several hypotheses regarding how PRRs are involved in autoimmune processes such as T1D exist. One hypothesis is that molecular mimicry may explain the contribution of the innate arm of the immune system in that T cells reacting to specific autoantigens arise because of recognition by PRR of cross-reactive epitopes on infectious agents.^{2,4} Recently, TLRs were shown to be important in regulating the expansion of regulatory T cells with subsequent suppression of the immune reaction.⁶ Regulatory T cells are important immune regulators involved in the balance of an immune response to microbes, as well as being essential for the maintenance of the tolerance to self and to prevent autoimmune disorders.⁷ Thus, TLRs and CD14 could be involved in development of autoimmune diseases in numerous ways.

Results from studies, exploring the relationship between genetic polymorphisms in the PRRs and T1D and allergic asthma, are conflicting. T1D has been found decreased in CD14-deficient mice⁸ and has been asso-

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Received 6 June 2008; revised 17 November 2008; accepted 18 November 2008

ciated with polymorphisms in *TLR2* in one study,⁹ but not another.¹⁰ Further, associations between other autoimmune diseases and *TLR4* are reported in some,^{11,12} but not all studies.^{13–15} Likewise, asthma has in some studies been associated with polymorphisms in *TLR2*,¹⁶ *TLR4*¹⁷ and *CD14*,¹⁸ but not in others.^{19–21} Epidemiological studies suggest a negative relation between asthma and T1D²² and certain genes appear to have associations in opposite directions for these two diseases.²³ To our knowledge, polymorphisms in these PRRs have not been compared in relation to T1D and allergic asthma, and the recent observation that PRRs are involved in regulation of Th1 versus Th2 responses raises the question of common genetic variants in these diseases.

Thus, the main aim of the present study was to determine whether common genetics variants in the PRRs *TLR2*, *TLR4* and *CD14* were associated with T1D and/or allergic asthma and whether or not possible associations were inversely correlated.

Results

TLR2

The percent genotyped (call rate) for the two *TLR2* SNPs was >95.8 and >99.6%, in the T1D_{child} and the 'Environment and Child Asthma' study (ECA) population, respectively, and slightly less in the T1D_{family} population.

Analysis performed for *TLR2* included analyses of allele association (Table 1), genotype association (Table 2) and haplotype association (Table 3).

The T allele in SNP (rs3804100; also reported as S450S or +1350) was significantly associated with T1D in the case-control analyses ($P=0.016$, odds ratio (OR)=1.45, 95% confidence interval (CI)=1.07–1.96), the association remaining significant also after correction for multiple testing ($p_{\text{c}}=0.03$; Table 1). The T allele in SNP (rs3804100) was also significantly associated with allergic asthma ($P=0.005$, OR=3.5, 95% CI=1.4–8.7), corrected P -value 0.009; Table 1), indicating T as a susceptibility allele for both T1D and asthma or vice versa, C as a protective allele for both diseases. The genotype homozygous for the susceptibility allele T was significantly associated both with T1D ($P=0.03$, OR=1.43, 95% CI=1.03–1.96) and allergic asthma ($P=0.008$, OR=3.55, 95% CI=1.39–9.03). The genotypes homozygous for the C allele (CC) and the heterozygous genotype CT were combined and used as reference genotype when estimating ORs (Table 2).

The genotype distribution for this SNP between patients with T1D as well as with allergic asthma compared to the control groups without diseases were significant with a global $P=0.025$ for T1D and $P=0.018$ for allergic asthma (Table 2). Haplotype construction of the two SNPs (rs3804099-rs3804100) in the Haploview program resulted in three haplotypes, all with frequencies $\geq 5\%$. One of the haplotypes that included the protective C allele (rs3804100), C-C was significantly negatively associated with both T1D ($P=0.016$) and allergic asthma ($P=0.006$), in accordance with the single allele analyses, and remained significant after correction for multiple testing (Table 3).

