

Influence of Polymorphisms in the *Interleukin-18* Gene on Allergic Rhinitis: A Meta-Analysis

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Keywords

Interleukin-18 polymorphisms · Allergic rhinitis · Meta-analysis

Abstract

Purpose: Reported associations of *interleukin-18* (*IL-18*) single-nucleotide polymorphisms (SNPs) with allergic rhinitis (AR) have been inconsistent, prompting a meta-analysis to obtain more precise estimates. **Methods:** We synthesized data from 8 articles and examined 3 *IL-18* SNPs. Two SNPs (rs360721 and rs187238), in linkage disequilibrium, were combined and termed RS1. The rs1946518 SNP was analyzed separately (termed RS2). The recessive, dominant, and co-dominant (multiplicative) genetic models were used to estimate ORs and 95% CIs. Subgroup analysis was ethnicity-based. Sources of heterogeneity were investigated with outlier treatment. Sensitivity analysis was used to assess robustness of the associative effects. Multiple comparisons were Holm-Bonferroni corrected. **Results:** All significant ($p^a < 0.05$) outcomes indicating increased risks were found in the dominant/codominant models in RS1 and RS2. Five

aspects of differences marked the significant African (RS1) and overall (RS2) outcomes: (i) magnitude of effect (ORs): greater (3.01–5.15) versus less (1.20–1.47); (ii) precision of effects (95% CIs): less (1.07–21.52) versus more (1.01–1.89); (iii) outlier treated: no versus yes; (iv) sensitivity outcomes: nonrobust versus robust (dominant model only); and (v) greater evidential strength for RS2 ($p^a = 0.002$) compared to RS1 ($p^a = 0.02$) rendered RS2 our core finding. These levels of statistical significance for RS1/RS2 enabled both to survive the Holm-Bonferroni correction. **Conclusions:** The core outcome indicating a 1.5-fold increased risk could render the *IL-18* polymorphisms useful in the clinical genetics of AR. Future studies that could focus on other *IL-18* SNPs may find deeper associations with AR than what we found here.

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Introduction

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa following exposure to an allergen. The disorder is clinically characterized by itching, sneezing, rhinorrhea (runny nose), and nasal congestion or stuffiness that prove reversible either spontaneously or as a result of treat-

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ment [1]. AR is a global health problem, with a prevalence of 9–42% among the general population [2]. The enduring prevalence of AR [3] renders this disease as the most common of all allergic disorders and constitutes one of the main causes of medical consultation, thus generating an important economic and social burden. Moreover, AR is also known to be a risk factor for the development of asthma [4]. The development of AR entails a complex interaction between genetic predisposition and environmental exposure to different factors, of which the most important is the implicated allergen. The genetics of AR may not be Mendelian, but based on twin studies, it exhibits an inheritability of 0.33–0.75 [5]. Yet, genetic studies are complicated in AR, for a number of reasons. First, monogenic forces contribute to the global effect of the disease. Second, polygenic interactions influence the final outcome. Lastly, gene-environment interactions have not yet been clearly established. To this, we add possible epigenetic effects, defined as those inheritable changes in gene expression outside the DNA sequence machinery [4]. Polymorphisms are variations in DNA sequence found in over 1% of the population, and those that result from a single base change are termed single-nucleotide polymorphisms (SNPs). From the perspective of human genetics, it seems that the great majority of genetic variations that contribute to complex diseases probably involve the interaction of several SNPs. SNPs in coding regions modify the structure of proteins by changing single amino acids. SNPs in promoter regions alter protein expression. Criteria for a given SNP as a disease contributor include the following [6]: (i) it must induce a change in function or expression of the gene product; (ii) linkage studies with strong statistical power [7] must confirm the association to the disease; and (iii) the mechanism of action must be biologically plausible, which is supported by *in vivo* studies [4]. Considerable research effort has focused on the implicating SNPs in allergy and asthma studies [8], and those that implicate SNPs in AR have curiously emphasized Asian populations. Most genetic studies in patients with AR have involved genes encoding for cytokines and their receptors, due to their involvement in the pathogenesis of AR [4]. Cytokines are a diverse group of immunomodulatory, signaling molecules with a wide range of functions in health and disease. They orchestrate host responses to infection and trauma, as well as the potentially damaging responses in allergic, inflammatory, and autoimmune disease. In sum, cytokines play an essential role in mediating allergic inflammation. Several SNPs in the cytokine family have been implicated in the pathogenesis of AR [9]. Interleukin-18 (IL-18) is among the possible factors that play a role in the development of

allergies. It is a proinflammatory cytokine that is involved in the T helper type 1-mediated immune response and inhibits immunoglobulin E synthesis, usually in synergy with IL-12 [10]. By contrast, IL-18 might initiate Th2 responses with the production of immunoglobulin E via the stimulation of IL-4 and IL-13 synthesis in mast cells and in basophil and eosinophil recruitment, such as allergic inflammation [11]. The gene for human *IL-18* is located on chromosome 11q22.2–22.3 (Locus ID 3606, NCBI, NIH). It is composed of 6 exons [12]. Database searches indicate close to 150 SNPs in the *IL-18* gene within the 5' and 3' untranslated regions, as well as various exons and introns [13]. Among them, –607 A/C (rs1946518), –137 C/G (rs187238) in the promoter region, and –140 C/G (previously –133 C/G, rs4988359) in intron 1 have been studied in detail with respect to etiopathogenesis of allergic diseases [14, 15] and transcriptional activity of IL-18 production [13]. Several studies have suggested that polymorphisms of *IL-18* and its receptor might be associated with allergic disorders in various populations. Inconsistent association between *IL-18* gene variants provide the opportunity to statistically synthesize these study findings using meta-analysis where we aim to obtain more precise estimates of the genetic role of *IL-18* SNPs in AR. Here, we examine the role of *IL-18* SNPs in the risk for AR, which might guide potential future directions in the genetics of allergy. This study aims to provide important information that could be useful in clinical decision-making.

