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RESEARCH LETTER

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IL-1RL1a serum levels and *IL1RL1* SNPs in the prediction of food allergy

Food allergy is a common disorder in the Western world, with increasing prevalence and substantial healthcare costs.[1] Food allergy is often accompanied by the presence of specific IgE against harmless proteins in food, but not all sensitized children show clinical reactions upon exposure. Therefore, double-blind placebo-controlled food challenges (DBPCFC) remain the gold standard to diagnose food allergy, yet this test is demanding. Biomarkers that can predict clinical response to food are urgently needed. These biomarkers may be based on genes associated with allergic disease.

Genetic single nucleotide polymorphisms (SNPs) in *interleukin-1-receptor-like 1* (*IL1RL1)* and serum levels of its soluble protein (IL-1RL1a or sST2) have repeatedly been associated with allergic phenotypes, including (allergic) asthma, eczema and eosinophilia.[2,3] Moreover, IL-1RL1a serum levels predict the development of eosinophilic asthma characterized by high FeNO in preschool wheezing children.[4] Also, IL-1RL1a serum levels increase during asthma exacerbations, suggesting this protein to be a marker of active inflammatory responses.[2,3,5] Disease-associated SNPs in *IL1RL1* correlate with *IL1RL1* mRNA and serum protein levels of IL-1RL1a.[6] Moreover, functional activation of the transmembrane variant of IL-1RL1 (IL-1RL1b) by the alarmin interleukin-33 (IL33) can lead to an IgE-mediated (type I) allergic response, including activation of B cells, Th2-helper cells and mast cells.[5] This type I allergic response plays a central role in food allergy,[5] and IL33 has been shown critical for the development of gastrointestinal food allergy in a mouse model.[7] After IgE-crosslinking of mast cells, activation, migration and degranulation are significantly enhanced by stimulation of IL-1RL1b,[8] further implicating IL1RL1 in food allergy pathogenesis. However, it is unknown whether soluble IL-1RL1a levels or *IL1RL1* SNPs are associated with food allergy[5,7] and could act as biomarkers of food allergy.

Therefore, here we investigate whether serum levels of IL-1RL1a and asthma- and allergy-associated polymorphisms in the *IL1RL1* locus associate with food allergy in children as diagnosed by a DBPCFC.

In children with a suspicion of food allergy referred to a tertiary food allergy centre, we measured IL-1RL1a in serum (0-3 months before food challenge) and genotyped *IL1RL1* SNPs. Next, we performed regression modelling between IL-1RL1a levels, *IL1RL1* SNPs and DBPCFC-confirmed current food allergy, food allergy at any time, severity of food allergy and IgE sensitization (sIgE > 0.35 kU/L). First, we tested for association with *any food*. Second, we specifically tested for association with allergy against specific food products, including the largest groups of patients for specific allergies, namely peanut, cow's milk and chicken egg allergy. Here we aimed to answer whether *IL1RL1* SNPs or IL-1RL1 serum protein levels could distinguish the children truly allergic (or sensitized) against a specific food from the ones suspected but not confirmed allergic (or sensitized) for the specific food tested. Lastly, we investigated whether *IL1RL1* SNPs associate with IL-1RL1a levels in serum of the DBPCFC-tested children. We applied logistic regression in case of a binary outcome variable and linear regression in case of a continuous variable; each model had age and gender as covariates next to IL-1RL1a levels or SNPs as predictors of a single outcome variable.