Table 1 TDT analysis and T1D (case-control dataset) and allergic asthma (case-control dataset) allele analysis of SNPs in *TLR2*

SNPs, rs no.	Alleles Maf	T1D trios					T1D 700 cases vs 555 controls				Allergic asthma 108 cases vs 494 controls		
		Overtr. allele	T/NT	%T	RR	P-value	Associated allele	Case-control (n alleles(%))	OR	P-value (p_{c})	Case-control (n alleles(%))	OR	P-value (p_{c})
Rs3804099	T>C 0.46	T	144/128	53	1.1	0.33	T	774/588 (58/55)	1.14	0.13	118/515 (60/56)	1.15	0.39
Rs3804100	T>C 0.09	T	48/37	56	1.3	0.23	T	1311/1013 (94/91)	1.45	0.016 (0.03)	199/879 (98/92)	3.49	0.005 (0.009)

Abbreviations: Maf, minor allele frequency; NT, nontransmitted; OR, odds ratio; p_{c} , corrected P -value according to the permutation test; RR, relative risk; SNP, single nucleotide polymorphisms; T1D, type 1 diabetes; T, transmitted; %T, percent transmission.

Maf in population-based control (ECA). OR (the negatively associated alleles are used as reference allele).

The permutation test is performed including only the two SNPs genotyped in *TLR2*.

n, number of associated alleles, (%) percent-associated alleles.

Table 2 Genotype distribution of *TLR2* polymorphism in the T1D (case-control dataset) and allergic asthma (case-control dataset)

Genotype	T1D case-control				Allergic asthma case-control			
	Case (%)	Control (%)	OR (95%CI)	P-value	Case (%)	Control (%)	OR (95% CI)	P-value
Rs3804100								
TT	614 (88)	463 (83)	1.43 (1.03–1.96)	0.03	97 (95)	404 (85)	3.55 (1.39–9.03)	0.008
CT+CC	86 (12)	92 (17)	Ref		5 (5)	74 (15)	Ref	

Abbreviations: CI, confidence interval; OR, odds ratio; T1D, type 1 diabetes.

Global P -value 0.025 for T1D and 0.018 for allergic asthma.

CT and CC are merged into one group, because few individuals have the genotype CC.

Table 3 TLR2 haplotype association (rs3804099-rs3804100) with T1D (trios and case-control datasets) and allergic asthma (case-control dataset)

Haplotypes	T1D trios					T1D case-control			Allergic asthma case-control		
	Freq	T/NT	%T	RR	P-value	Case-control (%)	OR	P-value (p_c)	Case-control (%)	OR	P-value (p_c)
C-C	0.07	38/46	45	0.8	0.38	87/97 (6.2/8.4)	1ref	0.016 (0.03)	6/77 (2.6/8.0)	1ref	0.006 (0.01)
C-T	0.35	148/155	49	1.0	0.68	507/411 (36/37)	1.38	0.7	78/345 (38/36)	2.90	0.62
T-T	0.58	165/147	53	1.1	0.33	806/606 (57/54)	1.48	0.10	124/538 (60/56)	2.96	0.34

Abbreviations: freq, haplotype frequency in population-based control (ECA); NT, nontransmitted; OR, odds ratio; p_c , corrected *P*-value according to the permutation test; RR, relative risk; %T, percent transmission; T, transmitted.

The permutation test is performed including the three haplotypes observed for TLR2. The underlined allele is the associated SNP rs3804100.

The transmission disequilibrium test (TDT) analysis in the T1D_{family} population demonstrated no significant association for this SNP. However, the C allele and the C-C haplotype were transmitted less frequently than the expected 50% transmission rate, 44 and 45% respectively, and relative risks of comparable magnitude and in the same direction as in the case-control analysis of T1D were observed (Tables 1 and 2).

Allele distribution or deviation in allele transmission was not significantly associated with T1D or allergic asthma for the other SNP (rs3804099) or other haplotypes (Tables 1 and 2).

TLR4 and CD14

In total, 12 SNPs were genotyped in *TLR4* and 4 SNPs were genotyped in *CD14*. None of the alleles were significantly associated with T1D or allergic asthma in any of the populations, after correction for multiple testing (Supplementary Table 1). Nine haplotype combinations for *TLR4* and four haplotype combinations for *CD14* were constructed using Haploview, but neither was significantly associated with T1D or allergic asthma (Supplementary Tables 2 and 3).

