

Association of *CARD15* polymorphisms with atopy-related traits in a population-based cohort of Caucasian adults

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Summary

Background Influences of microbial pathogens are crucial for the maturation of the immune system. Caspase-recruitment domain containing protein 15 (*CARD15*) is a cytosolic receptor involved in bacterial recognition by antigen-presenting cells. *CARD15* polymorphisms have been associated with Crohn's disease. Recently, associations with atopic phenotypes have been reported in children.

Objective Within a large population of German adults ($n = 1875$), we evaluated eight *CARD15* polymorphisms for associations with atopic phenotypes.

Methods Subjects were phenotyped by standardized questionnaires and interviews as well as total and allergen-specific IgE measurements. Genotyping was performed using matrix-assisted laser desorption/ionization – time of flight mass spectrometry. Haplotypes were estimated using the SAS/Genetics module.

Results Subjects with a T allele at rs1077861 had a decreased risk of developing asthma (odds ratio OR = 0.648, $P = 0.013$), whereas the presence of an A allele at rs3135500 was significantly associated with an increased risk (OR = 1.374, $P = 0.023$). In addition, a *CARD15* haplotype revealed to be protective against the development of asthma (OR = 0.326, $P = 0.003$). Subjects with an A allele at position rs5743266 or a T allele at rs2066842 had a significantly decreased risk of developing allergic rhinoconjunctivitis with ORs of 0.820 ($P = 0.049$) and 0.801 ($P = 0.025$). Polymorphism rs2066845 showed a significant association with increased total serum IgE (OR = 2.155, $P = 0.006$).

Conclusion Genetic variants of *CARD15* that might result in inappropriate immunomodulation are not only associated with autoimmune diseases but also with atopic disorders.

Keywords allergic rhinoconjunctivitis, asthma, atopic eczema, atopy, *CARD15*, haplotype, IgE, NOD2, SNP

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Introduction

Atopic diseases are multifactorial disorders caused by combined influences of genetic and environmental factors. Over the last decades, increasing prevalence rates have been observed in western countries that cannot be explained in genetic terms. Therefore, altered environmental and/or changed host factors are discussed to be involved in the development of atopic diseases (asthma, allergic rhinoconjunctivitis, atopic eczema) (reviewed in [1]). Several studies have shown that exposure to a farm environment and

microbial pathogens decreases the risk of developing atopic sensitization and respiratory atopy [2–9], supporting the so-called 'hygiene hypothesis'.

Innate immunity represents the first line of host defence recognizing a few highly conserved structures present in many different microorganisms. These microbial structures are called pathogen-associated molecular patterns (PAMPs) and include lipopolysaccharides (LPS), an essential cell wall component of Gram-negative bacteria. LPS is ubiquitous in the environment and also present indoors, e.g. as a constituent of house dust [10]. Various innate receptors participate in the recognition of PAMPs. Whereas toll-like receptors (TLRs), CD14 and MD2 are involved in their extracellular detection, caspase-recruitment domain containing protein 15 (*CARD15*, also named NOD2) functions as a cytosolic receptor for LPS and peptidoglycan (reviewed in

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[11]). *CARD15* is composed of an N-terminal caspase-recruitment domain, a centrally located nucleotide-binding oligomerization domain, and C-terminal leucine-rich repeats (LRRs) [12]. It is predominantly expressed in monocytes, granulocytes, dendritic cells and epithelial cells [13, 14], and its interaction with LPS and/or peptidoglycan has been shown to induce apoptosis and activate nuclear factor- κ B (NF- κ B) via the serine/threonine kinase RICK/Rip2 leading to subsequent activation of the inflammatory response [12].

It has been shown that the innate immune system is crucial for the activation of adaptive immunity [15, 16]. However, its role in the induction of T-helper type 2 (Th2) responses and regulation of Th2-driven diseases like atopic disorders is still unclear. Studies evaluating the role of LPS exposure for the development of asthma in children have delivered controversial results (reviewed in [15, 16]), and it has been suggested that the amount of LPS present during the sensitization phase determines whether Th1 or Th2 immunity is induced [17].

Genetic variations in innate immunity genes have been reported to be associated with a range of inflammatory disorders including atopic diseases (reviewed in [18]).

The *CARD15* gene is located on chromosome 16q12, a region that has been linked with several autoimmune diseases such as Crohn's disease, rheumatoid arthritis and lupus erythematosus [19–21]. Various studies showed associations between *CARD15* polymorphisms and Crohn's disease [22–24]. Recently, three single-nucleotide polymorphisms (SNPs) of *CARD15* were also found to display associations with allergic rhinoconjunctivitis and atopic eczema in a population of German schoolchildren [25].

