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# Differential modification of genetic susceptibility to childhood eczema by two probiotics

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**Abstract**

*Background*

In a double-blind, randomized, placebo-controlled birth cohort, we have recently shown a beneficial effect of *Lactobacillus rhamnosus* HN001 (HN001) for the prevention of eczema in children through to 6 years of age but no effect of *Bifidobacterium animalis* subsp *lactis* HN019 (HN019).

*Objective*

Among this cohort of children, we aim to investigate whether these probiotics could modify the expression of genetic predisposition to eczema conferred by genetic variation in susceptibility genes.

*Methods*

Thirty three eczema susceptibility SNPs (in eleven genes) were genotyped in 331 children of European ancestry.

*Results*

Children who carried a genetic variant that put them at a high risk of developing eczema were less likely to develop eczema if they had been randomised to the HN001 intervention group compared to those in the placebo group. HN019 was also able to protect against the effects of some SNPs. As well as modifying genetic susceptibility to childhood eczema, HN001 was also found to modify genetic susceptibility to eczema severity and atopy risk.

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*Conclusion and Clinical Relevance*

This is the first study to show an effect of a probiotic on reducing eczema risk amongst those with particular eczema-associated genotypes. Our findings suggest that *Lactobacillus rhamnosus* HN001 may be particularly effective in preventing eczema in children with specific high risk genotypes.

**Keywords**

Probiotics, *Lactobacillus rhamnosus* HN001, *Bifidobacterium animalis* subsp *lactis* HN019, eczema, atopy, infants, genetic susceptibility, SNP, genotype

## Introduction

Eczema is an intermittent inflammatory vesiculo-papular skin disease characterised by pruritus, erythema and oedema and when severe by lichenification. It is often associated with atopy and has a peak incidence in the first few years of life. It has been estimated that 15–30% of children are affected [1].

There is strong evidence in support of a genetic predisposition to eczema: the risk of childhood eczema is two to three times higher in children with a maternal or paternal history, [2, 3] and twin studies show concordance rates of 72–86 % in monozygotic compared with 21–23 % in dizygotic twin pairs [4-6]. Genetic studies have identified a number of candidate susceptibility genes for eczema. These genes fall into two main groups: genes that encode epidermal or epithelial structural proteins, such as DEFB1 and SPINK5 which are important for the skin barrier, and genes that encode for major elements in the immune system, such as CCL5, CD14, IL13, IL18 and PHF11 which are associated with a variety of immune functions and in orchestrating allergic inflammation.

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Epidemiological studies and clinical observation suggest that various other (non-genetic) factors are also associated with eczema prevalence and severity, including personal factors (social class, nutritional status, number of siblings, lifestyle, allergy status) and environmental exposures (house dust, moulds, cockroach infestation, occupational exposures, environmental tobacco smoke, heating systems, and aeroallergens) [7-9].

Recent studies have identified a further important risk factor for the development of eczema in children – the gut microflora [10-12]. The gut microflora plays an important role in the development of the immune system in early life [13]. Factors such as maternal microflora, maternal diet and medications, the mode of birth delivery, formula-feeding rather than breast feeding, and antibiotic use may all affect the child's acquisition and maintenance of a healthy gut microflora and subsequently lead to altered immune function and the possibility of developing allergic disorders such as eczema [11, 14]. A number of studies have demonstrated reduced diversity in the gut microflora of infants with eczema compared to those without [15-20]. Interventions that modulate the developing immune system or enhance microbial diversity in early life, such as probiotics, may therefore provide an effective means of preventing the development of eczema in infancy.

Probiotics as defined by the World Health Organisation are "live microorganisms which when administered in adequate amounts confer a health benefit on the host'. These benefits could be directly on host health or on disease prevention and/or treatment [21, 22]. Clinical trials have shown a wide range of beneficial effects of probiotics. For example, they have been reported to suppress diarrhoea [23], alleviate lactose intolerance [24], reduce irritable bowel symptoms [25], prevent inflammatory bowel disease [26], reduce postoperative complications [27], prevent infantile colic [28, 29] and exhibit antimicrobial activity against

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methicillin-resistant *Staphylococcus aureus* (MRSA) [30]. Recent studies have also shown a beneficial effect of probiotics in the prevention of eczema. The first of these was a small Finnish study showing that prenatal and then postnatal probiotic exposure using *Lactobacillus rhamnosus* for 6 months halved the frequency of eczema at 2, 4, and 7 years [11, 31, 32]. Other probiotic intervention studies have also found at least a partial reduction in eczema [33, 34] whilst others have found probiotics to have no effect [35-38].

In our own high risk birth cohort (parental history of allergic disease) we have shown that intervention with *Lactobacillus rhamnosus* HN001 halved the cumulative prevalence of eczema by age 2 years, and reduced the number of infants with SCORAD≥10 [39]. Furthermore, we went on to show that the protective effect of HN001 against eczema, when given for the first 2 years of life, extended to 4 years [40] and 6 years of age [41].

In this same cohort we aim to investigate if probiotics can modify the genetic predisposition to eczema and atopic sensitization conferred by genetic variation in susceptibility genes.

