

Lack of association between Toll-like receptor 2 and Toll-like receptor 4 polymorphisms and atopic eczema

To the Editor:

The etiology of atopic diseases (asthma, rhinoconjunctivitis, atopic eczema) is complex and multifactorial, involving both genetic and environmental factors. To explain the recent increase of their prevalence in Western societies, based on the hygiene hypothesis it has been speculated that an altered stimulation of the innate immune system might contribute to a dysbalance on the level of T_H2 and T_H1 immune responses. The innate immune system comprises a variety of pattern recognition receptors expressed on the cell surface, such as the Toll-like receptors (TLRs), or in intracellular compartments, like nucleotide-binding oligomerization domain protein 1 and 2.¹ TLRs are known to function as mediators between innate and adaptive immunity via induction of dendritic cell maturation and shifting the adaptive immune response toward T_H1 .² They are activated by a wide range of pathogen-associated and endogenous stimuli including agents associated with the development of allergic diseases.³ TLR4 is the principal receptor for bacterial endotoxin, the exposure to which has been suggested to protect from the development of asthma and atopy.⁴ TLR2 recognizes peptidoglycan, which predominates the cell wall of *Staphylococcus aureus*,¹ a pathogen with important implications for atopic eczema. Recently, it has been shown that peptidoglycan activates nuclear factor- κ B and induces abundant IL-8 production from keratinocytes via functionally TLR2.⁵ In humans, polymorphisms in TLRs have been shown to be associated with a variety of diseases.⁶ In previous studies, associations of the TLR2 polymorphisms rs4696480 with asthma⁷ and rs5743708 with severe atopic eczema⁸ as well as the TLR4 polymorphisms rs4986790 with

asthma⁹ and rs4986791 with a modified response to endotoxin¹⁰ have been reported.

To study the candidacy of TLR2 and TLR4 as atopic eczema susceptibility genes, we performed an association study using all haplotype-tagging single nucleotide polymorphisms (htSNPs; information retrieved from the Innate Immunity PGA, NHLBI Program in Genomic Applications: <http://innateimmunity.net/data/homology>, June 2005) as well as SNPs previously associated with atopic traits (Fig 1) in a large cohort of 275 German white parent-offspring trios who had been recruited in Munich and Bonn, Germany. Children and their parents were administered a standardized questionnaire based on the European Community Respiratory Health Survey on respiratory health. 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. In all subjects, total as well as specific IgE antibodies against common environmental allergens (grass, birch, rye, mugwort pollen, *Alternaria*, *Cladosporium*, cat, dog, and *Dermatophagoides pteronyssinus*) were measured (enzyme-immuno assay, CAP-FEIA; Pharmacia, Uppsala, Sweden). Subjects were classified as having asthma or allergic rhinoconjunctivitis when they reported a physician's diagnosis. Atopic eczema was diagnosed on the basis of a skin examination by experienced senior dermatologists using the UK diagnostic criteria. The clinical severity of atopic eczema was determined according to the SCORAD index. Specific sensitization was defined to be present if at least one of the specific IgE antibodies was positive (CAP class ≥ 1 , corresponding to ≥ 0.35 kU/L). Total serum IgE levels were considered dichotomously with the 50th percentile (246 kU/L), 66th percentile (466 kU/L), and 90th percentile (2000 kU/L) as cutoff levels.

Assays for SNP genotyping were developed for all TLR2 and TLR4 SNPs, and genotyping was performed by

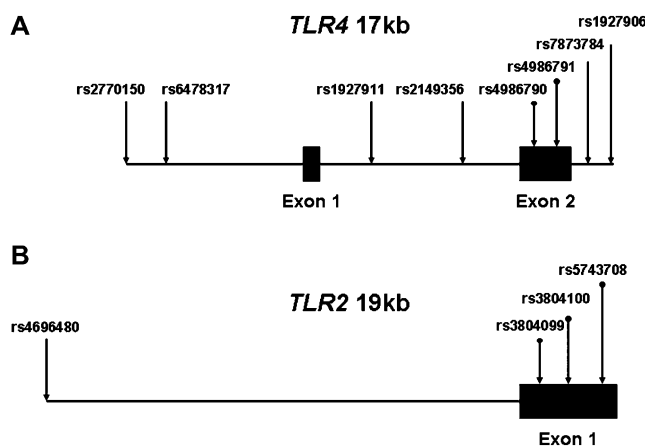


FIG 1. Gene structure of the TLR2 locus (A) and the TLR4 locus (B), according to ensembl v36 database (ENST00000339096/ENST00000260010). Location of genotyped SNPs is indicated by arrows above the line. Round-tailed arrows indicate exonic SNPs.

