

The Toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype

To the Editor:

Atopic dermatitis (AD) is a common chronic inflammatory skin disease that is part of the atopic syndrome. Although great progress has been achieved in understanding the immunopathogenesis of the disease, the etiology is still largely unknown. In many patients, the disease is complicated by their enhanced susceptibility to skin infections, which is associated with the permanent colonization of lesional and nonlesional skin with *Staphylococcus aureus* in more than 80% of affected patients with AD.¹ Nearly half of these patients show cutaneous colonization with superantigen-secreting (toxigenic) strains as well as the presence of IgE antibodies directed against these toxins. Both parameters correlate with more severe disease activity and high IgE antibody titers.²

These observations led to the hypothesis that the host immune defense against microbial pathogens is impaired in at least a subgroup of patients with AD. This immune response is initiated by the innate immune system, which recognizes pathogen-associated molecular patterns via pattern recognition receptors. Activation of the innate immune system not only triggers immediate innate effector functions but also critically controls initiation and development of adaptive immune responses. Thus innate and adaptive immune reactions are tightly linked and interconnected.

One class of pathogen-associated molecular pattern receptors comprises the Toll-like receptors (TLRs), a family of (currently) 10 members with distinct recognition profiles. Recent studies have indicated the critical roles of TLR-2 and TLR-4 in the recognition of bacterial and fungal components, including LPS, peptidoglycan, lipoteichoic acid, microbial lipoproteins, and zymosan from yeast cell walls. For each of these TLRs, 2 clinically relevant single nucleotide polymorphisms (SNPs) have been described; these result in changes of the amino acid sequences.

The first TLR-2 mutation, R753Q, is located within the intracellular portion of the receptor and has been particularly associated with *S aureus* infections.³ The second TLR-2 polymorphism investigated, R677W, led to altered intracellular mycobacterial signaling and was associated with lepromatous leprosy.⁴

For TLR-4, two clinically relevant polymorphisms, D299G and T399I, have been described. They were located within the extracellular domain and were found in patients with septic shock, particularly when such shock was due to gram-negative infection.⁵ Furthermore, these common cosegregating TLR-4 polymorphisms were linked to LPS hyporesponsiveness.⁶ We sought to determine whether AD patients would exhibit a higher frequency of such TLR SNPs and, if so, how the phenotype of these patients is characterized.

A total of 78 adult patients with mild to severe AD (age: median, 29 years; range, 14-78 years) who fulfilled the diagnostic criteria of Hanifin and Rajka and 39

healthy age matched control subjects (age: median, 32 years; range, 17-58 years) were analyzed. Each of the patients had a steroid-free interval of at least 4 weeks, and no patient received systemic or topical antibiotic treatment before the examination. Furthermore, none of the patients had acute bacterial superinfection.

The clinical severity of AD was determined according to the SCORAD index. Levels of total serum IgE and serum specific IgE to staphylococcal enterotoxins A through D and TSST-1 as well as to *Dermatophagoides pteronyssinus* were measured through use of the Pharmacia CAP system (Pharmacia and Upjohn, Uppsala, Sweden). DNA was isolated from the sera of patients and healthy control subjects through use of a Qiamp Blood Kit (Qiagen, Hilden, Germany). Primer sequences and the method for TLR-4 SNP determination have been described previously.⁷

For TLR-2 genotyping, 3 primers were used in 2 separate PCR-amplifications:

- TLR-2 Pst-753s: 5'-CAT TGA AAA GAG CCA CAA AAC TGT CTT TG-3'
- TLR-2 Aci-677s: 5'-GAT GCC TAC TGG GTG GAG AA-3'
- TLR-2 Pst-753as: 5'-CAG GTA GGT CTT GGT GTT CAT TAT C-3'.

The TLR-2 Pst-753as was designed to work in both reactions.

