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Fine mapping thymic stromal lymphopoietin confirms association with rs1898671 and atopic dermatitis onset and persistence.

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Abstract

Background: Atopic dermatitis (AD) is a common chronic relapsing skin disease. Genetic variants have been associated with skin barrier function and immune regulation. Thymic stromal lymphopoietin (TSLP), an immune regulator, has been previously associated with AD.

Objective: The goal of this study was to fine map TSLP and evaluate associations with the onset and persistence of AD.

Methods: TSLP variation was determined using targeted massively parallel sequencing in a longitudinal cohort of children with AD. Evaluations included linkage disequilibrium (LD) and the persistence of AD over as many as 10 years of follow-up. The association between the presence of AD and rs1898671 variation was evaluated in a second independent cohort.

Results: The minor variant frequency for rs1898671 was 23.5% (95% CI: 21.4, 25.8). This variant was not in LD with other TSLP variants in the longitudinal cohort (N=741). White children

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with AD were less likely to have rs1898671 variant (1.41 (1.20, 1.66)) than a genomAD control. Children with AD and the rs1898671 variant during follow-up were more likely to have a remission than children who were wildtype for rs1898671 (OR: 1.56; 95% CI: 1.26, 1.91). In the second cohort (N=585), the rs1898671 variant was less prevalent in those with AD than those without. The protective effect was found to be greater in rs1898671 heterozygotes (OR 1.91 (1.34, 2.75)) than homozygotes (OR: 1.28 (0.61, 2.70)).

Conclusion: TSLP and specifically rs1898671 is important in the pathogenesis of AD and could represent a potential clinical target for the development of therapies to treat individuals with AD.

Keywords

Atopic Dermatitis; eczema; gene association; genetics; filaggrin; thymic stromal lymphopoietin

Introduction

Atopic dermatitis (AD) is a common chronic relapsing disease that manifests as itchy, typically excoriated skin lesions that are often concentrated around the flexor surfaces of the extremities.[1,2] It was once thought to be solely a childhood illness, but recent studies show that AD can be a life-long disease that often persists into adulthood and even the initial diagnosis of AD can occur in adulthood.[3–7] In the US, the yearly prevalence of AD is about 10% in both children and adults with an annual cost of more than 4 billion dollars.[8–10] Despite our increasing understanding of the epidemiology of AD, the genetic risk factors that predispose to its development remain incompletely defined.

A family history of AD and other atopic illnesses such as asthma and seasonal allergies are associated with an increased risk of developing AD as a child.[11, 12] The heritability of AD is estimated to be as high as 84%.[13] The most commonly reported AD associated genetic variants are loss of function variants (LoF) in a skin barrier protein called filaggrin (*FLG*).[14] However, *FLG* LoF variants are only found in about 25–30% of those of European and Asian ancestry with AD and in only about 12% of individuals of American-African ancestry with AD exhibit *FLG* LoF variants.[3, 14–16]

Several studies have demonstrated that the heritability of AD is also linked to variations in genes that result in immune dysregulatrion.[17] *TSLP* is the gene that encodes for thymic stromal lymphopoietin (TSLP) protein, which is thought to be a master initiator of allergic inflammation at multiple barrier surfaces.[18–21] TSLP protein, predominantly expressed from stressed epithelial cells, promotes type 2 inflammation via a variety of cellular pathways.[18, 19] TSLP expression occurs upon skin skin barrier (e.g., FLG abnormality) disruption and subsequently promotes the type 2 immune responses that results in inflammation leading to AD.[14, 19, 21] However, which genetic variations in TSLP play a major role in the pathogenesis of AD remains poorly defined.