Parents and 716 children referred to the food challenge unit of the University Medical Centre Groningen between 2005 and 2014 were asked to participate, of whom 572 signed informed (parental) consent (Medical Ethics Committee Groningen, no. METc 2004-146). DBPCFCs were performed as previously described.[1] Children were classified as follows: having i) current food allergy if they had at least one positive DBPCFC at the time of testing, ii) food allergy at any time if they had one or more positive DBPCFCs at any time and iii) no food allergy if they had only negative DBPCFCs at any time. Severity of food allergy was defined on a scale from 0 to 12, based on symptoms registered on the day of positive DBPCFCs with 1 point for skin symptoms, 2 points for gastrointestinal symptoms and 3 points for upper airway, lower airway and/or cardiovascular/neurological symptoms.[8,10] Food-specific IgE (sIgE) was measured by CAP-FEIA (ImmunoDiagnostics, Uppsala, Sweden).

Serum samples from 513 children were available. IL-1RL1a protein levels in serum were determined with the R&D Quantikine® ELISA Human ST2/IL-1-R4 kit according to the manufacturer's instructions. IL-1RL1a measurements of samples with a coefficient of variance ≥15% (n = 14), or with values that exceeded the standard curve (n = 1), were excluded, leaving 498 children with valid IL-1RL1a values. IL-1RL1a levels were LN-transformed for normal-distributed data (see supplemental figure S1).

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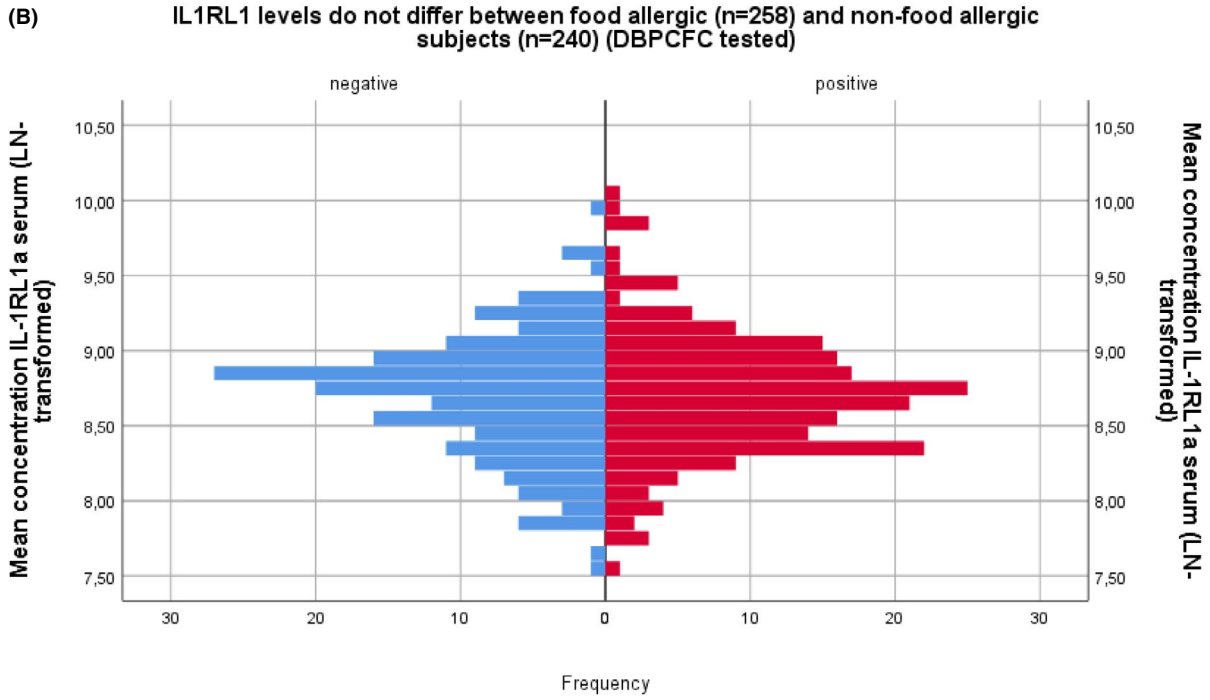
(A) clinical characteristics of food allergy

