Chromosome 11q13.5 variant associated with childhood eczema: An effect supplementary to filaggrin mutations

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Background: Atopic eczema is a common inflammatory skin disease with multifactorial etiology. The genetic basis is incompletely understood; however, loss of function mutations in the filaggrin gene (FLG) are the most significant and widely replicated genetic risk factor reported to date. The first genome-wide association study in atopic eczema recently identified 2 novel genetic variants in association with eczema susceptibility: a single nucleotide polymorphism on chromosome 11q13.5 (rs7927894) and a single nucleotide polymorphism (rs877776) within the gene encoding hornerin on chromosome 1q21.

Objective: To test the association of these 2 novel variants with pediatric eczema and to investigate their interaction with FLG null mutations.

Methods: Case-control study to investigate the association of rs7927894, rs877776 and the 4 most prevalent FLG null mutations with moderate-severe eczema in 511 Irish pediatric cases and 1000 Irish controls. Comprehensive testing for interaction between each of the loci was also performed.

Results: The association between rs7927894 and atopic eczema was replicated in this population (P = .0025, \( \chi^2 \) test; odds ratio, 1.27; 95% CI, 1.09-1.49). The 4 most common FLG null variants were strongly associated with atopic eczema (P = 1.26 \( \times 10^{-50} \); combined odds ratio, 5.81; 95% CI, 4.51-7.49). Interestingly, the rs7927894 association was independent of the well-established FLG risk alleles and may be multiplicative in its effect. There was no significant association between rs877776 and pediatric eczema in this study.

Conclusion: Single nucleotide polymorphism rs7927894 appears to mark a genuine eczema susceptibility locus that will require further elucidation through fine mapping and functional analysis. (J Allergy Clin Immunol 2010;125:170-4.)

Key words: Allergy, atopic dermatitis, atopy, inflammatory skin disease, skin barrier

Atopic eczema is a common inflammatory skin disease resulting in considerable morbidity in severely affected children. Genetic and environmental factors each contribute significantly to the etiopathogenesis of this disease, but our understanding of the role of genetic variation in atopic eczema remains limited, despite considerable research effort. An important breakthrough in understanding occurred with the identification of null mutations in the gene encoding filaggrin (FLG) on chromosome 1q21 as a major risk factor for atopic eczema. This insight has also served to focus attention on the role of epidermal barrier dysfunction as a key mechanism (perhaps the primary event) in eczema pathogenesis. FLG remains the most highly significant and widely replicated genetic risk factor for atopic eczema, with an estimated odds ratio (OR) of 3.12. However, even in the most severe case series, a maximum of 50% of cases carry FLG null alleles.

The first genome-wide association study in atopic eczema has recently identified a novel susceptibility variant: a single nucleotide polymorphism (SNP) on chromosome 11q13.5. The same study reported an additional susceptibility SNP located within the hornerin gene (HRNR), which encodes the protein hornerin, on chromosome 1q21, as well as again confirming the association of 4 prevalent FLG null mutations with atopic eczema in a European population.

We aimed to investigate the roles of these 2 novel risk variants and their interactions with the well described FLG null mutations in a collection of Irish children with moderate-to-severe eczema.

METHODS

Study participants

A total of 511 unrelated children of self-reported Irish ancestry with moderate-to-severe eczema were recruited from tertiary referral pediatric dermatology clinics based at 1 center (Our Lady’s Children’s Hospital, Dublin). The diagnosis of eczema was made by experienced dermatologists according to the United Kingdom diagnostic criteria with disease severity scored according to the Nottingham Eczema Severity Score.

A total of 1000 unselected control samples were obtained from the previously described population-based Trinity Biobank Control samples.

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Demographic and clinical data relating to the 511 Irish eczema cases and the 1000 individuals from the Irish control population are presented in Tables I and II.

The study was carried out in accordance with the Declaration of Helsinki Principles and approved by the Research Ethics Committee of Our Lady’s Children’s Hospital Crumlin (cases) and Trinity College Dublin (Trinity Biobank Control samples). All subjects or the subjects’ guardians gave written informed consent.

Genotyping methods

The 11q13.5 SNP (rs7927894) and the HRNR SNP (rs877776) were typed by the available predesigned Taqman allelic discrimination assays (Applied Biosystems, Foster City, Calif) according to the manufacturer’s recommended protocols.