Hardy-Weinberg equilibrium was not rejected for any SNPs among the children without the diseases. All analyses were also carried out after stratification by gender and no significant differences between boys and girls were detected. Stratifying on HLA risk groups in the T1D populations did not influence the results indicating that no interaction between HLA and the evaluated polymorphisms was found (data not shown).

Discussion

TLR2

In the present study, including both a T1D and an allergic asthma case-control material, we report a significant association between the *TLR2* rs3804100 T allele as well as the genotype homozygous for this allele, and both T1D and allergic asthma. The same tendency was found in the T1D_{family} but the transmission distortion did not reach statistical significance. The haplotype including the protective C allele showed significant protective association with both T1D and allergic asthma.

Supporting our finding of an association between T1D and the same *TLR2* polymorphism, one case-control study from Korea including 407 patients and 1142 controls found that the homozygous genotype composed of the minor allele (CC) of SNP rs3804100 showed weak

but significant protective association with T1D. The homozygous genotype composed of the haplotypes (rs3804099 and rs3804100) including the protective C allele in rs3804100 showed strong protection from developing T1D. Rs380499 was not found associated even if they reported strong linkage disequilibrium (LD; $r^2 = 0.9$) between the two SNPs.⁹ A study consisting of 70 families in a Basque population did not detect any evidence of disease association with T1D, as was the case in our T1D_{family} study. This study genotyped four SNPs (N199N = rs3804099, S450S = rs3804100, R674W and R753Q) in *TLR2*, but found only rs3804099 to be polymorphic in the investigated population.¹⁰ However, both our family study, and the study by Santin *et al.*¹⁰ had limited power (50% and less than 60% power, respectively) to detect an association with an OR = 1.45, even if minor allele frequency was set to 9 and 50%, respectively, for the polymorphic SNP.

The association between *TLR2* rs3804100 T allele and asthma reported in the present study has to our knowledge not previously been reported. Although Eder *et al.*²⁴ detected an association between a promoter SNP in *TLR2* and asthma in children of European farmers, they found no association between asthma and rs3804100. Also, Smit *et al.*²⁵ did not find any associations between *TLR2* SNPs and asthma in a population of Danish farmers, but they did not include the polymorphisms analyzed in the present study.

TLR4 and CD14

We found no association between the genotyped SNPs or constructed haplotypes in *TLR4* or *CD14* and T1D or allergic asthma (Supplementary Tables 1–3). Regarding *TLR4*, the two most studied SNPs (Asp299Gly = rs4986790 and Thr399Ile = rs4986791) have a low minor allele frequency (<0.05 in the CEU population in HapMap), requiring large sample sets to detect or invalidate a weak disease association. However, with our T1D_{child} study, we had more than 80% power to detect an association with an OR ≥ 1.5 with a minor allele frequency ≥ 0.03.

Genetic variations in *CD14* are among the most tested variations in relation to asthma and allergy, and the results have varied.²⁶ However, the present study is the first to our knowledge that investigates possible association between *CD14* and T1D. Although we cannot exclude a small effect of the tested polymorphisms in these two genes on development of T1D or allergic asthma, they do not seem to play any major role in the pathogenesis of these phenotypes in our populations.

Exposures to endotoxin, different allergens or virus have been studied as a possible trigger for developing asthma and T1D in genetically susceptible individuals. Interactions between CD14/TLRs and environmental factors have been reported in asthma studies.^{27,28} On the background of the knowledge of biological interactions between these genes, it is also possible that there exist gene–gene interactions between these genes. We have not investigated interactions in this study. Interaction studies require large sample sizes to have sufficient power. Gene–environment and gene–gene interactions could explain the lack of association to CD14 and TLR4 in this study and also the varying results in the literature regarding these genes and asthma and T1D.

