Functional and clinical translation of asthma and allergy associated genetic variants in IL33 and IL1RL1

Ketelaar, Marlies

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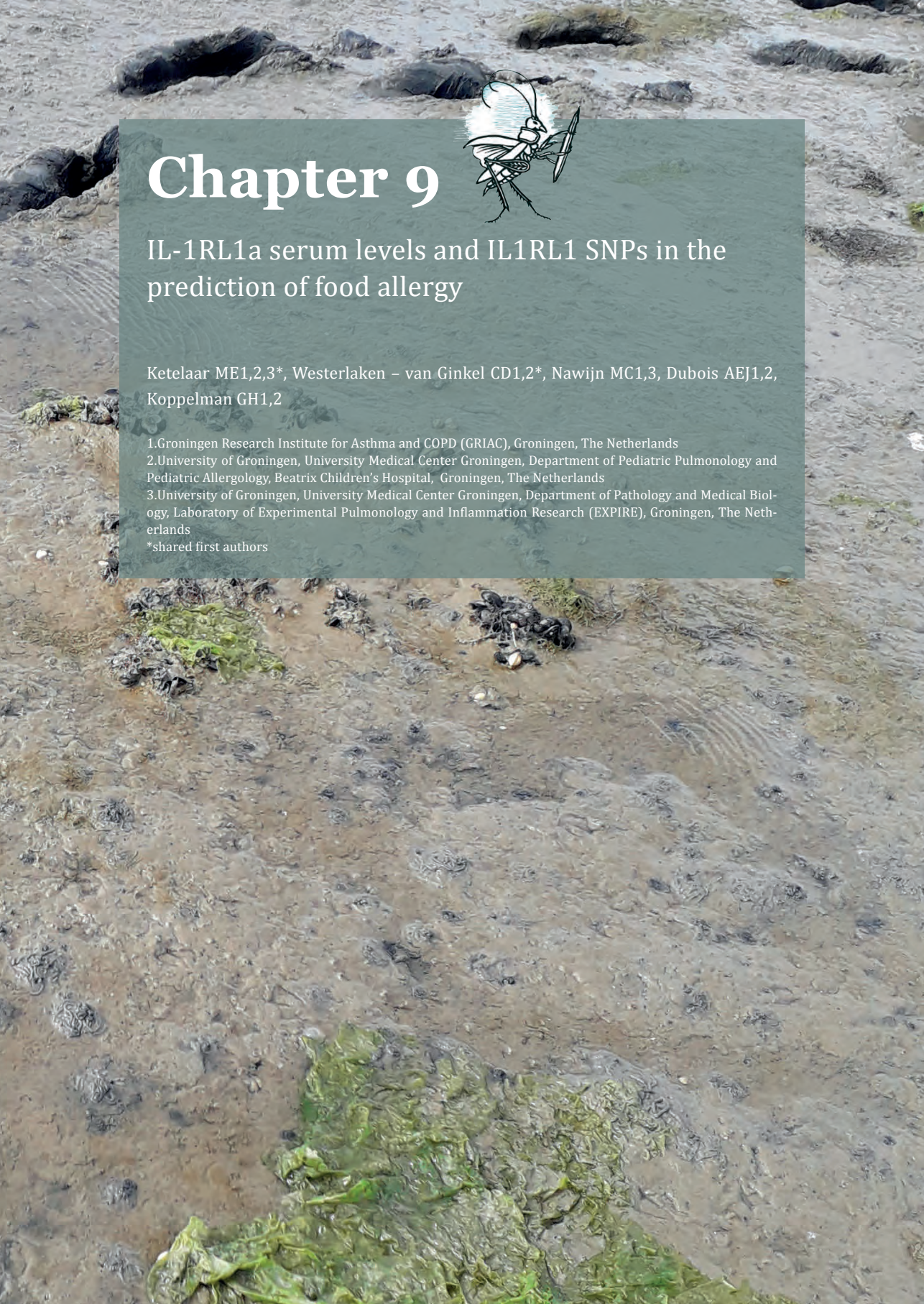


Chapter 9

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

Ketelaar ME1,2,3*, Westerlaken – van Ginkel CD1,2*, Nawijn MC1,3, Dubois AEJ1,2, Koppelman GH1,2

1.Groningen Research Institute for Asthma and COPD (GRIAC), Groningen, The Netherlands
2.University of Groningen, University Medical Center Groningen, Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, Groningen, The Netherlands
3.University of Groningen, University Medical Center Groningen, Department of Pathology and Medical Biology, Laboratory of Experimental Pulmonology and Inflammation Research (EXPIRE), Groningen, The Netherlands
*shared first authors