Sensitization for tested food	*Of which DBPCFC + (current)*	*Of which DBPCFC - (current)*	*Of which DBPCFC + (any time)*	*Of which DBPCFC - (any time)*
sIgE + 368	*221 (60.1%)*	*147 (39.9%)*	*264 (71.7%)*	*104 (28.3%)*
sIgE - 118	*28 (23.7%)*	*90 (76.3%)*	*48 (40.7%)*	*70 (59.3%)*
sIgE unknown 12	*9*	*3*	*8*	*4*

(B)



(C)- statistics

Predictor (serum)	Outcome	R^2 predictor	Effect	SE	P-value	N positive	N negative
(LN) IL-1RL1	**Current food allergy (any)**	**0.000**	**1.03 (OR)**	**0.26**	**0.90**	**258**	**240**
	Current food allergy (peanut)	**0.065**	**1.01 (OR)**	**0.37**	**0.97**	**79**	**84**
	Current food allergy (chicken egg)	**0.063**	**0.36 (OR)**	**0.74**	**0.17**	**31**	**39**
(LN) IL-1RL1	**Food allergy at any time**	**0.000**	**1.04 (OR)**	**0.24**	**0.88**	**320**	**178**
(LN) IL-1RL1	**Severity of food allergy**	**0.001**	**0.03 (beta)**	**0.46**	**0.94**	**258**	**N/A**
(LN) IL-1RL1	**Levels of blood sIgE (LN)**	**0.000**	**0.06 (beta)**	**0.25**	**0.82**	**Overall N=486**	
(LN) IL-1RL1	**Sensitization (sIgE+/-, any)**	**0.001**	**0.75 (OR)**	**0.33**	**0.39**	**368**	**118**
	Sensitization (sIgE+/-, peanut)	**0.019**	**0.59 (OR)**	**0.57**	**0.35**	**142**	**21**
	Sensitization (sIgE+/-, chicken egg)	**0.103**	**0.18 (OR)**	**0.87**	**0.049**	**53**	**17**

FIGURE 1 IL-1RL1a in serum and association with food allergy phenotypes. Figure 1—Serum IL-1RL1a levels were measured in samples taken within 3 months before the DBPCFC in a total of 498 children referred to a tertiary allergy centre. A, An overview of sensitization and DBPCFC food allergy status is shown. B, Of these children, n = 258 were DBPCFC positive at the time of testing (current food allergy) indicated in red in the figure. C, IL-1RL1a levels were also tested for association with food allergy at any time (n = 320 were reactive at any time), severity of food allergy, specific (s)IgE levels and/or sensitization (sIgE > 0.35 kU/L). No differences in serum IL-1RL1a were found for any of these food allergy phenotypes. Specific allergy for peanut was included. Other specific allergies can be found in supplemental table S5

Children were genotyped using the Illumina GSA beadchip (GSA-24v1-0). Non-Caucasian subjects, subjects with call rate <0.95 and with discordant sex were excluded. SNPs with call rate <0.90, MAF < 0.01 and SNPs out of Hardy-Weinberg equilibrium were excluded. Genotypes were imputed using IMPUTE2.0 against the 1000G-phase3 reference panel, and best-guess genotypes were derived (r^2 imputation 91.4%). Seven SNPs at the *IL1RL1* locus were selected. These represent six tagging SNPs that were selected from six distinct LD blocks (r^2 > 0.8) covering asthma- and allergy-associated *IL1RL1* signals in Caucasian population cohorts, as described by Grotenboer *et al*[2] (rs13431828, rs1041973, rs1420101, rs1946131, rs1921622, rs10204137). Furthermore, we added *IL1RL1* SNP

TABLE 1 *IL1RL1* SNPs and prediction of serum IL-1RL1a levels, food allergy and sensitization

