

Letter to the Editor

STAT6 and LRP1 polymorphisms are associated with food allergen sensitization in Mexican children

To the Editor:

Food allergy, affecting up to 8% of children in the United States,¹ is most often mediated by allergen-specific IgE antibodies in the blood. Food allergen sensitization is an intermediate phenotype measured by specific IgE blood tests or skin prick tests (SPTs) and used in the diagnosis of food allergy. Despite family history being a risk factor, no genetic variants have been conclusively identified for food sensitization or clinical food allergy.² By using the Mexico City Childhood Asthma Study, we examined associations between food sensitization (based on SPTs) and single nucleotide polymorphisms (SNPs) spanning 5 autosomal candidate genes reviewed by Hong et al² (*CD14* [cluster of differentiation 14], *IL10*, *IL13*, *SPINK5* [serine peptidase inhibitor, Kazal type 5 isoform], and *STAT6* [signal transducer and activator of transcription 6]).

We previously conducted a genome-wide association study of asthma in the Mexico City Childhood Asthma Study among 492

children with physician-diagnosed asthma (aged 5-17 years) and their parents, who were recruited from a pediatric allergy clinic in Mexico City.³ The children's clinical evaluation included SPTs to 6 major food allergens that are common in the Mexican diet (milk, egg, wheat, soy, peanuts, and tree nuts). An SPT result was declared positive if the largest diameter of the wheal exceeded 4 mm. Testing was considered valid if the reaction to the positive control (histamine) exceeded 6 mm and also exceeded 4 mm above the negative control (glycerin).⁴ There were 162 trios having an asthmatic child with a positive SPT result to at least 1 food allergen. We examined SNP associations with food sensitization in the 5 candidate gene regions in these 162 trios.² As all probands were asthmatic patients, it was not possible to adjust for asthma. These associations are generalizable to the asthmatic population, but not necessarily to the general population.

Genotyping was performed on the Illumina HumanHap 550v3 BeadChip, and standard quality control filters were applied.³ For this analysis, we selected SNPs spanning 5 candidate gene regions (20 kb upstream of the 5' end through 20 kb downstream of the 3' end), resulting in 343 SNPs (16 *CD14*, 52 *IL10*, 39 *IL13*, 28 *STAT6*, and 208 *SPINK5* SNPs). SNPs were directly genotyped

TABLE I. Associations of the 28 SNPs examined in or near *STAT6* (± 20 kb) in 492 trios with an asthmatic child and the subset of 162 trios with a food-sensitized child (at least 1 positive skin prick test result to 6 major food allergens)

SNP*	SNP type	Gene§	MAF†	MaCH Rsq value#	P value, asthma (n = 492 trios)	P value, food sensitization (n = 162 trios)
rs17119418	Intergenic	—	0.050	0.77	.085	.19
rs2035545	Intergenic	—	0.053	0.80	.19	.17
rs324010	Intergenic	—	0.41	0.92	.035	.010
rs324020	Intronic	<i>NAB2</i>	0.48	0.94	.034	.020
rs1059513‡	Intergenic	—	0.074	0.99	.35	.56
rs703817‡	3' UTR	<i>STAT6</i>	0.47	0.99	.026	.0076
rs324015‡	3' UTR	<i>STAT6</i>	0.31	0.99	.28	.084
rs3024975	Intronic	<i>STAT6</i>	0.060	0.81	.25	.30
rs3024974	Intronic	<i>STAT6</i>	0.060	0.80	.24	.29
rs841718‡	Intronic	<i>STAT6</i>	0.44	0.99	.072	.015
rs3024971	Intronic	<i>STAT6</i>	0.029	0.88	.077	.10
rs10783813	Intronic	<i>STAT6</i>	0.20	0.61	.57	.23
rs324011	Intronic	<i>STAT6</i>	0.47	0.97	.044	.055
rs167769‡	Intronic	<i>STAT6</i>	0.47	0.99	.044	.029
rs324013‡	Intergenic	—	0.39	0.99	.31	.41
rs12368672	Intergenic	—	0.44	0.90	.082	.066
rs11172106	Intergenic	—	0.41	0.91	.28	.36
rs12298170	Intergenic	—	0.41	0.88	.27	.31
rs12307379	Intergenic	—	0.020	0.69	.21	.011
rs7306742	Intergenic	—	0.020	0.70	.21	.012
rs11172113‡	Intronic	<i>LRP1</i>	0.40	0.99	.24	.21
rs4759044‡	Intronic	<i>LRP1</i>	0.34	0.99	.25	.0056
rs1385526	Intronic	<i>LRP1</i>	0.49	0.88	.24	.019
rs715948‡	Intronic	<i>LRP1</i>	0.27	0.99	.32	.011
rs4759277	Intronic	<i>LRP1</i>	0.41	0.97	.44	.066
rs1466535‡	Intronic	<i>LRP1</i>	0.43	0.99	.17	.0077
rs7968719	Intronic	<i>LRP1</i>	0.41	0.76	.18	.11
rs7398375	Intronic	<i>LRP1</i>	0.30	0.66	.37	.034

