Letter: Genetic Variation in the Inflammasome and Atopic Dermatitis Susceptibility

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Genetic variation in the inflammasome and atopic dermatitis susceptibility

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Short title: Inflammasome SNPs in atopic dermatitis

Abbreviations: AD, atopic dermatitis; CI, confidence interval; OR, odds ratio; NLR, nucleotide-binding domain and leucine-rich repeat; NLRP, nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin-domain containing protein; PDT, pedigree disequilibrium test; SNP, single nucleotide polymorphism

Atopic dermatitis (AD) is a common chronic inflammatory skin disease. The pathophysiological factors include genetic predisposition to skin barrier defects, dysregulated immunity and hypersensitive allergen response (Novak and Simon, 2011). The high levels of proinflammatory cytokines in AD initiate and maintain the inflammation (Bieber, 2008) and mediates cross-talk between innate and adaptive immune systems. The recently described cytosolic receptors of the nucleotide-binding domain and leucine-rich repeat (NLR) containing family have been shown to play a key role in innate immune regulation. The NLR family, pyrin domain containing proteins (NLRP) are recognized for forming a multiprotein complex referred to as the 'inflammasome', which activates the pro-inflammatory cytokines, interleukin (IL)-1β, -18 and -33 (Li et al, 2008). The above interleukins are important in innate immune functions and in regulation of adaptive immunity. Single nucleotide polymorphisms (SNPs) in *NLRP1* have been associated with vitiligo and related autoimmune conditions (Jin et al, 2007a; Jin et al, 2007b), while SNPs in *NLRP3* have been associated with food anaphylaxis and allergic asthma (Hitomi et al, 2009), rheumatoid arthritis (Kastbom et al, 2008), Crohn's disease (Schoultz et al, 2009; Villani et al, 2009) and malignant melanoma (Verma et al, 2012). Since atopic manifestations e.g. food allergies, asthma, allergic rhinitis, and allergic conjunctivitis are common, we investigate the significance of seven SNPs located in three different inflammasome genes (*NLRP1*, *NLRP3*, and *CARD8)* in the susceptibility of AD. All of the studied SNPs except those in NLRP1 are demonstrated to be of functional significance.

A Swedish AD family material (Bradley et al, 2000) including 1708 individuals (1260 affected) from 494 families with at least two affected siblings were included in the analysis. Measurements of total serum-IgE concentrations and IgE-specific antibodies for food and inhalant allergens were performed (Bradley et al, 2000). A Swedish control population without eczema comprising 732 healthy individuals was analyzed as reference population. We performed TaqMan genotyping for variants of *NLRP1* (rs6502867, rs12150220), *NLRP3* (rs35829419, rs10733113, rs10754558, rs4612666), and *CARD8* (rs2043211) using an ABI Prism 7500 Sequence Detection System, with the SDS 2.3 software for allelic discrimination (Applied Biosystems, Foster City, CA). None of the SNPs were in linkage disequilibrium ($r2 \leq 0.3$).

A pedigree disequilibrium test (PDT) was carried out to investigate evidence for association between the genetic variants and AD. Data for the family-based association analysis, including odds ratio (OR), 95% confidence interval (CI) and correction for multiple testing (10.000 permutations) were calculated using the UNPHASED program (v.3.1.4). All SNPs were shown to be in Hardy-Weinberg equilibrium in the control population. No statistical associations between variants and AD were found either overall or after stratification for different phenotypes (Table S1a). Since gender-specific associations have previously been reported for *NLRP3* SNPs (Schoultz et al, 2009), we further stratified for gender. The results showed significant association between *NLRP3* variant rs10733113 and raised total IgE antibodies among males (OR 0.44(0.25-0.77), p=0.02 corrected) (Table 1, S1b). No association among females was found (Table S1c). This *NLRP3* SNP has previously been implicated in Crohn's disease (Villani et al, 2009) and the *NLRP3* SNPs rs4612666 and rs10754558 have been associated with food-induced anaphylaxis and aspirin-induced asthma in a Japanese case-control study (Hitomi et al, 2009). However, we did not detect any AD association with these SNPs in our population.