To the editor

Food allergy is a common disorder in the Western world, with increasing prevalence and substantial healthcare costs (273). 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 (26). Moreover, IL-1RL1a serum levels predict the development of eosinophilic asthma characterized by high FeNO in preschool wheezing children (240). Also, IL-1RL1a serum levels increase during asthma exacerbations, suggesting this protein to be a marker of active inflammatory responses (26,274). Disease-associated SNPs in *IL1RL1* correlate with *IL1RL1* mRNA and serum protein levels of IL-1RL1a (239). 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 (274). This type I allergic response plays a central role in food allergy (274), and IL33 has been shown critical for the development of gastrointestinal food allergy in a mouse model (275). After IgE-crosslinking of mast cells, activation, migration and degranulation is significantly enhanced by stimulation of IL-1RL1b (138), further implicating IL1RL1 in food allergy pathogenesis. However, it is unknown whether soluble IL-1RL1a levels or *IL1RL1* SNPs are associated with food allergy (274,275) 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 center, we measured IL-1RL1a in serum (0-3 months before food challenge) and genotyped *IL1RL1* SNPs. Next, we performed regression modeling 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*. Secondly, 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 Center Groningen between 2005-2014 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 (273). 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, iii) No food allergy if they had only negative DBPCFCs at any time. Severity of food allergy was defined on a scale from 0-12, 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 1).

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.* (26) (rs13431828, rs1041973, rs1420101, rs1946131, rs1921622, rs10204137). Furthermore, we added *IL1RL1* SNP rs10185897, which is associated with atopic dermatitis/eczema (276), 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 8. For rs10185897, genotyped data was 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 1. 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, 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 2). *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 analyzed 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 3/4.

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 (26). 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 (273,277). 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 table 6 and 7), 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 2). 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 (273,277). 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 to DBPCFC confirmed peanut allergy. We did not find association of SNPs with clinical chicken egg allergy. Interestingly, these SNPs have potential functional consequences, i.e. 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 pediatric 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, i.e. 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, whilst 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 5), 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 (274). 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.

To take home

- IL-1RL1 serum protein is not predictive of food allergy, but *IL1RL1* SNPs may be predictive of specific food allergy phenotypes, such as peanut and chicken egg allergy.

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	**<0.001**
				Food allergy at any time (any food)	0.020	0.87 (OR)	0.17	0.44
				Peanut allergy (DBPCFC+)	0.028	0.81 (OR)	0.173	0.216
				Chicken egg allergy (DBPCFC+)	0.048	0.86 (OR)	0.293	0.589
				Sensitization (sIgE+/-, any food)	0.002	1.05 (OR)	0.25	0.84
				IgE sensitization @peanut	0.172	0.32 (OR)	0.566	**0.04**
				IgE sensitization @chicken egg	0.159	3.14 (OR)	1.150	**0.006**
rs13431828	2:102954653	T	0.16	**(LN) IL-1RL1 serum**	0.026	0.12 (B)	0.06	0.05
				Food allergy at any time (any food)	0.000	1.01 (OR)	0.29	0.97
				Peanut allergy (DBPCFC+)	0.044	0.53 (OR)	0.285	**0.03**
				Chicken egg allergy (DBPCFC+)	0.056	1.54 (OR)	0.444	0.332
				Sensitization (sIgE+/-, any food)	0.007	1.55 (OR)	0.41	0.29
				IgE sensitization @peanut	0.075	0.83 (OR)	0.818	0.821
				IgE sensitization @chicken egg	0.009	1.17 (OR)	0.676	0.815
rs10204137	2:102968212	G	0.38	**(LN) IL-1RL1 serum**	0.057	0.15 (B)	0.04	**<0.001**
				Food allergy at any time (any food)	0.007	1.12 (OR)	0.19	0.54
				Peanut allergy (DBPCFC+)	0.025	0.88 (OR)	0.179	0.465
				Chicken egg allergy (DBPCFC+)	0.046	1.24 (OR)	0.314	0.487
				Sensitization (sIgE+/-, any food)	0.000	1.00 (OR)	0.25	0.99
				IgE sensitization @peanut	0.082	1.42 (OR)	0.595	0.554
				IgE sensitization @chicken egg	0.114	0.39 (OR)	0.664	0.153
rs1041973	2:102955468	A	0.28	**(LN) IL-1RL1 serum**	0.009	-0.12 (B)	0.05	**0.01**
				Food allergy at any time (any food)	0.001	0.80 (OR)	0.22	0.30
				Peanut allergy (DBPCFC+)	0.049	0.55 (OR)	0.239	**0.01**
				Chicken egg allergy (DBPCFC+)	0.048	1.21 (OR)	0.358	0.598
				Sensitization (sIgE+/-, any food)	0.002	1.21 (OR)	0.30	0.53
				IgE sensitization @peanut	0.074	1.01 (OR)	0.687	0.99
				IgE sensitization @chicken egg	0.013	1.26 (OR)	0.580	0.688
rs10185897	2:102966790	A	0.16	**(LN) IL-1RL1 serum**	0.021	0.12 (B)	0.11	0.33
				Food allergy at any time (any food)	0.000	1.28 (OR)	0.49	0.61
				Peanut allergy (DBPCFC+)	-	-	-	-
				Chicken egg allergy (DBPCFC+)	-	-	-	-
				Sensitization (sIgE+/-, any food)	0.003	2.75 (OR)	0.82	0.22
				IgE sensitization @peanut	-	-	-	-
				IgE sensitization @chicken egg	-	-	-	-