## Methods

### *Study samples*

The study was originally designed as a double-blind, randomized, placebo-controlled trial of the effects of probiotic supplementation on the development of eczema and atopic sensitization in infants and has been described in detail previously [39]. In summary, daily supplementation with either *L. rhamnosus* HN001 (HN001), *B. animalis* subsp *lactis* HN019 (HN019), or placebo was from 35 weeks gestation until birth, continuing to 6 months after birth in the mothers if breastfeeding, and from birth till 2 years in all infants. Buccal swabs (Isohelix) were used to collect DNA from the children at 4 or 6 years of age, and the DNA

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was extracted from the buccal samples using Qiagen's DNA extraction kit and following the manufacturer's instructions. The genetic data and other data from 331 children of parental-reported sole European ancestry were used for this study. Of the 331 children, 114 were in the HN019 group, 108 were in the HN001 group and 109 were in the placebo group. Signed consent for the study, including buccal sampling for the extraction of DNA, was given by the mother of the child.

The study received ethical approval from the New Zealand Multi-region Ethics Committee.

*Outcome measures*

Outcome measures have been previously described [41]. Briefly, eczema prevalence and severity were assessed at follow-up visits at 3, 6, 12, and 18 months and 2, 4 and 6 years and eczema was defined using the UK Working Party's Diagnostic Criteria for atopic dermatitis [42]. For assessments at or before 2 years this was modified for use in infants [42]. Eczema severity was assessed using SCORing Atopic Dermatitis (SCORAD) [43] in all children with visible eczema regardless of their eczema diagnosis (as defined). SCORAD was analysed dichotomously using a cut-off ≥10 to exclude those with trivial rash. All staff was trained to apply SCORAD in a standardized way. ASCIA (Australasian Society of Clinical Immunology and Allergy) guidelines [44] were used in the conduct and assessment of skin prick test (SPT) reactions to egg white, peanut, cow's milk, cat pelt, *Dermatophagoides pteronyssinus,* and mixed grass pollen (Hollister-Stier, Spokane, USA) using Stallergenes 1mm lancets (Antony, France). SPT sensitization was defined as a mean wheal diameter ≥ 3mm to one or more allergens after subtraction of the negative control.

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*Genotyping*

SNPs and genes associated with eczema and replicated in at least two independent studies, as of January 2012, were selected for genotyping (Table 1). Thirty three common SNPs (minor allele frequency > 5%) in 11 genes were selected: Defensin, beta 1 (*DEFB1*), plant homeodomain finger protein 11 (*PHF11*), Serine peptidase inhibitor, Kazal type 5 (*SPINK5* alias LEKTI), Chromosome 11 open reading frame 30 (*C11orf30*), Chemokine (C-C motif) ligand 5 (*CCL5* alias RANTES), CD14 molecule (*CD14*), Chymase 1, mast cell (*CMA1* alias MCC), Interleukin 4 (*IL4*), Interleukin 4 receptor (*IL4R*), Interleukin 13 (*IL13*), and Interleukin 18 (*IL18*).

Genotyping was performed with the MassARRAY and iPlex systems of the Sequenom genotyping platform (Sequenom, San Diego, CA), which uses the MALDITOF primer extension assay [45, 46] according to manufacturers' recommendations. Assays were optimized in 24 samples consisting of 20 reference Centre d'Etude du Polymorphisme Humain (CEPH) samples and 4 blanks. All sample plates contained cases, controls, blanks, CEPH and duplicate samples. Quality control measures included independent double genotyping, and where available comparison of our CEPH genotypes to those in the HapMap (http://www.hapmap.org).

*Statistical analysis*

As the outcomes of interest (atopy, eczema and SCORAD≥10) were collected over a number of different time-points, we used the generalised estimating equations (GEE) model in the GENMOD procedure with the REPEATED statement in SAS to fit for association between gene and binary outcome data. The additive model was used for linearity of the genotype-phenotype relationship; each SNP was coded 0, 1, and 2 based on counting the numbers of

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tested allele of the genotypes [47]. We adjusted for six confounding variables (which were significantly associated with at least one of the three outcomes of interest): sex of child, gestational age, any pet at birth, maternal history of asthma, eczema or hay fever, paternal history of asthma, eczema or hay fever, and maternal or paternal family history of eczema. There was no indication of a deviation from Hardy Weinberg equilibrium (HWE), except for one SNP (rs1800972, *DEFB1*) which was excluded from further analysis. False discovery rate (FDR) q-values were calculated for the total number of tests on each outcome of interest using the 'qvalue' package in R [48, 49] with the null hypothesis fraction estimated by the smoothing method of Storey and Tibshirani [50]. A pre-specified FDR threshold was set at 0.05. SAS (V9.3 SAS Institute., Cary, NC, USA) and R (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org ) were used for statistical analyses.

**Results**

From the 33 selected SNPs, four SNPs (rs360717 in *IL18*, rs2243250 in *IL4*, rs1805010 in *IL4R*, and rs2569193 in *CD14*) were not genotyped as they did not fit into the sequenom multiplexes, two SNPs (rs3765526 and rs9526569 both in *PHF11*) were found to be subject to self-priming problems, and one SNP (rs1800972, *DEFB1*) was deviated from HWE. Therefore we had genotypic data for 26 SNPs which were all analysed for association with eczema, SCORAD≥10 and atopy and their interactions with probiotics. The results are summarised in Tables 2-4 (Note that only data for SNPs that showed statistically significant association in the SNP effect and/or SNPxTreatment interactions are shown. If a particular SNP is not listed in the Tables it indicates that this SNP showed no statistically significant associations).

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*Eczema*

From the 26 SNPs, 7 were found to be associated with eczema in our samples after correcting for multiple testing (Table 2): rs7927894 (*C11orf30*), rs1046295 and rs3794378 (*PHF11*), rs2107538 (*CCL5*), and rs1801275, rs2057768 and rs2107356 (*IL4R*). A number of other SNPs demonstrated a weaker association with eczema in that that they had odds ratios above 1 and p-values less than 0.05 but these results were not statistically significant after multiple testing correction (data not shown).