MAF, Minor allele frequency; UTR, untranslated region.

*SNPs were ordered by their position on chromosome 12.

†MAF was calculated among the 162 food allergen-sensitized children.

‡SNP was genotyped.

§The flanking regions around *STAT6* included SNPs in 2 other genes, *NAB2* and *LRP1*.

#SNPs were genotyped or imputed, and MaCH Rsq values indicate imputation quality.

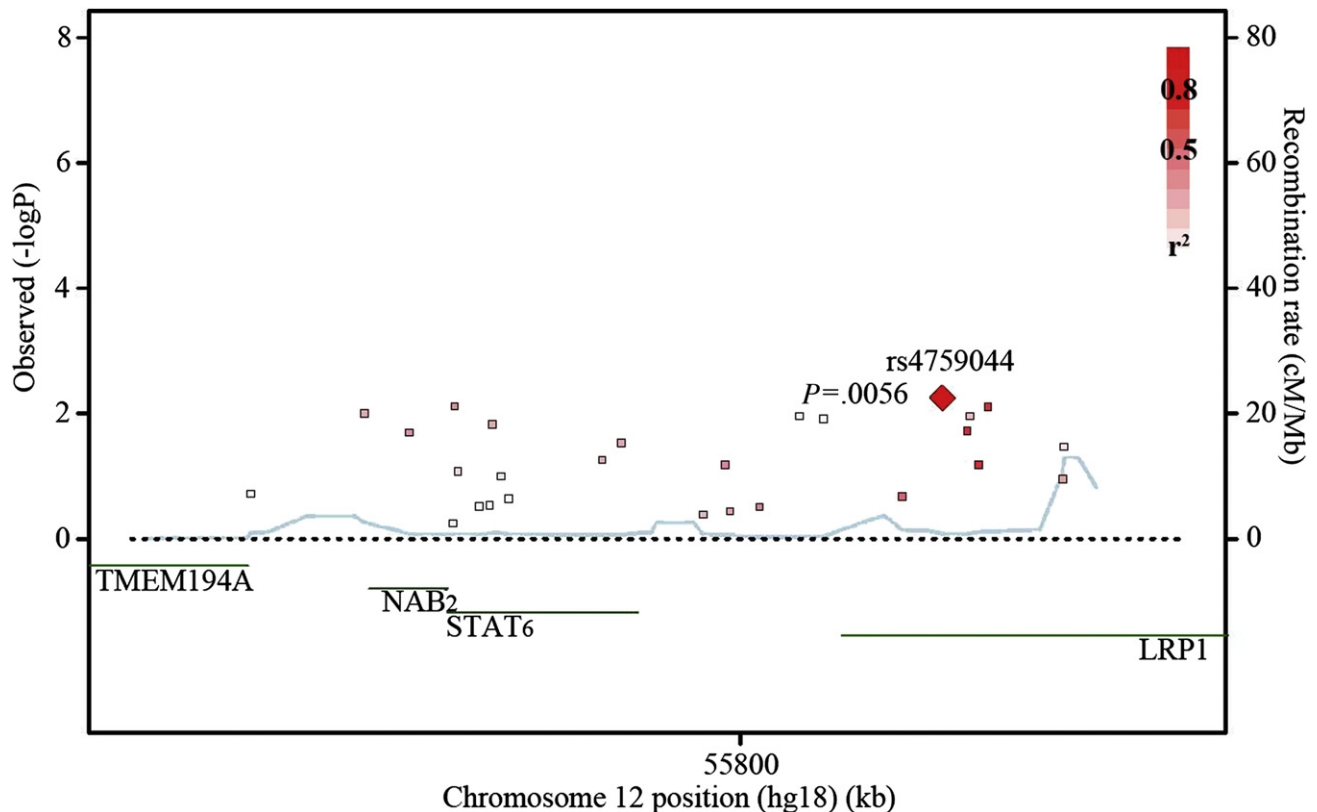


FIG 1. Association results and LD patterns of SNPs in or near *STAT6* (± 20 kb). Correlations between the SNP with the lowest *P* value (rs4759044) and surrounding SNPs are shown with reference to the HapMap CEU population, with darker red shading indicating higher r^2 values. Gene annotations are shown in green, and estimated recombination rates are shown in light blue.