Table 1 continued:

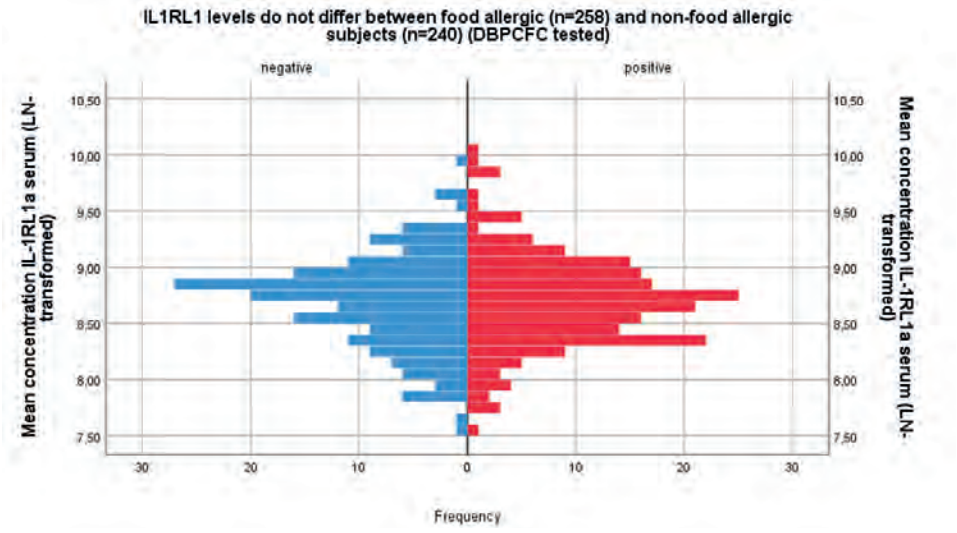
SNP	*Location (GRCh37.p13)*	*Tested allele*	*AF tested allele*	*Outcome*	*R^2 SNP*	*Effect*	*SE*	*P-value*
rs1921622	2:102966067	A	0.55	**(LN) IL-1RL1 serum**	0.181	-0.26 (B)	0.03	**<u>≤0.001</u>**
				Food allergy at any time (any food)	0.005	0.88 (OR)	0.18	0.46
				Peanut allergy (DBPCFC+)	0.020	0.95 (OR)	0.174	0.78
				Chicken egg allergy (DBPCFC+)	0.049	0.86 (OR)	0.306	0.630
				Sensitization (sIgE+/-, any food)	0.003	0.85 (OR)	0.24	0.50
				IgE sensitization @peanut	0.151	0.34 (OR)	0.623	0.80
				IgE sensitization @chicken egg	0.212	3.01 (OR)	0.820	**0.048**
rs1946131	2:102961929	A	0.09	**(LN) IL-1RL1 serum**	0.076	-0.29 (B)	0.06	**<u>≤0.001</u>**
				Food allergy at any time (any food)	0.000	0.69 (OR)	0.29	0.20
				Peanut allergy (DBPCFC+)	0.021	1.00 (OR)	0.33	0.99
				Chicken egg allergy (DBPCFC+)	0.046	0.83 (OR)	0.490	0.698
				Sensitization (sIgE+/-, any food)	0.002	0.93 (OR)	0.41	0.86
				IgE sensitization @peanut	0.075	1.32 (OR)	1.13	0.81
				IgE sensitization @chicken egg	0.007	0.96 (OR)	0.819	0.961

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 months 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 were performed on this variable. In* ***bold:*** *p-values <0.05.* *<u>Underlined:</u>* *p-values <0.0167 (adjusted cut-off corrected for the LD pattern in the region, correcting for three independent genetic signals).*

A. Clinical characteristics of food

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*	*R2 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
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 center. [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 the supplemental table 5.

