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BRIEF COMMUNICATION

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Association of *STAT6* gene variants with food allergy diagnosed by double-blind placebo-controlled food challenges

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Abstract

This study describes the role of two *STAT6* gene variants in food allergy using data of patients and their parents who underwent double-blind placebo-controlled food challenges (DBPCFCs). After quality control, 369 trios were analysed including 262 children (71.0%) with food allergy. Associations were tested by the Family based association test. The A alleles of both SNPs were associated with food allergy ($P = .036$ and $P = .013$ for rs324015 and rs1059513, respectively). Furthermore, these A alleles were associated with peanut allergy, higher sIgE levels to both peanut and cow's milk, more severe symptoms and higher eliciting doses during peanut and cow's milk DBPCFCs (all $P < .05$). In silico analysis indicates that the identified risk variants increase *STAT6* expression which stimulates the differentiation of CD4 + T cells to the Th2 subset. In conclusion, *STAT6* variants may be involved in the pathophysiology of food allergy and their role seems to be independent of the allergenic food.

KEYWORDS
children, clinical reactivity, peanut allergy, sensitization

1 | INTRODUCTION

Multiple studies have provided evidence that food allergy is partly genetically determined.[1] One potentially important but less well-studied gene in this context is signal transducer and activator of transcription 6 (*STAT6*), which stimulates the differentiation of naïve CD4 + T cells to the Th2 subset.[2] Two SNPs within *STAT6* have been reported to be associated with IgE concentrations[3-6] and sensitization to foods,[7] a history of nut allergy[8] and persistence of cow's milk allergy(CMA).[9] These last studies were based on a history of nut allergy or open food challenges for cow's milk allergy, both known to have high false-positive rates. Therefore, we used the double-blind placebo-controlled food challenge (DBPCFC), the gold standard, to investigate the genetics of food allergy. This study aimed to investigate the association of two selected SNPs in *STAT6*, rs324015 and rs1059513, with food allergy defined by any positive DBPCFC. In two subgroups of children who had a DBPCFC for the two most frequently tested allergenic foods (peanut or cow's milk), we studied the association with (i) peanut and CMA; (ii) peanut or cow's milk-specific IgE (sIgE); (iii) the dose sensitivity to the tested food; and (iv) the severity of the food-allergic reaction during the DBPCFC.

2 | METHODS

The GENEVA cohort included 421 trios (parents and child) in which the child had a DBPCFC as part of regular tertiary paediatric allergy care because of a history consistent with an IgE-mediated reaction after ingestion of a food. A subgroup of the GENEVA cohort was described previously.[10,11] Recruitment took place at the University

Medical Center Groningen from 2005 onwards. This study was ethically approved (METc 2004-146), and written informed (parental) consent was obtained.

All DBPCFCs with positive or negative results were included. The DBPCFCs were performed as previously described,[12] and sIgE for foods tested in DBPCFC was measured by CAP-FEIA (ImmunoDiagnostics, Uppsala, Sweden). A severity score was calculated based on symptoms registered on the active day of positive DBPCFCs ranging from 0 to 12 with 1 point for skin symptoms, 2 points for gastrointestinal symptoms and 3 points for upper airway, lower airway and/or cardiovascular/neurological symptoms.[13] The eliciting dose was defined as the last dose in milligrams of protein of the allergenic food ingested by the patient on the active day of a positive DBPCFC.

The two SNPs were selected because these had previously been associated with sensitization to food or a history of food allergy[3–8]. DNA was extracted from buccal swabs, saliva (Oragene Saliva Self-Collection Kits OG-575 DNA-Genotek, Ottawa, Canada) or EDTA blood (DNA Investigator Kit, Qiagen, Venlo, the Netherlands). Genotyping was performed by competitive allele-specific PCR by LGC Genomics (LGC, Teddington, UK).

