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Review

Original Articles

M. Omraninava[1], M. M. Eslami[2], S. Aslani[3], B. Razi[2], D. Imani[4], S. Feyzinia[5,6]

Interleukin 13 gene polymorphism and susceptibility to asthma: a meta-regression and meta-analysis

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Key words

Asthma; Meta-analysis; IL-13; polymorphism; genetic susceptibility.

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Impact statement

According to this meta-analysis, IL13 gene rs20541 and rs1800925 polymorphisms were associated with an increased susceptibility to asthma in the overall analysis and in the Caucasian and Asian populations.

Summary

Background*. Previously, lots of studies researched the association of interleukin (IL) 13 gene polymorphisms and the risk of asthma, yielding incongruent outcomes.* ***Objective.*** *To resolve the inconsistency among the different studies, we performed the most up-to-date meta-analysis of IL13 gene rs20541 and rs1800925 polymorphisms and susceptibility to asthma.* ***Methods****. After a systematic literature search up to September 2020, the pooled odds ratio (OR) and their corresponding 95% CI were extracted to determine the association level.* ***Results****. Overall, 45 (containing 10572 cases and 11575 healthy controls) and 31 (containing 10139 cases and 13304 healthy controls) case-control studies for rs20541 and rs1800925 polymorphisms, respectively, were retrieved. Pooled analysis indicated statistically significant association of rs20541 with asthma in the overall analysis. According to the subgroup analysis, significant association was detected between rs20541 polymorphism in European population across dominant model, allelic model, and GA vs GG model. A strongly significant association between rs20541 polymorphism and asthma risk was identified in Asian population under all genetic models except heterozygote model. There was significant association between rs20541 polymorphism and asthma risk in dominant, allelic and heterozygote models for Caucasians. The rs1800925 single-nucleotide polymorphism (SNP) was associated with asthma risk in some genetic models for the overall, Asian, and European populations.* ***Conclusions****. Both rs20541 and rs1800925 polymorphisms of IL13 gene confer a risk factor for asthma in different populations.*

Introduction

Asthma is a chronic inflammatory disorder of the airways that is characterized by reversible airflow obstruction and bronchospasms, affecting 300 million people worldwide and estimated to approach 400 million by 2025 (1, 2). It is a multifactorial disease caused by interactions between multiple genetic and environmental factors, and heritability of asthma has been estimated as 35 to 75% (3, 4). Therefore, genetic susceptibility may play a critical role in the pathogenesis of asthma. A growing body of research has reported more than 100 genes affect predisposing to asthma (5).
T helper (Th) 2 cytokines, including interleukin (IL)-4 and IL1-3 play an important role in the pathogenesis of asthma (6, 7). IL-13 induces many cellular responses, including overexpression of adhesion molecules, antibody class switching to IgE, development of airway hyper-responsiveness (AHR), and proliferation of goblet cells (8, 9). *In vitro* experiments by Walter *et al*. indicated that despite inflammation and Th2 active response, the challenge of IL-13-deficient mice by allergen failed to develop allergen-induced AHR (10). Also, Huang *et al*. demonstrated that IL-13 expression level was raised in the allergen-challenged broncho-alveolar lavage (BAL) of asthmatic patients (11).
The *IL13* gene is located on chromosome 5q31 that codifies for a 13-kDa glycoprotein (12). Two common single nucleotide polymorphisms (SNPs) of *IL13* gene have been studied extensively in susceptibility to asthma (13). The polymorphism of rs20541 (R130Q or Arg130Gln) is located in exon 4, and results in reduced affinity of IL-13 for IL-13 receptor, and enhanced expression of IL-13 in subjects with asthma (14, 15). The other SNP, rs1800925 (1112C/T), is located in the promoter region of *IL13* gene and affects the expression of IL-13 by changing the binding of Signal transducer and activator of transcription (STAT) transcription factors to the promoter region of IL-13 (16, 17). Numerous studies examined the association between these polymorphisms and susceptibility to asthma, but the results are inconsistent and conflicting. As a result, we performed the most up-to-date meta-analysis to determine the association between *IL13* gene rs20541 and rs1800925 polymorphisms and the risk of asthma.