The 4 filaggrin loss-of-function variants that are most common in the Irish population (R501X, 2282del4, R2447X, and S3247X) were largely genotyped by using Taqman allelic discrimination assays, as described previously,12 or with modified and simplified protocols (Campbell and McLean, July 2009).

Statistical genetic analysis

Allele and genotype frequencies were compared by using the $\chi^2$ test, the Fisher exact test, and logistic regression analysis, performed with the statistical analysis package STATA (StataCorp LP, College Station, Tex). Logistic regression models the log odds of disease as a linear function of variables encoding allele or genotype effects at the relevant locus or loci. For the allele model, a genotype variable coded as 0, 1, or 2 (according to the number of mutant alleles carried at a locus) was included in the regression equation. For the genotype model, a variable coding for homozygosity with respect to the mutant alleles was also included in the regression equation.

Tests for interaction were performed by including the main effects of rs7927894 and the relevant FLG mutation together with their interaction variables in the logistic regression equation.13 Because very few homozygous FLG mutant individuals were observed, we considered only a single main effect of FLG homozygosity together with either a genotype effect at rs7927894 (resulting in 2 interaction variables, ie, a 2-df interaction test) or an allele effect at rs7927894 (resulting in a single interaction variable, ie, a 1-df interaction test).

The fit of different models to the data was compared via a Wald test (which tests for interaction between the genotype and allele models was not statistically significant (chi-square test, $P = .318$)). The results of logistic regression analyses to investigate the OR of disease are shown in Table IV. Separate analyses performed using allele-based and genotype models demonstrated the overall OR when allele frequencies were compared between case and control groups (allele model) and the distinct ORs relating to the heterozygotes and homozygotes for each genetic variant and the combined FLG null genotype (genotype model).

Tests comparing the fit of the genotype versus the allele models are shown in this article’s Table E1 in the Online Repository at www.jacionline.org. The OR associated with the rs7927894 SNP was estimated at 1.27 by using the allele model; the genotype model gave an OR of 1.27 for heterozygote individuals and 1.63 for homozygotes (Table IV). The genotype model did not fit significantly better than the allele model ($P = .95$; the allele model had lower AIC and BIC). In comparison, the combined FLG null genotype showed an OR of 5.81 (95% CI, 4.51-7.49) by using the allele model, 5.23 (95% CI, 3.96-6.91) for heterozygotes and 140 (95% CI, 19.18-1021) for homozygotes, in keeping with semidominant inheritance. However, the low number of homozygous individuals meant that the difference between the genotype and allele models was not statistically significant ($P = .12$), although the genotype model did give the lower AIC, suggesting that it might be preferred over the allele model.

The 11q13.5 and FLG loci are independent and multiplicative

Additional analyses performed to test the statistical significance of the rs7927894 SNP having controlled for the presence/absence of the strongly significant FLG null genotype indicated that rs7927894 still shows a statistically significant effect ($P = .0025$) with an OR of 1.22 (95% CI, 1.02-1.26). A mathematical model combining the effects of rs7927894 and FLG null genotype in a linear model fits the data significantly better ($P = .0258$) than allowing for FLG alone. Furthermore, tests for interaction between each of the FLG and rs7927894 risk alleles (Table E1, results based on the cross-classification of genotypes shown in this article’s Table E2 in the Online Repository at www.jacionline.org) showed no evidence of statistically significant epistatic effects on the basis of either Wald tests from logistic regression or comparison of AIC and BIC values. Hence these data

Abbreviations used

AIC: Akaike information criterion
BIC: Bayesian information criterion
FLG: Filaggrin gene
HRNR: Hornerin gene
OR: Odds ratio
SNP: Single nucleotide polymorphism

Power calculation

Using the program QUANTO14 and assuming a population prevalence of 15%, we estimated that our sample had 71% power to detect the previously reported 11q13.5 (rs7927894) effect (OR, 1.22; allele frequency, 0.363) at 2-sided $P$ value .05 (1-sided $P$ value, .025). Our sample had only 43% power to detect the previously reported HRNR (rs877776) effect (maximum OR, 1.2; allele frequency, 0.158). We had >70% power to detect an interaction effect of OR of 1.64 or above between the 11q13.5 SNP (rs7927894) and FLG assuming a combined FLG mutation frequency of 0.078 and a FLG OR of 3 (at the lower end of data from comparable studies published previously).15

RESULTS

Replication of the 11q13.5 locus association but not the HRNR association

Genotyping results for the 11q13.5 SNP (rs7927894), the HRNR SNP (rs877776), and the 4 FLG null mutations, R501X, 2282del4, S3247X, and R2447X,12,16 are shown in Table III, in addition to their corresponding allele frequencies. The FLG null mutations each showed strong and significant association with pediatric eczema in this case/control study when analyzed individually and as a combined null genotype (Fisher exact test, $P = 1.26 \times 10^{-50}$). The rs7927894 SNP also showed a significant association with eczema ($\chi^2$ test, $P = .0025$), but the rs877776 SNP was not significant ($\chi^2$ test, $P = .318$).

