# *FCRL3* Gene Association with Asthma and Allergic Rhinitis

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## SUMMARY

This study sought to determine if there is an association between the single nucleotide polymorphism rs7528684 of the Fc receptor-like-3 (*FCRL3*) gene and asthma or allergic rhinitis (AR). Based on previous studies in an Asian population, we hypothesized that participants with an AA genotype of *FCRL3* would be more likely to have asthma and/ or allergic rhinitis. To test the hypothesis, surveys were administered to participants, and genotyping was performed on spit samples via PCR, restriction digest, and gel electrophoresis. Our results identified a statistically significant association between the GG genotype of *FCRL3* and an increased risk of asthma with comorbid AR. Additionally, we found a statistically significant association between the presence of other allergies with asthma and comorbid AR. These results suggest that having the GG genotype of *FCRL3* or having a history of other allergies predisposes one to having asthma with comorbid AR. The results of the study are important in more clearly identifying asthma and AR-related genes such as *FCRL3* and the variation in such genes across ethnicities. These findings could lead to earlier diagnosis and advancements in targeted therapies for the highly prevalent diseases of asthma and allergic rhinitis.

## INTRODUCTION

Asthma is a chronic lung disease that causes inflammation of the airways, which can result in coughing, wheezing, and difficulty breathing (1). Thirteen percent of children under eighteen years of age and 13.4% of adults eighteen or older have asthma in the United States (2). These numbers translate to over 25 million people, 6 million of them children (3). In addition, 40 to 60 million Americans have allergic rhinitis (AR), a sensitivity to inhaled particles such as pet dander, pollen, or dust that can occur seasonally or throughout the year, causing sinus congestion, coughing, sneezing, nasal and ocular itching, and rhinorrhea (4). About two-thirds of asthma cases are allergy related (1). Asthma and AR can also occur simultaneously, worsening symptoms for both (5). In most cases, the exact cause of asthma and a possible cure are unknown (3). Patients must instead rely on various treatments that can provide relief from their symptoms (1). However, around 50% of children with asthma in the United States have uncontrolled asthma, meaning their symptoms can occur frequently and in intense

episodes, potentially resulting in death (6). Further study into asthma and allergic rhinitis and their genetic determinants is essential for improving diagnosis, increasing awareness of symptom control options, and implementing effective, targeted treatment strategies.

Cookson and Moffatt noted that the etiology of asthma is 50% genetic and 50% environmental (7). Numerous studies have researched the genetic component of asthma, particularly genes connected to the immune system, because asthma and AR are considered to result from an overly-sensitive immune response (1). The Fc receptor-like-3 (or *FCRL3*) gene in particular has been associated with autoimmune diseases like rheumatoid arthritis, autoimmune thyroid disease, and systemic lupus erythematosus (8). This gene is believed to play an important role in the regulation of the immune system by promoting the proliferation, activation, and survival of B-cells while suppressing the differentiation of plasma cells and inhibiting the production of antibodies (9). *FCRL3* is expressed in B-cells, particularly memory B-cells, as well as T-cells and cells of the lymphoid organs and spleen (10).

Due to the relation of *FCRL3* with various autoimmune diseases, Gu et al. (2015, 2019) have researched single nucleotide polymorphisms (SNPs) in *FCRL3* in patients with asthma and/or AR, along with healthy controls in the Chinese Han population. They found a significant association between the SNP rs7528684 (-169 A/G) and AR, as well as asthma with comorbid AR, and concluded that SNPs of *FCRL3* may be related to regulating B- and T-cell proliferation and function, although the actual pathways are unclear, and it is unknown if these SNPs increase or decrease function (11, 12). However, based on a study by Swainson et al. (2010), it is possible that these SNPs may result in increased *FCRL3* expression, leading to T regulatory cell dysfunction, thus causing the development of an unregulated immune response (8).