Supplemental: see also online

Supplemental figure 1:

Distribution of IL1-RL1 serum protein levels:

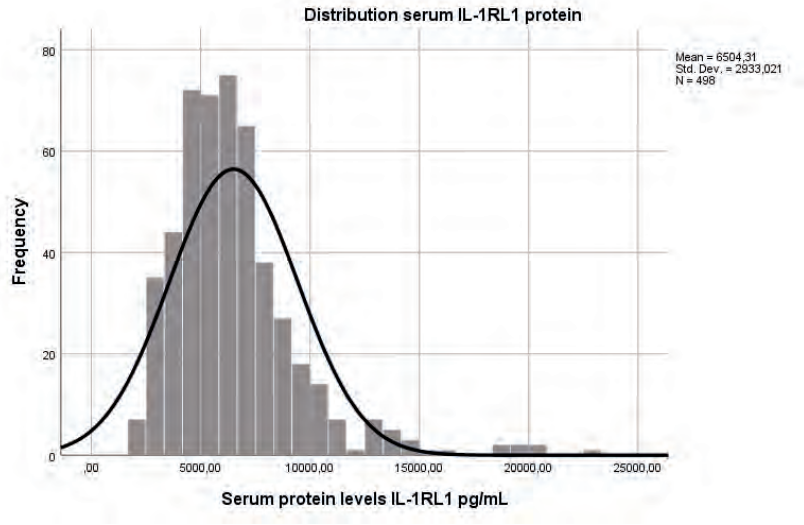


Figure S1a: Before transformation
Shown is the distribution of serum IL-1RL1 levels in n=498 children of this study. Because of skewed distribution, we LN-transformed the data (see S1b).

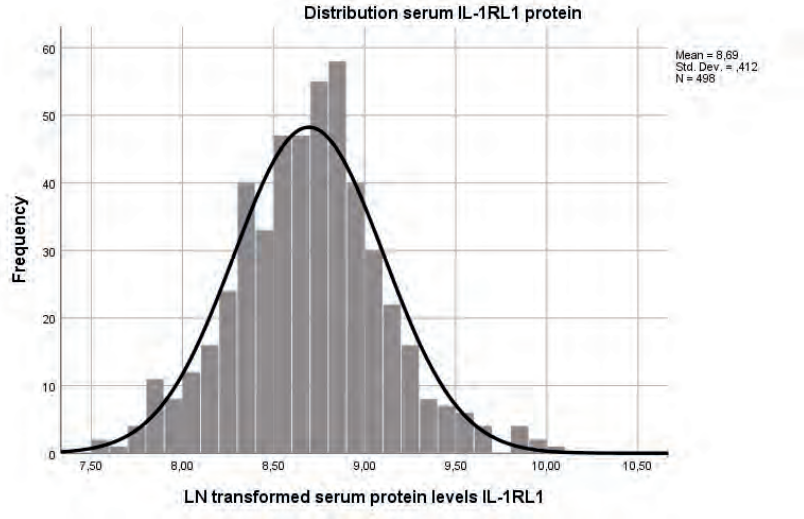
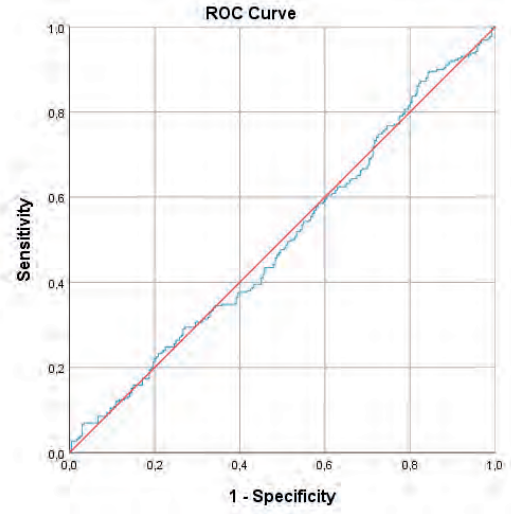


Figure S1b: After LN-transformation
Shown is the distribution of serum IL-1RL1 levels in n=498 children of this study after LN-transformation.

Supplemental figure 2: ROC for IL-1RL1 serum levels as predictor of food allergy (DBPCFC+/-)



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 center, of which n=258 were DBPCFC positive at the time of testing (current food allergy). The ROC curve shows that there was no difference in prediction of food allergy (DBPCFC+) due to IL-1RL1a levels (blue curve) or compared to chance (red reference curve). See also the figure in the main text.