Materials and Methods

Selection of Studies

We searched MEDLINE using PubMed, Google Scholar, and Science Direct for association studies as of November 17, 2019. The terms used were “cytokine,” “interleukin,” “IL-18,” “polymorphism,” “allergy,” and “rhinitis” as medical subject heading and text, restricted to the English language. References cited in the retrieved articles were also screened manually to identify additional eligible studies. In case of duplicate articles, we selected the one with a later date of publication. Inclusion criteria were (i) case-control design evaluating the association between *IL-18* SNPs and risk AR; (ii) available *IL-18* genotype frequencies in the presence and absence of AR; and (iii) sufficient genotype frequency data to enable calculation of the ORs and 95% CIs. Exclusion criteria were (i) studies not involving AR, (ii) review articles, (iii) non-English studies, and (iv) studies whose genotype or allele frequencies were unusable/absent or, when available, combined with SNPs in other genes, preventing proper data extraction.

SNP Groupings and Data Extraction

The included articles covered several *IL-18* SNPs, from which we examined 3: (i) –137G/C (rs187238), (ii) –133 C/G or –140 C/G (rs360721), and (iii) –607A/C (rs1946518). Observed phenotypic

Table 1. Characteristics of the included studies in *IL-18* AR meta-analysis

K	Cheng, 2014	First author	Reference	Year	Country	Ethnicity	<i>IL-18</i> polymorphisms studied	Case	Control	Power ($\alpha = 0.05$; OR 1.5)	CB
1	○	Nieters	[35]	2004	Germany	Caucasian	rs187238, rs1946518	316	321	71.2	9
2	✓	Lee	[36]	2006	South Korea	Asian	rs1946518	160	166	43.6	7
3	✓	Sebelova	[37]	2007	Czechoslovakia	Caucasian	rs187238, rs1946518, rs360721	539	312	80.2	9
4	○	Ibrahim	[38]	2012	Egypt	African	rs187238, rs360721	20	5	6.7	7
5	○	Wu	[39]	2012	China	Asian	rs187238, rs1946518	71	120	26.5	7
6	○	Birbian	[40]	2013	India	Asian	rs187238	323	87	37.9	7
7	○	Jumpasri	[41]	2014	Thailand	Asian	rs360721	150	50	23.0	7
8	○	Tungtrongchitr	[42]	2017	Thailand	Asian	rs187238, rs1946518, rs360721	30	30	11.9	7

K, number designation of the study; studies included in the Cheng et al. [21] meta-analysis are marked (✓) and those that were not are marked (○). Value in bold indicates statistical power above the threshold of 80%. AR, allergic rhinitis; *IL-18*, interleukin-18; CB, Clark-Baudouin score.

associations in AR [14] have been attributed to the proximity of these 3 SNPs. NCI LDLink (<https://ldlink.nci.nih.gov/>) results show that these SNPs are in linkage disequilibrium (LD) based on both European (CEU) and Han (CHB) populations [16]. LD is defined as the correlation between alleles located near each other [17], which is measured in terms of D' and r^2 with values of 1.0 indicating complete LD [18, 19]. In this study, the basis for SNP combining rested on strong to complete LD (0.88–1.0) of both D' and r^2 . The depth of LD between rs187238 and rs360721 merited combination, which we termed RS1 (Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000506010), but not rs1946518 (termed RS2), which was separately analyzed (online suppl. Table S2). The RS1 combination increased the number of studies, thus, aggregate statistical power [20], for the forest plot analysis (online suppl. Table S1). Two investigators (P.T. and N.P.) independently extracted data and arrived at a consensus. The following information was obtained from each publication: whether the article was covered in a previous meta-analysis [21], first author's name, year of the study, country of origin, ethnicity, genotyping platform, *IL-18* SNPs (rs number), and the Clark-Baudouin (CB) score (Table 1).

Data Distribution, Power Calculations, and Hardy-Weinberg Equilibrium

Distribution behavior of the data was assessed with the Shapiro-Wilks test using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Gaussian (normal) distribution ($p > 0.05$) warranted descriptive and inferential expressions of mean $\pm$ SD as well as the parametric approach. Otherwise, the median with interquartile range and nonparametric tests were used. Using the G*Power program [22], we evaluated statistical power as its adequacy bolsters the level of associative evidence. Assuming an OR of 1.5 at a genotypic risk level of $\alpha = 0.05$ (two-sided), power was considered adequate at $\geq 80\%$. Using the application in <https://ihg.gsf.de/cgi-bin/hw/hwa1.pl>, we assessed the Hardy-Weinberg equilibrium (HWE) and reported the p value of the controls from the Pearson's goodness-of-fit χ^2 test (online suppl. Table S1).

