

A Genome-Wide Association Study in Koreans Identifies Susceptibility Loci for Allergic Nickel Dermatitis

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Dear Editors,

Allergic contact dermatitis (ACD) is one of the frequently faced problems in general dermatology, and among its various causes, nickel is known to be the most common allergen in the general population [1]. The prevalence of nickel allergy has constantly been growing in many countries and presents a major health and socioeconomic issue [2]. ACD is thought to develop through the interactions between genetic and environmental factors. Historically, for several decades many different approaches have been used to study the genetic factors in ACD, which are well reviewed by Schnuch et al. [3]. In addition, many studies have recently been performed to identify functionally relevant polymorphisms in ACD. Polymorphisms in genes encoding filaggrin, N-acetyltransferase, glutathione-S-transferases, manganese superoxide dismutase, angiotensin-converting enzyme, TNF and interleukin (IL)-16 have been studied widely, showing varying results in their

relevance to the pathogenesis of ACD [3]. The aim of this study was to find novel gene polymorphisms in individuals with nickel dermatitis by a genome-wide association study (GWAS) in the Korean population.

A total of 98 people were enrolled. All patients underwent a patch test with nickel according to the International Contact Dermatitis Research Group guidelines [4]. Test methods, interpretations of test reactions and results are described elsewhere in detail [4]. Among the tested patients, 24 had at least one or more positive reactions to nickel, 52 had no reactions to nickel and 22 had irritant reactions. In the GWAS, the 24 patients positive to the nickel patch test and the 52 control subjects negative to the patch test were examined. The 22 people who showed irritant reactions were excluded from the data analysis. All nickel-positive and control subjects in the GWAS were genotyped by using Axiom Genome-Wide Assay Chip Plate[®] (Affymetrix, Santa

Clara, Calif., USA). Filtering of genotyping data was performed. Single nucleotide polymorphisms (SNPs) with a minor allele frequency of <0.1 and SNPs with a Hardy-Weinberg equilibrium test *p* value of <1 × 10⁻³ were excluded from the subsequent statistical analysis. SNP with a minimum *p* value of <1 × 10⁻⁴ in the GWAS was considered having a suggestive association with nickel dermatitis. In case/control association analysis, Fisher's exact test was used. Data filtering and statistical analysis were performed by using PLINK statistical software (v1.07; <http://pnug.mgh.harvard.edu/~purcell/plink/>) [5].

In this GWAS, we were not able to detect any SNPs with a significant association (*p* value <1 × 10⁻⁷). However, we found two novel SNPs with a possible association with a minimum *p* value of <1 × 10⁻⁴. These genes were identified as NTN4 and PELI1. SNP rs2367563(A) which is located in NTN4 (*netrin 4*) had a *p* value of 3.7 × 10⁻⁶ and SNP rs6733160(C) of PELI1 (*pellino*

Table 1. Association results for novel SNPs by GWAS

Gene	Description	Locus (chr.)	Strongest SNP (risk allele)	Chromosome position	NCBI AccID	GenBank ID	Odds ratio	p value
<i>Nickel 1+ and 2+ included in data analysis</i>								
NTN4	netrin 4	12q22	rs2367563(A)	96057184	NM_021229	59277	0.169	3.7×10^{-5}
PELI1	pellino homolog 1 (Drosophila)	2p13.3	rs6733160(C)	64364632	NM_020651	57162	6.444	7.7×10^{-5}
<i>Nickel 2+ included in data analysis</i>								
PELI1	pellino homolog 1 (Drosophila)	2p13.3	rs6733160(C)	64364632	NM_020651	57162	9.276	7.1×10^{-6}

homolog 1) had a p value of 7.7×10^{-5} . We then classified the 24 patch test-positive people into two groups according to their positive grades. Of 7 patients 1 was positive to the nickel patch test whereas of 17 patients 2 were positive. When only individuals with a 2-fold positive reaction to nickel were exclusively included in the data analysis, only SNP of PELI1 showed a lower p value (7.1×10^{-6}) and a higher odds ratio (9.276). Additionally, we used publicly available data, SNP database of the National Center for Biotechnology Information (NCBI), to find out whether SNPs are located in the coding regions. As a result, both of our novel SNPs were located in noncoding regions (intron), which does not cause amino acid changes. However, these SNPs could be linked to other undefined functional changes such as mRNA splicing alterations or influences on binding sites of transcription factors. We propose that further functional studies of these SNPs should be conducted in the near future. Information about the two identified SNPs of different genes is described in table 1.