Supplemental table 1: Population characteristics

		Children with DBPCFC overall 2003-2014	Consented and enrolled in study	Children with serum IL-1RL1a
N		716	572	498
Age (months +/- stdev)		81.0+/-58.3	77.4+/-55.6	79.5+/-56.0
Gender N male (% male)		437 (61.0%)	348 (60.8%)	306 (61.4%)
Eczema ever-child N (% yes)		608 (84.9%)	495 (87.1%)	435 (87.5%)
Rhinitis ever-child N (% yes)		254(35.5%)	209 (37.7%)	168 (38.6%)
Asthma ever-child N (% yes)		293 (40.9%)	264 (46.2%)	232 (46.7%)
Food allergic at any time N (% positive)		412 (57.5%)	361 (63.4%)	320 (64.3%)
Food allergic at current N (% positive)			295 (51.8%)	258 (51.8%)
Food allergic at current N (peanut, % positive)				79 (48.5%)
Food allergic at current N (chicken egg, % positive)				31 (44.3%)
Food allergic at current N (cow's milk, % positive)				86 (61.4%)
Sensitized to tested food (sIgE >0.35 kU/L, % positive)				368 (488 tested=75.4%)
sIgE tested food (median[range] kU/L)			2.19 [0.0-101.0]	2.28 [0.0-101.0]
Serum IL-1RL1a (median[range] pg/mL)			6049.20 [1948.0-22536.0]	6056.40 [1948.0-22536.0]
Genotype freq (%)- rs1420101 (N available)			(N=309)	(N=263)
	T:T		16.8	16.3
	T:C		47.9	49.0
	C:C		35.3	34.6
Genotype freq (%)- rs13431828 (N available)			(N=309)	(N=263)
	T:T		1.6	1.5
	T:C		14.6	14.1
	C:C		83.8	84.4
Genotype freq (%)- rs10204137 (N available)			(N=304)	(N=258)
	G:G		10.9	10.9
	G:A		44.1	43.4
	A:A		45.1	45.7
Genotype freq (%)- rs1041973 (N available)			(N=309)	(N=263)
	A:A		3.6	3.8
	C:A		26.0	25.5
	C:C		69.6	70.7
Genotype freq (%)- rs1921622 (N available)			(N=304)	(N=258)
	G:G		18.8	18.1
	G:A		50.7	51.7
	A:A		30.6	30.1
Genotype freq (%)- rs10185897 (N available)			(N=161)	(N=112)
	A:A		0.0	0.0
	C:A		16.1	15.2
	C:C		83.9	84.8
Genotype freq (%)- rs1946131 (N available)			(N=309)	(N=263)
	A:A		0.3	0.4
	G:A		19.1	18.6
	G:G		80.6	81.0

Characteristics of the study population are shown, 1a) indicating the number (N) of children data was available for. 1b) showing a cross table of sensitization versus food allergy (DBPCFC outcome)
freq=frequency; *DBPCFC*= double-blind placebo-controlled food challenge; tested food allergens included cow's milk, wheat, fish, shellfish, chicken egg, peanut, cashew, almond, hazelnut, walnut, pistachio, coconut, sesame, and soy bean; *food allergic:* Positive DBPCFC for the tested food. *sIgE*=specific IgE against the tested food allergen; *stdev*=standard of deviation.

Supplemental table 2: Association of IL1RL1 SNPs with eczema or asthma

SNP	*Location (GRCh37.p13)*	*Tested allele*	*AF tested allele*	*Outcome*	*R^2 SNP*	*Effect*	*SE*	*P-value*
rs1420101	2:102957716	T	0.36	**Eczema ever**	0.015	0.926	0.243	0.751
				Asthma ever	0.198	1.050	0.180	0.788
rs13431828	2:102954653	T	0.16	**Eczema ever**	0.017	1.293	0.438	0.558
				Asthma ever	0.202	0.743	0.296	0.315
rs10204137	2:102968212	G	0.38	**Eczema ever**	0.018	0.869	0.255	0.582
				Asthma ever	0.226	1.004	0.191	0.983
rs1041973	2:102955468	A	0.28	**Eczema ever**	0.015	1.019	0.311	0.953
				Asthma ever	0.198	0.933	0.228	0.762
rs10185897	2:102966790	A	0.16	**Eczema ever**	0.067	0.450	0.549	0.146
				Asthma ever	0.292	0.760	0.488	0.574
rs1921622	2:102966067	A	0.55	**Eczema ever**	0.022	0.826	0.250	0.444
				Asthma ever	0.215	1.218	0.185	0.287
rs1946131	2:102961929	A	0.09	**Eczema ever**	0.016	0.851	0.403	0.688
				Asthma ever	0.203	1.467	0.311	0.218

IL1RL1 SNPs were used as univariate predictor of eczema (ever in history, yes=260, no=41) or asthma (ever in history, yes=151, no=150) in a logistic regression model using age and gender as covariates.