Sixteen genetic variants showed an interaction with HN001 in that children with these variants who were in the HN001 treatment group showed a significantly reduced risk of eczema compared with the placebo group: rs7927894 (*C11orf30*), rs2738182, rs5743399 and rs5743409 (*DEFB1*), rs1046295 and rs3794379 (*PHF11*), rs1423001, rs3756688 and rs3815735 (*SPINK5*), rs1800825 (*CCL5*), rs2569190 (*CD14*), rs1800875 (*CMA1*), rs20541 (*IL13*), and rs1801275, rs2057768 and rs2107356 (*IL4R*).

Three genetic variants showed an interaction with HN019 in that children with these variants who were in the HN019 treatment group showed a significantly reduced risk of eczema compared with the placebo group: rs2738182 and rs5743399 (*DEFB1*), and rs2107356 (*IL4R*).

*SCORAD≥10*

From the 26 SNPs, 9 were found to be associated with SCORAD≥10 in our samples after correcting for multiple testing (Table 3): rs1046295 and rs3794378 (*PHF11*), rs2107538 (*CCL5*), rs1800875 (*CMA1*), rs187238, rs360718, rs360721 and rs795467 (*IL18*), and

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rs2107356 (*IL4R*). A number of other SNPs demonstrated a weaker association in that they had odds ratios above 1 but these results were not statistically significant (data not shown).

Thirteen genetic variants showed an interaction with HN001 in that children with these variants who were in the HN001 treatment group showed a significantly reduced risk of SCORAD≥10 compared with the placebo group: rs5743399 (*DEFB1*), rs1046295, rs3794378 and rs3794379 (*PHF11*), rs2287772 and rs4529181 (*SPINK5*), rs1800825 (*CCL5*), rs2569190 (*CD14*), rs1800875 (*CMA1*), rs20541 (*IL13*), and rs1801275, rs2057768 and rs2107356 (*IL4R*).

Six genetic variants showed an interaction with HN019 in that children with these variants who were in the HN019 treatment group showed a significantly reduced risk of SCORAD≥10 compared with the placebo group: rs5743399 (*DEFB1*), rs1046295 and rs3794378 (*PHF11*), rs2287772 and rs4529181 (*SPINK5*), and rs360721 (*IL18*).

*Atopy*

From the 26 SNPs, 10 were found to be associated with atopy in our samples after correcting for multiple testing (Table 4): rs5743399 and rs5743409 (*DEFB1*), rs1046295 and rs3794378 (*PHF11*), rs1423001, rs2303067 and rs3815735 (*SPINK5*), rs1800825 and rs2107538 (*CCL5*), and rs1800875 (*CMA1*).

Sixteen genetic variants showed an interaction with HN001 in that children with these variants who were in the HN001 treatment group showed a significantly reduced risk of atopy compared with the placebo group: rs7927894 (*C11orf30*), rs2738182 and rs5743399 (*DEFB1*), rs1046295 and rs3794379 (*PHF11*), rs1423001, rs2287772, rs3756688, rs3815735

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and rs4529181 (*SPINK5*), rs1800825 (*CCL5*), rs2569190 (*CD14*), rs1800875 (*CMA1*), rs20541 (*IL13*), and rs1801275 and rs2107356 (*IL4R*).

Three genetic variants showed an interaction with HN019 in that children with these variants who were in the HN019 treatment group showed a significantly reduced risk of atopy compared with the placebo group: rs5743399 (*DEFB1*), rs3815735 (*SPINK5*), and rs2107356 (*IL4R*).

**Discussion**

Using a double-blind, randomized, placebo-controlled trial we have previously shown that in a high risk birth cohort, maternal supplementation from 35 weeks gestation and until 6 months post-delivery if still breast-feeding, and infant supplementation until 2 years with HN001 provided statistically significant protection against eczema development at age 2, 4 [39, 40] and 6 years and an effect on atopic sensitization which became significant by age 6 years [41]. We did not observe any effects with HN019.

In our present study we sought to extend our previous research and determine whether probiotic intervention could modify genetic predisposition to eczema conferred by susceptibility polymorphisms. We selected key eczema susceptibility genes and SNPs from the literature and genotyped these SNPs in our cohort. Many of the SNPs in our dataset showed associations with eczema as well as SCORAD≥10 and atopy. It is important to recognise that our definition of eczema is highly specific for eczema, while SCORAD≥10 which is based on visible rash on the day of assessment, and is more sensitive. We recognise that not all the SNPs showed statistically significant associations in our population which was probably a result of a lack of power due to our relatively small sample size. However, most of

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the SNPs had odds ratios for eczema, atopy and SCORAD≥10 that were greater than 1.0. Additional analysis showed that children who carried a genetic variant that put them at a higher risk of developing eczema were in fact less likely to develop eczema if they had been in the HN001 intervention group compared to those in the placebo group. HN019 was also able to protect against the effects of some SNPs but these effects were less likely to reach significance than HN001 effects. As well as modifying genetic susceptibility to childhood eczema, probiotics were also found to modify the related outcomes SCORAD≥10 and atopy.