Increased expression of TSLP has been strongly associated with AD as well as other allergic diseases including asthma, allergic rhinitis, and food allergy.[18–25] A 2013 publication by Noti *et al* demonstrated that *TSLP* variation was associated with the production of a specific lineage of circulating basophils in patients with eosinophilic esophagitis.[26] TSLP also has

been shown to be upregulated in keratinocytes in response to local pro-inflammatory triggers.[27] In 2010, Gao *et al* reported an association between two *TSLP* single nucleotide polymorphisms (SNPs) (rs1898671, rs2416259) and eczema herpeticum, which is an infectious complication of AD most often seen in those with severe AD.[22] Recently, siRNA-mediated knockdown of *FLG* expression was shown to induce TSLP expression in epidermal keratinocytes.[28] Using chip-based technology and tagging SNPs, we previously demonstrated that *TSLP* variant rs1898671 is associated with less persistent AD.[29] The goal of this study was to more carefully evaluate the association between TSLP variants using fine mapping of the region to identify other potentially causal SNPs and to investigate SNPs in linkage disequilibrium with the tagging SNP rs1898671. We evaluated haploblocks in the region of rs1898671 to further investigate the association with AD onset and persistence.

Methods:

Population

The Pediatric Eczema Elective Registry (PEER; www.thepeerprogram.com) is a United States nationwide cohort of more than 8,000 subjects with pediatric-onset AD. The current study represents the subcohort of PEER children who provided a genetic sample (PEER DNA cohort).[30, 31] Both self-described race and ancestry informative markers were previously used to define race and were found in this cohort to be highly correlated.[30] PEER DNA enrollment occurred between November of 2004 and January 2015. At the time of enrollment, children were two to seventeen years old, had a physician-confirmed diagnosis of AD, and had used pimecrolimus cream for at least 6 months.[30] Subjects were followed for up to 10 years and during that time were not required to (and most did not) continue therapy with pimecrolimus.[32] Full details of the PEER cohort have been previously reported.[3, 30, 32]

A second cohort, not related to the PEER cohort, called the *Genetics of Atopic Dermatitis* (GAD) cohort was also studied to evaluate the effect of *TSLP* variation on the likelihood of having AD. All subjects were examined by a dermatologist from the following Dermatology practice locations: University of Pennsylvania Perelman School of Medicine, Children's Hospital of Philadelphia, Pennsylvania State University/Hershey Medical Center and Washington University at St Louis School of Medicine. All subjects had a history and an exam consistent with AD (cases) or no history of AD (controls). There was no age limitation, but enrollment was restricted to African-Americans or white patients. All patients provided written informed consent approved by their appropriate Institutional Review Board.

Genetic analysis

DNA was collected using Oragene DNA collection kits (DNA Genotek, Ottawa Canada) as previously reported.[30] For the PEER Cohort *TSLP* and *FLG* gene was sequenced for all subjects using targeted massively parallel sequencing (MPS). The average coverage for TSLP by region varied from 115 to 181. The reliability of this technique for *FLG* sequencing, was previously reported.[15] Raw sequencing data were aligned and mapped to the reference genome GRCh37 using the Burrows-Wheeler Aligner.[33] Single nucleotide variant and insertion/deletion (indel) calling was accomplished using the Genome Analysis

Toolkit (GATK) HaplotypeCaller, after following GATK Best Practices realignment and recalibration.[34–37] For the GAD cohort, *TSLP* rs1898671 was assayed using TaqMan technique. Population based gene variation was confirmed using Genome Aggregation Database, gnomAD, (https://gnomad.broadinstitute.org/). Tissue specific gene expression in the skin was confirmed using Genotype-Tissue Expression (GTEx) portal (https://gtexportal.org/home/snp/rs1898671).