Genetic factors underlying the development of contact allergy have been investigated for several decades. Various genetic approaches had been applied to reveal genetic susceptibilities in contact allergy-prone individuals and families. The majority of recent genetic studies of contact allergy have been focused on finding potentially relevant polymorphisms. The probable functionally relevant polymorphisms in contact allergic patients have been found in genes encoding filaggrin, N-acetyltransferase 1 and 2, manganese superoxide dismutase, angiotensin-converting enzyme, glutathione-S-transferases M1 and T1, TNF and IL-4 or IL-16. Detailed reviews of these polymorphisms are be-

yond our scope but are well reviewed in another paper [3].

In our GWAS, we found two new SNPs associated with nickel dermatitis. SNPs are located in the NTN4 and PELI1 genes. SNPs of these genes, in aspect of ACD, have never been reported before. We have extensively reviewed these two novel genes and found that PELI1 is probably a biologically relevant gene related to nickel dermatitis.

Netrin-4 is a member of the netrin family, which is an extracellular matrix molecule that has homology with laminin amino-terminal domains. Netrin-4 is known to promote neurite extension and is also involved in regulation of pulmonary airway branching, vasculogenesis patterning and endothelial proliferation in pathological angiogenesis [6]. However, the current understandings of netrin-4 molecule lack any biological relevance to nickel dermatitis.

Pellino-1 is a member of the E3 ubiquitin ligase family which attaches ubiquitin to a lysine on target proteins via an isopeptide bond. Pellino-1 is also involved in TLR/IL-1R signaling. Recently, human TLR4 has been found to be crucial in the development of contact allergy to nickel [7]. Nickel can directly activate human TLR4, which results in proinflammatory intracellular signal transduction cascades and subsequent stimulation of the transcription factor nuclear factor- κ B (NF- κ B)-dependent genes. Pellino-1 regulates activation of NF- κ B by catalyzing polyubiquitylation of IL-1 receptor-associated kinase (IRAK) molecules in the TLR signaling pathways [8]. In addition, pellino-1 interacts with Smad6, a TGF- β 1-induced inhibitory protein, and subsequently inhibits NF- κ B activation [9]. Such studies support the view that pellino-1 is a crucial element in the regulation

of innate immune signaling. Therefore, it is possible that the SNP of PELI1 influences the pellino-1 functions and impairs its NF- κ B-regulatory actions, which eventually results in an unregulated inflammatory response in the clinical appearance of nickel dermatitis.

Herein, we have found two novel gene polymorphisms of nickel dermatitis patients by GWAS. A variety of SNPs related to ACD has been reported up to now. However, most of the reports have been related to the three functionally relevant steps in ACD: factors influencing the availability of the allergen, factors interfering with inflammatory processes, and immunological factors [3]. As far as we know, this study is the first to report SNPs of genes related to ubiquitination in nickel dermatitis. Furthermore, pellino-1 is involved in the innate immune system and notch signaling, and it is also the first to report SNPs of genes related to innate immunity in nickel dermatitis. Yet, there are some limits in our current study. Further investigations will be needed to see whether these PELI1 SNPs are general polymorphisms in ACD or only specific to nickel dermatitis. Despite thorough investigations of NTN4, we were not able to find any functionally relevant relationships to ACD. Nevertheless, NTN4 might be involved in ACD, and further studies of these novel genes in aspects of ACD are awaited. The number of enrolled subjects was relatively small, and the p values of novel SNPs were also not small enough to confirm the absolute relationships between the SNPs and nickel dermatitis. Even so, we think it is noteworthy to report SNPs of PELI1 and NTN4 in nickel dermatitis as there has been no previous literature addressing these genes.

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Disclosure Statement

The authors have declared no conflicts of interest.

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