We aimed to validate these observations in a large population-based cross-sectional cohort of adults and to evaluate eight polymorphisms with minor allele frequencies ≥ 0.05 (except for rs2066845, which has been consistently associated with Crohn's disease) covering the whole *CARD15* gene concerning associations with atopy-related phenotypes.

Material and methods

Study population

Subjects were recruited from the KORA C (Cooperative Health Research in the Augsburg Region) and KORA S2000 survey, which were large population-based cross-sectional studies on Caucasian adults carried out in 1994–1995 and 1999–2001 in the city and region of Augsburg, South Germany. The sampling frame and study design have been described previously [26, 27], and followed the guidelines of the WHO MONICA Project [28]. Briefly, KORA C is based on a random, cross-sectional sample stratified for age and sex studied in 1994/1995 ($n = 4356$). In the sera of these subjects, allergen-specific IgE antibodies to common aeroallergens (grass and birch pollen, house dust mite, cat, and *Cladosporium*) were determined by the fluorescence enzyme immunoassay technique (CAP-FEIA, Pharmacia, Uppsala, Sweden). The cut-off point for RAST reactivity was set at 0.35 kU/L. For KORA C, we aimed to recruit an enriched sample of 1600 subjects out of the 4178 individuals who had valid RAST results.

Subjects were selected, stratified by age and sex, so as to provide 50% with and 50% without a positive RAST, and,

furthermore, so that within these groups, 50% reported respiratory atopy ($n = 1417$). To account for an estimated response of 63%, a sample of 2539 subjects was approached. One thousand five hundred and thirty-seven subjects participated (60.5%), of whom 50.2% exhibited a positive RAST result, and 53.9% of this group and 43.1% of those with negative RAST result reported symptoms of atopy. DNA samples were available from 1407 subjects. KORA S4 studied an independent population-based cohort of 4261 subjects. From KORA S4, 227 individuals suffering from atopic eczema and 227 matched controls were selected. Finally, we ended up with $n = 1991$ persons aged 25–74 years in our study population for case-control analysis. DNA samples were available from 1875 subjects. All study methods were approved by the ethics committee of the 'Bayerische Landesärztekammer' Munich.

A written and informed consent was obtained from all participants prior to the beginning of the study.

Phenotyping

Subjects completed a standardized questionnaire that included demographic data and core questions of the European Community Respiratory Health Survey on respiratory health [29]. In addition, all probands were interviewed in a standardized manner to report on symptoms of atopic diseases, basic family data and parental history of atopic diseases.

All subjects received a conventional lancet skin prick test (SPT) and total and specific IgE measurements (enzyme-immuno assay: CAP-FEIA, Pharmacia, Uppsala, Sweden) using a sample of common allergens (grass, birch, rye, mugwort pollen, *Alternaria*, *Cladosporium*, cat, dog and *Dermatophagoides pteronyssinus*). Saline and histamine were used as controls (ALK-Scherax, Hamburg, Germany). Subjects were classified as having allergic rhinoconjunctivitis or asthma when they reported a doctor's diagnosis of hayfever or asthma, respectively. Specific sensitization was defined to be present if against at least one of the allergens tested, specific IgE antibodies could be detected (CAP class ≥ 1 , corresponding to ≥ 0.35 kU/L), or if a positive SPT reaction (weal diameter ≥ 3 mm after subtraction of the negative control) was observed.

Genetic analyses

SNP information for this paper was retrieved from the Innate Immunity PGA, NHLBI Program in Genomic Applications, URL: <http://innateimmunity.net/data/homology>.

Genotyping analyses were carried out by using the MassARRAY system (Sequenom, San Diego, CA, USA). Briefly, genomic DNAs were amplified by PCR using HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). Genotyping assays were carried out by using 5 ng of genomic DNA. PCR primers were used at 167 nM final concentrations for a PCR volume of 6 μ L. The PCR condition was 95 °C for 15 min for hot start, followed by denaturing at 95 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 1 min for 45 cycles and finally, incubation at 72 °C for 10 min. PCR products were first treated with shrimp alkaline phosphatase (SAP, Amersham, Freiburg, Germany) for 20 min at 37 °C to