T1D versus allergic asthma

Epidemiological studies have shown positive associations between the occurrence of T1D and asthma suggesting a common etiology,^{29,30} as well as inverse relationships between the two diseases suggesting distinction in disease etiology with Th1- or Th2-dominated immune responses^{31–33} as a plausible explanation. A possible reason for the discrepancies may be the existence of common genetic dispositions in the immune system occurring before CD4 differentiation, thereby influencing both T1D and allergic asthma development, as well as different or opposite genetic effects operating later in the immune-mediated cascade directly affecting Th1–Th2 differentiation.²³ As such, the joint association between the *TLR2* and T1D and allergic asthma may represent a common susceptibility pathway before CD4 differentiation, or a pathway involving the regulatory T cells. This is of interest in light of the fact that for both diseases, the prevalence is increasing,^{34,35} warranting studies exploring possible common factors influencing disease susceptibility.

In the present study, specific hypotheses were tested based on assumed immunologic role of the genes and earlier association studies either with T1D, other autoimmune diseases, or asthma. There is no consensus on the optimal way to correct for multiple testing, and whether correction should account for the number of polymorphisms in one gene, the number of polymorphisms in the entire study or ultimately for all tests ever performed on the material. With the hypothesis driven analyses in the present study, we believe that a Bonferroni's correction would be overly conservative, thus implying the permutation test as a prudent correction for number of genotyped polymorphisms in each gene.³⁶

One limitation in the present study is that we have not genotyped all SNPs in the reported genes. Thus, the whole gene or the complete haplotype structure in the genes may not have been accurately represented or reconstructed. However, information from HapMap and other studies^{9,10,37} show that both SNP frequency and LD pattern vary to a large extent between different populations for these genes. The genes include many SNPs with minor allele frequency below 5% that demand very large sample sizes to detect minor contributions to disease. This emphasizes the importance of studies performed in different populations and materials with sufficient power to detect low frequent alleles as susceptibility alleles.

In conclusion, the present study demonstrates a common association between a *TLR2* polymorphisms

and T1D and allergic asthma. This suggests that *TLR2* is a susceptibility locus common for the two diseases. More studies are needed to elucidate how genetic variation in *TLR2* might affect the pathogenesis of these diseases.

Materials and methods

Subjects

Two independent T1D populations were used for analyses. The T1D family dataset (T1D_{family}) comprised 357 trios (one affected child with parents) (55.5% boys) recruited between 1993 and 1997 by the Norwegian Childhood Diabetes Study Group including all pediatric departments in Norway. Furthermore, 700 children (51.4% boys) with T1D (T1D_{child}) were included from the Norwegian Childhood Diabetes Registry consecutively recruited between 2002 and 2007. The controls analyzed against the 700 T1D patients consists of 555 children without T1D (53.5% boys) born in 1992–1993. Briefly, 108 children (75% boys) diagnosed with allergic asthma (see diagnostic criteria described below) were analyzed against a control group consisting of 496 children without allergy and asthma (54% boys). Both the asthma cases and the T1D controls and asthma controls were selected from a general Norwegian population-based birth cohort study, the ECA.³⁸ The control groups used in the T1D and asthma analyses are not totally identical, as the T1D control group included children without a diagnosis of T1D, whereas the asthma control group included children without a diagnosis of allergy or asthma. More than 95% of the cases and controls were of Norwegian ethnicity. To further rule out the possibility of population stratification, we looked at the allele frequencies of all individuals with a name that could potentially indicate non-Norwegian ethnicity. No differences in allele frequencies were detected when comparing these few individuals with the rest of the group (data not shown).

In the ECA study, healthy term babies were enrolled during 1992–1993, and 1215 invited subjects attended a follow-up investigation at 10 years. These children were selected for the 10-year follow-up on the basis of having either (1) lung function measurements at birth, a population found to be representative for the entire birth cohort³⁸ or (2) being included in a nested case–control study identified through recurrent or no physician diagnosed bronchial obstruction by 2 years of age, respectively. Individuals included in the present study comprise those children with available DNA, outlined in Table 4.

All studies were approved by the Regional Committee for Medical Ethics, the Norwegian Data Inspectorate and performed in accordance with the latest version of the Declaration of Helsinki. Informed consent was obtained from all participants or their parents.