No research on the association between asthma and/ or AR and the SNP rs7528684 has been reported to date in populations outside of Asia, which is important because the relationship between SNPs in various immune system-related genes and asthma can vary greatly between ethnic groups (11). The purpose of this study was to determine if there is an association between the SNP rs7528684 of *FCRL3* and asthma and/or allergic rhinitis in a North American population. The results indicate an association between the GG genotype of *FCRL3* and an increased risk of asthma with comorbid AR, as well as an association between participants who had other

allergies and an increased risk of asthma with comorbid AR. The results of the study will aid in identifying asthma and AR-related genes such as *FCRL3* and determining the variation in such genes across ethnicities, which could lead to earlier diagnosis and advancements in targeted therapies (7).

## RESULTS

There were 38 participants in the study, including 17 healthy controls who had neither asthma nor AR. Summaries of the demographic results obtained from the surveys completed by the study population as well as the demographic characteristics in relation to asthma alone, allergic rhinitis (AR) alone, and asthma with comorbid AR were compiled **(Table 1, Table 2)**. The patients were divided into three age ranges: young adult (16-26), adult (27-55), and senior adult (56+). The type of location (suburban, city, multi, or rural) that participants lived in for 7 or more years was also recorded **(Table 1)**. We collected this information in order to determine a possible correlation between where one has lived and asthma and/or AR. None of the participants were related or lived together. Two participants did not provide information on their AR diagnosis or other allergies **(Table 2,** designated as (-)). Only information about a diagnosis of asthma was known. Both of these participants did not have asthma. Their samples were not included in the genotype or risk ratio calculations.

| Characteristic | | Percentage Breakdown % (#) |
|---|---|---|
| Gender: | Male | 28.95% (11) |
| | Female | 71.05% (27) |
| Age: | 16-26 years | 60.05% (23) |
| | 27-55 years | 28.95% (11) |
| | 56+ years | 10.53% (4) |
| Race: | White | 73.68% (28) |
| | Asian | 15.79% (6) |
| | Multi-racial | 7.89% (3) |
| | Black | 2.63% (1) |
| Location: | Suburb | 81.57% (31) |
| | City | 7.89% (3) |
| | Multi | 7.89% (3) |
| | Rural | 2.63% (1) |
| Other Allergies: | No | 73.68% (28) |
| | Yes | 21.05% (8) |
| | No Answer | 5.26% (2) |

*Other Allergies* means allergies other than asthma or AR reported; *No Answer* indicates no answer given for other allergies.

**Table 1:** General demographic characteristics of the study population.

There was a significant association between males and asthma with comorbid AR (RR 5.2, 95% CI 1.12-24.08). Males were 5.2 times as likely to have asthma with comorbid AR compared to females. Additionally, there was a significant association between other allergies and asthma with comorbid AR (RR 7, 95% CI 1.56-31.51). Participants with other allergies were 7 times as likely to have asthma with comorbid AR compared to participants without other allergies. No other demographic such as age, race, or location was significantly associated with asthma, AR, or asthma with comorbid AR.

In the study population, we found 16 (42.11%) individuals with an AA genotype, 17 (44.74%) with an AG genotype, and 5 (13.16%) with a GG genotype. Frequency distributions of each genotype with regards to asthma, AR, asthma with comorbid

AR, or neither asthma nor AR, were calculated, along with the corresponding risk ratios and confidence intervals **(Table 3, Table 4)**.

| Characteristic | | Asthma (#) | AR (#) | Asthma-AR (#) |
|---|---|---|---|---|
| Gender (male/female) | | 0/2 | 0/11 | 4/2 |
| Age: | 16-26 years | 2 | 5 | 5 |
| | 27-55 years | 0 | 5 | 1 |
| | 56+ years | 0 | 1 | 0 |
| Race: | White | 0 | 8 | 5 |
| | Asian | 0 | 2 | 1 |
| | Multi-racial | 1 | 1 | 0 |
| | Black | 1 | 0 | 0 |
| Location: | Suburb | 2 | 10 | 3 |
| | City | 0 | 0 | 2 |
| | Multi | 0 | 1 | 0 |
| | Rural | 0 | 0 | 1 |
| Other Allergies: | No | 1 | 8 | 2 |
| | Yes | 1 | 3 | 4 |
| | No Answer | 0 | - | 0 |

*AR*, allergic rhinitis; *Other Allergies*, indicated experience of allergies other than asthma and/or AR; *No Answer* indicates no answer given for other allergies; - indicates unknown.