or imputed by using MaCH (<http://www.sph.umich.edu/csg/abecasis/MACH/index.html>) with HapMap phase II release 21 reference haplotypes combined from European Americans (CEU, Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain collection), Africans (YRI, Yoruba in Ibadan, Nigeria), and East Asians (CHB [Han Chinese in Beijing, China] and JPT [Japanese in Tokyo, Japan]).

PBAT v3.61 (<http://www.biostat.harvard.edu/~clange/default.htm>) was used to conduct family-based association tests on additive SNP genotype dosage values (estimated reference allele counts with a fractional value ranging from 0 to 2.0). To correct for multiple testing, Bonferroni correction for the total number of SNPs ($P < \alpha/M$, where M is the number of independent tests) is too conservative, given the correlation patterns among SNPs. Therefore, we applied the widely used Nyholt correction ($P < \alpha/M_{\text{eff}}$), which adjusts for the effective number of independent tests (M_{eff}) based on the correlation matrix of pairwise linkage disequilibrium (LD) among the SNPs.^{5,6} This method gave a significance threshold of $P < .0011$. A less conservative significance threshold, based on the number of candidate genes examined, is $P < .01$. Association results and LD patterns were plotted together by using SNAP (<http://www.broadinstitute.org/mpg/snap/>).

In the 162 trios with a food allergen-sensitized child, no SNP associations were statistically significant at $P < .0011$. However, 3 genotyped SNPs in or near *STAT6* were associated with food sensitization at $P < .01$ (Table I), 2 intronic SNPs in the nearby *LRP1*

(low-density lipoprotein receptor-related protein 1) gene, and a 3' untranslated *STAT6* SNP. Their minor allele frequencies were lower in the food-sensitized children (39.5% for the top SNP rs4759044) compared with the children with no sensitization to the 6 food allergens (42.2% for rs4759044). The *STAT6* and *LRP1* SNPs are in moderate LD in CEU (Fig 1; $r^2 = 0.34$ -0.37) and weaker LD in YRI ($r^2 = 0.08$ -0.24) and CHB + JPT ($r^2 = 0.12$ -0.13). Of 28 SNPs in this region, none were associated with asthma at $P < .05$, despite a 3-fold greater sample size and thus more statistical power (Table I). There was minimal evidence for SNP associations with food sensitization in the *CD14*, *IL10*, *IL13*, or *SPINK5* regions (results not shown).

Another 3' untranslated *STAT6* SNP (rs324015) was previously associated with the risk for, and severity of, nut allergy in white atopic children from the United Kingdom,⁷ but this association was not corroborated for severity of food allergy in Japanese children.⁸ In our study of Mexican children, rs324015 gave a *P* value of .072, but its adjacent SNP (rs703817) was associated with food sensitization at $P = .0076$. The implicated SNPs may tag underlying functional or regulatory variants, and it is not surprising that association patterns vary across populations with dissimilar LD patterns.

LRP1 and its protein product have been largely implicated in neurologic processes. *STAT6* SNPs have been associated with food allergy,^{7,8} asthma,⁹ total IgE,¹⁰ and eosinophilic esophagitis.¹¹ *STAT6* plays a central role in mediating IL4 and IL13 signals for IgE antibody production.² Given its biological role, *STAT6*

polymorphisms are more likely than *LRP1* polymorphisms to influence food sensitization. Nonetheless, the association patterns between *STAT6* and *LRP1* SNPs reflect moderate LD with low recombination across the region (Fig 1). While our findings might not be generalizable to the general population, we provide evidence that *STAT6* and *LRP1* polymorphisms are associated with food sensitization in asthmatic patients who are at particularly high risk for developing sensitization. Follow-up studies in Hispanics and people of other ethnicities could help to refine these associations with food sensitization and clinical food allergy.

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