SNP association results suggested that HN019 had a greater effect in reducing the risk of eczema than was indicated by our previous publications investigating clinical effects. A possible explanation for this is that this probiotic strain may not work on eczema and atopy in general but only on specific types. For example, it is possible that HN019 is more effective against eczema/atopy caused by genetic variants rather than types caused by environmental risk factors e.g. exposure to irritants/allergens. Furthermore, the results of our present study suggest that the effect of HN019 may be specific to the type of gene involved. HN001 interacts with genes that code for epithelial structure/function and with genes that code for immune function, while HN019 interacts more commonly with genes that code for epithelial structure/function and less so with those involved in immune function. Thus, a probiotic such as HN001 which has stronger and more frequent interactions with genes associated with both immune function and epithelial structure would be expected to have a greater clinical effect than HN019, specifically on a disease such as eczema that involves both immune and epithelial function. Indeed, the differential effect of these two probiotics on eczema risk associated with particular high risk genotypes is similar to the differential effect observed on eczema prevalence in our previous studies [39-41].

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There is no known cure for eczema. Existing treatments use emollients and in more difficult cases topical corticosteroids, oral corticosteroids and immune-modulating agents but these may have unwanted side effects. The results here show that probiotics, and HN001 in particular, may be a particularly effective way of preventing eczema in those individuals with a genetic predisposition.

The mechanisms for the possible preventive effects of probiotics on eczema are unclear, as are the reasons why different probiotics appear to have different effects. While it is important not to over-speculate on the basis of a single study, the results of this present study present some interesting hypotheses. For instance, genetic variation in genes encoding proteins involved in the immune response such as *CCL5, CD14, IL13, IL18* and *PHF11* are known to modulate T helper subsets that influence the onset and severity of allergic disease. It is possible that some probiotics may help to re-adjust the immune system away from a pro-allergic response. Interestingly, in an animal model of a Th2-skewed immune response, oral exposure to HN001 shifted the peripheral blood cytokine patterns back toward a more balanced Th1/Th2 response [51].

Probiotics may also be working to overcome the defects in skin barrier as a result of genetic variation in genes such as *DEFB1* and *SPINK5*. It has been shown that HN001 can enhance intestinal barrier function in an *in vitro* model of tight junction integrity [52], and gut barrier function has been implicated in a number of conditions, including allergic disease [53, 54]. Therefore it was interesting that SNPs associated with skin barrier function were associated with HN001 treatment effects. The *DEFB1* gene encodes Human β-defensin-1 (hBD-1) which is constitutively expressed throughout the body, including in skin keratinocytes, respiratory tract cells, peripheral blood mononuclear cells, and gut epithelial cells. It appears

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to have a range of functions, including antiviral, antimicrobial and immune-regulatory properties in addition to its role in the skin, and reduced hBD-1 levels are associated with increased risk of infections. Thus, probiotic interactions with hBD-1 and eczema may be complex, and may employ both immune and skin barrier pathways. Interestingly, lipopolysaccharide (LPS) is also known to be an inducer of *DEFB1* expression. As the *CD14* molecule is an LPS binding protein, the finding that *CD14* SNP (rs2569130) was also associated with a probiotic treatment effect might be suggestive of a role of the Gram-negative bacterial signalling pathway in infant-onset eczema.

The different possible mechanisms of probiotic efficacy are not mutually exclusive. Indeed, the ability to influence several pathways simultaneously may explain why only a handful of probiotic bacteria, including HN001, have shown significant and long-lasting efficacy against allergic disease. In the light of these latest results, further research is required to elucidate the mechanisms of probiotic benefits in eczema and atopy.

To conclude, probiotics have been previously reported to be beneficial in prevention of eczema, but this is the first study that has shown probiotic supplementation can have an effect in modifying genetic susceptibility to eczema in high risk children. Unravelling the genetic effect of probiotics is still in its infancy as is understanding the precise function of the genes associated with allergic disease and studies focusing on the mechanisms by which maternal and/or infant supplementation of probiotics exerts an effect during early life are clearly necessary. Determining the precise mechanisms of probiotic action may lead to more specific and efficacious therapeutic strategies in the prevention or treatment of eczema and atopic disease.

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**Conflict of interests**

The authors declare no conflict of interest.

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Table 1 Eczema susceptibility genes

Table 2 Effect of SNP and Treatment Interaction on Eczema

Table 3 Effect of SNP and Treatment Interaction on SCORAD≥10

Table 4 Effect of SNP and Treatment Interaction on Atopy

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Table 1 Eczema susceptibility genes

| Gene symbol | Gene name | Gene function | SNP | References |
|---|---|---|---|---|
| C11orf30 | Chromosome 11 open reading frame 30 | Unknown | rs7927894 | [1, 2] |
| DEFB1 | Defensin, beta 1 | antimicrobial peptide of epithelia | rs5743399<br>rs5743409<br>rs2738182 | [3, 4] |
| PHF11 | plant homeodomain finger protein 11 | Component of protective structures of the epidermis, natural moisturizing factor | rs1046295<br>rs3794379<br>rs3794378 | [5-9] |
| SPINK5 | Serine peptidase inhibitor, Kazal type 5 | modulator of proteolytic activities in the epidermis | rs2303067<br>rs3815735<br>rs4529181<br>rs2287772<br>rs1423001<br>rs3756688 | [10-13] |
| CCL5 | Chemokine (C-C motif) ligand 5 | activation of eosinophilic granulocytes,<br>IgE synthesis | rs2107538<br>rs1800825 | [14, 15] |
| CD14 | CD14 molecule | endotoxin receptor complex, release of proinflammatory cytokines | rs2569190 | [16-18] |
| CMA1 | Chymase 1, mast cell | induction of eosinophilic granulocyte infiltration, release of pro-inflammatory mediators | rs1800875 | [19-21] |
| IL4R | Interleukin 4 receptor | induction of Th2 T-cell differentiation,<br>IgE synthesis | rs1801275<br>rs2057768 | [22-25] |