**Table 2:** Frequencies of demographic characteristics of study population in relation to asthma alone, AR alone, and asthma with comorbid AR (Asthma-AR).

| Genotype | Asthma (%) | AR (%) | Asthma-AR (%) | None (%) |
|---|---|---|---|---|
| AA | 0 | 4 (26.67%) | 2 (13.33%) | 9 (60%) |
| AG | 2 (11.76%) | 7 (41.18%) | 2 (11.76%) | 6 (35.29%) |
| GG | 0 | 0 | 2 (50%) | 2 (50%) |

*None*, neither asthma or AR.

**Table 3:** Frequencies and percentages of genotypes of SNP rs7528684 in relation to asthma alone, AR alone, and asthma with comorbid AR (Asthma-AR).

| Genotype | Asthma (RR (95%CI); p-value) | AR (RR (95%CI); p-value) | Asthma-AR (RR (95%CI); p-value) |
|---|---|---|---|
| AA | 0.07 (0.00-38.48; 0.13) | 0.8 (0.28-2.25; 0.33) | 0.7 (0.15-3.34; 0.33) |
| AG | 22.47 (0.04-12450; 0.07) | 1.96 (0.69-5.53; 0.10) | 0.56 (0.12-2.68; 0.23) |
| GG | 0.39 (0.00-205.6; 0.38) | 0.07 (0.00-32.93; 0.09) | 4* (1.05-15.3; 0.03) |

*RR*, risk ratio; *CI*, confidence interval; *, statistically significant (95% Confidence Interval (Taylor series) and p-value (chi-square test)).

**Table 4:** Risk Ratios for genotypes in relation to asthma alone, AR alone, and asthma with comorbid AR (Asthma-AR).

| GG Genotype | | **Asthma-AR** | | |
|---|---|---|---|---|
| | | **Present** | **Not Present** | **Total** |
| | **Present** | 2 | 2 | 4 |
| | **Not Present** | 4 | 28 | 32 |
| | **Total** | 6 | 30 | 36 |

**Table 5:** Risk Ratio of FCRL3 GG Genotype and Asthma with comorbid AR (Asthma-AR).

Risk ratios containing counts of zero were substituted with 0.1 to allow completion of the calculation, conducted using OpenEpi, online software for epidemiological calculations (13). The GG genotype was significantly associated with an increased risk for asthma with comorbid AR (RR 4, 95% CI 1.05-15.3) **(Table 4)**. The risk ratio calculation and analysis was completed using a risk ratio table **(Table 5)**.

## DISCUSSION

Asthma and allergic rhinitis cause significant morbidity worldwide, with etiologies linked to both genetics and the environment. The hypothesis for this study was developed based on previous research studying the association between SNPs of *FCRL3* and asthma as well as AR in a Chinese Han population. Unlike previous research, this study was based in a predominantly Caucasian population. It was hypothesized that the AA genotype would be associated with an increased risk of asthma and/or AR. However, in this North American study, the AA genotype seemed to be associated with a decreased risk for asthma, AR, and asthma with comorbid AR, although this was not statistically significant. Previous research had indicated a possible protection against asthma and/or AR with the GG genotype in the Chinese Han population, but in this United States study, the GG genotype was significantly associated with an increased risk for asthma with comorbid AR (11). People with the GG genotype were 4 times as likely to have asthma with comorbid AR than people without the GG genotype (RR 4, 95% CI 1.05-15.3). Previous studies have mentioned the possible differences across ethnicities in the expression of genes related to the immune system (7). Results from this study suggest that those differences are present in *FCRL3*. The variation in results between ethnicities may be due to the multi-factorial nature of asthma and AR. Asthma and AR involve multiple genes and proteins interacting with each other and with the environment, which can result in different consequences for various populations (7).

Another significant result was that participants with allergies other than asthma or AR were 7 times as likely to have asthma with comorbid AR as compared to participants without other allergies (RR 7, 95% CI 1.56-31.51). This result corroborates previous research that indicates AR is a complex allergic disease associated with other atopic diseases such as asthma, eczema, and food allergies (14). Additionally, males were 5.2 times as likely to have asthma with comorbid AR as compared to females (RR 5.2, 95% CI 1.12-24.08). Although this significant result may have been due to a small sample size rather than an actual correlation, a higher incidence of asthma with comorbid AR in males has been described in an Italian population of a similar age range to this study (15).