SNP	Location (GRCh37.p13)	Tested allele	AF tested allele	Outcome	R^2 SNP	Effect	SE	*P*-Value
rs1420101	2:102957716	T	0.36	(LN) IL-1RL1 serum	0.386	−0.37 (B)	0.03	**<.001**
				Food allergy at any time (any food)	0.020	0.87 (OR)	0.17	.44
				Peanut allergy (DBPCFC+)	0.028	0.81 (OR)	0.173	.216
				Chicken egg allergy (DBPCFC+)	0.048	0.86 (OR)	0.293	.589
				Sensitization (sIgE±, any food)	0.002	1.05 (OR)	0.25	.84
				IgE sensitization @peanut	0.172	0.32 (OR)	0.566	**.04**
				IgE sensitization @chicken egg	0.159	3.14 (OR)	1.150	**.006**
rs13431828	2:102954653	T	0.16	(LN) IL-1RL1 serum	0.026	0.12 (B)	0.06	.05
				Food allergy at any time (any food)	0.000	1.01 (OR)	0.29	.97
				Peanut allergy (DBPCFC+)	0.044	0.53 (OR)	0.285	**.03**
				Chicken egg allergy (DBPCFC+)	0.056	1.54 (OR)	0.444	.332
				Sensitization (sIgE±, any food)	0.007	1.55 (OR)	0.41	.29
				IgE sensitization @peanut	0.075	0.83 (OR)	0.818	.821
				IgE sensitization @chicken egg	0.009	1.17 (OR)	0.676	.815
rs10204137	2:102968212	G	0.38	(LN) IL-1RL1 serum	0.057	0.15 (B)	0.04	**<.001**
				Food allergy at any time (any food)	0.007	1.12 (OR)	0.19	.54
				Peanut allergy (DBPCFC+)	0.025	0.88 (OR)	0.179	.465
				Chicken egg allergy (DBPCFC+)	0.046	1.24 (OR)	0.314	.487
				Sensitization (sIgE±, any food)	0.000	1.00 (OR)	0.25	.99
				IgE sensitization @peanut	0.082	1.42 (OR)	0.595	.554
				IgE sensitization @chicken egg	0.114	0.39 (OR)	0.664	.153
rs1041973	2:102955468	A	0.28	(LN) IL-1RL1 serum	0.009	−0.12 (B)	0.05	**.01**
				Food allergy at any time (any food)	0.001	0.80 (OR)	0.22	.30
				Peanut allergy (DBPCFC+)	0.049	0.55 (OR)	0.239	**.01**
				Chicken egg allergy (DBPCFC+)	0.048	1.21 (OR)	0.358	.598
				Sensitization (sIgE±, any food)	0.002	1.21 (OR)	0.30	.53
				IgE sensitization @peanut	0.074	1.01 (OR)	0.687	.99
				IgE sensitization @chicken egg	0.013	1.26 (OR)	0.580	.688
rs10185897	2:102966790	A	0.16	(LN) IL-1RL1 serum	0.021	0.12 (B)	0.11	.33
				Food allergy at any time (any food)	0.000	1.28 (OR)	0.49	.61
				Peanut allergy (DBPCFC+)	-	-	-	-
				Chicken egg allergy (DBPCFC+)	-	-	-	-
				Sensitization (sIgE±, any food)	0.003	2.75 (OR)	0.82	.22
				IgE sensitization @peanut	-	-	-	-
				IgE sensitization @chicken egg	-	-	-	-
rs1921622	2:102966067	A	0.55	(LN) IL-1RL1 serum	0.181	−0.26 (B)	0.03	**<.001**
				Food allergy at any time (any food)	0.005	0.88 (OR)	0.18	.46
				Peanut allergy (DBPCFC+)	0.020	0.95 (OR)	0.174	.78
				Chicken egg allergy (DBPCFC+)	0.049	0.86 (OR)	0.306	.630
				Sensitization (sIgE±, any food)	0.003	0.85 (OR)	0.24	.50
				IgE sensitization @peanut	0.151	0.34 (OR)	0.623	.80
				IgE sensitization @chicken egg	0.212	3.01 (OR)	0.820	**.048**