Associations were tested by the family-based association test (FBAT 2.0.4 using the additive model[14]) which is robust to population stratification and tests for Mendelian errors. The FBAT is based on the transmission disequilibrium test which compares the alleles transmitted to affected offspring with the expected distribution of alleles among offspring. We did not apply correction for multiple testing as these data reflect a validation of previously identified associations. However, we tested in a two-tailed approach as literature reported conflicting direction of effects. Linkage disequilibrium (LD) between the studied variants and Hardy-Weinberg equilibrium was calculated using Haploview 4.2.[15] Trios were excluded when (i) the outcome of the DBPCFC was inconclusive (n = 18); (ii) ≥2 Mendelian errors were detected (n = 6) or (iii) when one of the members of the trio had a call rate lower than an arbitrary cut-off of 50% (n = 28, the latter two criteria included data of previously published gene variants[10,11]).

The functional consequences and LD patterns were checked in the Finnish and British population using 1000 genomes phase 3 via ensembl.org[16] (r^2 threshold .8). Expression quantitative trait loci (eQTL) characteristics were studied by genenetwork.nl/bloodeqtl-browser,[17] and expression and genomic annotation was studied in the online Haploreg[18] and GTEx project[19] databases.

3 | RESULTS

Of the 369 children, 14.1% of the DNA was extracted from blood. Call rates and HWE *P* -values were 98.9% and 0.57 for rs324015, and 98.2% and 0.94 for rs1059513, respectively. Allele frequencies (AF) of both risk alleles were concordant with literature (AF of minor allele A of rs324015 = 0.26 with reported AF = 0.31,[8] 0.24-0.29[20]; AF of major allele A of rs1059513 = 0.91 with reported AF = 0.91,[3] 0.93-0.92[20]). The two SNPs were independent (R^2 = .03, D' = 1.00).[15]

Of all children, 262 (71.0%) had at least one positive DBPCFC and were thereby defined as having food allergy. Baseline characteristics are shown in Table 1, and genetic association results with a $P < .10$ are shown in Table 2. The A alleles of both SNPs were significantly associated with being allergic to at least one food. In the subgroup of 205 children tested for peanut, both A alleles were associated with peanut allergy, higher sIgE levels to peanut and more severe symptoms and greater eliciting doses during the peanut DBPCFC. In the subgroup of 117 children tested for CMA, the A allele of rs324015 was significantly associated with higher sIgE levels to cow's milk and the A allele of rs1059513 was significantly associated with more severe symptoms and greater eliciting doses during the cow's milk DBPCFC.

Of both SNPs, only rs1059513 is in LD with another variant, rs3024971 (R^2 = 1.0).[16] Both genotyped SNPs are localized in the 3′ untranslated region of *STAT6*,[16] compatible with a role in post-transcriptional gene expression by influencing polyadenylation, translation efficiency and stability of mRNA.[21] Locations of both rs324015 and rs3024971 are characterized by enhancer histone marks in multiple tissues which influence the accessibility to the transcriptional machinery and can be modified by environmental exposures.[18,22] Both genotyped SNPs and rs3024971 are listed in the eQTL browser as CIS-eQTLs for *STAT6* in peripheral blood in which the A alleles increase expression of *STAT6* (rs324015 minor allele A: Z-score 49.74, P = 9.91E-198 and rs1059513 major allele A: Z-score 16.12, P = 1.83E-58, rs3024971 major allele A: Z-score = 15.93, P = 4.03E-57).[17] Furthermore, rs324015 is described as a single-tissue eQTL in oesophageal mucosa (effect size 0.20 for the A allele, P = 3.8E-7) and *STAT6* is highly expressed in whole blood, skin and small intestines.[19]

4 | DISCUSSION

We show for the first time that both A alleles of rs324015 and rs1059513 are associated with food allergy and peanut allergy as diagnosed by DBPCFCs, IgE sensitization to peanut and cow's milk, as well as more severe allergic reactions. We therefore conclude that *STAT6* genetic polymorphisms may be involved in the pathophysiology of food allergy and their role seems to be independent of the causal allergenic food.