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| | | | | |
|---|---|---|---|---|
| | | | rs2107356 | |
| IL13 | Interleukin 13 | induction of Th2 T-cell differentiation, IgE synthesis | rs1800925 rs20541 | [26-28] |
| IL18 | Interleukin 18 | pro-inflammatory cytokine | rs360718 rs187238 rs360721 rs795467 | [29-31] |


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Table 2 Effect of SNP and Treatment Interaction on Eczema

| Gene | SNP | Tested Allele | SNP effect | | | SNP x Treatment effect | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | OR (95% CI) | P value | Q value | | OR (95% CI) | P value | Q value |
| C11orf30 | rs7927894 | C | 1.48 (1.14-1.91) | **0.0028*** | **0.0242** | HN001 | 0.72 (0.56-0.94) | **0.0138*** | **0.0429** |
| | | | | | | HN019 | 0.75 (0.58-0.97) | 0.0258 | 0.0629 |
| | | | | | | Placebo | 1.00 | | |
| DEFB1 | rs2738182 | T | 1.16 (0.90-1.49) | 0.2618 | 0.2959 | HN001 | 0.63 (0.49-0.82) | **4.77e-04*** | **0.0135** |
| | | | | | | HN019 | 0.73 (0.58-0.93) | **0.0111*** | **0.0412** |
| | | | | | | Placebo | 1.00 | | |
| DEFB1 | rs5743399 | T | 1.34 (0.99-1.81) | 0.0556 | 0.1112 | HN001 | 0.61 (0.41-0.91) | **0.0143*** | **0.0429** |
| | | | | | | HN019 | 0.59 (0.40-0.87) | **0.0070*** | **0.0321** |
| | | | | | | Placebo | 1.00 | | |
| DEFB1 | rs5743409 | T | 1.23 (0.94-1.60) | 0.1344 | 0.1872 | HN001 | 0.56 (0.39-0.80) | **0.0016*** | **0.0208** |
| | | | | | | HN019 | 0.77 (0.55-1.08) | 0.131 | 0.1872 |
| | | | | | | Placebo | 1.00 | | |
| PHF11 | rs1046295 | G | 1.48 (1.11-1.96) | **0.0068*** | **0.0321** | HN001 | 0.66 (0.48-0.92) | **0.0127*** | **0.0429** |
| | | | | | | HN019 | 0.77 (0.57-1.05) | 0.0969 | 0.1512 |
| | | | | | | Placebo | 1.00 | | |
| PHF11 | rs3794378 | C | 1.57 (1.16-2.12) | **0.0031*** | **0.0242** | HN001 | 0.69 (0.48-1.00) | 0.0489 | 0.1031 |
| | | | | | | HN019 | 0.75 (0.54-1.05) | 0.0948 | 0.1509 |
| | | | | | | Placebo | 1.00 | | |
| PHF11 | rs3794379 | C | 1.67 (1.07-2.60) | 0.0239 | 0.0621 | HN001 | 0.74 (0.60-0.90) | **0.0030*** | **0.0242** |
| | | | | | | HN019 | 0.87 (0.72-1.05) | 0.1446 | 0.1979 |
| | | | | | | Placebo | 1.00 | | |
| SPINK5 | rs1423001 | C | 1.34 (1.04-1.72) | 0.0232 | 0.0621 | HN001 | 0.72 (0.55-0.93) | **0.0132*** | **0.0429** |
| | | | | | | HN019 | 0.86 (0.67-1.10) | 0.2314 | 0.2777 |
| | | | | | | Placebo | 1.00 | | |
| SPINK | rs37566 | T | 1.31 (1.02- | 0.0364 | 0.083 | HN0 | 0.71 (0.55- | **0.0104** | **0.040** |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 88 | | 1.69) | | 5 | 01 | 0.92) | * | **6** |
| | | | | | | | HN019 | 0.91 (0.71-1.15) | 0.4224 | 0.4576 |
| | | | | | | | Placebo | 1.00 | | |
| | SPINK5 | rs3815735 | T | 1.35 (1.05-1.75) | 0.0201 | 0.0581 | HN001 | 0.69 (0.53-0.90) | **0.0063*** | **0.0321** |
| | | | | | | | HN019 | 0.84 (0.65-1.07) | 0.1592 | 0.2105 |
| | | | | | | | Placebo | 1.00 | | |
| | CCL5 | rs1800825 | T | 1.74 (0.94-3.22) | 0.0782 | 0.1386 | HN001 | 0.75 (0.61-0.91) | **0.0038*** | **0.0254** |
| | | | | | | | HN019 | 0.88 (0.73-1.05) | 0.1592 | 0.2105 |
| | | | | | | | Placebo | 1.00 | | |
| | CCL5 | rs2107538 | T | 2.00 (1.34-3.00) | **7.10e-04*** | **0.0135** | HN001 | 0.59 (0.35-1.01) | 0.0543 | 0.1112 |
| | | | | | | | HN019 | 0.63 (0.38-1.04) | 0.0710 | 0.1288 |
| | | | | | | | Placebo | 1.00 | | |
| | CD14 | rs2569190 | A | 1.05 (0.80-1.36) | 0.7388 | 0.7484 | HN001 | 0.64 (0.45-0.91) | **0.0126*** | **0.0429** |
| | | | | | | | HN019 | 0.89 (0.66-1.20) | 0.4339 | 0.4636 |
| | | | | | | | Placebo | 1.00 | | |
| | CMA1 | rs1800875 | A | 1.32 (1.01-1.71) | 0.0414 | 0.0897 | HN001 | 0.63 (0.46-0.85) | **0.0026*** | **0.0242** |
| | | | | | | | HN019 | 0.92 (0.71-1.19) | 0.5213 | 0.5350 |
| | | | | | | | Placebo | 1.00 | | |
| | IL13 | rs20541 | C | 1.04 (0.78-1.40) | 0.7750 | 0.7750 | HN001 | 0.68 (0.54-0.85) | **8.59e-04*** | **0.0135** |
| | | | | | | | HN019 | 0.89 (0.72-1.09) | 0.2599 | 0.2959 |
| | | | | | | | Placebo | 1.00 | | |
| | IL4R | rs1801275 | A | 1.48 (1.10-1.99) | **0.0099*** | **0.0406** | HN001 | 0.73 (0.59-0.91) | **0.0058*** | **0.0321** |
| | | | | | | | HN019 | 0.88 (0.72-1.09) | 0.2445 | 0.2858 |
| | | | | | | | Placebo | 1.00 | | |
| | IL4R | rs2057768 | G | 1.46 (1.12-1.90) | **0.0051*** | **0.0306** | HN001 | 0.69 (0.54-0.89) | **0.0039*** | **0.0254** |
| | | | | | | | HN019 | 0.79 (0.63-0.99) | 0.0385 | 0.0858 |
| | | | | | | | Placebo | 1.00 | | |
| | IL4R | rs21073 | A | 1.78 (1.36- | **2.69e-** | **0.002** | HN0 | 0.57 (0.41- | **8.66e-** | **0.013** |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 56 | 2.33) | **05*** | **1** | 01 | 0.79) | **04*** | **5** |
| | | | | | | | HN019 | 0.65 (0.48-0.90) | **0.0084*** | **0.0364** |
| | | | | | | | Placebo | 1.00 | | |