(Continues)

TABLE 1 (Continued)

SNP	Location (GRCh37.p13)	Tested allele	AF tested allele	Outcome	R^2 SNP	Effect	SE	*P*-Value
rs1946131	2:102961929	A	0.09	(LN) IL-1RL1 serum	0.076	-0.29 (B)	0.06	**<u><.001</u>**
				Food allergy at any time (any food)	0.000	0.69 (OR)	0.29	.20
				Peanut allergy (DBPCFC+)	0.021	1.00 (OR)	0.33	.99
				Chicken egg allergy (DBPCFC+)	0.046	0.83 (OR)	0.490	.698
				Sensitization (sIgE±, any food)	0.002	0.93 (OR)	0.41	.86
				IgE sensitization @peanut	0.075	1.32 (OR)	1.13	.81
				IgE sensitization @chicken egg	0.007	0.96 (OR)	0.819	.961

Table 1-IL1RL1 SNPs were used as univariate predictor of serum IL-1RL1a levels (LN transformed), food allergy at any time (any food) and sensitization (IgE > 0.350kU/L) to the tested food allergen of the DBPCFC. IgE and IL-1RL1a measured within a 3-month period before the DBPCFC in GENEVA. Age and gender were used as covariates. Peanut and chicken egg were included as specific allergies. More allergy phenotypes can be found in the supplemental material. '-'= less than n = 5 per analysis group with data available, therefore no analysis was performed on this variable. In bold: *P*-values < 0.05. Underlined: p-values < 0.0167 (adjusted cut-off corrected for the LD pattern in the region, correcting for three independent genetic signals)

rs10185897, which is associated with atopic dermatitis/eczema,[9] but not in high linkage disequilibrium (r^2 < 0.8) with any of the six *IL1RL1* SNPs above. Between all 7 SNPs, there still was moderate LD (r^2 > 0.3), see also supplemental Table S8. For rs10185897, genotyped data were used, as it was not successfully imputed. Analyses were performed in SPSSv25.0 (IBM, Chicago, USA). The statistical cut-off was corrected for multiple testing, taking into account the presence of moderate LD between the selected SNPs, therefore correcting for 3 tests: alpha cut-off 0.05/3 = 0.0167.

Cohort characteristics are shown in supplemental Table S1. First, we studied any food allergy. Of the children suspected of food allergy, 368 had sIgE sensitization against the tested food, while 118 were not sensitized to this food. Of the children that were sIgE sensitized, 264 (71.7%) had a positive DBPCFC at any time, while of the children that were not sIgE sensitized, and 48 (40.7%) had a positive DBPCFC at any time (See Figure 1). The distribution of IL-1RL1a levels in serum of children did not differ between DBPCFC-positive and -negative children, nor were serum IL-1RL1a levels associated with any other measure of food allergy or sIgE sensitization against food allergens (Figure 1 and supplemental Figure S2). *IL1RL1* SNPs were also tested for association with measures of food allergy and IgE sensitization. The *IL1RL1* SNPs did not explain any variance in current food allergy (*any food*), food allergy at any time, severity of food allergy, levels of blood sIgE or sensitization (*any food allergen*) (Table 1). Next, we analysed specific allergies, including peanut, cow's milk and chicken egg allergy. Here, we found that one *IL1RL1* SNP specifically associates with IgE sensitization against chicken egg allergen (rs1420101). Another *IL1RL1* SNP associated with peanut allergy (rs1041973), but no association of *IL1RL1* SNPs with DBPCFC-confirmed chicken egg allergy was found. Among these *IL1RL1* SNPs, we confirmed a strong association with IL-1RL1 protein levels (pQTLs) in serum of these children. See also Table 1 and supplemental Tables S3/S4.