Supplemental table 3: Association of SNPs with food allergy (DBPCFC+ for any food or specific foods)

SNP	*Location (GRCh37.p13)*	*Tested allele*	*AF tested allele*	*Outcome*	*R^2 SNP*	*Effect*	*SE*	*P-value*
rs1420101	2:102957716	T	0.36	**Any food**	0.020	0.87 (OR)	0.17	0.44
				Peanut	0.028	0.807	0.173	0.216
				Cow's Milk protein	0.003	1.012	0.239	0.961
				Chicken egg	0.048	0.854	0.293	0.589
rs13431828	2:102954653	T	0.16	**Any food**	0.000	1.01 (OR)	0.29	0.97
				Peanut	0.044	0.530	0.285	**0.026**
				Cow's Milk protein	0.004	1.148	0.415	0.740
				Chicken egg	0.056	1.539	0.444	0.332
rs10204137	2:102968212	G	0.38	**Any food**	0.007	1.12 (OR)	0.19	0.54
				Peanut	0.025	0.877	0.179	0.465
				Cow's Milk protein	0.004	1.058	0.259	0.829
				Chicken egg	0.046	1.243	0.314	0.487
rs1041973	2:102955468	A	0.28	**Any food**	0.001	0.80 (OR)	0.22	0.30
				Peanut	0.049	0.552	0.239	**0.013**
				Cow's Milk protein	0.003	1.052	0.325	0.875
				Chicken egg	0.048	1.208	0.358	0.598
rs10185897	2:102966790	A	0.16	**Any food**	0.000	1.28 (OR)	0.49	0.61
				Peanut	-	-	-	-
				Cow's Milk protein	-	-	-	-
				Chicken egg	-	-	-	-
rs1921622	2:102966067	A	0.55	**Any food**	0.005	0.877 (OR)	0.18	0.46
				Peanut	0.020	0.953	0.174	0.782
				Cow's Milk protein	0.002	0.985	0.234	0.948
				Chicken egg	0.049	0.863	0.306	0.630
rs1946131	2:102961929	A	0.09	**Any food**	0.000	0.69 (OR)	0.29	0.20
				Peanut	0.021	1.000	0.327	0.999
				Cow's Milk protein	0.005	0.938	0.408	0.875
				Chicken egg	0.046	0.827	0.490	0.698

IL1RL1 SNPs were used as univariate predictor of DBPCFC tested food allergy, either for any food (yes=258, no=240) or specific foods, including peanut (yes=162, no=129), milk protein (yes=73, no=77) or chicken egg (yes=50, no=60). '-'= *less than n=5 per analysis group with data available, therefore no analysis performed. In* ***bold:*** *p-values <0.05. Underlined: p-values <0.0167 (adjusted cut-off corrected for the LD pattern in the region).*

Supplemental table 4: Association of SNPs with IgE sensitization (for any food or specific foods)

SNP	*Location (GRCh37.p13)*	*Tested allele*	*AF tested allele*	*Outcome*	*R^2 SNP*	*Effect*	*SE*	*P-value*
rs1420101	2:102957716	T	0.36	**Any food**	0.002	1.05 (OR)	0.25	0.84
				Peanut	0.172	0.318	0.566	**0.043**
				Cow's Milk protein	0.074	1.367	0.325	0.336
				Chicken egg	0.159	3.141	1.150	**0.006**
rs13431828	2:102954653	T	0.16	**Any food**	0.007	1.55 (OR)	0.41	0.29
				Peanut	0.075	0.831	0.818	0.821
				Cow's Milk protein	0.070	1.711	0.668	0.421
				Chicken egg	0.009	1.171	0.676	0.815
rs10204137	2:102968212	G	0.38	**Any food**	0.000	1.00 (OR)	0.25	0.99
				Peanut	0.082	1.422	0.595	0.554
				Cow's Milk protein	0.107	0.566	0.358	0.112
				Chicken egg	0.114	0.387	0.664	0.153
rs1041973	2:102955468	A	0.28	**Any food**	0.002	1.21 (OR)	0.30	0.53
				Peanut	0.074	1.012	0.687	0.987
				Cow's Milk protein	0.063	1.215	0.468	0.678
				Chicken egg	0.013	1.262	0.580	0.688
rs10185897	2:102966790	A	0.16	**Any food**	0.003	2.75 (OR)	0.82	0.22
				Peanut	-	-	-	-
				Cow's Milk protein	0.136	1.378	1.027	0.755
				Chicken egg	-	-	-	-
rs1921622	2:102966067	A	0.55	**Any food**	0.003	0.847 (OR)	0.24	0.50
				Peanut	0.151	0.337	0.623	0.80
				Cow's Milk protein	0.071	0.805	0.323	0.502
				Chicken egg	0.212	3.076	0.820	**0.048**
rs1946131	2:102961929	A	0.09	**Any food**	0.002	0.93 (OR)	0.41	0.86
				Peanut	0.075	1.316	1.128	0.807
				Cow's Milk protein	0.060	0.949	0.569	0.927
				Chicken egg	0.007	0.961	0.819	0.961

IL1RL1 SNPs were used as univariate predictor of current IgE sensitization (yes/no, specific IgE>0.35 kU/L) against a food allergen, either for any food (yes=368, no=118) or specific foods, including peanut (yes=149, no=27), cow's milk protein (yes=75, no=91) or chicken egg (yes=20, no=55).