Methods

Clinical methods. T1D patients were diagnosed according to EURODIAB criteria.³⁹ Allergic asthma was diagnosed based on having two of the three following criteria in the presence of at least one positive skin prick test against a standard panel of food and inhalant allergens:^{38,40} (1) symptoms, (2) medication, (3) a doctor's diagnosis by the age of 10.

Table 4 Individuals/materials used in this study

Type1 diabetes		The ECA study		
T1D _{family}	T1D _{child}	Children without T1D (born in 1992–1993), T1D controls	Children without asthma and allergy (born in 1992–1993), asthma controls	Children with allergic asthma, allergic asthma cases
357 Trios ^a	700	555	494	108

Abbreviation: ECA, Environment and childhood asthma.

^aMother, father and affected child.

DNA extraction and genotyping. DNA was extracted from peripheral whole blood using a salting out protocol for the T1D materials and with a magnetic bead-based approach (Magnapure, Roche, Basel, Switzerland) for the ECA material. We performed genotyping using the SNPlex genotyping system on an ABI3730 DNA Analyzer, a capillary electrophoresis-based assay that enables simultaneous genotyping of up to 48 SNPs in a single sample (<https://products.appliedbiosystems.com/>) or using TaqMan genotyping system analyzed on an ABI 7900HT real-time PCR (Applied Biosystems, Foster City, CA, USA). Briefly, 16 SNPs were genotyped using this SNPlex system whereas two additional *TLR4* SNPs were genotyped in T1D with the TaqMan genotyping system. Genotyping of HLA class II genes was performed as earlier described.⁴¹

SNP selection. Single nucleotide polymorphism selection was based on data from HapMap accessible at time of selection⁴² and performed according to the following criteria. All validated SNPs in *TLR4* and *CD14* including 2000 bp flanking the transcribed genes with a minor allele frequency greater than 5% according to HapMap⁴³ Caucasian/European (CEU) population (Utah residents with ancestry from northern and western Europe) were identified. The LD patterns from HapMap CEU population were evaluated to avoid SNPs in strong LD ($r^2 \geq 0.8$). However, because of the known risk of genotyping failure in the SNPlex genotyping system some SNPs in LD ($r^2 \geq 0.8$) were included.

For *TLR2* (encoded at 4q32), we analyzed two synonymous SNPs in the single exon of the gene (rs3804099, rs3804100). A third SNP, rs7656411, originally included, failed for technical reasons. These SNPs were selected because they were previously included in association studies of T1D.^{9,10} The LD between these two SNPs in our population-based controls was $r^2 = 0.09$.

In *TLR4* (encoded at 9q32-33), the following 10 SNPs were genotyped in the SNPlex system; rs10759930, rs2737191, rs2770150, rs1927914, rs1927911, rs5030728, rs11536889, rs11536891, rs11536897 and rs1554973. These 10 SNPs include 4 SNPs in the 5' flanking region, 2 intronic SNPs and 4 in the 3' flanking region. Two additional nonsynonymous SNPs in the third exon with minor allele frequencies below 5% were genotyped in the T1D populations because of previously association studies to other autoimmune diseases (rs4986791 (THR399Ile) and rs4986790 (Asp299Gly)).^{11–15} According to HapMap data we captured 62% of the gene.

In *CD14* (encoded at 5q31), we genotyped four SNPs (rs4914, rs2569190, 5744455, rs2569191); one synonymous SNP in the only exon and three SNPs in the 5' flanking region. These SNPs are assumed to capture the majority of the known common variations in the gene.

Statistical analyses

Tests for association in the T1D and asthma populations were performed in Haploview 3.22,⁴⁴ using Pearson's χ^2 -test. ORs for the haplotype association were calculated using logistic regression in SPSS for Windows (version 15.0; SPSS, Chicago, IL, USA). The allele-wise and haplotype-wise TDT in the family material was performed using the UNPHASED application implemented in the UNPHASED software version 2.4.⁴⁵ Haplotypes were constructed in Haploview 3.22 that uses an accelerated EM algorithm.⁴⁶ Haploview 3.22 was also used to check the alleles in the control group for Hardy–Weinberg equilibrium. When *P*-values in allele and haplotype analysis were less than 0.05, 10,000 permutations were executed in Haploview to assess association including correction for multiple testing. Permutation refers to random rearrangements of the data. Under the null hypothesis, all permutations are equally likely. Sum statistics are calculated for each permutation and compared with the statistic in the original data. The *P*-value is the proportion of replicate statistics larger or equal to the observed statistic.

