Letter to the Editor

Evaluation of food allergy candidate loci in the Genetics of Food Allergy study

To the Editor:

Food allergy (FA) is a common health problem with a strong genetic etiology. Twin studies estimated FA heritability at about 80%,[1,2] but our knowledge of the genes underlying FA is still sparse. A recent genome-wide association study (GWAS) on FA published in the *Journal of Allergy and Clinical Immunology* investigated 850 cases from the Canadian Peanut Allergy Registry (CanPAR) and 926 Australian controls.[3] The CanPAR study successfully identified a new FA locus on chromosome 11q13, near the genes *C11orf30* (chromosome 11 open reading frame 30) and *LRRC32* (leucine-rich repeat-containing 32), and proposed additional risk variants for further investigation that did not meet the threshold of genome-wide significance.[3]

Here, we aimed to evaluate the findings of the CanPAR study in an independent study population, the German Genetics of Food Allergy (GOFA) study. The GOFA study consists of 902 cases with FA, including 342 with peanut allergy, and 3668 control individuals of unknown phenotype from 2 German population-based studies, the Heinz Nixdorf Recall Study (n = 2682) and the Study of Health in Pomerania (n = 986). A GWAS on the GOFA study has recently been published and identified 5 genome-wide significant susceptibility loci for FA and peanut allergy.[4] Most GOFA study cases were diagnosed by double-blind, placebo-controlled food challenges (Table I), the current gold standard for the diagnosis of FA.[5,6] Detailed information on clinical phenotypes, study population, genotyping, and statistical analyses is provided in this article's Methods section in the Online Repository at www.jacionline.org. After quality control, 866 cases including 336 peanut-allergic children and 3358 controls were included in our case-control association study on FA.

In line with the CanPAR study,[3] we have previously reported genome-wide significant association of the *C11orf30/LRRC32* locus with FA.[4] Analyzing the CanPAR lead single nucleotide polymorphism (SNP), rs7936434, in the GOFA study revealed association with FA ($P = 1.6 \times 10^{-7}$) and with peanut allergy ($P = .0024$; Table II), which was significant after correction for the number of markers tested. Since rs7936434 is in high linkage disequilibrium (LD) with the previously reported, best-associated SNP of the GOFA study, rs2212434 ($r^2 = 0.89$), both variants represent the same locus. In addition, we investigated the other 7 independent SNPs, rs115218289, rs72827854, rs144897250, rs7475217, rs744597, rs523865, and rs78048444, which were suggestive of association with FA in the CanPAR GWAS. Two additional variants reported in that study (rs56151068 and rs139462954) were in very high LD with the lead variant rs72827854 ($r^2 > 0.92$) on chromosome 17, thus representing the same association. All candidate SNPs were either genotyped or imputed with high quality ($r^2 > 0.75$) in the GOFA study. None of the 7 candidate SNPs was associated with FA in our study (Table II). Because all CanPAR cases were recruited through peanut allergy, we then tested whether the reported associations were peanut-specific. However, restricting the analysis to the subset of peanut-allergic children from the GOFA study did not change the results (Table II). We used the Genetic Power calculator[7] to assess the power of our study sample for replication. Based on the allele frequencies and the reported effect sizes, the GOFA study provided nearly 100% power to detect association of the candidate variants with FA at the Bonferroni-corrected significance threshold of $P < .00714$. The power to detect a peanut-specific effect was reduced for only 2 low-frequency variants (rs115218289 and rs78048444) but still exceeded 80% for a nominal significance level (see Table E1 in this article's Online Repository at www.jacionline.org).

TABLE I. Characterization of the GOFA study

Characteristic	GOFA study
Total number of samples	902
Sex	64% males
Age (y), mean ± SD*	4.8 ± 3.6
Diagnosis, n (%)	
Double-blind, placebo-controlled food challenge	650 (72)
Oral food challenge	125 (14)
Severe allergic reaction plus elevated allergen-specific serum IgE (>0.35 kU/L)	127 (14)
Food allergies, n (%)	
Hen's egg	504 (56)
Peanut	352 (39)
Cow's milk	276 (31)

*Mean age at last visit.

In the CanPAR study, 7 loci suggestive for association with FA were reported, which were not confirmed in independent study populations included in the original report.[3] Because nonreplication is not necessarily due to false-positive results in the discovery study, we evaluated these 7 candidate loci in the GOFA study and did not verify the CanPAR study results. One potential cause of nonreplication is lack of power due to small study size. This is unlikely to be the case in the GOFA study because power calculations demonstrated that our study sample had excellent power to replicate the reported genetic effects in FA. Even in the subanalysis of low-frequency variants in peanut allergy, our sample was sufficiently powered to detect effects at nominal significance.