'-'= less than n=5 per analysis group with data available, therefore no analysis performed. In ***bold:*** *p-values <0.05. Underlined: p-values <0.0167 (adjusted cut-off corrected for the LD pattern in the region).*

Supplemental table 5: Association of IL-1RL1 serum protein levels with food allergy (DBPCFC+) or IgE sensitization (sIgE>0.350kU/L) for any food or specific foods

PREDICTOR	*Outcome*	*R^2 predictor*	*Effect*	*SE*	*P-value*	*N positive*	*N negative*
(LN) IL-1RL1 serum	**Current food allergy, any (DBPCFC+)**	0.000	1.03 (OR)	0.26	0.90	258	240
	Peanut allergy (DBPCFC+)	0.065	1.01	0.37	0.97	79	84
	Cow's milk protein allergy (DBPCFC+)	0.013	1.20	0.45	0.69	72	68
	Chicken egg allergy (DBPCFC+)	0.063	0.36	0.74	0.17	31	39
	IgE sensitization @ Any food	0.001	0.75 (OR)	0.33	0.39	368	118
	IgE sensitization @ Peanut	0.019	0.59	0.57	0.35	142	21
	IgE sensitization @ Cow's milk protein	0.149	0.74	0.50	0.55	86	54
	IgE sensitization @ Chicken egg	0.103	0.18	0.87	**0.049**	53	17

IL-1RL1 serum protein levels were used as univariate predictor of DBPCFC tested food allergy, or for IgE sensitization (IgE>0.350kU/L). Either for any food (DBPCFC+=258, DBPCFC-=240) (IgE+=368, IgE-=118) or specific foods, including peanut (DBPCFC+=79, DBPCFC-=84) (IgE+=142, IgE-=21); cow's milk protein (DBPCFC+=72, DBPCFC-=68) (IgE+=86, IgE-=54); or chicken egg (DBPCFC+=31, DBPCFC-=39) (IgE+=53, IgE-=17)

*In **bold:** p-values <0.05.*

Supplemental table 6: Power calculation for association between IL1RL1 SNPs and food allergy (DBPCFC tested)

SNP	*Location (GRCh37.p13)*	*Tested allele*	*AF tested allele*	*Outcome*	*R^2 SNP*	*Actual effect size*	*SE*	*Power to detect significant effect at alpha=0.05*
rs1420101	2:102957716	T	0.36	**Any food**	0.020	0.87 (OR)	0.17	62%
rs13431828	2:102954653	T	0.16	**Any food**	0.000	1.01 (OR)	0.29	11%
rs10204137	2:102968212	G	0.38	**Any food**	0.007	1.12 (OR)	0.19	58%
rs1041973	2:102955468	A	0.28	**Any food**	0.001	0.80 (OR)	0.22	26%
rs10185897	2:102966790	A	0.16	**Any food**	0.000	1.28 (OR)	0.49	41%
rs1921622	2:102966067	G	0.45	**Any food**	0.005	1.14 (OR)	0.18	72%
rs1946131	2:102961929	A	0.09	**Any food**	0.000	0.69 (OR)	0.29	46%

Power calculations are shown at alpha=0.05 for the effect of SNP on food allergy (DBPCFC+ any food allergy)

Supplemental table 7: Power calculation for association between IL-1RL1a serum protein levels and food allergy