For the analysis of the TLR-2 polymorphism R753Q, we used the Pst I RFLP. Determination of the R677W polymorphism was performed by melting curve analysis after TLR-2-specific gene amplification through use of the following sequence-specific hybridization probes on the LightCycler instrument (Roche Diagnostics, Basel, Switzerland):

- sensor LC red 640: 5'-TCT TCA TAA GCG GGA CTT CAT TC-p anchor: 5'-GAA CTT CAA TCC CCC CTT CAA GTT G-Fl.

Positive controls were included.

In our control population, the TLR-2 polymorphism R753Q had a prevalence of 2.5%, which is identical to the frequencies of the TLR-4 D299G and T399I polymorphisms; in contrast, none of the controls expressed TLR-2 R677W. Of the 78 patients with AD, 9 (11.5%) carried the R753Q SNP, whereas the R677W SNP was absent in this group. The TLR-4 mutations occurred in 12% of our AD population. Taken together, 23.5% of all patients with AD were identified as carriers of TLR polymorphisms (Fig 1). None of the patients carried polymorphisms in both the TLR-2 gene and the TLR-4 gene.

Patients carrying the TLR-2 R753Q allele variant exhibited a distinct phenotype characterized by severe to moderate AD (SCORAD score: median, 55.8 points; range, 30.3 to 90.5 points). None of these patients had a SCORAD score of <30 points, and the median SCORAD score of the nonpolymorphic AD group was 44.8 points (min-max values, 14.9 to 89 points). In contrast, patients with AD carrying the TLR-4 polymorphism did not differ from the patients with AD with nonvariant TLR-4 genotypes.

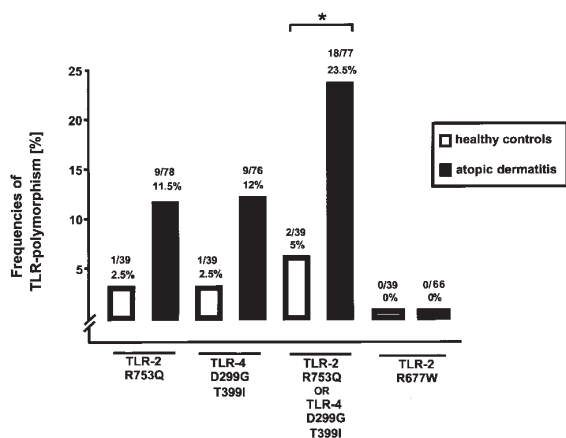


FIG 1. Frequencies of TLR-2 and TLR-4 SNPs in 78 patients with AD and 39 healthy controls. Statistical analysis by 2-tailed χ^2 test; $P = .01$.

Similar results that have recently been shown for bronchial asthma were not associated with other common TLR-4 polymorphisms tested.⁸ On the other hand, Werner et al⁹ found a nonsignificant trend toward an even lower risk of asthma among the TLR-4 D299G/T399I carriers. In contrast, all patients with TLR-2 R753Q showed higher levels of total serum IgE (Fig 2, A) as well as of superantigen-specific IgE (Fig 2, B) than the nonpolymorphic AD group. In addition, the highest levels of *D pteronyssinus*-specific IgE antibody titers were also observed in this group (median, 100 kU/L); next highest were the levels in the patients with AD with nonvariant TLR-2 genotype (median, 12.25 kU/L) and the patients with AD carrying the TLR-4 SNPs (median, 1.9 kU/L; Fig 2, C).