Methodological Quality of the Studies

We used the CB scale to evaluate methodological quality of the included studies [23]. The CB criteria include p values, statistical power, correction for multiplicity, comparative sample sizes between cases and controls, genotyping methods, and the HWE. In this scale, scores of <5 , 5–6, and ≥ 7 represent low, moderate, and high quality, respectively.

Meta-Analysis

Given the hypothesis of association between *IL-18* SNPs and risk of AR, we estimated the ORs with 95% CIs for each study by comparing cases with controls. We used genetic models following that of Pereira et al. [24], which included (i) recessive: *var-var* versus *het + wt-wt*; (iii) dominant: *var-var + het* versus *wt-wt*; and (iii) codominant (multiplicative): *var* versus *wt*. Using raw data for frequencies, study-specific risks (ORs) of AR were estimated and pooled ORs were calculated by comparing the effects on the same baseline. Multiple comparisons were corrected with the Holm-Bonferroni test. Subgrouping was ethnicity-based. Strength of evidence was assessed with 2 indicators: first, the p value is contextualized in terms of the Bayes Factor (BF), where $p^a = 0.05$ and 0.002 correspond to the minimum BF of ≥ 0.15 and 0.006, suggesting moderate and strong evidence, respectively [25]. The contextual meaning of BF rests on its support from the null and alternate hypotheses, which, in contrast to the p value, addresses the null only [26]. The BF rests on the likelihood paradigm [27], indicative of how strong the hypotheses are supported by the data [26]. Thus, the likelihoods between absence (null hypothesis) and presence (alternate hypothesis) of association of *IL-18* with AR are compared. Second, although heterogeneity is unavoidable [28], homogeneity (or close to it) is preferred. The reason for this preference is that conclusions made in the milieu of homogeneity (nonheterogeneity) have greater evidential strength than those that were heterogeneously derived. Thus, occurrence of heterogeneity between studies was estimated with the χ^2 -based Q test [29], the significance threshold of which was set at $p^b < 0.10$. Heterogeneity was also quantified with the I^2 statistic that measures variability

between studies [30]. I^2 values of $>50\%$ have more variability than those $\leq 50\%$ with 0% indicating zero heterogeneity or homogeneity. Evidence of functional similarities in population features of the studies warranted the use of fixed-effects model [31]; otherwise, the random-effects model [32] was used. Outliers are sources of heterogeneity detectable with the Galbraith plot [33]. Outlier-treated data were then re-analyzed, which dichotomized the comparisons into pre-outlier (PRO) and post-outlier (PSO). Sensitivity analysis involves omitting one study at a time and reassessing the resulting effect on robustness of the pooled OR. We assessed publication bias for comparisons that (i) had ≥ 10 studies only [34] and (ii) were associatively significant ($p^a \leq 0.05$). Except for heterogeneity estimation [29], two-sided p values of ≤ 0.05 were considered significant. Data for the meta-analysis were analyzed using Review Manager 5.3 (Cochrane Collaboration, Oxford, England), SIGMASTAT 2.03, and SIGMA-PLOT 11.0 (Systat Software, San Jose, CA, USA).

Results

Search Results and Study Features

Figure 1 outlines the study selection process in a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)-sanctioned flowchart. Initial search resulted in 20 citations, followed by a series of omissions that eventually yielded 8 articles for inclusion [35–42]. Of the 8 included articles, 6 [35, 38–42] were not included in the previous meta-analysis on the same topic [21]. The 8 articles had a 13-year publication span (2004–2017), 5 [35, 37–39, 42] of which examined >1 *IL-18* SNP (Table 1). LD facilitated SNP combination that raised the number of studies to 15 (online suppl. Table S1). Of the 15, 8, 5, and 2 studies were Asian, Caucasian, and African, respectively. Unduplicated sample sizes for Asian and Caucasian were 734 cases/453 controls and 628 cases/860 controls, with statistical powers of 91.7 and 96.8%, respectively. However, sample sizes and power for the African studies were insignificant (20 cases/5 controls, statistical power: 6.7%). Online supplementary Table S1 shows the aggregate total for all 15 studies with 1,362 cases and 1,313 controls translating to a statistical power of 99.9%. Nonnormal distribution (Shapiro-Wilks test: $p < 0.01$) of the CB scores (median 7.0, interquartile range 7.0–8.0) indicated that methodological quality of the component studies was high. Control frequencies deviated from the HWE were found in 2 studies [36, 37] only in RS2. This meta-analysis followed the PRISMA guidelines (online suppl. Table S3).