Outcome:

AD resolution without therapy (remission) was evaluated in the PEER Cohort based on the self-reported outcome of whether or not a child's skin was AD symptom-free during the previous six-months while the child was not using medication to treat their AD.[30] AD disease activity was based on the survey question: "During the last six months would you say that your child's skin disease (AD) has shown: complete disease control, good disease control, limited disease control, or uncontrolled disease". Symptom free was defined as an affirmative response to "complete disease control". This response has been shown to correlate with other tools used to evaluate symptom control and is likely a marker of long-term disease severity.[38] The absence of medication use was determined by series of questions and pictures asking about specific medications as well as the quantity of the medication prescribed. A single Patient Oriented Eczema Measure (POEM) score (0–7 (mild); 8–16 (moderate); and 17–28 (severe)), a tool used to measure eczema severity as reflected by the patient, was obtained on enrollment into the GAD cohort.[39]

Haplotype Blocks:

Linkage disequilibrium (LD) was assessed for the full PEER DNA Cohort and separately for both the African American and white sub cohorts. LD was first estimated using R^2 values between rs1898671 and all other co-occurring SNPs. The software Haploview (https://www.broadinstitute.org/haploview/haploview) was used to visualize haplotype blocks and estimate haplotype frequencies assuming the Gabriel algorithm.[40]

Statistical Analyses:

Covariate frequencies were summarized using means or medians as appropriate. Initial comparisons were conducted using Chi-Square or logistic regression. The association between the "remission" outcome and the *TSLP* variant was evaluated using an additive model for the variant and generalized estimating equations (GEE) for binary outcomes assuming an independence working correlation structure to account for the correlation among repeated measures per participant (one survey every six months for up to 10 years). Initial enrollment occurred over a ten-year period and observation is still ongoing for some of the subjects. At any given survey, approximately 60% of subjects responded. Missingness was evaluated visually and felt to be completely at random and consistent with GEE modeling specifications. Weighted GEE models to account for survey response missingness were also conducted and the results were nearly identical (not presented). All analyses were conducted with Stata version 15.1 (StataCorp, College Station, Texas) or R (https://www.r-project.org/).

Results:

The PEER DNA cohort included an analysis of 741 children with an MPS analysis of the *TSLP* gene. From the full PEER DNA cohort of 741 children, 326 children were of African-American ancestry, 379 were white, 21 children of other ancestries, and 15 children multiple ancestries. 53.2% (394) of the children were female, the average age of AD onset was 1.99 (sd: 2.76) years, and 78.6% and 56.4% of the children had completed five and the full 10 years of PEER enrollment, respectively. Overall 44.4% of children had at least one six-month period of AD remission. MPS revealed 156 variants in *TSLP.* This study focused on rs1898671.

The minor variant frequency (MVF) for rs1898671 (C substituted by T) was 23.5% (95% CI: 21.4, 25.8) for the full cohort, 9.8% (7.6, 12.4) for children of African-American ancestry, and 34.9% (31.6, 38.3) for white children. For comparison, we obtained population based frequencies from the Genome Aggregation Database, gnomAD, (https://gnomad.broadinstitute.org/), and found that European (non-Finnish) controls have MVF 27.5% (26.3, 29.7) and African controls have 9.8% (8.7, 11.1). In other words, white PEER DNA children with AD are less likely to have rs1898671 variant (1.41 (1.20, 1.66)) than the more general population but African-American children are equally likely (1.00 (0.73, 1.13) as the genomAD African population. Per GTEx (https://gtexportal.org/home/snp/rs1898671), the measured *TSLP* mRNA in skin of the lower leg and suprapubic regions is increased by rs1898671 variation.

At enrollment children, with rs1898671 were more likely to have a food, animal and medication allergies than children in PEER who did not have the variant, but were not more likely to have asthma, seasonal allergies, concomitant *FLG* LoF variant, or an earlier AD disease onset (Table 1). Compared to white children, African-American children with the rs1898671 variant also had many more additional *TSLP* variants (Table 2). However, these variants do not appear to be in linkage with rs1898671 (Table 2). MVF for all of the other variants as well as the most common (≥ 10%) TSLP variants not co-occuring with rs1898671 are reported in Table 2 and Supplement Table 1–3). Overall very few common variants were noted. One haplotype block was identified for the white children (Figure 1) and no haplotype blocks were identified for African-American children.