remove excess dNTPs and afterwards for 10 min at 85 °C to inactivate SAP. ThermoSequenase (Amersham) was used for the base extension reactions. Extension primers were used at a final concentration of 5.4 µM in 10 µL reactions. The base extension reaction condition was 94 °C for 2 min, followed by 94 °C for 5 s 52 °C for 5 s and 72 °C for 5 s for 40 cycles. All reactions (PCR amplification, base extension) were carried out in a Tetrad PCR thermal cycler (M.J. Research). The final base extension products were treated with SpectroCLEAN resin (Sequenom) to remove salts in the reaction buffer. This step was carried out with a Multimek 96-channel autopipette (Beckman Coulter, Krefeld, Germany), and 16 µL of resin-water suspension was added into each base extension reaction, making the total volume 26 µL. After a quick centrifugation (644 g, 3 min) in a Sigma 4K15 centrifuge (Sigma, Osterode, Germany), 10 nL of reaction solution was dispensed onto a 384-format SpectroCHIP (Sequenom) prespotted with a matrix of 3-hydroxypicolinic acid (3-HPA) by using a SpectroPoint nanodispenser (Sequenom). A modified Bruker Biflex matrix-assisted laser desorption ionization-time-of-flight mass spectrometer (Sequenom) was used for data acquisitions from the SpectroCHIP. Genotyping calls were made in real time with MASSARRAY RT software (Sequenom).

Statistics

Descriptive statistics for quantitative and qualitative values are given by mean ± standard deviation (SD) and relative frequencies or absolute numbers, respectively. For descriptive statistics, SPSS version 11.5 was used. The association of quantitative traits with SNP genotype was tested with *t*-tests for two independent samples or Satterthwaite tests using the SAS statistical software package version 8.2. Deviation from Hardy–Weinberg equilibrium was tested with PROC ALLELE in SAS/Genetics for any of the SNPs under consideration. Linkage disequilibrium was assessed with procedure ldmax in the software package GOLD. The patterns of linkage disequilibrium between markers were visualized using the GOLD 1.0 package (<http://www.well.ox.ac.uk/asthma/GOLD>). We used Lewontin's measure of disequilibrium D' .

Haplotype frequencies were estimated from genotype data using the EM algorithm [30, 31]. To evaluate associations with quantitative and qualitative traits, haplotype trend regression models were applied, including estimated probabilities of haplotypes in a linear and logistic regression approach as independent variables [31], respectively. Further independent variables were study population (KORA C or KORA S2000), age, family size and sex. Because smoking is an essential influence factor on IgE levels and atopic diseases [32], smoking status was included as an independent variable in multiple regressions. All haplotype analyses were implemented using the SAS/Genetics module.

The same covariates were included in logistic regressions testing associations of qualitative traits asthma, atopic eczema, allergic rhinoconjunctivitis and elevated total IgE levels with SNP genotypes. IgE levels were considered as elevated at values greater than 100 kU/L, a level that has been associated with sensitization and asthma risk [33, 34]. Results are reported considering backward elimination and stepwise

variable selection. Odds ratios are given with 95% confidence intervals, (CIs) and any *P*-values given are two sided.

P-values for different traits are not adjusted for multiple testing, because the comparisons do not represent completely independent tests. Single-analysis results are strongly supported by haplotype analyses and are in line with previous observations [25].

Results

As regression analyses revealed no significant population effect, both studies were analysed together. 8.9% ($n = 167$) of the individuals were diagnosed with asthma, 12.9% ($n = 242$) were suffering from AE and 26.0% ($n = 488$) from allergic rhinoconjunctivitis. IgE serum levels of more than 100 kU/L were detected in 32.2% ($n = 604$). DNA samples were available from 1875 individuals. The phenotypic characteristics of the study population are shown in Table 1.

Between 1693 and 1818 samples (average call rate 95.3%) were genotyped successfully for the respective *CARD15* polymorphisms. The location of the genotyped *CARD15* SNPs and their frequencies are provided in Table 2 and Fig. 1. All analysed SNPs were in Hardy–Weinberg equilibrium. Calculation of the linkage disequilibrium in the gene showed a strong degree of LD (D') across the gene (Fig. 2).

Analyses revealed a significant association of two SNPs with asthma. For subjects with a T allele at position rs1077861, we observed a decreased risk of developing asthma with an OR of 0.648 (95% CI = 0.461–0.911, $P = 0.013$). The presence of an A allele at rs3135500 was associated with an increased risk for asthma (OR = 1.374, 95% CI = 1.045–1.809, $P = 0.023$).