Meta-Analysis Outcomes

Significant ($p^a < 0.05$) associations were found in RS1 African subgroup (Table 2) and overall RS2 (Table 3), all

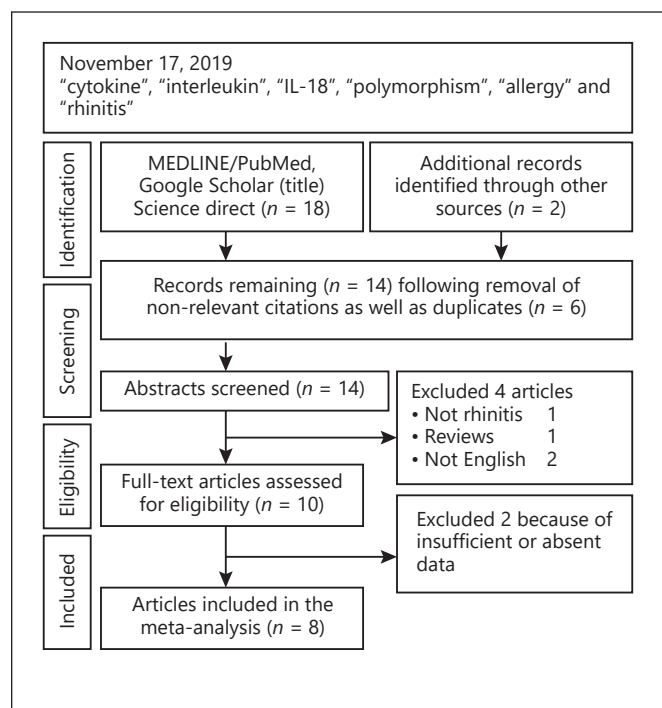


Fig. 1. Summary flowchart of literature search. IL-18, interleukin-18.

in the dominant and codominant models indicating increased risks (OR >1.0). The differences are that pooled outcomes in RS1 had higher magnitude (ORs 3.01–5.15) but less precision (95% CIs 1.07–21.52) than RS2 (ORs 1.20–1.47, 95% CIs 1.01–1.89). Furthermore, the outlier-derived (PSO) RS2 had a higher significant associative p^a value (0.002) than RS1 (0.02). Yet, both 0.002 and 0.02 p^a values survived the Holm-Bonferroni correction but not the modestly significant codominant outcomes in RS1 and RS2 ($p^a = 0.04$). The other 2 ethnic (Asian and Caucasian) subgroups (RS1 and RS2) and Hardy-Weinberg compliant in RS2 (Tables 2, 3) outcomes were nonsignificant ($p^a > 0.05$).

Impact and Mechanism of Outlier Treatment

The impact of outlier treatment is shown in Table 3, which is bisected into left and right panels, indicating PRO and PSO, respectively. Two features describe this table. First, all bolded (associatively significant) values were found in the PSO panel and none in the PRO panel; second, predominance of random effects in PRO and fixed effects in PSO. Table 4 summarizes the impact of outlier treatment wherein we compare PRO with PSO based on 3 key meta-analytical parameters: (1) signifi-

Table 2. Overall and subgroup outcomes for *IL-18* polymorphism associations with allergic rhinitis in RS1

	<i>n</i>	Test of association			Risk	Test of heterogeneity		
		OR	95% CI	<i>p</i> ^a		<i>p</i> ^b	<i>I</i> ² , %	AM
Overall								
Recessive	10	0.92	0.70–1.22	0.58	De	0.36	8	F
Dominant	10	0.94	0.73–1.21	0.65	De	0.05	47	R
Dominant*	9	0.92	0.80–1.07	0.30	De	0.15	38	F
Codominant	10	0.96	0.77–1.19	0.72	De	0.02	53	R
Codominant*	9	0.94	0.83–1.05	0.27	De	0.10	40	F
Asian								
Recessive	5	0.68	0.33–1.39	0.29	De	0.26	24	F
Dominant	5	0.73	0.53–1.01	0.06	De	0.27	22	F
Codominant	5	0.76	0.58–1.01	0.06	De	0.11	46	F
Caucasian								
Recessive	3	0.95	0.70–1.29	0.75	De	0.59	0	F
Dominant	3	0.97	0.82–1.15	0.72	Null	0.29	19	F
Codominant	3	0.97	0.85–1.11	0.68	Null	0.29	20	F
African								
Recessive	2	1.86	0.35–9.95	0.47	In	0.15	52	F
Dominant	2	5.15	1.23–21.52	0.02 ✓	In	0.34	0	F
Codominant	2	3.01	1.07–8.47	0.04	In	0.23	32	F

* Indicates post-outlier; De, decreased risk; In, increased risk; ORs 0.97–1.03 were considered null; R, random effects; F, fixed effects. ✓ Survived the Holm-Bonferroni correction. IL, interleukin 18; RS1, rs187238/rs360721; *p*^a, *p* value for association; *p*^b, *p* value for heterogeneity; *I*², measure of variability; AM, analysis model; *n*, number of studies; values in bold indicate significant associations of RS1 with AR.

cance: 8 vs. 22%; (2) random effects: 42 vs. 0%; and (3) fixed effects: 58 vs. 100%. These features underpin the impact of outlier treatment in uncovering significance and streamlining study variability. An example of the mechanism of outlier treatment is presented for the dominant model in the RS2 overall analysis (Fig. 2–4). Figure 2 shows the PRO forest plot, with an increased risk pooled OR (1.34), nonsignificant (*p*^a = 0.10) and heterogeneous (*p*^b = 0.02, *I*² = 64%). The Galbraith plot identified the outlying study [37], found below the –2 confidence limit (Fig. 3). In Figure 4, the PSO outcome (outliers omitted) showed reduced heterogeneity (*p*^b = 0.23, *I*² = 30%) and intensified increased risk (OR 1.47, 95% CI 1.15–1.89) with acquired significance (*p*^a = 0.002). This operation is numerically summarized in Table 3.

Sensitivity Analysis and Publication Bias

Table 5 shows that only the overall dominant significant outcome was robust. None of the 4 significant associative outcomes had >10 studies, which precluded publication bias testing.