Children with the rs1898671 variant, were more likely to have a remission period (Table 3 and Figure 2). Children with the rs1898671 variant at any given survey were more likely to have a remission than children who were wildtype for rs1898671 (OR: 1.56; 95% CI: 1.26, 1.91) (Table 3)). This effect was greater for African-American than white children and appears to be greater in those with a *FLG* LoF variant (Table 3). In addition to rs1898671, the SNPs in the haplotype for the white children included rs10062929, rs2289276, and rs11466741 (Figure 2). Neither of the haplotypes nor the individual SNPs were associated with remission (Table 3).

At this time, the GAD cohort includes 585 individuals including 337 individuals with AD and 238 individuals without AD, respectively. The average age of enrollment was 33.6 years (sd: 21.7), the average age of disease onset for those with AD was 6.8 years (sd: 13.8).

56.7% were female and 49.2% were African-American. The rs1898671 MVF was 21.6% (18.9, 23.7) for the full GAD, 12.0% (9.4, 14.9) for African-Americans and 31.9% (27.8, 36.1) for whites. Individuals with rs1898671 were less likely to have AD (1.48 (1.12, 1.96)). This effect is not significantly different between African-Americans and for whites (p=0.150). The rs1898671 variant AD protective effect was found to be greater in heterozygotes (1.91 (1.34, 2.75)) than homozygotes (1.28 (0.61, 2.70)). Individuals with AD and the rs1898671 variant tend to have a later age of onset of their AD (wildype-6.2 years (sd: 11.8), heterozygous-7.6 years (sd:16.6) years and homozygous-9.0 years (sd:19.6)). The median POEM score was of 12 (moderate eczema severity) (25%: 6 (mild), 75%:17 (severe)) for GAD group with AD and was not associated with rs1898671 variation.

Discussion:

TSLP is important for the activation of type 2 inflammation at barrier surfaces. It has previously been shown to be associated with multiple allergic disorders. Based on prior studies, mostly chip based studies, we focused on rs1898671 to examine its association with AD. We replicated prior findings using massively parallel sequencing and demonstrated that rs1898671 variant, which was originally selected as a tagging SNP, is not in linkage disequilibrium with other *TSLP* variants found in the PEER DNA cohort.[22] It is more frequently found in white individuals than those of African-American ancestry. We can now confirm that children with AD who have rs1898671 have a less severe course of AD as manifested by more frequent remissions. The variant may also be associated with decreased incidence of AD, as noted in both the white PEER DNA cohort as compared to gnomAD and the first assessment of the GAD cohort, which is a new cohort of individuals with AD that is not related to the PEER cohort. Based on the clinical observations and GTEx, the rs1898671 variant may have a direct effect on the production of TSLP in the skin. Finally, the effect of *FLG* LoF, which has been shown to increase the prevalence and persistence of AD, appears to be modified by rs1898671.[15, 30] These findings are unique and important because we can now confirm that the rs1898671 variant and not another *TSLP* variant in linkage disequilibrium (LD) with re1898671 is associated with both an decreased risk of AD and decreased AD persistence/severity.

The rs1898671 variant is located in an intron of the *TSLP* gene on chromosome 5(110408002 (GRCh37)). It represents a single nucleotide change from C to T and potentially falls on two *TSLP* transcripts. The *TSLP* gene is transcribed into a long form (exons one to four) and short form (exons three and four) protein.[41, 42] Increased TSLP production is associated with rs1898671. The rs1898671 variant is within about 300 bp of exon three and the initiation sequence for the short form of the TSLP protein (sfTSLP). sfTSLP protein is constitutively expressed at the mRNA level in human keratinocytes and is likely the predominate form produced by keratinocytes.[41] It appears to act as an antimicrobial peptide creating a skin barrier defense and is not secreted in response to inflammatory influences.[41, 42] The exact functions of the two forms of TSLP are not fully understood. It is possible that the isoforms act in antagonistic ways; however, due to technical difficulties, in most studies, these two proteins are often not differentiated.[41–43]