In previous studies, the A allele of rs1059513 was described as a risk variant, associated with asthma,[23] atopic dermatitis,[24] higher IgE levels[3,6] and sensitization to common food and inhalant allergens.[7] The A allele of rs324015 was previously associated with an increased risk for atopic asthma in a meta-analysis[25] and with eosinophilia in local inflammatory sites.[4] In contrast, this A allele was also described as the protective allele for nut allergy in 300 British subjects.[8] Interestingly, a gene-gene interaction between the A allele of rs324015 and GT dinucleotide repeat polymorphisms in *STAT6* exon 1 was reported to influence the risk of any allergic disease in 168 Japanese subjects.[26] Such a gene-gene interaction could explain these potentially conflicting results, or they might be due to an as yet unidentified gene-environment interaction, similar to that previously described for *CD14*.[27]

TABLE 1 Descriptive statistics of the study population

	DBPCFC confirmed food allergy 71.0% (n = 262)	DBPCFC confirmed tolerant 29.0% (n = 107)	Total (n = 369)
Male, % (n)	56.9 (149)	61.7 (66)	58.3 (215)
Age in months at first DBPCFC, median, Q1-Q3[a]	78.0, 44.0-131.0	72.0, 34.5-135.0	76.0, 41.0-131.0
Number of foods tested in DBPCFC			
Median, Q1-Q3[a]	2.0, 1.0-3.0	1.0, 1.0-1.0	1.0, 1.0-2.0
Any positive DBPCFC[b] % (n/n tested)			
Peanut	75.8 (122/161)	(0/44)	59.5 (122/205)
Cow's milk	78.0 (64/82)	(0/35)	54.7 (64/117)
Hen's egg	62.9 (44/70)	(0/20)	48.9 (44/90)
Hazelnut	56.5 (39/69)	(0/6)	52.0 (39/75)
Cashew	93.0 (53/57)	(0/7)	82.8 (53/64)
Walnut	87.0 (20/23)	(0/6)	69.0 (20/29)
Soy	34.8 (8/23)	(0/3)	30.8 (8/26)
Almond	9.1 (1/11)	(0/2)	7.7 (1/13)
Wheat	(0/4)	(0/2)	0 (0/6)
Lupine seed	(0/3)	(0/2)	0 (0/5)
Pistachio	(0/1)	(0/2)	0 (0/3)
Sesame	100 (2/2)		100 (2/2)
Pine nut	100 (2/2)		100 (2/2)
Macadamia	(0/1)	(0/1)	0 (0/2)
Brazil nut	100 (1/1)		100 (1/1)
Atopic comorbidities % (n/n tested)			
Atopic dermatitis	89.5 (230/257)	83.7 (87/104)	87.8 (317/361)
Asthma	58.5 (151/258)	48.5 (50/103)	55.7 (201/361)
Rhinoconjunctivitis	50.2 (127/253)	32.0 (32/100)	45.0 (159/353)
sIgE[b] (KU/L) median, Q1-Q3[a] (n)			
Peanut	6.5, 2.0-40.0 (162)	2.0, 0.6-5.5 (43)	5.1, 1.3-24.9 (205)
Cow's milk	5.2, 0.7-18.6 (83)	0.3, 0.3-0.7 (34)	1.9, 0.3-13.7 (117)
Severity of reaction[b] mean, SD (n)			
Peanut	3.7, 2.3 (113)		
Cow's milk	3.5, 2.3 (61)		
Eliciting dose[b] (mg protein) median, Q1-Q3[a] (n)			
Peanut	69.9, 3.5-348.0 (109)		
Cow's milk	1750.0, 70.0-1750.0 (58)		

DBPCFC, double-blind placebo-controlled food challenge; sIgE, specific IgE; SD, standard deviation; Q1-Q3, first and third quartile.
[a]Variables which were defined as not normally distributed after visual inspection of the Q-Q plot are presented by median and quartiles (Q1-Q3).
[b]When children had a DBPCFC for multiple foods, they are listed for each food-specific variable.

Such a gene-environment interaction is likely to be mediated by epigenetic modifications.[22] The functional consequences of the A allele of rs324015 as presented in this article lend further credence to its role as a risk variant for food allergy.