Discussion

Summary of Findings

The findings of this study showed 4 significant (*p*^a < 0.05) associations indicating increased risks (OR > 1.0). Two outcomes were found in the African subgroup (RS1) and 2 in the overall PSO (RS2) for the dominant/codominant models. In the dominant model, evidential strength appears greater for RS2 (robust) compared to RS1 (nonrobust) with contrasting aggregate statistical powers (α = 0.05, OR 1.5), the values of which were at either ends of the scale (Table 5). Increased risk effects in RS1 were 5-fold (but less precise) compared to the modulated, but more precise 1.5-fold in RS2. The highly significant (*p*^a = 0.002) RS2 outcome was equivalent to a BF of 0.006 indicating strong evidence for association [26]. These lines of evidence favoring RS2 (overall dominant PSO) designate this finding as the core outcome. That RS2 (along with RS1) survived the Holm-Bonferroni correction indicates stability and minimized the risk for type 1 error. Outlier and sensitivity treatments played salient roles in designating the RS2 core outcome. By design, meta-analytical features such as

Table 3. Overall and subgroup outcomes for *IL-18* polymorphism associations with AR in RS2

	PRO				PSO				Effect of outlier treatment								
	test of association			risk	test of heterogeneity			n	test of association			test of heterogeneity					
	OR	95% CI	p ^a	p ^b	I ² , %	AM	OR		95% CI	p ^a	p ^b	I ² , %	AM				
Overall	5	0.87	0.56–1.36	0.54	De	66	R	4	1.07	0.84–1.37	0.59	in	0.65	0	F	RNS	EH
Recessive	5	1.34	0.94–1.92	0.10	In	64	R	4	1.47	1.15–1.89	0.002 ✓	in	0.23	30	F	GS	RH
Dominant	5	1.02	0.79–1.30	0.90	Null	67	R	3	1.20	1.01–1.43	0.04	in	0.63	0	F	GS	EH
Codominant																	
Asian	3	0.77	0.27–2.16	0.62	De	81	R	2	1.20	0.80–1.80	0.37	in	0.70	0	F	RNS	EH
Recessive	3	1.30	0.98–1.72	0.07	In	0	F	–	–	–	–	–	–	–	–	–	–
Dominant	3	0.97	0.56–1.69	0.91	Null	81	R	2	1.17	0.95–1.45	0.14	in	0.37	0	F	RNS	EH
Codominant																	
Caucasian	2	1.00	0.73–1.36	0.98	Null	0	F	–	–	–	–	–	–	–	–	–	–
Recessive	2	1.36	0.55–3.34	0.51	In	89	R	–	–	–	–	–	–	–	–	–	–
Dominant	2	1.05	0.88–1.24	0.60	In	46	F	–	–	–	–	–	–	–	–	–	–
Codominant																	
HWC	3	0.77	0.27–2.16	0.62	De	81	R	2	1.20	0.80–1.80	0.37	in	0.70	0	F	RNS	EH
Recessive	3	1.30	0.98–1.72	0.07	In	0	F	–	–	–	–	–	–	–	–	–	–
Dominant	3	0.97	0.56–1.69	0.91	Null	81	R	2	1.17	0.95–1.45	0.14	in	0.37	0	F	RNS	EH
Codominant																	

Values in bold indicate significant associations. P Indicates robustness and survived the Holm-Bonferroni correction. * Core outcome. IL-18, interleukin-18; AR, allergic rhinitis; *n*, number of studies; RS2, rs1946518; –, not applicable; *p*^a, *p* value for association; *p*^b, *p* value for heterogeneity; *I*², measure of variability; AM, analysis model; Sig, significance; Het, heterogeneity; HWC, Hardy-Weinberg compliant; PRO, pre-outlier; PSO, post-outlier; In, increased risk; De, decreased risk; ORs 0.97–1.03 were considered Null; R, random effects; F, fixed effects; GS, gained significance; RNS, retained nonsignificance; EH, eliminated heterogeneity; RH, reduced heterogeneity.