Another obstacle to the genetic investigation of FA is the difficulty in obtaining a reliable diagnosis. It is well recognized that the prevalence of FA is grossly overestimated if based on patients' history of an adverse reaction to food.[5] Even if the results of allergy tests were taken into account, the prevalence of FA was overestimated by a factor of 3 compared with challenge-proven FA. Thus, current medical guidelines recommend standardized oral food challenges as the gold standard to diagnose FA,[5,6] which are, however, demanding and expensive to perform. The strength of the GOFA study lies in its large sample size in combination with a well-defined FA phenotype that was based on oral food challenges.

Differences between the CanPAR study and the GOFA study results might be further explained by different inclusion criteria used for the 2 studies. For the CanPAR study, all patients were recruited through peanut allergy, whereas the GOFA study also investigated patients with allergic responses against other food allergens such as hen's egg and cow's milk. Interestingly, the only allergen-specific locus identified so far, the HLA locus for peanut allergy,[8] was also identified at genome-wide significance in

TABLE II. The GOFA study association results of the SNPs reported in the CanPAR GWAS

				CanPAR study			GOFA study						
				Peanut allergy			FA				Peanut allergy		
SNP	Chr	Gene region	Alleles*	MAF	OR (95% CI)	*P* value	MAF	OR (95% CI)	*P* value	Same risk allele†	OR (95% CI)	*P* value	Same risk allele†
rs115218289	2	*DLX2/ITGA6*	A/C	0.02	0.18 (0.10-0.32)	1.8×10^{-8}	0.026	0.89 (0.63-1.26)	.50	Yes	0.97 (0.59-1.60)	.91	Yes
rs744597	4	*ARHGAP24*	T/C	0.40	0.61 (0.50-0.74)	4.0×10^{-7}	0.425	1.04 (0.93-1.16)	.47	No	1.02 (0.87-1.19)	.84	No
rs78048444	7	*CHCHD3/EXOC4*	G/A	0.02	0.22 (0.12-0.39)	5.4×10^{-7}	0.024	0.99 (0.70-1.39)	.93	Yes	0.52 (0.26-1.04)	.065	Yes
rs7475217	10	*CTNNA3*	T/C	0.38	1.64 (1.35-1.98)	3.6×10^{-7}	0.415	1.05 (0.95-1.17)	.35	Yes	0.99 (0.84-1.16)	.90	No
rs7936434‡	11	*C11orf30/LRRC32*	C/G	0.49	1.58 (1.32-1.90)	5.2×10^{-7}	0.490	1.34 (1.20-1.49)	1.6×10^{-7}	Yes	1.28 (1.09-1.51)	.0024	Yes
rs144897250	11	*MMP12/MMP13*	A/C	0.02	6.20 (3.09-12.45)	2.9×10^{-7}	0.016	0.99 (0.64-1.54)	.96	No	0.69 (0.32-1.49)	.34	No
rs72827854	17	*SKAP1*	T/C	0.09	2.16 (1.61-2.90)	2.6×10^{-7}	0.107	1.17 (0.99-1.39)	.067	Yes	1.24 (0.97-1.59)	.080	Yes
rs523865	20	*ANGPT4*	G/A	0.23	0.57 (0.46-0.71)	4.4×10^{-7}	0.230	1.03 (0.90-1.17)	.70	No	1.05 (0.87-1.27)	.59	No

Chr, Chromosome; *MAF*, minor allele frequency; *OR*, odds ratio.
*Minor/major allele.
†As compared with the risk allele in the CanPAR study.
‡Only rs7936434 reached genome-wide significance in the CanPAR meta-analysis after including independent study populations.

peanut-allergic children of the GOFA study, highlighting the power of this study to identify allergen-specific associations.

Another source of variation may originate from the use of different genotyping arrays. The CanPAR GWAS used the same genotyping array for cases and controls, whereas in the GOFA study, cases and controls were genotyped on different Illumina arrays. However, for genotype imputation, only markers genotyped on both arrays were used, which is state-of-the art.[9] In addition, we applied stringent quality control to ensure high data quality as confirmed by a genomic inflation factor lambda of 1.03 for FA and peanut allergy.[4]

Finally, confounding environmental factors and population-specific genetic characteristics might hamper the replication of disease-associated SNPs. Both studies, the CanPAR study and the GOFA study, investigated study populations of Caucasian ethnic origin, which is reflected in very similar marker allele frequencies (Table II). Likewise, differences in LD patterns, which may be observed if populations of diverse ethnic origin are being compared, should be minor between the 2 studies.

Problems may particularly arise from population admixture and population stratification, which may yield false-positive association results if not strictly controlled for. Accordingly, all GOFA study cases and controls originated from Germany and were ethnically homogeneous, as confirmed by STRUCTURE. A lambda close to 1 pointed to the absence of population structure in our data set.

In summary, evaluation of the CanPAR study lead variant on chromosome 11q13 in the GOFA study revealed that both studies identified the same FA locus. We found no evidence supporting the association of the 7 candidate loci reported in the CanPAR GWAS. Combined efforts to achieve larger sample sizes with well-defined, standardized phenotypes and the integration of other genomic resources, including gene expresssion/regulation data, will be required to identify novel genetic associations with FA.

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