Two polymorphisms revealed significant associations with allergic rhinoconjunctivitis. Subjects with an A allele at position rs5743266 or a T allele at rs2066842 had a significantly decreased risk of developing allergic rhinocon-

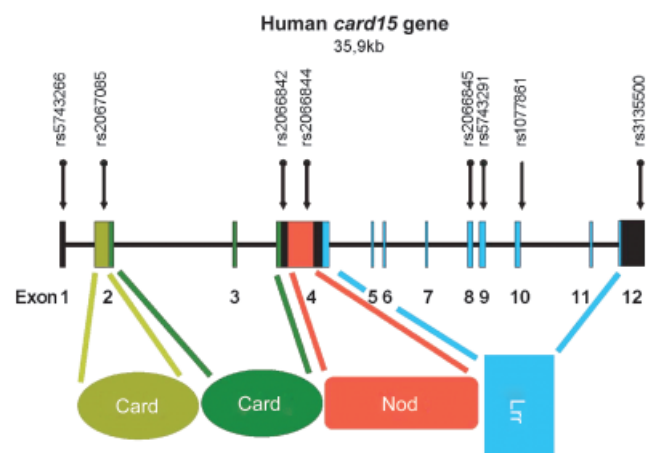


Fig. 1. Structure of the Caspase-recruitment domain containing protein 15 (*CARD15*) gene and protein. The *CARD15* gene extends more than 35 kb of genomic DNA and contains 12 exons. All polymorphisms analysed are indicated by arrows (round-tailed arrows indicate exonic SNPs) and are described in public databases (<http://www.innateimmunity.net>). The *CARD15* protein consists of 1040 amino acids and contains two N-terminal caspase-recruitment domains (CARD), a centrally located nucleotide-binding oligomerization domain (NOD) and multiple C-terminal leucine-rich repeats (LRRs).

conjunctivitis with ORs of 0.820 (95% CI = 0.672–0.998, $P = 0.049$) and 0.801 (95% CI = 0.600–0.973, $P = 0.025$).

For polymorphism rs2066845, we observed an increased risk of elevated IgE levels with ORs of 1.87 (95% CI = 1.088–3.214, $P = 0.024$) and 2.155 (95% CI = 1.254–3.704, $P = 0.006$) for IgE cut-off 100 kU/L and IgE percentile 66%, respectively. Geometric means (kU/L) were 50.6 in GG wild types vs. 63.7 in GC and CC genotypes.

For haplotype analysis, 1369 samples were available in which genotyping was successful in each of the eight *CARD15* polymorphisms. The EM algorithm showed five out of 256 possible haplotypes exceeding a frequency of 3%. Ninety-five percent CIs for frequencies are small because of the large study population indicating a sufficiently high accuracy (Table 3).

Analyses revealed that haplotype GCCCGGTG is a significant protective factor for the development of asthma (OR = 0.326, 95% CI = 0.154–0.690, $P = 0.0034$). Haplotype results are in accordance with the above-mentioned association of SNP rs1077861 (presence of T allele) and rs3135500 (presence of a G allele) with asthma.

No significant associations between AE and any single polymorphism or haplotype of *CARD15* were observed. Table 4 provides an overview of all significant association results.

Table 1. Phenotype characteristics of adults included in analyses ($n = 1875$)

	Total study population ($n = 1875$)
Male gender	866 (46.2%)
Mean ($\pm$ SD) age	49.0 ($\pm$ 13.6)
Asthma	167 (8.9%)
% with specific sensitization	76.3
Allergic rhinoconjunctivitis	488 (26.0%)
% with specific sensitization	81.8
Atopic eczema	242 (12.9%)
% with specific sensitization	50.8
Total serum IgE > 100 kU/L	604 (32.2%)
Mean ($\pm$ SD) ln(total serum IgE)	3.9 ($\pm$ 1.4)

Discussion

In this study, we analysed the effects of eight polymorphisms in the *CARD15* gene on atopic phenotypes by using a population-based cohort of 1875 German adults. *CARD15* polymorphisms have been associated with Crohn's disease [22–24], and recently associations of three SNPs with allergic rhinoconjunctivitis and AE in children have been reported [25].

Within the large population of adults analysed in this study, the strongest associations with *CARD15* SNPs could be observed for respiratory atopy. Polymorphisms rs1077861 and rs3135500 showed significant relationships with asthma, whereas rs5743266 and rs2066842 were associated with allergic rhinoconjunctivitis. In addition, the presence of a C allele at polymorphism rs2066845 revealed to be significantly associated with elevated total IgE levels. This polymorphism, which is located in exon 8 and creates an amino acid substitution, alters the LRR of *CARD15*, and therefore seems to be a good candidate for a functional SNP. Furthermore, in previous studies, the presence of a C allele has been reported to be associated with an increased risk of developing Crohn's disease and allergic rhinoconjunctivitis [22–25].