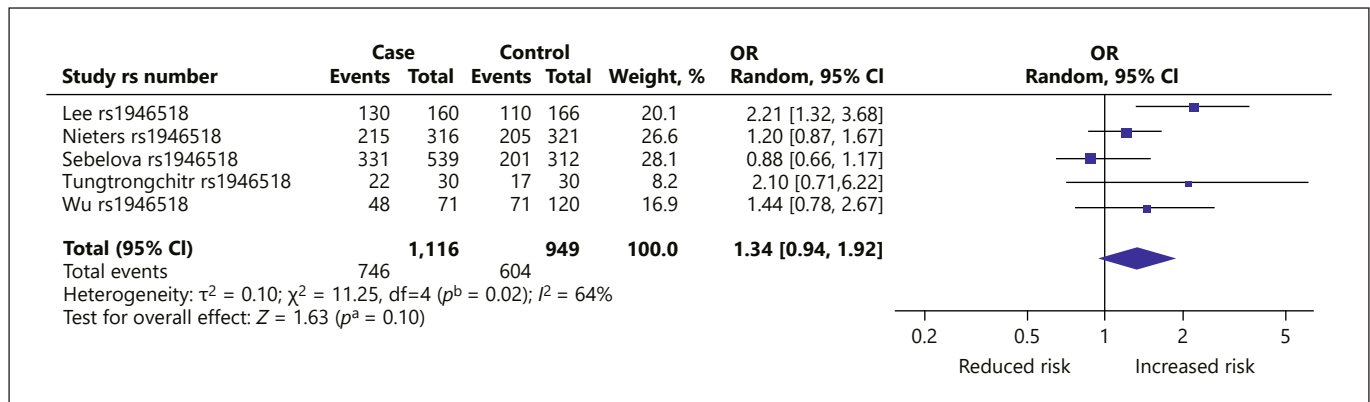


Fig. 2. Forest plot of the PRO outcome in the overall analysis for the dominant genetic model. Diamond denotes the pooled OR, here, indicating increased risk (1.34). Squares indicate the OR in each study. Horizontal lines on either side of each square represent the 95% CIs. The Z test for overall effect is nonsignificant ($p^a = 0.10$). The χ^2 test outcome shows presence of heterogeneity ($p^b = 0.02$, $I^2 = 64\%$). I^2 , a measure of variability expressed in %.

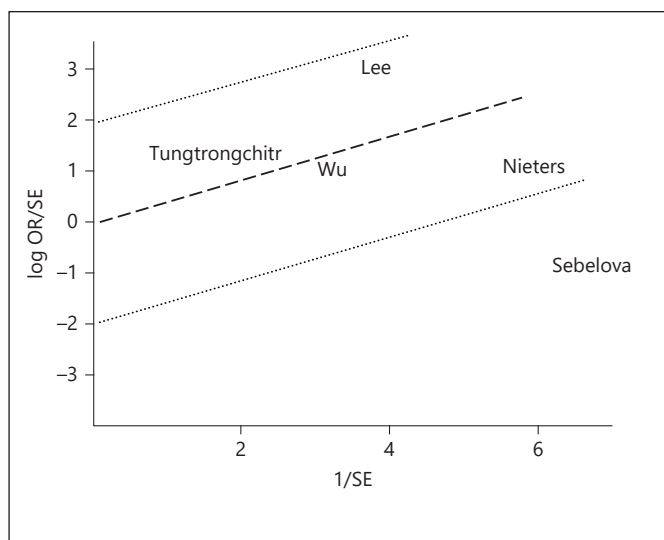


Fig. 3. Galbraith plot in the overall analysis for the dominant genetic model in *IL-18*. The study below the -2 confidence limit is the outlier. Log OR, logarithm of standardized OR.

outlier and sensitivity treatments are not present in the component single-study outcomes. Thus, statistical features such as magnitude, precision, and robustness of effects underpin the value of meta-analysis in synthesizing primary study outcomes and providing insight into associations of SNPs with AR. Conflicting outcomes between primary studies may be due to small sample sizes, hence, lack of power. Underpowered outcomes appear to be common in candidate gene studies [43] and are prone to the risk of type 1 error.

Meta-Analyses Comparison

Although this is the first meta-analysis to examine associations between *IL-18* SNPs and risk of AR, we compare our study to a previous meta-analysis that examined the same *IL-18* polymorphisms and allergic disease, a broad term that included allergic asthma, allergic dermatitis, and AR [21]. Our meta-analysis focused on AR only, which included 8 articles, and translated to 15 studies based on LD relationships among the *IL-18* SNPs. The 15 studies in our meta-analysis were sufficient in number to enable subgroup analysis. In contrast, the Cheng meta-analysis [21] examined 2 articles [36, 37] and 2 SNPs (rs1946518 and rs187238) with no mention of LD relationships. In either SNP, none of their AR pooled ORs were significant ($p^a > 0.05$) in all the genetic models that they used. In contrast, our meta-analysis yielded 4 significant ($p^a < 0.05$) pooled ORs and subjected to the Holm-Bonferroni correction. These differences and our more detailed investigation of the *IL-18*-AR associations present in itself the novelty of our study.

IL-18 and AR

This study takes the approach of systematically compiling evidence to help establish *IL-18* SNP associations with AR. These associations are best served with the validating role of functional studies of *IL-18*. Polymorphisms in the *IL-18* promoter region affect its expression. Changes in *IL-18* expression through genetic polymorphisms could lead to the exacerbation or suppression of allergic responses. A previous study demonstrated an increased expression of *IL-18* in nasal secretions of AR patients due to allergen exposure [44]. The most important