We show in peanut- and cow's milk-allergic cases associations between the A alleles of rs1059513 and rs324015 and a greater eliciting dose. This implies that these A alleles are associated with lower clinical sensitivity (allergic reaction at higher allergen dosages) and that peanut-allergic subjects carrying these alleles are at lower risk for allergic reactions. Interestingly, a higher eliciting dose was previously associated with earlier resolution of peanut/tree nut allergy and CMA.[28] Therefore, our results are consistent with another report describing the association between the A genotype in rs324015 and an earlier age of developing tolerance for CMA.[9] However, the A allele is the risk variant for having food allergy and is associated with more severe food allergy. This confirms recent insights regarding the independence of severity and dose sensitivity in food allergy.[29]

Other *STAT6* SNPs which are independent from the 2 SNPs reported here (ie, rs167769, rs324011, rs12368672, rs2598483 and

TABLE 2 The Family-based association test (FBAT) results with a $P < .10$. Associations of significance are shown in bold

Trait		#fam	Z	*P*	Risk (ref)
Rs324015					
Food allergy	DBPCFC confirmed food allergy	221	**2.097**	**.036**	A(g)
Peanut allergy	DBPCFC confirmed peanut allergy	122	**2.365**	**.018**	A(g)
	sIgE	89	**2.063**	**.039**	A(g)
	Severity	64	**2.131**	**.033**	A(g)
	ED	54	**2.558**	**.011**	A(g)
Cow's milk allergy	DBPCFC confirmed cow's milk allergy	72	1.781	.075	A(g)
	sIgE	58	**2.812**	**.005**	A(g)
	Severity	43	1.693	.090	A(g)
Rs1059513					
Food allergy	DBPCFC confirmed food allergy	103	**2.488**	**.013**	A(g)
Peanut allergy	DBPCFC confirmed peanut allergy	60	**2.412**	**.016**	A(g)
	sIgE	41	**2.195**	**.028**	A(g)
	Severity	31	**2.265**	**.024**	A(g)
	ED	29	**2.168**	**.030**	A(g)
Cow's milk allergy	Severity	18	**2.428**	**.015**	A(g)
	ED	15	**2.261**	**.024**	A(g)

DBPCFC, double-blind placebo-controlled food challenge; sIgE, specific IgE (log-transformed to improve distribution; LN(sIgE+1)); ED, eliciting dose (log-transformed to improve distribution; LN(ED+1)); #fam, numbers of informative families; Z, Z-score; *P*, *P* -value; Risk(ref), risk allele (reference allele).

rs703817) have been associated with other allergy-related phenotypes such as total IgE, atopic dermatitis, eosinophilic esophagitis, atopy or asthma.[5,6,30-32] However, evidence on their association with IgE-mediated food allergy was lacking. Therefore, these SNPs were not included in our study, as our aim was to replicate and validate SNPs previously associated with IgE-mediated food allergy. We therefore cannot exclude that these other SNPs may also be involved in (food) allergy and IgE production. We acknowledge that most of our associations reach borderline significance and are not corrected for multiple testing. More accurate phenotype definition by use of the DBPCFC will have reduced the power requirement somewhat, as was our experience with a similar study of the filaggrin gene.[29] Nevertheless, these results require replication to ascertain whether these associations are truly independent of sensitization to foods or atopic comorbidities and should thus be confirmed in a larger study population with lower prevalences of the latter conditions.

The A alleles of both SNPs are risk variants for food allergy, and these A alleles are associated with higher expression of *STAT6* in several tissues.[17,19] By inducing expression of GATA-3, STAT6 enhances expression of the Th2 cytokine genes IL-4, IL-5 and IL-13 which stimulates differentiation of naïve CD4+ T cells to the Th2 subset.[2,22] These cytokines subsequently activate mast cells, macrophages and eosinophils to promote allergic responses. In activated B cells, STAT6 promotes immunoglobulin class switching to IgE and expression of antigen presenting cell surface molecules.[2] To summarize, *STAT6* genetic polymorphisms may be involved in the pathophysiology of food allergy and their role seems to be independent of the causal allergenic food.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

CDvG, AEJD and GHK involved in conception of the idea for the study. AEJD and GHK gave expert advice and supervised collection of data. CDvG and MEP collected the study data. CDvG analysed the data and prepared the manuscript. CDvG, MEP, AEJD and GHK actively contributed to writing, editing and evaluation of the manuscript.

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