TABLE I. Phenotype characteristics of all individuals (parents and affected) included in analyses

	Trios (n = 275)	
	Parents	Offspring
Male sex, %	50.4	44.0
Mean ($\pm$ SD) age, y	55.1 ($\pm$ 9.2)	19.5 ($\pm$ 11.1)
Asthma, %	9.9	30.3
Atopic eczema, %	11.6	100
Extrinsic atopic eczema, %	3.7	63.4
Mean SCORAD	NA	44.6
Allergic rhinoconjunctivitis, %	28.6	50.9
Mean ($\pm$ SD) ln(total serum IgE)	3.16 ($\pm$ 4.11)	4.93 ($\pm$ 4.01)

using the MassARRAY system (Sequenom, San Diego, Calif). The Hardy-Weinberg equilibrium for genotype frequency distributions was tested using the χ^2 goodness-of-fit test. Pairwise linkage disequilibrium between each pair of SNP loci was also evaluated using the χ^2 test.

Evidence of association with the binary traits atopic eczema, moderate to severe atopic eczema (cutoff median SCORAD score, 44.6 points), asthma, allergic rhinoconjunctivitis, total serum IgE, and allergic sensitization was evaluated using the classical transmission/disequilibrium test implemented in Haploview 3.2 (J. Barrett and J. Maller, Whitehead Institute, Cambridge, Mass). Haplotype frequencies were estimated from htSNP genotype data using the expectation maximization (EM) algorithm implemented in Haploview, and haplotypes were evaluated for transmission distortion. Power analysis showed that our sample of 275 families has a power of >80% to detect a deviation of 20% in the allel transmission rate to offspring with atopic eczema on a Bonferroni-corrected significance level.

Parental genotype frequencies were found to be consistent with Hardy-Weinberg equilibrium. There were no significant deviations from expected transmission rates

in our family-based study for any of the SNPs under consideration (Table I). The SNPs within *TLR2* and *TLR4* were in strong linkage disequilibrium with each other. The 4 *TLR2* SNPs and 8 *TLR4* SNPs defined 5 haplotypes with frequencies >5% for each gene, which accounted for 96.4% and 94.5% of all observed haplotypes. None of the individual SNPs showed associations with atopic eczema or severity of atopic eczema in our population of German white families (Table II), and there was no evidence of haplotype transmission disequilibrium. In particular, we could not replicate the reported association of the *TLR2* polymorphism rs5743708 with severe atopic eczema, which was likely to be false-positive because of a low number of cases and an unbalanced study design.⁹ However, because there is substantial variation in frequencies of both genetic and nongenetic risk factors among different populations, our results cannot be generalized to other populations. Analysis of the subgroups of children with asthma, rhinitis, or allergic sensitization likewise did not reveal evidence of association with *TLR2* or *TLR4* polymorphisms. However, the number of children with these traits was lower, and the children were selected on the basis of atopic eczema. Therefore, the current sample might lack statistical power to replicate associations with asthma.

In conclusion, we have performed a comprehensive evaluation of the *TLR2* and *TLR4* locus in a large family-based study, which eliminates the possibility of spurious associations on the basis of population stratification. We have found no evidence that common *TLR2* or *TLR4* variants or haplotypes are associated with atopic eczema in our study population. Our data indicate that *TLR2* and *TLR4* do not represent major atopic eczema susceptibility loci in German white subjects.

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TABLE II. Association analysis of *TLR2* and *TLR4* SNPs in the trio cohort*