Gao *et al* reported an association between two *TSLP* SNPs (rs1898671, rs2416259) and eczema herpeticum.[22] In addition, we previously noted that rs1898671 was associated with less persistent AD and was not associated with the timing of onset of AD. [26, 44] These studies relied on genotyping techniques and could not properly differentiate rs189867 from other variants that could potentially be in LD. In both studies, the presence of the rs1898671 appeared to be associated with a milder clinical phenotype. A recent study in Korea of another set of *TSLP* SNPs showed an alteration in the onset of AD and co-morbidities like asthma and allergic rhinitis in those with *TSLP* variation.[45] TSLP variation has been associated with eosinophilic esophagitis another illness that is associated with AD.[26, 46] TSLP has also been shown in experimental human keratinocyte models and mouse models to be associated with skin inflammation like that seen in AD.[23]

Our study does have limitations. The PEER study is a cohort study and follow-up data are obtained by survey. It is possible that the survey data do not properly reflect the severity of AD. However, it is unlikely that misclassification of severity is different between genotypes (which are unknown to the participant) thereby minimizing this potential bias. Outcome data, like POEM, were obtained during an office visit usually scheduled for AD treatment. It is likely that the POEM results are biased towards more severe findings (i.e., differential information bias) and, since the GAD cohort is a one-time evaluation, could explain why no clinical differences in POEM were noted in our study. It is also important to note that neither PEER nor GAD are random samples of the US population so it is possible that our results are not generalizable. However, many of the rs1898671 findings first noted in PEER were reproduced in the GAD cohort. As discussed isoforms of TSLP exist that have different functions. Differentiating the isoform of TSLP is technically difficult, was not assessed in this report, and is necessary to properly differentiate TSLP function. In addition, allergen reactivity testing and measurements of serum IgE, which could be influenced by rs1898671 variation was not measured in this study.

In summary, rs1898671 genetic variation in those with AD appears to have a direct influence on the persistence of AD and, in whites, it appears to decrease the incidence of AD. Others have shown that the presence of rs1898671 in those with AD diminishes the likelihood of AD associated HSV infection and other atopic illnesses, such as asthma and eosinophilic esophogitis.[22] We demonstrated that rs1898671 is not in LD with other co-existing TSLP variants; thus it is likely to have a direct effect on TSLP production or function. Based on the location of rs1898671 and GTEx database, it is likely that the variant has an effect on the function or production of the sfTSLP.

Current biologic therapies for AD due target the general immunologic pathway that includes lymphocyte activation by TSLP. Clinicians may begin to use *FLG* LoF genotyping, *TSLP* genotyping, or the AD polygenic risk score to personalize AD treatment as part of their treatment plan[30, 31, 47]. However, at this time it is best to proceed with caution. Very few studies have formally evaluated the influence of genetic variation on longitudinal AD severity and very few have evaluated the influence that genetic variation has on treatment[30, 31]. Studies have also shown that genetic testing for genes associated with AD is technically difficult[16, 48, 49] Additional study is necessary before clinical personalized treatment is based AD genetic testing. Based on our current findings it is fair to conclude

that TSLP, and specifically rs1898671 is an important genetic variant in the pathogenesis and long-term prognosis of AD and could represent a potential clinical target for the development of therapies to treat individuals with AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

(AD)	Atopic dermatitis
(CI)	Confidence interval
(FLG)	Filaggrin
(GEE)	Generalized estimating equations
(GAD)	Genetics of Atopic Dermatitis
(LD)	Linkage disequilibrium
(LoF)	Loss of function
(MPS)	Massively parallel sequencing
(MVF)	Minor variant frequency
(OR)	Odds Ratio
(POEM)	Patient Oriented Eczema Measure
(PEER)	Pediatric Eczema Elective Registry
(SNP)	Single nucleotide polymorphisms
(TSLP)	Thymic stromal lymphopoietin