As haplotype analyses may be of higher informative value to draw associations between phenotypes and genetic variation than SNPs [35], we also assessed the effects of haplotypes in our cross-sectional study population of unrelated subjects. Haplotype analysis showed that the *CARD15* haplotype GCCCGGTG is highly significantly protective against the development of asthma. This haplotype is the only one with a T allele on position rs1077861, which was also significant in the single analysis (Table 3).

These data indicate that genetic variants in the *CARD15* gene may contribute to susceptibility to atopic diseases.

Exposure to microbial stimuli is important for the perinatal development of the immune system with innate immunity playing a crucial role in activating and directing adaptive immunity [11]. Epidemiologic data show that during early

Table 2. *CARD15* polymorphisms, dbSNP accession number, localization, call rates, allele frequencies and genotyping primers

SNP ID	Location	Call rate	Allele frequency	Direction	PCR primer	Extension Primer
rs5743266	UTR 5'/exon 1	95.2	G 65.64	Fwd	ACGTTGGATGGAATTTCCCTTGAAGGTGGG	CGGGTAGGCCTTGTGCAGAA
			A 34.06	Rev	ACGTTGGATGATCAAAGCCATTAGGCTGG	
rs2067085	Exon 2	93.9	C 59.57	Fwd	ACGTTGGATGTCCTGAGAACTCTGTGATGC	CCGATCTTCACACCGTC
			G 40.43	Rev	ACGTTGGATGGTGATGAAATCAGGTTGCCG	
rs2066842	Exon 4	97.0	C 68.68	Fwd	ACGTTGGATGAGCACAGTGTCCGCATCGTC	ATGTGGGCATGGCTGGA
			T 31.32	Rev	ACGTTGGATGAGAATGTCTGGAGGTCTGG	
rs2066844	Exon 4	96.7	C 94.97	Fwd	ACGTTGGATGATGGAGTGAAGTGCTTGCG	TCTGAGAAGGCCCTGCTC
			T 05.03	Rev	ACGTTGGATGAGTGCCAGACATCTGAGAAG	
rs2066845	Exon 8	96.7	G 97.95	Fwd	ACGTTGGATGGTCTGTTGACTCTTTGGCC	CTCGTACCCACTCTGTTGC
			C 02.05	Rev	ACGTTGGATGTGATCACCCAAGGCTTCAGC	
rs5743291	Exon 9	90.3	G 90.18	Fwd	ACGTTGGATGAGAGAATCCCCAAACTCAC	CTGATGCTGGCAAAGAAC
			A 09.82	Rev	ACGTTGGATGAACAACATTGGCAGTGTGGG	
rs1077861	Intron 10	96.3	A 70.82	Fwd	ACGTTGGATGAATTGCTGGCCTTTGGAAGG	GAGCGGCCAGATCTTA
			T 29.18	Rev	ACGTTGGATGAAGGCAAGCTGTTCTACCAAG	
rs3135500	UTR 3'/exon 12	97.0	G 59.83	Fwd	ACGTTGGATGTGTGATGCTGTGCAAAATG	CTTAAGACAGTAAACAAAGTGA
			A 40.17	Rev	ACGTTGGATGGCCATGTTGTCTATAAGAG	

CARD15, caspase-recruitment domain containing protein15; SNP, single-nucleotide polymorphisms.