The results of logistic regression analyses to investigate the OR of disease are shown in Table IV. Separate analyses performed using allele-based and genotype models demonstrated the overall OR when allele frequencies were compared between case and control groups (allele model) and the distinct ORs relating to the heterozygotes and homozygotes for each genetic variant and the combined FLG null genotype (genotype model).

Tests comparing the fit of the genotype versus the allele models are shown in this article’s Table E1 in the Online Repository at www.jacionline.org. The OR associated with the rs7927894 SNP was estimated at 1.27 by using the allele model; the genotype model gave an OR of 1.27 for heterozygote individuals and 1.63 for homozygotes (Table IV). The genotype model did not fit significantly better than the allele model ($P = .95$; the allele model had lower AIC and BIC). In comparison, the combined FLG null genotype showed an OR of 5.81 (95% CI, 4.51-7.49) by using the allele model, 5.23 (95% CI, 3.96-6.91) for heterozygotes and 140 (95% CI, 19.18-1021) for homozygotes, in keeping with semidominant inheritance. However, the low number of homozygous individuals meant that the difference between the genotype and allele models was not statistically significant ($P = .12$), although the genotype model did give the lower AIC, suggesting that it might be preferred over the allele model.
TABLE I. Characterization of 511 Irish pediatric eczema cases

| Male sex, n (%) | 315/511 (61.6) |
|----------------|----------------|
| Age (y), mean (SD) | 4.17 (3.96) |
| No. in age group (%) | | |
| <2 y | 201 (39.3) |
| 2-5 y | 141 (27.6) |
| 5-10 y | 104 (20.4) |
| 10-15 y | 55 (10.8) |
| >15 y | 10 (2.0) |
| Nottingham Eczema Severity Score, mean (SD) | 11.29 (4.60) |
| No. of cases with coexistent asthma (%) | 132/511 (25.8) |
| Serum IgE level (kU/L), mean (SD) | 2130.56 (5510.41) |

Nottingham Eczema Severity Score, range, 0 to 15, where 9 to 11 defines moderate and 12 to 15 severe disease.10

TABLE II. Characterization of Irish control population

| Male sex, n (%) | 296/1000 (29.6) |
|----------------|----------------|
| Age (y), mean (SD) | 33.34 (15.54) |
| Mean age range in years, n (%) | | |
| <25 y | 212 (21.2) |
| 25-50 y | 620 (62.0) |
| >50 y | 158 (15.8) |

The control population represents 1000 consecutive Trinity Biobank Control samples, derived from Irish adult blood donors.

demonstrate that the risk of disease in individuals having FLG null mutations and risk alleles at rs7927894 is well fitted by a multiplicative model, with the OR multiplied by 5.21 for possession of a single FLG null allele (or 138 for possession of 2 FLG null alleles, although note the wide associated CI [18.92-1009.42]) and 1.22 times for each rs7927894 mutation (therefore 5.21\times 1.22 = 7.75 for an individual with a single FLG null allele and homozygous for mutations in rs7927894).

DISCUSSION

Many candidate gene studies in atopic eczema have been performed on relatively small case collections and have generated data that have not been replicated in further studies.17,18 However, in 1 of 5 reported microsatellite-based genome screens, the 1q21 locus was identified, and we have shown that a large component of this signal was a result of FLG null mutations.4 No additional loci have been identified with any certainty by other screening studies, and therefore the recent genome-wide association study, performed on German, French, and East European cases,8 provided evidence for this genetic association. Furthermore, the frequency of the rs7927894 risk allele is similar in our Irish population control group to that reported by Esparza-Gordillo et al5; 14.5% of the Irish controls were homozygotes, compared with approximately 13% in the European control population. The combination of such a prevalent risk allele with, it appears, a substantial OR illustrates the potential importance of this novel susceptibility locus in eczema.