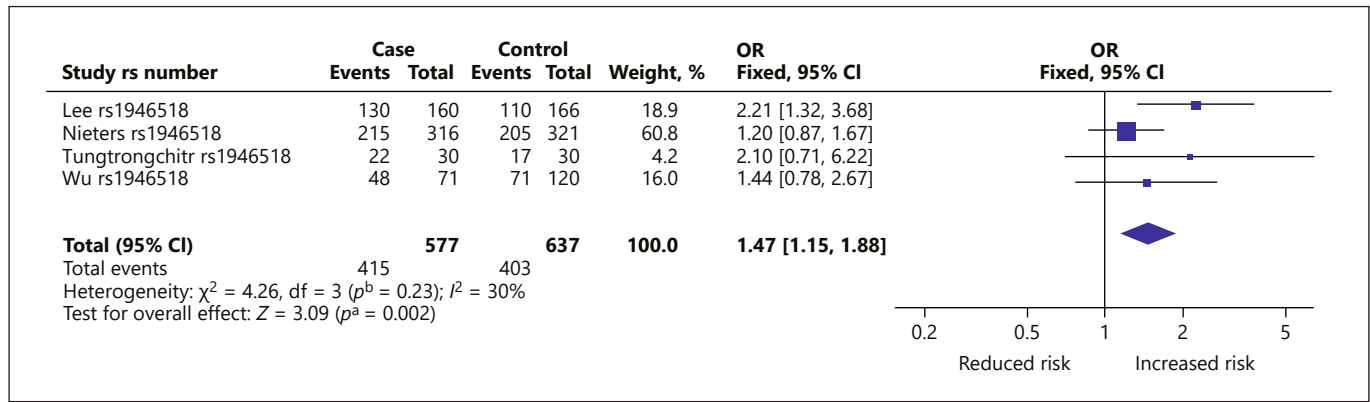


Fig. 4. Forest plot PSO outcome in the overall analysis for the dominant genetic model. Diamond denotes the pooled OR indicating increased risk (1.47). Squares indicate the OR in each study. Horizontal lines on either side of each square represent the 95% CIs. The Z test for overall effect shows significance ($p^a = 0.002$). The χ^2 test outcome is nonheterogeneous ($p^b = 0.23$, $I^2 = 30\%$). I^2 , a measure of variability expressed in %.

Table 4. Impact of outlier treatment for all *IL-18* associations with AR in RS1 and RS2

Parameter	Indicator	PRO	PSO
Number	Comparisons	24	9
Significance, %	$p^a \leq 0.05$	8	22
Heterogeneity, %	Random effects	42	0
Nonheterogeneity, %	Fixed effects	58	100

IL-18, interleukin-18; AR, allergic rhinitis; RS1, rs187238/rs360721; RS2, rs1946518; p^a , p value for association; PRO, pre-outlier; PSO, post-outlier.

Table 5. Sensitivity analysis for significant *IL-18* associations with AR in RS1 and RS2

Comparison (n)	Genetic model	p^a	Sensitivity outcome
African (2) 1	Dominant	0.02	[38]
African (2) 1	Codominant	0.04	[38]
Overall (4) 2	Dominant	0.002	Robust*
Overall (3) 2	Codominant	0.04	[35, 36, 42]

* Core outcome. IL, interleukin-18; AR, allergic rhinitis; RS1, rs187238/rs360721; RS2, rs1946518; PSO, post-outlier; p^a , p value for association; numbers in brackets indicate the articles/studies (reference) that contributed to nonrobustness of the comparison; n, sample size.

polymorphisms in promoters 1 and 2 are -137 G/C (rs187238) and -133 C/G (rs360721), both found in transcription binding sites (GATA-3 [guanine adenine thymine adenine sequence-binding protein 3] and NF-1 [neurofibromin 1], respectively). GATA-3 induces the Th2 phenotype [45], while NF-1 activates the transcription of a number of immune regulatory proteins, such as tumor necrosis factor receptor 2, transforming growth factor- β 1, and interleukin-1 β [46]. The Caucasian subgroup RS1 associations (rs187238/rs360721) in our study indicating non-significant outcomes (ORs 0.95–0.97) are in agreement with other findings. Sebelova et al. [37] found no significant differences between controls and AR patients in rs1946518 (-607 A/C), rs187238 (-137 G/C), and rs360721 (-133 C/G) in an adult Czech population, when analyzed separately. However, when the 3 SNPs were combined in haplotype analysis, associations

emerged. These contrasts appear with our Caucasian RS1 null outcomes. In further contrast, a German population [14] with both SNPs was found to be associated with specific sensitization to common allergens and AR. Moreover, elevated transcription activity of IL-18 on account of -607 /allele C (rs1946518) or -137 /allele G (rs187238) was found in a Swedish population [10]. For the African subgroup, the magnitude of association (5.2-fold) found in our RS1 analysis resulted from the combined SNPs (rs187238/rs360721). Regarding RS2 (rs1946518), our findings with up to 1.5-fold increased risk appear to agree with the study of Lee et al. [36], who found the frequency of rs1946518 (-607 A/C) to be significantly greater in patients with AR than in controls. In all, these RS1 and RS2

SNP data suggest variation in *IL-18* associations with AR that maybe modulated by ethnic differences in the study populations [39, 42].

Strengths and Limitations

Interpreting our findings should consider its limitations and strengths. Strengths include (i) to our knowledge, this study provides the latest comprehensive synthesis of the available data on *IL-18* associations with AR with attention to the number of included studies and use of an array of meta-analysis treatments that increased the rigor of our approach; (ii) aggregate sample sizes of the significant outcomes translated to a level of statistical power above the set threshold (80%); (iii) efficiency of outlier treatment was key to generating associative significance and eliminating or reducing heterogeneity (PSO outcomes in Table 3); (iv) stability of the core overall outcome rested on surviving the Holm-Bonferroni correction; and (v) the core outcome was robust. On the other hand, limitations include (i) the individual component studies except one [37] were underpowered; (ii) ethnic subgroup analysis was unable to delineate effects of the *IL-18* SNPs between Asians and Caucasians in RS1 and RS2; (iii) the significant African outcomes in RS1 were beset with low number of studies ($n = 2$) and low statistical power (6.7%); this warrants caution when interpreting the findings for this subgroup; (iv) the significant outcomes (except the core finding) were nonrobust; and (v) the core (overall dominant PSO) outcome contained an HW-noncompliant study [36], which, when omitted, marginalized the statistical significance ($p^a = 0.07$) but generated homogeneity ($I^2 = 0\%$).