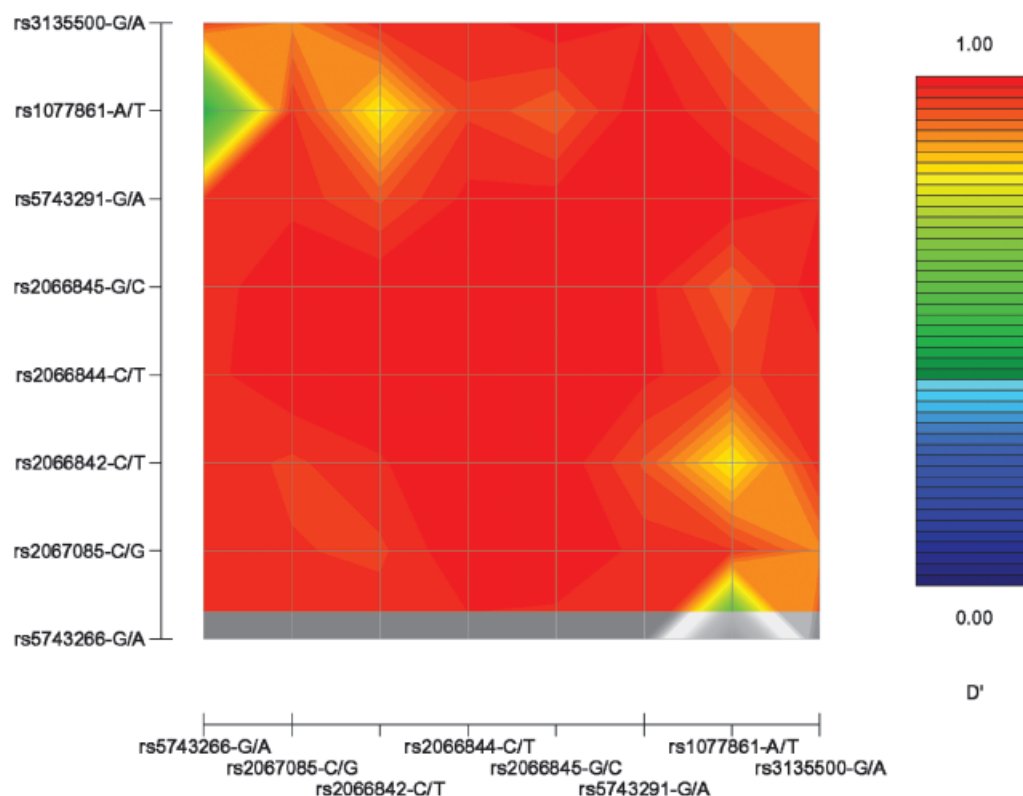


Fig. 2. GOLD linkage disequilibrium map of the CARD15 SNPs applying a normalized correlation coefficient. Pairwise estimations of Lewontin's measure of disequilibrium D' are shown from unrelated subjects on a scale of 1 (complete linkage disequilibrium: red) to 0 (blue). Marker positions are shown as a schematic rather than as actual distances apart.

Table 3. CARD15 haplotypes and haplotype frequencies with 95% confidence intervals

Haplotype	Frequency	Standard error	95% confidence limits	
A-C-T-C-G-G-A-G	0.21431	0.00670	0.20117	0.22744
A-C-T-T-G-G-A-G	0.04689	0.00345	0.04013	0.05366
G-C-C-C-G-G-T-G	0.23882	0.00696	0.22517	0.25247
G-G-C-C-G-A-A-A	0.09240	0.00473	0.08313	0.10168
G-G-C-C-G-G-A-A	0.27280	0.00727	0.25854	0.28706

Haplotypes with frequencies <3% were not considered.

CARD15, caspase-recruitment domain containing protein 15.

childhood, microbial exposure can influence the induction and expression of atopic diseases, particularly in the respiratory tract [36, 37]. In infants, lower environmental exposure to LPS has been found to be associated with an increased prevalence of allergic sensitization [9, 38, 39]. Intravenous application of LPS has been reported to cause a shift of the T cell immune response towards the Th2-type response [40]. Recently, associations between a polymorphism of TLR2 and asthma [41] and severe AE [42] have been reported. Thus, it has been suggested that a reduced or altered microbial stimulation contributes to an impaired down-regulation of the dominating neonatal Th2-like immune responses to allergens in the inductive phase of atopic diseases [43].

However, in recent years, it has become clear that the Th1/Th2 paradigm might be an oversimplification (reviewed in [44]). In addition, impaired innate immunity has also been

Table 4. Results of population-based association analyses of the CARD15 variants and atopic phenotypes

SNP ID	Location	Amino acid exchange	Asthma	Rhino-conjunctivitis	Total IgE
rs5743266	UTR 5'/exon 1	None	NS	0.049	NS
rs2067085	Exon 2	S178S	NS	NS	NS
rs2066842	Exon 4	P268S	NS	0.025	NS
rs2066844	Exon 4	R702W	NS	NS	NS
rs2066845	Exon 8	G908R	NS	NS	0.006
rs5743291	Exon 9	V944I	NS	NS	NS
rs1077861	Intron 10	None	0.013	NS	NS
rs3135500	UTR 3'/exon 12	None	0.023	NS	NS
G-C-C-C-G-G-T-G			0.003	NS	NS

NS, not significant; CARD15, caspase-recruitment domain containing protein 15; SNP, single-nucleotide polymorphisms.