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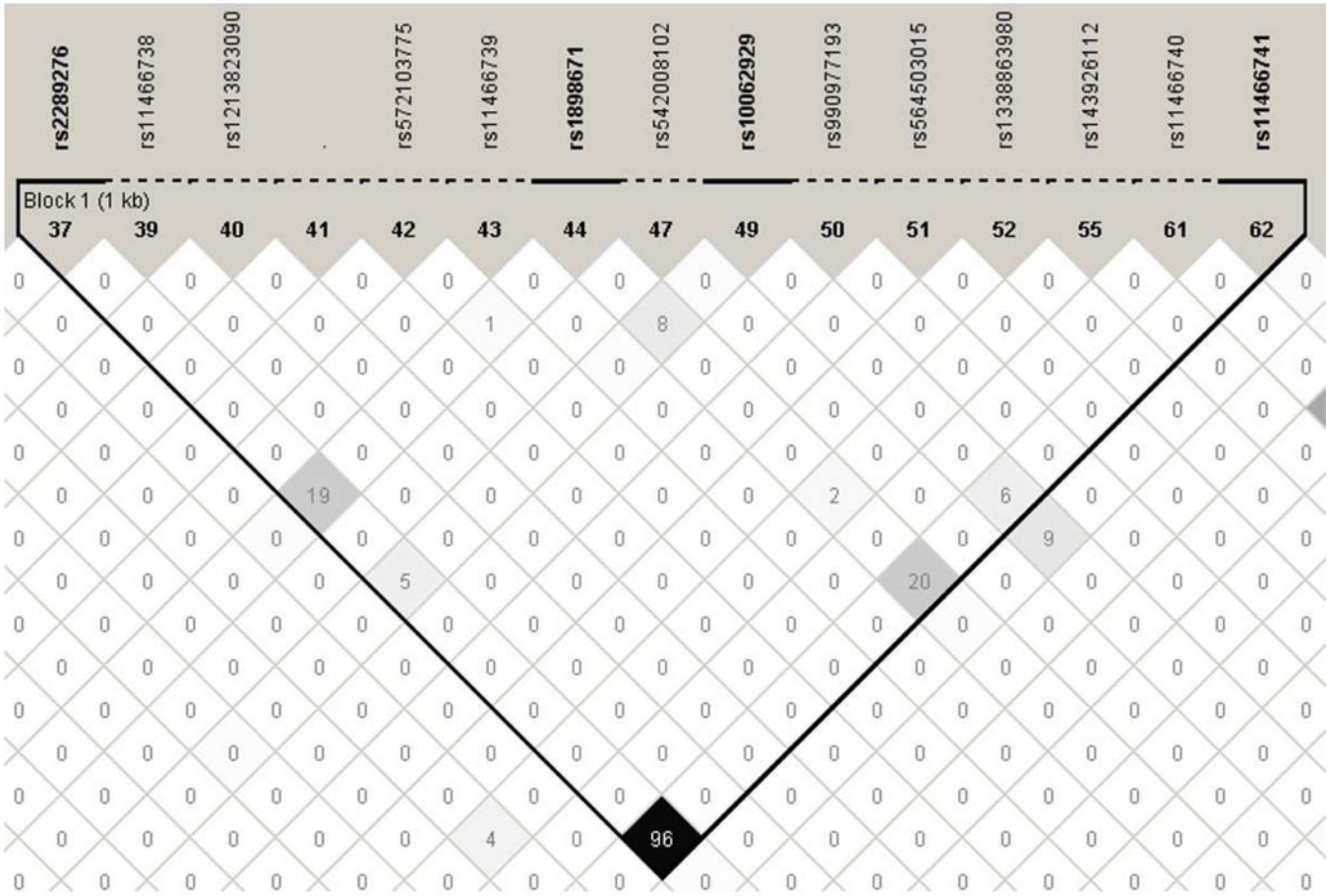

	rs2289276	rs1898671	rs10062929	rs11466741	Haplotype Frequency
1	1	1	1	1	0.2373
2	1	2	1	1	0.34898
3	1	1	1	2	0.00635
4	2	1	1	1	0.00128
5	2	1	1	2	0.26776
6	1	1	2	1	0.13832