When correcting for multiple testing, the number of genotyped SNPs per gene were included. Thus, for *TLR2* allele association analyses, the permutation test was performed including only the two SNPs genotyped in this gene and for the *TLR2* haplotype association analyses, the permutation test was performed including the three haplotypes observed for *TLR2*. Genotype data in families were checked for Mendelian inconsistencies using the program implemented in Progeny (www.progenygenetics.com) and rechecked in Haploview 3.22. Families where markers showed Mendelian errors were removed from the analyses for that marker. Disease-only analyses were used to estimate interaction with HLA. Power analyses were performed in R (<http://www.rproject.org/>) based on an implementation of the two sample binomial model.⁴⁷ Such power calculations depend on the model chosen. For our purposes, it has been reasonable and fair to make optimistic assumptions (Hardy–Weinberg holds and the SNP is causal) as the calculations will indicate that some other studies may be underpowered. With our T1D_{child} study, we had >80% power to replicate a possible association with an OR of 1.45 with a minor allele frequency of 9%.

Acknowledgements

This work has been supported by The National Program for Research in Functional Genomics in Norway (FUGE), in The Research Council of Norway, University of Oslo, The Norwegian Diabetes Association, Eastern Norway Regional Health Authority, Western Norway Regional Health Authority and University of Bergen. We are grateful to all the children and their parents for participating in the study.

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Supplementary Information accompanies the paper on Genes and Immunity website (<http://www.nature.com/gene>)

ESM Table1. TDT analysis and case-control analysis of SNPs in *TLR4* and *CD14*.

<i>TLR4</i>	<i>T1D family study</i>							<i>T1D case control study</i>				<i>Allergic asthma case control study</i>			
<i>rs no.</i>	<i>Alleles</i>	<i>maf</i>	<i>Overtr. allele</i>	<i>T/NT</i>	<i>% T</i>	<i>RR</i>	<i>p-value</i>	<i>Assoc. allele</i>	<i>Case/contr [n(%)]</i>	<i>OR</i>	<i>p-value</i>	<i>Assoc. allele</i>	<i>Case/contr [n(%)]</i>	<i>p-value</i>	
Rs10759930	C>T	0.37	C	167/143	54	1.2	0.17	T	510/407 (37/37)	1.01	0.89	T	79/350 (39/37)	0.59	
Rs2737191	A>G	0.27	G	129/127	50	1.0	0.90	G	387/303 (28/27)	1.03	0.71	A	151/691 (73/73)	0.87	
Rs2770150	T>C	0.28	T	130/129	50	1.0	0.95	C	394/305 (28/27)	1.04	0.63	T	151/694 (73/73)	0.89	
Rs1927914	T>C	0.35	C	149/131	53	1.1	0.28	T	917/718 (66/65)	1.05	0.55	T	138/620 (66/65)	0.66	
Rs1927911	C>T	0.30	T	133/109	55	1.3	0.12	C	1001/778(72/70)	1.11	0.25	C	147/671 (71/70)	0.82	
Rs5030728	G>A	0.29	A	140/139	50	1.0	0.95	A	415/325 (30/29)	1.03	0.77	G	150/675 (72/71)	0.70	
Rs11536889	G>C	0.14	G	93/67	58	1.4	0.04	G	1205/946(87/86)	1.06	0.61	C	35/147 (17/16)	0.62	
Rs11536891	T>C	0.17	T	80/79	50	1.0	0.93	T	1185/923(85/83)	1.14	0.25	T	182/795 (88/83)	0.12	
Rs11536897	G>A	0.05	A	20/17	54	1.2	0.62	G	1332/1051(95/95)	1.00	0.49	G	200/916 (96/96)	0.82	
Rs1554973	T>C	0.25	C	117/112	51	1.0	0.74	T	1048/830 (75/75)	1.00	0.99	T	161/713 (78/75)	0.34	
Rs4986790*	A>G	0.04						G	93/189*(4/4)	1.06	0.69				
Rs4986791*	C>T	0.04						T	92/193*(4/4)	1.00	0.87				