shown to be associated with Th1-dominated autoimmune diseases [18]. Thus, it has been suggested that poor stimulation of the immune system in early life rather acts by decreased activation of T regulatory cells, resulting in reduced immune suppression, than by impairment of immune deviation [45]. *CARD15*, which is expressed primarily in antigen-presenting cells, serves as an intracellular receptor for bacterial LPS and/or other bacterial products and transduces signals leading to activation of NF- κ B and transcription of regulatory genes as well as enhancement of apoptosis [12, 14, 23, 46]. Deletion of its LPS-responsive LRR domain has been shown to enhance NF- κ B activation [12], whereas polymorphisms in the LRR domain lead to a decreased NF- κ B

response [23]. In addition, activation of *CARD15* in its mutated form may give rise to an abnormal activation-induced cell death of T cells [47]. Recently, evidence has been provided that *CARD15* mediates the release of anti-inflammatory cytokines from mononuclear cells stimulated with peptidoglycan [48]. Thus, *CARD15* polymorphisms could contribute to dysregulation of immune function through altered detection/binding of bacterial antigens and/or altered NF- κ B signalling, immune cell apoptosis and cytokine balancing.

However, the effects of microbial stimuli on atopic diseases appear to be complex. In addition to individual differences in the ability to respond to microbial exposure, cause-and-effect relationships seem to differ depending on the time, dose and frequency of exposure. While there is now circumstantial evidence suggesting that proper interactions with bacterial stimuli in early life may have a preventive effect on allergy and asthma, it has also been shown that microbial exposures adversely affect established disease (reviewed in [49]). Longitudinal prospective studies and analyses of gene-environment interactions would help to further evaluate the complex relationships between microbial exposures and atopic diseases.

In conclusion, our data confirm and expand previous observations that *CARD15* gene polymorphisms not only confer increased susceptibility to autoimmune diseases but also significantly contribute to the development of atopic disorders. Allergy and autoimmunity both result from dysregulation of the immune system [50], and their genetic basis is represented by a complex network of interacting genes or common genetic variants rather than a few disease-causing mutations. It may therefore be speculated whether certain alleles in innate immunity genes, like *CARD15*, impair proper interactions with bacterial stimuli in early life and result in an inappropriate immunomodulation, which in turn, depending on the individual genetic susceptibility, permits patterns of immune response predisposing to either autoimmunity or allergy.

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References

- 1 Von Mutius E. The environmental predictors of allergic disease. *J Allergy Clin Immunol* 2000; 105:9–19.
- 2 Braun-Fahrlander C, Gassner M, Grize L et al. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. *Clin Exp Allergy* 1999; 29: 28–34.
- 3 Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Böhm O, Von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000; 30: 187–93.
- 4 Riedler J, Braun-Fahrlander C, Eder W et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; 358:1129–33.
- 5 Kilpeläinen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. *Clin Exp Allergy* 2000; 30:201–8.
- 6 Ernst P, Cormier Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. *Am J Respir Crit Care Med* 2000; 161:1563–6.
- 7 Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. *Clin Exp Allergy* 2000; 30:194–200.
- 8 Portengen L, Sigsgaard T, Omland Ø, Hjort C, Heederik D, Doekes G. Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. *Clin Exp Allergy* 2002; 32:247–53.
- 9 Braun-Fahrlander C, Riedler J, Herz U et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347:869–77.
- 10 Wickens K, Douwes J, Siebers R et al. Determinants of endotoxin levels in carpets in New Zealand homes. *Indoor Air* 2003; 13: 128–35.
- 11 Athman R, Philpott D. Innate immunity via toll-like receptors and nod proteins. *Curr Opin Microbiol* 2004; 7:25–32.
- 12 Inohara N, Nunez G. The NOD: a signaling module that regulates apoptosis and host defense against pathogens. *Oncogene* 2001; 20:6473–81.
- 13 Gutierrez O, Pipaon C, Inohara N et al. Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor – kappa B activation. *J Biol Chem* 2002; 277:41701–5.
- 14 Kobayashi K, Inohara N, Hernandez LD et al. RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. *Nature* 2002; 416:194–9.
- 15 Douwes J, Pearce N, Heederik D. Does environmental endotoxin exposure prevent asthma? *Thorax* 2002; 57:86–90.
- 16 Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R. Toll-like receptors control activation of adaptive immune responses. *Nat Immunol* 2001; 2:947–50.
- 17 Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002; 196:1645–51.
- 18 Lazarus R, Vercelli D, Palmer LJ et al. Single nucleotide polymorphisms in innate immunity genes: abundant variation and potential role in complex human disease. *Immunol Rev* 2002; 190:9–25.
- 19 Hugot JP, Laurent-Puig P, Gower-Rousseau C et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996; 379:821–3.
- 20 Jawaheer D, Seldin MF, Amos CI et al. A genomewide screen in multiplex rheumatoid arthritis families suggests genetic overlap with other autoimmune diseases. *Am J Hum Genet* 2001; 68: 927–36.
- 21 Tsao BP, Cantor RM, Grossman JM et al. Linkage and interaction of loci on 1q23 and 16q12 may contribute to susceptibility to SLE. *Arthritis Rheum* 2002; 46:2929–36.
- 22 Hugot JP, Chamaillard M, Zouali H et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; 411:599–603.