These data suggest that the TLR-2 polymorphism R753Q increases the susceptibility to infections and chronic colonization with various pathogens, including *S aureus*. It is known that the extracellular domain of TLR-2 directly interacts with peptidoglycan derived from *S aureus*, and TLR-2 $-/-$ mice are highly susceptible to *S aureus* infection. Very recently, it was discovered that epidermal keratinocytes in AD are deficient in their production of certain defensins with potent antimicrobial activity.¹⁰ With respect to the underlying mechanisms, it has been documented that increased levels of IL-4 and IL-13 inhibit defensin gene expression. Therefore, this TLR-2 missense mutation is associated with a certain phenotype of AD, defined by markedly elevated IgE antibody levels, increased disease severity, and susceptibility to colonization with *S aureus*. Carrying this polymorphism is a novel example of the complex gene-environment-interaction in chronic AD. Future studies will be directed at further analyzing the molecular effect of this polymorphism and assessing whether this SNP might be a useful predictive marker for a severe phenotype in at least a subgroup of patients with AD.

Parviz Ahmad-Nejad, MD^a
Salima Mrabet-Dahbi, BS^b
Kristine Breuer, MD^c
Martina Klotz, MD^d

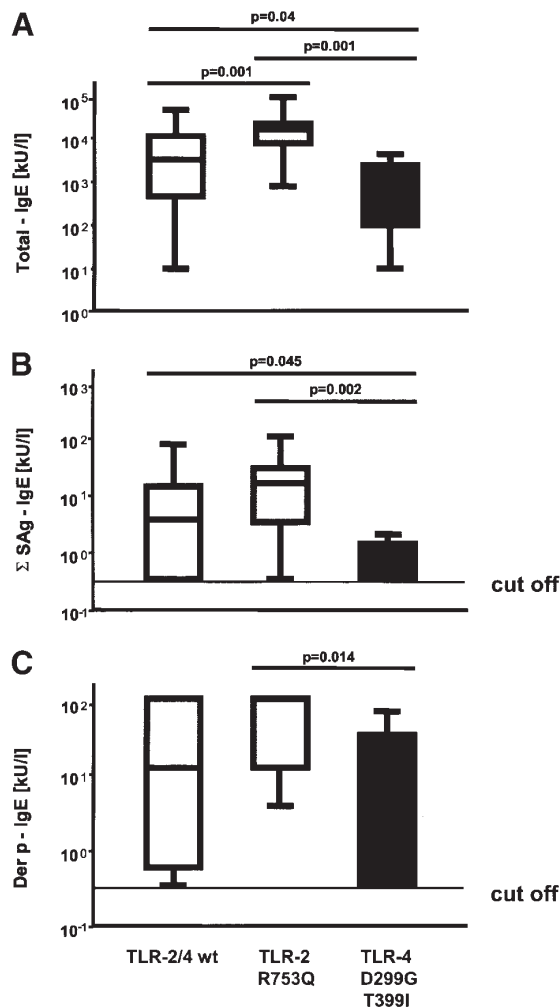


FIG 2. Levels of total serum IgE (A), superantigen-specific IgE (B), and anti-Der p IgE (C) in patients with AD. A total of 60 subjects carried the TLR-2/4 wild-type variant, 9 subjects carried the TLR-2 R753Q variant, and 9 subjects had the cosegregating TLR-4 D299G/T399I missense mutations. Anti-staphylococcal enterotoxin (SE) A, anti-SEB, anti-SEC, anti-SED, and TSST-1-specific IgE values were added and are depicted as the sum of superantigen-specific IgE (B). The lowest limit of antigen-specific IgE sensitivity was 0.35 kU/L (cut off). Boxes represent first quartile, median, and third quartile; bars represent min-max values. Significance between experimental groups was calculated through use of a 2-tailed Mann-Whitney *U* test. *P* values of <.05 were regarded as statistically significant.

Thomas Werfel, MD^c

Udo Herz, PhD^b

Klaus Heeg, MD^d

Michael Neumaier, MD^a

Harald Renz, MD^b

^aInstitute for Clinical Chemistry

University Hospital Mannheim

University of Heidelberg

^bDepartment of Clinical Chemistry and Molecular Diagnostics

Central Laboratory

University Marburg

^cDepartment of Dermatology

Hannover Medical School

^d*Institute of Medical Microbiology and Hygiene
University Marburg
Germany*

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