In a separate study design we included one randomly selected AD patient from each family, resulting in 454 patients and compared with 732 healthy Swedish controls from the same geographical area. Comparison between cases and controls was performed using logistic regression analysis, and STATA v.10 statistical package. P values <0.05 were subjected to Bonferroni's correction. The commonly occurring *NLRP1* SNP rs12150220 revealed strong association in overall comparison, also following corrections for multiple testing (OR 0.54(0.39-0.76), p=0.0001, p=0.0021 corrected, Table 2), indicating a role in AD susceptibility. Upon gender stratification, associations were found for both sexes, most prominent among males (OR 0.45(0.27-0.76), p=0.003) compared to females (OR 0.61(0.39-0.96), p=0.033). However, only borderline significance among males remained after Bonferroni correction (p=0.063). This missense coding SNP (Leu155His), due to its localization between PYRIN and NACHT domains of *NLRP1*, might have an important function during oligomerization with other proteins. The consequence of this allelic variant was analyzed by Polyphen and was predicted to be "probably functionally damaging". Furthermore, the SNP is located in a

highly conserved region through primate evolution (Jin et al, 2007b) indicating this region to be critical for the NLRP1 function. Moreover, the *CARD8* SNP rs2043211, a suggested binding partner in the NLRP3 inflammasome and inhibitor of NFkB activity was found to be associated with AD among females (OR 0.55(0.32-0.95), p=0.033), however not following correction. This SNP has previously been shown to be associated with rheumatoid arthritis (Kastbom et al, 2010) and in combination with *NLRP3* variant rs35829419 with Crohn's disease (Roberts et al, 2010). Interestingly, CARD8 interacts with NOD2 that has been associated with atopic traits (Kabesch et al, 2003).

Interaction analyses of rs10733113 and rs12150220, which showed significant associations to AD in this study, did not reach overall significant association in the pedigree approach (p=0.81, 0.91(0.64-1.30)) nor strengthen the association in the case-control design (p=0.017, 0.62(0.42-0.92)). No further changes were noted following gender stratifications.

Genome-wide association studies (GWAS) in AD have failed to identify studied chromosomal regions (Hirota et al, 2012; Paternoster et al, 2012), but this does not entirely exclude the significance of these genes. The SNPs were selected as disease associations have already been reported for each of them in other relevant inflammatory conditions. Inflammasomes have emerged as promising candidates for different skin pathologies and the inflammatory cytokines, IL-1β and IL-18 are key molecules orchestrating atopic skin inflammation (Homey, 2006). A critical role for an additional inflammatory cytokine, IL-33 in AD has recently been reported (Savinko et al, 2012) and SNPs in IL-33, IL-33R as well as IL-18R are of potential interest in this regard (Akhabir and Sandford, 2010). In this large and comprehensive study, we have analyzed seven commonly occurring inflammasome SNPs using two study designs: a family based PDT analysis comprising 1708 individuals and a case control design comprising 454 cases and 732 controls. One limitation of case-control studies, the most commonly used approach for SNP association studies is the sensitiveness to population stratifications. The family-based design is more robust to confounding factors and variance due to the common genetic background among the family members. However PDTs require more patients to detect the same level of association as case-control studies. *NLRP1* rs12150220 displayed strong associations with overall susceptibility to AD using the case-control design, which however could not be confirmed in the PDT

analysis prompting a cautious interpretation of our results. The associated risk is probably quite modest and NLRP1 might be one of several players, demanding an additional triggering risk locus/factor. The role of rare susceptibility variants cannot be ruled out, although their contribution to the disease might be limited. However, to confirm the relevance of these *NLRP* SNPs and AD, additional cohorts are warranted.