Figure 1:

rs1898671 TSLP Haplotypes in white PEER cohort children including the only haploblock (1=not present, 2=variant present)

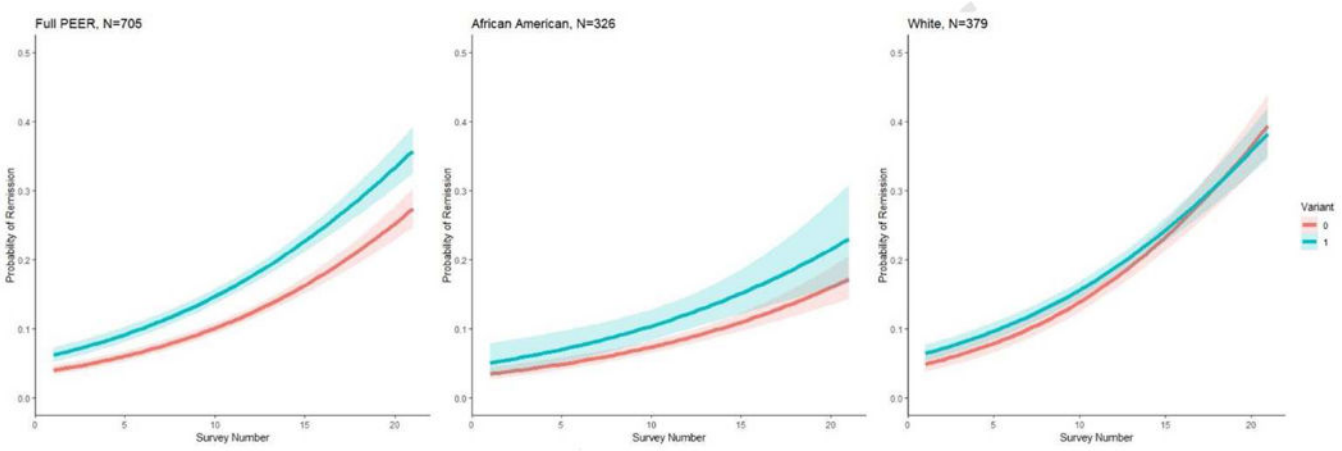

Figure 2:

Likelihood of remission (overtime, each survey represents approximately a six-month interval) for children with and without the rs1898671 variant in the PEER cohort for the full cohort, white children, and children of African ancestry.

Table 1:

Phenotype characteristics by rs1898671 (C>T) genotype from the PEER cohort (chi –square p-value)

	Frequency of those without rs1898671 and attribute	**Frequency of those with rs1898671 and attribute**
Sex (female)	51.2%	54.5%
African-American	59.6%	20.3% p<0.0001
Asthma	52.9%	55.2%
Seasonal allergies	69.4%	68.5%
Food allergy	28.5%	37.3% p=0.012
Peanut allergy	14.1%	16.6%
Milk allergy	6.7%	10.8% p=0.047
Animal allergy	26.5%	36.3% p=0.004
Dog allergy	17.3%	26.1% p=0.004
Cat allergy	22.4%	29.8% p=0.023
Medication Allergy	48.2%	51.7% p=0.004
Any FLG variant	22.6%	25.8%
Age AD onset	2.02 years	1.89 years

Table 2:

Linkage disequilibrium as estimated by R^2 for *TSLP* variants if they co-occurred with rs1898671. R^2 is listed for the full PEER DNA cohort and then for children of African-American ancestry or Caucasian ancestry. Variants are listed by RSID and in order of location on the *TSLP* gene per GRCh37.