Conclusions

We have shown that the significant associations generated in this study were genetic model dependent and ethnic-selective, but with one core finding of the 35 comparisons, this result is admittedly modest. However, the 3 layers of statistical evidence that strengthens the core outcome may be sufficient to render the *IL-18* rs1946518 SNP useful as a prognostic biomarker for AR. In any case, such utility may need more studies to further delineate ethnic differences as well as other *IL-18* SNPs that might yield more evidence of associations. Yet, the complexity of AR involves interactions between genetic and nongenetic factors allowing for the likelihood of environmental involvement. Gene-gene and gene-environment interactions have been reported to have roles in associations of

other SNPs. All but one [39] of the 8 articles mentioned gene-environment interaction. One article [35] examined other *IL* genes (i.e., 2, 4, 5, 6, 10, 12, 13) as well as tumor necrosis factor and also mentioned haplotypes. Additional well-designed studies exploring other parameters would confirm or modify our results in this study and add to the existing knowledge about the association of the *IL-18* SNPs and AR.

Statement of Ethics

Ethical approval was not obtained for this study as the data were not directly collected from patients.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

N.P. and P.T.: conceptualization. N.P. and P.T.: formal analysis. P.T., N.P., and H.J.: investigation. N.P., P.T., and H.J.: methodology. N.P., P.T., and H.J.: resources. O.P.: supervision and validation. P.T. and N.P.: writing – original draft. O.P.: writing – review and editing.

Data Availability Statement

All relevant data are within the paper and its supporting information files.

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IL-18 AR Supplementary file

S1 Table Genotype data of the included studies that examined Interleukin-18 polymorphism associations with allergic rhinitis

	First author	Ethnicity	<i>IL-18</i> Poly- morphism	Case			Control			minor allele frequency	HWE
				<i>wt-wt</i>	<i>var- wt</i>	<i>var- var</i>	<i>wt-wt</i>	<i>var- wt</i>	<i>var- var</i>		
RS1											
1	Ibrahim	African	rs187238	2	7	11	3	1	1	0.300	0.241
2	Ibrahim (L)	African	rs360721	11	11	1	6	1	1	0.188	0.095
3	Birbian	Asian	rs187238	235	81	7	52	28	7	0.241	0.258
4	Jumpasri (L)	Asian	rs360721	120	30	3	43	9	1	0.104	0.526
5	Nieters	Caucasian	rs187238	157	138	23	172	130	19	0.262	0.389
6	Sebelova	Caucasian	rs187238	286	216	37	161	125	26	0.284	0.803
7	Sebelova	Caucasian	rs360721	281	206	52	150	130	32	0.311	0.626
8	Tungtrongchitr	Asian	rs187238	22	7	1	24	5	1	0.117	0.295
9	Tungtrongchitr (L)	Asian	rs360721	23	8	2	26	6	1	0.121	0.400
10	Wu	Asian	rs187238	57	10	4	83	32	5	0.175	0.402
RS2											
1	Lee	Asian	rs1946518	30	86	44	56	58	52	0.488	0.00012
2	Nieters	Caucasian	rs1946518	101	161	54	116	157	48	0.394	0.665
3	Sebelova	Caucasian	rs1946518	208	259	72	111	164	37	0.381	0.044
4	Tungtrongchitr	Asian	rs1946518	8	15	7	13	12	5	0.367	0.447
5	Wu	Asian	rs1946518	23	39	9	30	49	41	0.546	0.053

IL-18: interleukin-18; (L): Laplace-corrected genotype data; wt: wild-type; var: variant; HWE: Hardy-Weinberg Equilibrium; values in bold indicate deviations from the HWE

S2 Table Linkage disequilibrium (LD) matrix of Interleukin -18 single nucleotide polymorphisms based on CEU and CHB populations

RS_number		rs360721	rs187238	rs1946518
D' / r ²		-133G>C	-137G>C	-607A>C
rs360721	-133G>C	1.0 / 1.0	1.0 / 0.88	0.83 / 0.18
rs187238	-137G>C	1.0 / 0.88	1.0 / 1.0	1.0 / 0.23
rs1946518	-607A>C	0.83 / 0.18	1.0 / 0.23	1.0 / 1.0

IL-18: interleukin-18; CEU: European population source for genomic dataset; CHB: Han Chinese population source for genomic dataset; G: guanine; A: adenine; C: cytosine; D' / r² are measures of LD

S3 Table PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving a rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search, and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in the systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe the method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing the risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	NA
Summary	13	State the principal summary measures (e.g., risk ratio, the difference in means).	8

measures			
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8-9
Risk of bias across studies	15	Specify any assessment of the risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8-9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8-9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9
Risk of bias within studies	19	Present data on the risk of bias of each study and, if available, any outcome level assessment (see item 12).	NA
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9-10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-10
Risk of bias across studies	22	Present results of any assessment of the risk of bias across studies (see Item 15).	NA
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policymakers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence and implications for future research.	14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); the role of funders for the systematic review.	NA