One limitation of the study is the small study sample size of 38 drawn from a primarily Caucasian population located in the southeastern United States. Research by Gu et al. (2015, 2019) was performed in a predominantly Chinese Han population with a sample size of 1140 and 506, respectively (11, 12). Based on power analysis for a one sample, dichotomous outcome, future studies with sample sizes of around 383 participants in a larger, more racially and geographically diverse population would enhance the significance of the results. Additionally, this study did not take into account different types of asthma or AR, primarily because it sought only to identify a general association between a *FCRL3* SNP and asthma and/or allergic rhinitis in a population where no such study had been reported to date. Therefore, the proportion of participants with asthma who had atopic asthma is unknown. Furthermore, the physician diagnosis of asthma and AR was self-reported by the participants, and could be subject to reporting error. Finally, the sample was restricted to people sixteen years of age and above and thus did not include a younger pediatric population.

The results of this study could be important in identifying genes related to asthma and allowing earlier diagnosis and targeted therapy. For instance, if one can identify a risk of asthma early on, physicians can prescribe immunotherapy for allergic rhinitis to prevent the development of asthma later in life (16). To establish significance of the findings, we recommend further study of *FCRL3* and asthma as well as allergic rhinitis in a larger, more diverse population, with a wider age range and physician-documented diagnoses.

## MATERIALS AND METHODS

Volunteers were recruited from a suburban Atlanta school's junior and senior classes as well as teachers during school assembly. Volunteers included both those without a history or diagnosis of allergies and those with allergies, particularly asthma and/or allergic rhinitis. Participants gave their informed consent through consent forms. A 1% NaCl solution was used to collect saliva samples. During the study, the researcher was blinded to sample identification.

DNA extraction was completed by centrifuging the spit samples, mixing together 50 µL of DNA and 200 µL of 10% Chelex, and placing mixed samples in a dry bath at 95ºC for 10 minutes. *FCRL3* SNP rs7528684 (-169A/G) was analyzed using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). The PCR tubes were each labeled with the participant identification number. The PCR-RFLP methodology was modeled after a study by Jin et al (2015) (17). The tubes were placed in a rack over ice, with 12.5 µL GoTaq Green Master Mix (Promega), 2.5 µL of the forward primer, 2.5 µL of the reverse primer, and 7.5 µL of DNA pipetted into each tube (Forward: 5'-CCCTTCACTACCTTGTCTTCACAC-3'; Reverse: 5'-GGGTGGAACCTCTTTGATTGC-3'). The tubes were placed into the thermal cycler with the following conditions: pre-denaturation at 95ºC for 5 minutes, 95ºC for 30 seconds, annealing at 58ºC for 20 seconds, extension at 72ºC for 60 seconds (40 cycles), and a final extension at 72ºC for 10 minutes.

Restriction enzyme digestion was performed with a mix of 7.5 µL of PCR product, 36 µL nuclease-free water, 5 µL 10X Buffer Tango, and 1 µL FaqI (BsmFI) restriction enzyme (New England Biolabs). The reaction tube was then incubated for 60 minutes at 65ºC and another 20 minutes at 80ºC to deactivate the enzyme. The products were detected using 2% agarose gel electrophoresis. The finished gels were stained overnight on a rocker in 1X FastBlast stain (Bio Rad).

Two samples from each subject were genotyped to confirm results. Samples were genotyped by the number of

lines in each lane of the gel **(Figure 1)**. There were three possible genotypes: AA, GG, and AG. The AA genotype corresponded to one fragment of 296 bp; the GG genotype to two fragments of 184 bp and 112 bp; and the AG genotype to three fragments of 296 bp, 184 bp, and 112 bp. Results were used to determine the frequency distribution of genotypes and alleles for each group (asthma, AR, asthma with comorbid AR, none), which was recorded in a Google spreadsheet. Results were found by calculating risk ratios and determining confidence intervals using OpenEpi.

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