<i>CD14</i> <i>rs no.</i>	<i>T1D family study</i>							<i>T1D case control study</i>				<i>Allergic asthma case control study</i>			
	<i>Alle les</i>	<i>maf</i>	<i>Overtr. allele</i>	<i>T/NT</i>	<i>% T</i>	<i>RR</i>	<i>p-value</i>	<i>Assoc. allele</i>	<i>Case/contr [n(%)]</i>	<i>OR</i>	<i>p-value</i>	<i>Assoc. allele</i>	<i>Case/contr [n(%)]</i>	<i>OR</i>	<i>p-value</i>
Rs4914	C>G	0.13	G	61/55	53	1.1	0.58	C	1226/965 (88/87)	1.03	0.54	C	185/839 (90/88)		0.50
Rs2569190	G>A	0.42	G	156/146	52	1.1	0.57	A	593/469 (43/42)	1.02	0.83	G	127/555 (62/58)		0.26
Rs5744455	C>T	0.26	T	130/126	51	1.0	0.80	T	396/288 (28/26)	1.14	0.16	T	66/257 (32/27)		0.13
Rs2569191	T>C	0.42	T	153/144	52	1.1	0.60	T	597/465 (43/42)	1.03	0.72	T	126/555 (61/58)		0.33

Maf= Minor allele frequency in population based control (ECA); T = transmitted; NT = nontransmitted; %T = percent transmission; RR = relative risk; OR = odds ratio (the alleles not associated are used as ref. allele);

*For these two SNPs we used another control group consisting of 2322 blood donors.

ESM Table2 *TLR4* haplotype association with T1D (case-control) and allergic asthma (case-control)

<i>Haplotype*</i>	<i>T1D case control study</i>			<i>Allergic asthma case control study</i>	
	<i>Case/Control (%)</i>	<i>OR</i>	<i>p-value</i>	<i>Case/Control (%)</i>	<i>p-value</i>
CGCTCAGTGT	385/296 (28/27)	1 ref	0.60	55/255 (26/27)	0.94
TATTCGGTGT	327/255 (24/23)	0.99	0.77	47/208 (23/22)	0.82
TATTCGCTGT	182/149 (13/14)	0.94	0.78	34/146(17/15)	0.65
CATCTGGCGC	143/123(10/11)	0.89	0.51	17/120 (8/13)	0.08
CATCTGGTGT	125/105 (9/10)	0.92	0.64	23/87 (11/9)	0.38
CATCTGGCAC	65/58 (5/5)	0.86	0.54	8/39 (4/4)	0.88
CATCCGGTGC	70/39 (5/4)	1.38	0.07	7/32 (3/3)	0.99
CATCTGGTGC	59/48 (4/4)	0.95	0.86	13/43(6/5)	0.29
CATCCAGTGT	14/19 (1/2)	0.57	0.12	2/16 (1/2)	0.45

*SNP order in haplotype; rs10759930-rs2737191-r2770150-rs1927914-rs1927911-rs5030728-rs11536889-rs11536891-rs11536897-rs1554973

ESM Table3 *CD14* haplotype association with T1D (case-control) and allergic asthma (case-control)

<i>Haplotype*</i>	<i>T1D case control study</i>			<i>Allergic asthma case control study</i>	
	<i>Case/Control (%)</i>	<i>OR</i>	<i>p-value</i>	<i>Case/Control (%)</i>	<i>p-value</i>
CACC	594/470 (43/42)	1 ref	0.71	78/403 (38/42)	0.35
CGCT	233/210 (17/19)	1.1	0.13	39/185 (19/19)	0.94
CGTT	392/285 (28/26)	0.9	0.15	65/258 (32/27)	0.15
GGCT	164/139 (12/13)	0.9	0.59	22/114 (10/12)	0.62

*SNP order in haplotype; rs4914-rs2569190-rs5744455-rs2569191