Conflict of interest

The authors declare no conflict of interest

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Table 1. Results of Pedigree disequilibrium test for *NLRP3* rs10733113 variant in families with atopic dermatitis.

		p-value	N affected	OR (95% CI)
AD/Williams[a]	**AD**	0.50	1260	0.93 (0.76-1.15)
	Males	0.27	450	0.83 (0.61-1.14)
	Females	0.85	723	0.97 (0.75-1.26)
AD+specific IgE	**AD**	0.64	669	0.94 (0.73-1.21)
	Males	0.34	297	0.84 (0.58-1.20)
	Females	0.95	372	0.99 (0.71-1.38)
Early onset of AD[b]	**AD**	0.58	881	0.94 (0.74-1.19)
	Males	0.25	348	0.81 (0.57-0.16)
	Females	0.94	533	1.01 (0.76-1.34)
Severe AD[c]	**AD**	0.53	483	0.91 (0.68-1.22)
	Males	0.18	210	0.74 (0.48-1.15)
	Females	0.86	273	0.97 (0.67-1.40)
AD+Asthma	**AD**	0.64	611	0.94 (0.73-1.20)
	Males	0.34	269	0.84 (0.58-1.2)
	Females	0.95	342	0.99 (0.71-1.38)
AD+Rhinoconj[d]	**AD**	0.80	851	0.97 (0.77-1.23)
	Males	0.21	343	0.79 (0.55-1.12)
	Females	0.62	508	1.09 (0.78-1.52)
AD+Raised total IgE	**AD**	0.03	355	0.71 (0.52-0.98)
	Males	**0.002**[*#]	137	0.44 (0.25-0.77)
	Females	0.53	218	0.88 (0.58-1.33)
AD+Food Allergy	**AD**	0.04	215	0.65 (0.43-1.00)
	Males	0.04	96	0.52 (0.26-1.03)
	Females	0.14	119	0.67 (0.38- 1.16)

AD; atopic dermatitis, OR; odds ratio, CI; confidence interval

[a] AD based on clinical examination according to the UK Working Party's Diagnostic Criteria, [b] ≤2 years, [c] severity scoring ≥4, [d] AD+Rhinoconjunctivitis

corrected p value 0.02

Table 2. Genetic association of *NLRP3, NLRP1* and *CARD8* variants with atopic dermatitis in cases and controls.

			p-value	Controls	Cases	OR (95% CI)
NLRP3	**rs35829419**	**AD**	0.704	732	454	1.29 (0.34-4.84)
		Males	0.332	370	178	2.22 (0.44-11.13)
		Females	0.698	361	276	0.62 (0.056-6.89)
	rs10733113	**AD**	0.427	732	441	1.39 (0.62-3.14)
		Males	0.258	370	166	1.90 (0.62-5.77)
		Females	0.899	361	275	1.08 (0.33-3.59)
	rs10754558	**AD**	0.363	684	452	1.18 (0.83-1.69)
		Males	0.192	342	177	1.45 (0.83-2.54)
		Females	0.877	341	275	0.96 (0.60-1.54)
	rs4612666	**AD**	0.872	687	420	1.04 (0.67-1.61)
		Males	0.759	344	164	1.11 (0.57-2.18)
		Females	0.942	342	256	0.98 (0.54-1.76)
NLRP1	**rs6502867**	**AD**	0.147	724	435	1.46 (0.88-2.42)
		Males	0.116	366	172	1.95 (0.85-4.50)
		Females	0.697	357	263	1.14 (0.60-2.16)
	rs12150220	**AD**	0.0001*[a]	730	436	0.54 (0.39-0.76)
		Males	0.003*[b]	368	174	0.45 (0.27-0.76)
		Females	0.033*[c]	361	262	0.61 (0.39-0.96)
CARD-8	**rs2043211**	**AD**	0.321	732	452	0.82 (0.56-1.21)
		Males	0.334	370	177	1.31 (0.76-2.27)
		Females	0.033*[d]	361	275	0.55 (0.32-0.95)

AD; atopic dermatitis, OR; odds ratio, CI; confidence interval
Corrected p values; [a] 0.0021, [b] 0.063, [c] 0,693, [d] 0,693