Our data show that IL-1RL1a serum levels and the tested *IL1RL1* SNPs are not associated with food allergy phenotypes in children when testing for any food. This is in contrast to the reported strong association of *IL1RL1* with asthma and other allergic disorders including allergic rhinitis, eczema and the allergy-associated phenotype of blood eosinophils.[2] This is especially intriguing, as we also included an *IL1RL1* SNP (rs10185897) specifically based on its potential association with eczema, and food allergy and eczema were previously reported to show a high correlation in children.[1,10] This could suggest that food allergy and asthma/eczema do not have a common underlying molecular pathway involving the IL-1RL1 pathway. However, our power was limited to detect small genetic effects (see also supplemental Tables S6 and S7), and the original study that found an association of the rs10185897 SNP with eczema had a much larger study cohort (>2000 patients[9]), which might explain why we could not replicate the association of this *IL1RL1* SNP with eczema and lacked an association with food allergy (supplemental Table S2). Nevertheless, in this same cohort, previous studies have shown a genetic association for other candidate genes, such as *Filaggrin*, showing associations with eczema, food allergy and asthma.[1,10] Another explanation could be that, although the food allergy phenotype is strictly-defined in the current cohort (DBPCFC tested), we initially tested association with *any* food, and not for specific food products. Indeed, when testing for specific food products, we found an *IL1RL1* SNP associated with IgE sensitization against chicken egg, as well an *IL1RL1* SNP associated with DBPCFC-confirmed peanut allergy. We did not find association of SNPs with clinical chicken egg allergy. Interestingly, these SNPs have potential functional consequences, that is are known eQTL and pQTL in several tissues of asthma cohorts,[2-4,6] as well as contain potential transcription factor binding sites,[3] and one of these SNPs (rs1041973) is a non-synonymous SNP.[6] We now confirm this pQTL function in the current paediatric food allergy cohort as well. In these children, the allele associated with higher levels of serum IL-1RL1a was associated with decreased risk of chicken egg sensitization, that is the decoy receptor IL-1RL1a seems to be protective, which we see in other allergic

disorders.[2-4,6] Interestingly, however, for peanut the direction of effect was opposite; the *IL1RL1* allele associated with higher levels of IL-1RL1a was associated with higher risk of peanut sensitization/allergy. This could potentially suggest different underlying pathogenic mechanisms, including a potential explanation for the lack of association with DBPCFC-confirmed chicken allergy, while we did see *IL1RL1* SNPs associated with clinical peanut allergy. However, we did not find a direct association between serum IL-1RL1a levels and clinical peanut allergy, and only a weak association between serum IL-1RL1a levels and IgE sensitization for chicken egg (supplemental Table S5), likely due to a lack of power when investigating these specific subgroups.

In conclusion, our results indicate that serum protein levels of IL-1RL1a as biomarker do not predict clinical responses to food in food allergic children, but that *IL1RL1* SNPs associate with very specific food allergies such as peanut and chicken egg. Nevertheless, although we studied a well-defined population of allergic children (using the golden standard DBPCFC), the authors acknowledge that our study had limited power to conclusively ascertain genetic and protein effects. Therefore, these data need to be confirmed in larger studies.

Understanding the genetic association of *IL1RL1* genetic variation with different allergic diseases is relevant, as multiple monoclonal antibodies targeted at the IL33/IL-1RL1 pathway are currently under development.[5] Our data would suggest to prioritize testing these novel drugs in asthma, hay fever and eczema; but when considering food allergy, then our data suggest to test specific food allergies as phenotype.

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CONFLICT OF INTEREST

The authors report that no conflict of interest exists for the currently submitted work.

AUTHOR CONTRIBUTION

MEK and CDvG performed the analyses of this manuscript and had the lead in the writing phase of this work. MCN was involved in analysis and writing phase of this work. AEJD and GHK were involved in the design, analysis and writing phase of this work.

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DATA SHARING STATEMENT

Data available on request due to privacy/ethical restrictions.

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