- 23 Ogura Y, Bonen DK, Inohara N et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; 411:603–6.
- 24 Hampe J, Grebe J, Nikolaus S et al. Association of NOD2 (CARD 15) genotype with clinical course of Crohn's disease: a cohort study. *Lancet* 2002; 359:1661–5.
- 25 Kabesch M, Peters W, Carr D, Leupold W, Weiland SK, Von Mutius E. Association between polymorphisms in caspase recruitment domain containing protein 15 and allergy in two German populations. *J Allergy Clin Immunol* 2003; 111:813–7.
- 26 Weidinger S, Klopp N, Wagenpfeil S et al. Association of a *STAT 6* haplotype with elevated serum IgE levels in a population-based cohort of caucasian adults. *J Med Genet* 2004; 41:658–63.
- 27 Illig T, Bongardt F, Schöpfer A et al. The endotoxin receptor TLR 4 polymorphism is not associated with diabetes or components of the metabolic syndrome. *Diabetes* 2003; 52:2861–4.
- 28 Bothig S. WHO MONICA Project: objectives and design. *Int J Epidemiol* 1989; 18:S29–37.
- 29 Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994; 7: 954–60.
- 30 Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol* 1995; 12:921–7.
- 31 Slatkin M, Excoffier L. Testing for linkage disequilibrium in genotypic data using the Expectation-Maximization algorithm. *Heredity* 1996; 76:377–83.
- 32 Jarvis D, Chinn S, Luczynska C, Burney P. The association of smoking with sensitivity to common allergens: results from the European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1999; 104:934–40.
- 33 Busse WW, Rosenwasser LJ. Mechanisms of asthma. *J Allergy Clin Immunol* 2003; 111:S799–804.
- 34 Leynaert B, Neukirch C, Kony S et al. Association between asthma and rhinitis according to atopic sensitization in a population-based study. *J Allergy Clin Immunol* 2004; 113:86–93.
- 35 Stephens JC, Schneider JA, Tanguay DA et al. Haplotype variation and linkage disequilibrium in 313 human genes. *Science* 2001; 293:489–93.
- 36 Holt PG, Macaubas C, Prescott SL, Sly PD. Microbial stimulation as an aetiological factor in atopic disease. *Allergy* 1999; 49:12–6.
- 37 Holt PG, Sly PD. Interactions between respiratory tract infections and atopy in the aetiology of asthma. *Eur Respir J* 2002; 19:538–45.
- 38 Gereda JE, Leung DY, Thatayatikom A et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; 355:1680–3.
- 39 Gehring U, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J. House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med* 2002; 166:939–44.
- 40 Lauw FN, ten Hove T, Dekkers PE, de Jonge E, van Deventer SJ, van Der Poll T. Reduced Th1, but not Th2, cytokine production by lymphocytes after in vivo exposure of healthy subjects to endotoxin. *Infect Immun* 2000; 68:1014–8.
- 41 Eder W, Klimecki W, Yu L et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004; 113:482–8.
- 42 Ahmad-Nejad P, Mrabet-Dahbi S, Breuer K et al. The toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype. *J Allergy Clin Immunol* 2004; 113:565–7.
- 43 Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; 353:196–200.
- 44 Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology* 2004; 112:352–63.
- 45 O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. *Nat Med* 2004; 10:801–15.
- 46 Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J Biol Chem* 2001; 276: 4812–8.
- 47 Beutler B. Autoimmunity and apoptosis: the Crohn's connection. *Immunity* 2001; 15:5–14.
- 48 Netea MG, Kullberg BJ, de Jong D et al. NOD2 mediates anti-inflammatory signals induced by TLR2 ligands: implications for Crohn's disease. *Eur J Immunol* 2004; 34:2052–9.
- 49 Liu AH, Murphy JR. Hygiene hypothesis: fact or fiction? *J Allergy Clin Immunol* 2003; 111:471–8.
- 50 Rottem M, Gershwin ME, Shoenfeld Y. Allergic disease and autoimmune effectors pathways. *Dev Immunol* 2002; 9:161–7.