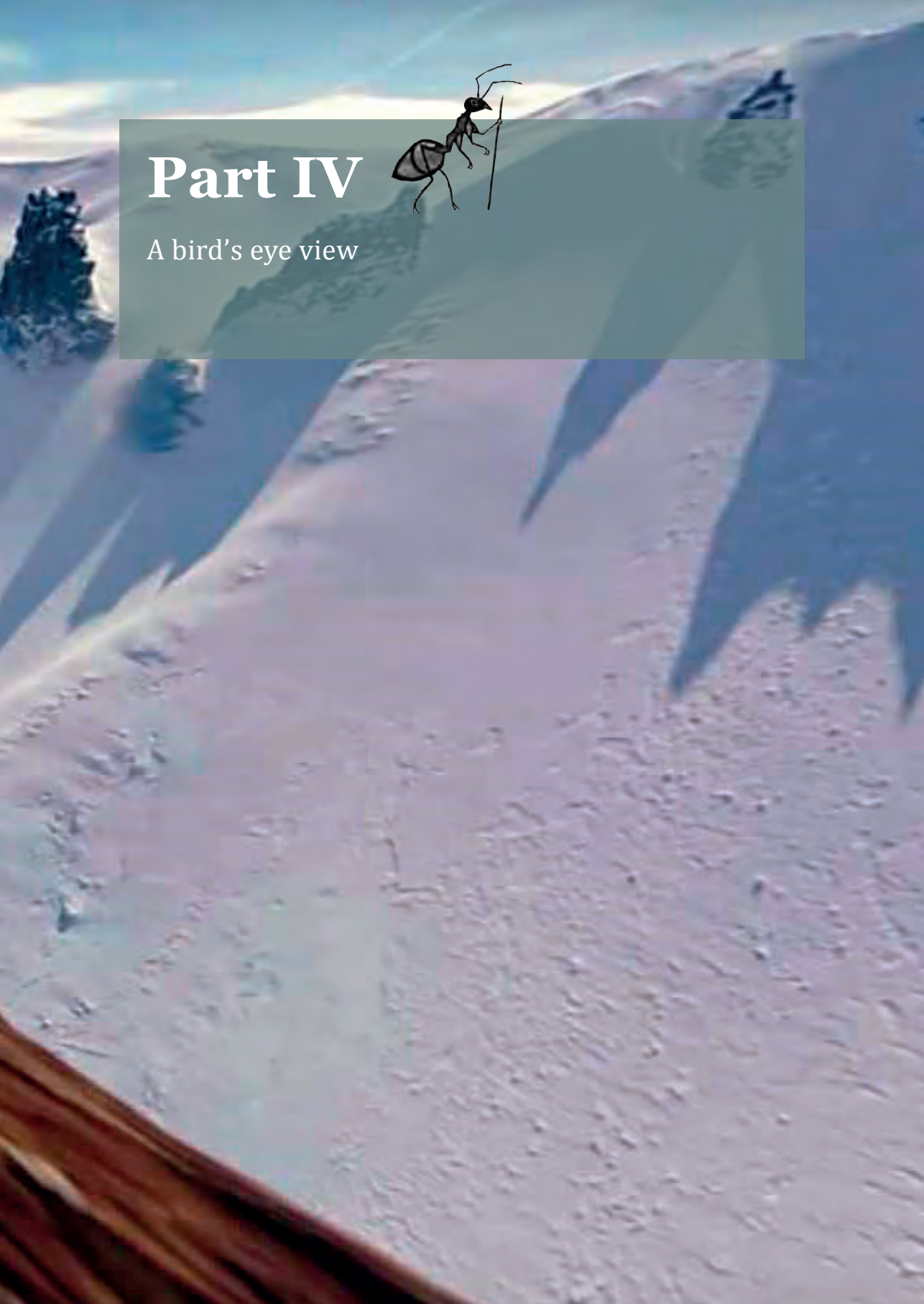
Predictor	*Outcome*	*R^2 predictor*	*Effect*	*SE*	*Power to detect significant effect at alpha=0.05*
(LN) IL-1RL1 serum	**Current food allergy**	**0.000**	**1.03 (OR)**	**0.26**	**52%**
(LN) IL-1RL1 serum	**Food allergy at any time**	**0.000**	**1.04 (OR)**	**0.24**	**54%**
(LN) IL-1RL1 serum	**Severity of food allergy**	**0.001**	**0.03 (beta)**	**0.46**	**51%**
(LN) IL-1RL1 serum	**Levels of blood sIgE (LN)**	**0.000**	**0.06 (beta)**	**0.25**	**58%**
(LN) IL-1RL1 serum	**Sensitization (sIgE+/-)**	**0.001**	**0.75 (OR)**	**0.33**	**82%**

Power calculations are shown at alpha=0.05 for the effect of IL-1RL1a serum protein levels on food allergy measures. See also main figure 1 for results of the association analyses.

Supplemental table 8: LD pattern of the IL1RL1 SNPs used

RS_number	*rs13431828*	*rs1041973*	*rs1420101*	*rs1946131*	*rs1921622*	*rs10204137*	*rs10185897*
rs13431828	1						
rs1041973	0.565	1					
rs1420101	0.09	0.001	1				
rs1946131	0.024	0.171	0.084	1			
rs1921622	0.178	0.025	0.487	0.133	1		
rs10204137	0.252	0.066	0.34	0.095	0.087	1	
rs10185897	0.302	0.458	0.094	0.098	0.106	0.109	1

Overview of the LD pattern of the 7 selected SNPs in this paper using R^2. In *italics* R^2>0.3 is shown (moderate LD), which leaves 3 signals/blocks. No SNPs had LD R^2>0.8, as we originally had selected the SNPs based on these tagging distinct LD blocks using an R^2>0.8 cut-off. The LD was determined based on the 1000G EUR population.



Part IV

A bird’s eye view