RSID	Location	Full Peer R^2	African American R^2	African American MVF (%)	White R^2	White MVF (%)
375939272	110406225	0.0002	0.0010	0.5 (0.1,1.3)		
189331165	110406489	0.0006	0.0002	0.6 (0.2,1.6)		
560721535	110406605	0.0023	0.0020	0.5 (0.1,1.3)		
185194032	110406650	0.0088	0.0109	0.2 (0.0,0.8)	0.0071	33.1(29.9,34.4)
186519906	110406923	0.0020	0.0079	0.2 (0.0,0.8)	0.0005	0.1 (0.0,0.7)
188567463	110407103	0.0002	0.0010	0.5 (0.1,1.3)	0.0005	0.1 (0.0,0.7)
532579260	110407131	0.0015	0.0013	0.3 (0.0,1.1)		
755621192	110407189	0.0000	0.0031	0.3 (0.0,1.1)		
141800763	110407492	0.0000	0.0031	0.3 (0.0,1.1)		
1898671	**110408002**	reference	reference	9.8 (7.6, 12.4)	reference	34.9 (31.6, 38.3)
148296322	110408003	0.0006	0.0002	0.6 (0.2,1.6)		
1016936592	110408158	0.0015	0.0013	0.3 (0.0,1.1)		
560518251	110408275	0.0023	0.0013	0.3 (0.0,1.1)	0.0029	0.1 (0.0,0.7)
148396476	110408710	0.0006	0.0002	0.6 (0.2,1.6)		
374175217	110408804	0.0031	0.0026	0.6 (0.2,1.6)		
368498616	110408815	0.0015	0.0013	0.3 (0.0,1.1)		
571508866	110409065	0.0002	0.0006	0.2 (0.0,0.8)	0.0023	0.4 (0.0,1.0)
201709945	110409391	0.0015	0.0013	0.3 (0.0,1.1)		
140872604	110409408	0.0076	0.0109	0.2 (0.0,0.8)		
1046714887	110409740	0.0015	0.0013	0.3 (0.0,1.1)		
532541421	110409819	0.0015	0.0013	0.3 (0.0,1.1)		
540379350	110409894	0.0000	0.0031	0.3 (0.0,1.1)		
532704183	110411182	0.0023	0.0018	0.5 (0.1,1.3)		
141535387	110411311	0.0031	0.0329	0.5 (0.1,1.3)		
374549851	110411336	0.0002	0.0010	0.5 (0.1,1.3)		
192309314	110412049	0.0006	0.0002	0.6 (0.2,1.6)		
146408762	110412321	0.0020	0.0219	0.3 (0.0,1.1)		
11466749	110412585	0.0308	0.0132	11.0(8.7,13.7)	0.0910	15.1 (12.7,17.7)
115625984	110413287	0.0006	0.0002	0.6 (0.2,1.6)		
966395563	110413332	0.0015	0.0013	0.3 (0.0,1.1)		

Table 3:

The association of rs1898671 and remission in the full PEER cohort and among those who are African-American and white and based on the presence of *FLG* LoF. The odd ratios are adjusted for age onset, sex, race if appropriate and the presence of *FLG* LoF when appropriate.

	PEER: White or African-American	***FLG* LoF**	**No *FLG* LoF**
Combined	1.56 (1.26,1.91) p<0.0001 N=705	1.98 (1.33,2.98) p=0.001 N=170	1.43 (1.12,1.82) p=0.003 N=535
White	1.20 (0.94,1.53) N=379	1.81 (1.16,2.84) p=0.007 N=120	1.19 (0.94,1.53) N=259
African-American	1.83 (1.12,2.98) p=0.018 N=326	_____* N=50	1.94 (1.18,3.20) p=0.011 N=276

* insufficient sample for estimate.