The gene or gene product affected by the rs7927894 risk allele defined by this anonymous SNP remains to be identified and functionally characterized; rs7927894 lies in an intergenic region between 2 annotated genes, chromosome 11 open reading frame 30 (C11orf30) and leucine rich repeat containing 32 (LRRC32) (UCSC Genome Browser, Human Genome March 2006 assembly, http://genome.ucsc.edu). C11orf30 encodes the EMSY protein, which has been shown to bind the BRCA2 breast cancer susceptibility protein22 and may therefore play a role in epithelial differentiation. The second nearby gene, LRRC32 (also known as glycoprotein A repetitions predominant (GARP), has recently been shown to be a cell surface molecule expressed on regulatory T cells,23 and may therefore be a more likely candidate for atopic eczema susceptibility, given the important role of T-cell–mediated inflammation in atopic eczema.24 Although rs7927894 is located in the same haplotype block as C11orf30,6 providing tentative genetic evidence that this is the causative gene, it is possible that nearby genes such as LRRC32 may be regulated by sequences within this haplotype block. However, it is also possible that this SNP, or more likely, causative variants in linkage disequilibrium with it, may be involved in long-range control of more distant genes, well removed from either C11orf30 or LRRC32. Defects in noncoding long-range control elements, in many cases acting over distances spanning many intervening genes, are an emerging disease mechanism.25 Finally, it should be noted that there are some expressed sequence tag clusters close to rs7927894 (UCSC Genome Browser) that may represent further uncharacterized candidate genes.

Intriguingly, rs7927894 has also been identified as a susceptibility factor for Crohn disease.6 Thus, beyond skin-specific physical barrier genes, genes having an effect on susceptibility to epithelial inflammation may have similar functions in diverse inflammatory diseases affecting both skin and the gastrointestinal
mucosal epithelia. There is limited evidence of an increased prevalence of atopic eczema in children with inflammatory bowel disease, and this cannot be explained by a differential prevalence of FLG null mutations.26 Although currently highly speculative, it is tempting to suggest that the 11q13.5 gene may contribute to a T_{H}1-dominant immune profile, as seen in Crohn disease and chronic atopic eczema lesions rather than the T_{H}2-dominant lesions of acute atopic eczema.24

In conclusion, although we have replicated the association of rs7927894 with eczema, much further work remains in identifying the causative variant or variants at this locus as well as functional analyses to determine how these sequence changes contribute to the pathogenesis of this complex disease.

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Key messages

- This replication of a novel variant on chromosome 11q13.5 (rs7927894), carried out in a large, carefully phenotyped collection of pediatric eczema cases, confirms the locus to be of importance.
- The rs7927894 SNP is prevalent in the general population, and supplementary to, the well established FLG null mutations.