* Remain significant after applying multiple testing correction using false discovery rate.

Table 3 Effect of SNP and Treatment Interaction on SCORAD≥10

| | | | SNP effect | | | SNP x Treatment effect | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gene | SNP | Tested Allele | OR (95% CI) | P value | Q value | | OR (95% CI) | P value | Q value |
| DEFB1 | rs5743399 | T | 1.29 (0.97-1.73) | 0.0836 | 0.1304 | HN001 | 0.60 (0.40-0.90) | **0.0125*** | **0.0443** |
| | | | | | | HN019 | 0.58 (0.40-0.85) | **0.0054*** | **0.0281** |
| | | | | | | Placebo | 1.00 | | |
| PHF11 | rs1046295 | G | 1.51 (1.13-2.01) | **0.0048*** | **0.0281** | HN001 | 0.67 (0.48-0.92) | **0.0140*** | **0.0455** |
| | | | | | | HN019 | 0.69 (0.51-0.94) | **0.0172*** | **0.0479** |
| | | | | | | Placebo | 1.00 | | |
| PHF11 | rs3794378 | C | 1.78 (1.31-2.40) | **1.88e-04*** | **0.0073** | HN001 | 0.59 (0.41-0.86) | **0.0053*** | **0.0281** |
| | | | | | | HN019 | 0.60 (0.43-0.84) | **0.0026*** | **0.0281** |
| | | | | | | Placebo | 1.00 | | |
| PHF11 | rs3794379 | C | 1.54 (0.99-2.38) | 0.0560 | 0.1081 | HN001 | 0.74 (0.60-0.90) | **0.0032*** | **0.0281** |
| | | | | | | HN019 | 0.88 (0.73-1.06) | 0.1903 | 0.2249 |
| | | | | | | Placebo | 1.00 | | |
| SPINK5 | rs2287772 | T | 1.22 (0.92-1.61) | 0.1637 | 0.2059 | HN001 | 0.58 (0.41-0.84) | **0.0033*** | **0.0281** |
| | | | | | | HN019 | 0.66 (0.46-0.92) | **0.0157*** | **0.0479** |
| | | | | | | Placebo | 1.00 | | |
| SPINK5 | rs4529181 | G | 1.19 (0.91-1.57) | 0.2043 | 0.2378 | HN001 | 0.60 (0.42-0.85) | **0.0044*** | **0.0281** |
| | | | | | | HN019 | 0.66 (0.47-0.93) | **0.0162*** | **0.0479** |
| | | | | | | Placebo | 1.00 | | |
| CCL5 | rs1800825 | T | 1.63 (0.85-3.11) | 0.1424 | 0.1851 | HN001 | 0.78 (0.64-0.95) | **0.0133*** | **0.0451** |
| | | | | | | HN019 | 0.88 (0.74-1.06) | 0.1859 | 0.2231 |
| | | | | | | Place | 1.00 | | |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | bo | | | |
| CCL5 | rs2107538 | T | 2.26 (1.49-3.43) | **1.31e-04*** | **0.0073** | HN001 | 0.68 (0.41-1.14) | 0.1411 | 0.1851 |
| | | | | | | HN019 | 0.55 (0.33-0.93) | 0.0251 | 0.0644 |
| | | | | | | Placebo | 1.00 | | |
| CD14 | rs2569190 | A | 1.07 (0.82-1.39) | 0.6274 | 0.6613 | HN001 | 0.62 (0.44-0.86) | **0.0042*** | **0.0281** |
| | | | | | | HN019 | 0.85 (0.63-1.15) | 0.2934 | 0.3223 |
| | | | | | | Placebo | 1.00 | | |
| CMA1 | rs1800875 | A | 1.41 (1.08-1.84) | **0.0105*** | **0.0405** | HN001 | 0.69 (0.51-0.94) | **0.0172*** | **0.0479** |
| | | | | | | HN019 | 0.86 (0.66-1.13) | 0.2699 | 0.3007 |
| | | | | | | Placebo | 1.00 | | |
| IL13 | rs20541 | C | 0.93 (0.70-1.24) | 0.6405 | 0.6661 | HN001 | 0.70 (0.56-0.88) | **0.0023*** | **0.0281** |
| | | | | | | HN019 | 0.85 (0.69-1.05) | 0.1338 | 0.1831 |
| | | | | | | Placebo | 1.00 | | |
| IL18 | rs187238 | C | 1.66 (1.17-2.37) | **0.0050*** | **0.0281** | HN001 | 0.69 (0.42-1.12) | 0.1329 | 0.1831 |
| | | | | | | HN019 | 0.61 (0.40-0.94) | 0.0264 | 0.0644 |
| | | | | | | Placebo | 1.00 | | |
| IL18 | rs360718 | C | 1.63 (1.14-2.31) | **0.0068*** | **0.0295** | HN001 | 0.71 (0.44-1.16) | 0.1702 | 0.2074 |
| | | | | | | HN019 | 0.66 (0.43-1.02) | 0.0596 | 0.1081 |
| | | | | | | Placebo | 1.00 | | |
| IL18 | rs360721 | G | 1.60 (1.16-2.21) | **0.0044*** | **0.0281** | HN001 | 0.67 (0.42-1.06) | 0.0880 | 0.1346 |
| | | | | | | HN019 | 0.60 (0.41-0.89) | **0.0109*** | **0.0295** |
| | | | | | | Placebo | 1.00 | | |
| IL18 | rs795467 | A | 1.63 (1.14-2.31) | **0.0068*** | **0.0295** | HN001 | 0.71 (0.44-1.16) | 0.1702 | 0.2074 |
| | | | | | | HN019 | 0.66 (0.43-1.02) | 0.0596 | 0.1081 |
| | | | | | | Placebo | 1.00 | | |
| IL4R | rs1801275 | A | 1.42 (1.05-1.92) | 0.0246 | 0.0644 | HN001 | 0.73 (0.59-0.92) | **0.0066*** | **0.0295** |
| | | | | | | HN019 | 0.92 (0.74-1.13) | 0.4143 | 0.4488 |
| | | | | | | Placebo | 1.00 | | |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | bo | | | |
| IL4R | rs2057768 | G | 1.29 (0.99-1.67) | 0.0577 | 0.1081 | HN001 | 0.71 (0.55-0.92) | **0.0106*** | **0.0405** |
| | | | | | | HN019 | 0.83 (0.66-1.04) | 0.1044 | 0.1508 |
| | | | | | | Placebo | 1.00 | | |
| IL4R | rs2107356 | A | 1.48 (1.13-1.93) | **0.0045*** | **0.0281** | HN001 | 0.56 (0.40-0.78) | **6.85e-04*** | **0.0178** |
| | | | | | | HN019 | 0.75 (0.55-1.03) | 0.0712 | 0.1148 |
| | | | | | | Placebo | 1.00 | | |