Gene	dbSNP rs#	Alleles	MAF (%)	Atopic eczema		Asthma		Rhinoconjunctivitis	
				T:U	P value	T:U	P value	T:U	P value
<i>TLR2</i>	rs4696480	T $\rightarrow$ <u>A</u>	48.2	141:134	.6729	47:47	1.0000	73:68†	.6737
<i>TLR2</i>	rs3804099	T $\rightarrow$ <u>C</u>	42.6	137:129	.6238	51:47†	.6862	73:61†	.2999
<i>TLR2</i>	rs3804100	T $\rightarrow$ <u>C</u>	7.8	46:36	.2695	12:08	.3711	21:17†	.5164
<i>TLR2</i>	rs5743708	G $\rightarrow$ <u>A</u>	3.8	24:18	.3545	07:04†	.3657	13:07†	.1797
<i>TLR4</i>	rs2770150	<u>T</u> $\rightarrow$ C	27.8	71:65	.6069	40:32	.3458	57:53	.7029
<i>TLR4</i>	rs6478317	<u>A</u> $\rightarrow$ G	32.8	73:71	.8676	42:40	.8252	61:56†	.6439
<i>TLR4</i>	rs1927911	<u>C</u> $\rightarrow$ T	26.9	64:57	.5245	40:36	.6464	52:49	.7653
<i>TLR4</i>	rs2149356	<u>C</u> $\rightarrow$ A	32.0	74:72	.8685	41:40	.9115	60:58†	.8539
<i>TLR4</i>	rs4986790	A $\rightarrow$ <u>G</u>	6.1	25:21	.5553	11:11	1.0000	20:16	.5050
<i>TLR4</i>	rs4986791	C $\rightarrow$ <u>T</u>	6.6	26:23	.6682	12:12	1.0000	21:17	.5164
<i>TLR4</i>	rs7873784	<u>G</u> $\rightarrow$ <u>C</u>	15.7	43:42	.9136	25:22†	.6617	35:31†	.6225
<i>TLR4</i>	rs1927906	A $\rightarrow$ <u>G</u>	9.8	36:30	.4602	16:14†	.7150	29:23	.4054

MAF, Minor allele frequency; dbSNP rs#, reference marker.

*Alleles are listed in chromosomal order, as genotyped in the family collection Alleles are coded as major allele $\rightarrow$ minor allele. T:U ratio refers to the ratio of transmitted and untransmitted alleles of the allele underlined except for †(opposite allele overtransmitted).

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Oral phenylephrine: An ineffective replacement for pseudoephedrine?

To the Editor:

In 1976, a US Food and Drug Administration (FDA) review panel concluded that oral phenylpropanolamine, pseudoephedrine, and phenylephrine are safe and

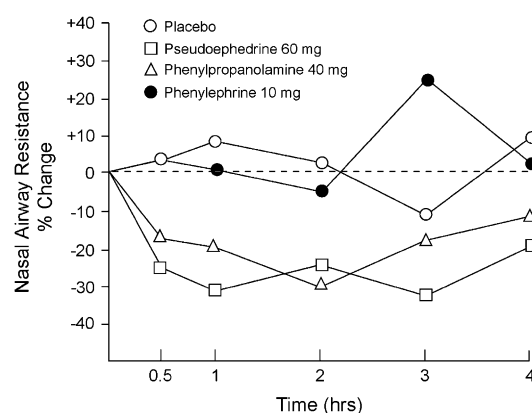


FIG 1. Percent change in nasal airway resistance in 20 patients with chronic nasal stuffiness after single doses of placebo and 3 oral decongestants administered in a randomized, double-blind, crossover manner on different days. Changes were significant at each time point after pseudoephedrine and phenylpropanolamine but not after phenylephrine. Drawn from data presented by Bickerman.⁵ This graph was previously published¹¹ and is reproduced with permission of *Pharmacotherapy*.

effective for nonprescription relief of nasal congestion caused by the common cold, allergic rhinitis, and sinusitis.¹ They are α -adrenergic agonists that decrease nasal mucosal swelling by vasoconstriction.

In 2000, phenylpropanolamine was voluntarily removed from all products because hemorrhagic strokes were associated with its use.² Recently, 34 states have enacted restrictions on the availability of pseudoephedrine because it is used to manufacture methamphetamine illegally. Some states require products containing this decongestant to be sold by a pharmacist or "behind the counter," whereas others restrict the quantity sold. Effective September 30, 2006, an amendment to the USA Patriot Act (HR 3889, Title VII) will require all stores to keep pseudoephedrine products behind the counter and purchasers will have to show a photo identification and sign a log book to obtain them.

Although these actions are unlikely to prevent patients from obtaining pseudoephedrine-containing products from pharmacists, it is increasingly difficult to obtain them from grocery, discount, and convenience stores. In response, Pfizer, Inc (Morris Plains, NJ), introduced a replacement product containing 10 mg phenylephrine (Sudafed-PE) that cannot be converted to methamphetamine and can be sold without restriction. Other manufacturers are following suit.

Phenylephrine, at the FDA-approved dose of 10 mg for adults, is unlikely to provide relief of nasal congestion. It has poor oral bioavailability because of extensive first-pass metabolism in the gut and liver.³ Only 38% of the dose reaches the systemic circulation,³ compared with 90% of a pseudoephedrine dose.⁴ Moreover, in a randomized, double blind, placebo-controlled, crossover study of 3 oral decongestants in 20 patients with chronic nasal stuffiness, phenylephrine was no more effective than placebo in reducing nasal airway resistance⁵ (Fig 1).