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| Model                          | df | No. of observations | AIC       | BIC       | Nested model [AIC, BIC] | \(P\) value from Wald test from logistic regression comparing model with nested model |
|-------------------------------|----|---------------------|-----------|-----------|-------------------------|-----------------------------------------------------------------------------------------------|
| rs7927894 (genotype)          | 3  | 1505                | 1924.11   | 1940.06   | rs7927894 (allele) [1922.12, 1932.75] | .95                                                                                           |
| rs7927894 (allele)            | 2  | 1505                | 1922.12   | 1932.75   | rs7927894 (allele) [1906.99, 1922.92] | .22                                                                                           |
| rs87776 (genotype)            | 3  | 1491                | 1906.99   | 1922.92   | rs87776 (allele) [1906.59, 1917.20] | .22                                                                                           |
| rs87776 (allele)              | 2  | 1491                | 1906.59   | 1917.20   | rs7927894 (allele) [1892.75, 1903.37] | .34                                                                                           |
| R501X (allele)                | 2  | 1496                | 1795.79   | 1806.42   | Combined FLG (allele) [1635.00, 1645.59] | .12                                                                                           |
| 2282del4 (allele)             | 2  | 1505                | 1829.93   | 1840.56   | rs7927894 (allele) [1817.17, 1833.10] | .88                                                                                           |
| R2447X (allele)               | 2  | 1497                | 1891.09   | 1901.71   | R2447X (allele) [1859.39, 1870.92] | .74                                                                                           |
| S3247X (genotype)             | 3  | 1498                | 1893.92   | 1908.86   | Combined FLG (allele) [1635.00, 1645.59] | .12                                                                                           |
| S3247X (allele)               | 3  | 1498                | 1892.75   | 1903.37   | Combined FLG (allele) [1635.00, 1645.59] | .12                                                                                           |
| Combined FLG (genotype)       | 2  | 1475                | 1633.12   | 1649.01   | Combined FLG (allele) [1635.00, 1645.59] | .12                                                                                           |
| rs7927894 (genotype) + R501X  | 6  | 1488                | 1789.70   | 1821.53   | rs7927894 (genotype) [1817.17, 1833.10] | .88                                                                                           |
| (heterozygote)                |    |                     |           |           | rs7927894 (allele) [1787.97, 1809.19] | .31                                                                                           |
| + 2df interaction             |    |                     |           |           | rs7927894 (allele) [1785.99, 1801.90] | .47                                                                                           |
| rs7927894 (allele) + R501X (het) | 4  | 1488                | 1787.46   | 1808.68   | rs7927894 (allele) [1819.17, 1840.41] | .99                                                                                           |
| + 1df interaction             |    |                     |           |           | rs7927894 (allele) [1817.17, 1833.10] | .88                                                                                           |
| rs7927894 (genotype) + 2282del4 (het) + 2df interaction | 6  | 1493                | 1823.15   | 1855.00   | rs7927894 (allele) [1819.17, 1840.41] | .99                                                                                           |
| rs7927894 (allele) + 2282del4 (het) + 1df interaction | 4  | 1493                | 1819.15   | 1840.39   | rs7927894 (allele) [1819.17, 1840.41] | .99                                                                                           |
| rs7927894 (allele) + R2447X (het) + 1df interaction | 4  | 1491                | 1880.40   | 1901.63   | rs7927894 (allele) [1817.17, 1833.10] | .88                                                                                           |
| rs7927894 (genotype) + S3247X (het) + 2df interaction | 6  | 1490                | 1882.11   | 1913.94   | rs7927894 (allele) [1817.17, 1833.10] | .88                                                                                           |
| rs7927894 (allele) + S3247X (het) + 1df interaction | 4  | 1490                | 1878.68   | 1899.91   | rs7927894 (allele) [1817.17, 1833.10] | .88                                                                                           |
| rs7927894 (genotype) + FLG (het) + 2df interaction | 6  | 1427                | 1619.32   | 1650.90   | rs7927894 (allele) [1817.17, 1833.10] | .74                                                                                           |
| rs7927894 (allele) + FLG (het) + 1df interaction | 4  | 1427                | 1615.84   | 1636.89   | rs7927894 (allele) [1817.17, 1833.10] | .71                                                                                           |
**TABLE E2.** Cross-classification of genotypes used for the interaction analysis

|            | Controls |            | Cases |            |
|------------|----------|------------|-------|------------|
|            | rs7927894 | rs7927894  |       | rs7927894  |
|            | AA       | Aa         | aa    | AA         | Aa         | aa    |
| R501X      |          |            |       |            |            |       |
| AA         | 335      | 482        | 134   | 114        | 198        | 72    |
| Aa         | 14       | 20         | 10    | 28         | 62         | 19    |
| aa         | 0        | 0          | 0     | 0          | 1          | 1     |
| 2282del4   |          |            |       |            |            |       |
| AA         | 335      | 479        | 138   | 116        | 210        | 77    |
| Aa         | 14       | 22         | 6     | 27         | 52         | 17    |
| aa         | 0        | 0          | 0     | 2          | 2          | 2     |
| R2447X     |          |            |       |            |            |       |
| AA         | 346      | 497        | 144   | 138        | 248        | 91    |
| Aa         | 3        | 5          | 0     | 6          | 10         | 3     |
| aa         | 0        | 0          | 0     | 0          | 0          | 0     |
| S3247X     |          |            |       |            |            |       |
| AA         | 343      | 496        | 142   | 136        | 245        | 90    |
| Aa         | 5        | 6          | 2     | 7          | 15         | 3     |
| aa         | 1        | 0          | 0     | 0          | 0          | 2     |
| Combined FLG null genotype |          |            |       |            |            |       |
| AA         | 312      | 448        | 126   | 79         | 138        | 49    |
| Aa         | 36       | 53         | 18    | 47         | 92         | 29    |
| aa         | 1        | 0          | 0     | 11         | 21         | 10    |

AA, Wild-type homozygous individuals for each genetic variant; Aa, wild-type/mutant heterozygous individuals; aa, individuals who are homozygous for each of the genetic variants tested.