* Remain significant after applying multiple testing correction using false discovery rate.

Table 4 Effect of SNP and Treatment Interaction on Atopy

| | | | SNP effect | | | SNP x Treatment effect | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gene | SNP | Tested Allele | OR (95% CI) | P value | Q value | | OR (95% CI) | P value | Q value |
| C11orf30 | rs7927894 | C | 1.19 (0.93-1.52) | 0.1735 | 0.2461 | HN001 | 0.72 (0.56-0.93) | **0.0126*** | **0.0396** |
| | | | | | | HN019 | 0.75 (0.58-0.97) | 0.0293 | 0.0618 |
| | | | | | | Placebo | 1.00 | | |
| DEFB1 | rs2738182 | T | 1.22 (0.95-1.57) | 0.1156 | 0.1810 | HN001 | 0.74 (0.59-0.94) | **0.0125*** | **0.0396** |
| | | | | | | HN019 | 0.76 (0.60-0.97) | 0.0252 | 0.0561 |
| | | | | | | Placebo | 1.00 | | |
| DEFB1 | rs5743399 | T | 1.49 (1.09-2.03) | **0.0117*** | **0.0396** | HN001 | 0.52 (0.35-0.76) | **7.47e-04*** | **0.0115** |
| | | | | | | HN019 | 0.51 (0.35-0.75) | **5.37e-04*** | **0.0115** |
| | | | | | | Placebo | 1.00 | | |
| DEFB1 | rs5743409 | T | 1.39 (1.08-1.80) | **0.0108*** | **0.0396** | HN001 | 0.82 (0.60-1.14) | 0.2376 | 0.3038 |
| | | | | | | HN019 | 0.87 (0.63-1.20) | 0.4016 | 0.4592 |
| | | | | | | Placebo | 1.00 | | |
| PHF11 | rs1046295 | G | 1.63 (1.26-2.11) | **1.78e-04*** | **0.0115** | HN001 | 0.65 (0.48-0.87) | **0.0047*** | **0.0262** |
| | | | | | | HN019 | 0.73 (0.54-0.98) | 0.0360 | 0.0720 |
| | | | | | | Placebo | 1.00 | | |
| PHF11 | rs3794378 | C | 1.57 (1.18-2.08) | **0.0019*** | **0.0148** | HN001 | 0.72 (0.51-1.03) | 0.0696 | 0.1234 |
| | | | | | | HN0 | 0.73 (0.53- | 0.0603 | 0.114 |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 19 | 1.01) | | | 7 |
| | | | | | | Placebo | 1.00 | | | |
| PHF11 | rs3794379 | C | 1.10 (0.73-1.64) | 0.6571 | 0.6834 | HN001 | 0.79 (0.65-0.96) | **0.0173*** | **0.0465** |
| | | | | | | HN019 | 0.88 (0.73-1.06) | 0.1938 | 0.2606 |
| | | | | | | Placebo | 1.00 | | | |
| SPINK5 | rs1423001 | C | 1.36 (1.06-1.73) | **0.0156*** | **0.0435** | HN001 | 0.69 (0.54-0.89) | **0.0042*** | **0.0252** |
| | | | | | | HN019 | 0.77 (0.60-0.98) | 0.0332 | 0.0681 |
| | | | | | | Placebo | 1.00 | | | |
| SPINK5 | rs2287772 | T | 1.33 (1.02-1.75) | 0.0389 | 0.0759 | HN001 | 0.65 (0.47-0.89) | **0.0083*** | **0.0345** |
| | | | | | | HN019 | 0.81 (0.59-1.12) | 0.1996 | 0.2639 |
| | | | | | | Placebo | 1.00 | | | |
| SPINK5 | rs2303067 | A | 1.52 (1.18-1.97) | **0.0012*** | **0.0134** | HN001 | 0.72 (0.54-0.96) | 0.0232 | 0.0561 |
| | | | | | | HN019 | 0.96 (0.72-1.27) | 0.7546 | 0.7546 |
| | | | | | | Placebo | 1.00 | | | |
| SPINK5 | rs3756688 | T | 1.33 (1.04-1.71) | 0.0234 | 0.0561 | HN001 | 0.68 (0.52-0.87) | **0.0027*** | **0.0182** |
| | | | | | | HN019 | 0.79 (0.62-1.01) | 0.0652 | 0.1211 |
| | | | | | | Placebo | 1.00 | | | |
| SPINK5 | rs3815735 | T | 1.39 (1.08-1.78) | **0.0104*** | **0.0396** | HN001 | 0.64 (0.50-0.84) | **8.82e-04*** | **0.0115** |
| | | | | | | HN019 | 0.72 (0.56-0.92) | **0.0084*** | **0.0345** |
| | | | | | | Placebo | 1.00 | | | |
| SPINK5 | rs4529181 | G | 1.27 (0.98-1.66) | 0.0757 | 0.1301 | HN001 | 0.67 (0.49-0.92) | **0.0133*** | **0.0399** |
| | | | | | | HN019 | 0.83 (0.60-1.13) | 0.2366 | 0.3038 |
| | | | | | | Placebo | 1.00 | | | |
| CCL5 | rs1800825 | T | 2.86 (1.48-5.54) | **0.0018*** | **0.0148** | HN001 | 0.77 (0.64-0.93) | **0.0065*** | **0.0330** |
| | | | | | | HN019 | 0.86 (0.72-1.02) | 0.0901 | 0.1495 |
| | | | | | | Placebo | 1.00 | | | |
| CCL5 | rs2107538 | T | 2.06 (1.38-3.07) | **3.74e-04*** | **0.0115** | HN001 | 0.78 (0.48-1.27) | 0.3155 | 0.3845 |
| | | | | | | HN019 | 0.70 (0.43-1.14) | 0.1563 | 0.2258 |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| CD14 | rs2569190 | A | 1.36 (1.04-1.78) | 0.0242 | 0.0561 | Placebo | 1.00 | | | |
| | | | | | | HN001 | 0.59 (0.43-0.82) | **0.0014*** | **0.0137** |
| | | | | | | HN019 | 0.80 (0.59-1.08) | 0.1393 | 0.2050 |
| CMA1 | rs1800875 | A | 1.43 (1.10-1.86) | **0.0072*** | **0.0330** | Placebo | 1.00 | | | |
| | | | | | | HN001 | 0.68 (0.51-0.90) | **0.0071*** | **0.0330** |
| | | | | | | HN019 | 0.79 (0.60-1.03) | 0.0767 | 0.1301 |
| IL13 | rs20541 | C | 0.96 (0.72-1.27) | 0.7515 | 0.7546 | Placebo | 1.00 | | | |
| | | | | | | HN001 | 0.68 (0.54-0.85) | **6.31e-04*** | **0.0115** |
| | | | | | | HN019 | 0.89 (0.73-1.09) | 0.2714 | 0.3414 |
| IL4R | rs1801275 | A | 1.12 (0.84-1.49) | 0.4348 | 0.4777 | Placebo | 1.00 | | | |
| | | | | | | HN001 | 0.77 (0.62-0.95) | **0.0146*** | **0.0422** |
| | | | | | | HN019 | 0.91 (0.74-1.12) | 0.3813 | 0.4476 |
| IL4R | rs2107356 | A | 1.36 (1.04-1.79) | 0.0259 | 0.0561 | Placebo | 1.00 | | | |
| | | | | | | HN001 | 0.60 (0.43-0.84) | **0.0028*** | **0.0182** |
| | | | | | | HN019 | 0.66 (0.48-0.92) | **0.0127*** | **0.0396** |
| | | | | | | Placebo | 1.00 | | | |

* Remain significant after applying multiple testing correction using false discovery rate.

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