Methods

This study was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement (18). The current meta-analysis does not contain any studies with human participants or animals performed by any of the authors.

Publication search

Two major electronic databases (Scopus and PubMed/Medline) were searched systematically to identify potential studies published up to September 2020. The combination of following key words and Medical Subject Headings (Mesh) terms were applied: ("asthma" (Mesh) OR "asthmatic") AND ("interleukin-13" OR "IL-13" OR "IL13" OR "rs20541" OR "rs1800925") AND ("single nucleotide polymorphism" OR "SNP" OR "polymorphisms" OR "mutation" OR "variation"). No restrictions were placed on language, sample size, population, or publication date. To avoid losing of eligible studies, reference list of the reviews and qualified studies were also scanned.

Inclusion and exclusion criteria

The following inclusion criteria were explored to identify eligible studies: 1) studies evaluating the association between *IL13* gene polymorphism (rs20541 and/or rs1800925) and susceptibility to asthma as the main outcome, 2) subjects diagnosed as having asthma based on the medical history/lung function or the final diagnosis by physician (additionally, we did not distinguish the studies for the grade of asthma severity), 3) studies with sufficient data to extract or calculate odds ratio (OR) and 95% confidence interval (CI), 4) studies which reported genotype distributions and allele frequency and 5) studies with cohort and case-control design. The reviews, meta-analysis, duplicates, case reports, book chapters, and animal studies all were excluded.

Data extraction

Two authors independently and in accordance with a standardized extraction form extracted the following information: the first author's name, journal and year of publication, country of origin, ethnicity, number of subjects in the case and the control groups for each gender, mean or range of age, genotyping method, genotype counts in the case and the control group. Any discrepancies between two authors were solved by discussion.

Quality assessment

We used Newcastle-Ottawa Scale (NOS) in order to evaluate methodological quality of the eligible studies (19). In this scale, selection, comparability of case/controls, exposure/outcome, age and gender were evaluated and scored ranging from 0 to 9 stars. We defined a range in which studies with scores of 0-3, 4-6, or 7-9 was regarded as low, moderate, or high-quality, respectively.

Statistical analysis

We used Pearson's χ^2 test to estimate the deviation from Hardy-Weinberg equilibrium (HWE) in the control group. Pooled OR and 95% CI were calculated to quantitatively evaluate the risk of asthma in the Dominant model (TT + CT *vs* CC), Recessive model (TT *vs* CT + CC), Allelic model (T *vs* C), Homozygote contrast (TT *vs* CC), and Heterozygote contrast (CT *vs* CC) for rs1800925 and Dominant model (AA + GA *vs* GG), Recessive model (AA *vs* GA + GG), Allelic model (A *vs* G), Homozygote contrast (AA *vs* GG), and Heterozygote contrast (GA *vs* GG) for rs20541. Between-study heterogeneity was identified by Q statistics (P-value < 0.1 was considered statistically significant) and I^2-test (I^2 values of 25%, 50%,

and 75% were described as low, moderate, and high heterogeneity, respectively). Studies were pooled using the DerSimonian-Laird algorithm in the presence of heterogeneity (I^2 > 50% and Q statistics < 0.1 (random effects model)) (20). On the other hand, the Mantel-Haenszel algorithm was used in the absence of heterogeneity (I^2 < 50% and Q statistics > 0.1 (fixed effects model)) (21). In order to assess the predefined sources of heterogeneity among included studies, subgroup analysis and meta-regression analysis based on year of population, the continent of the study population, and genotyping method were performed. Publication bias in this study was measured by Egger's regression asymmetry test and Begg's adjusted rank correlation test (21, 22). Finally, to show statistical stableness and the impact of every individual study on the pooled OR, the sensitivity analysis was conducted. Statistically analyses were carried out using STATA (version 14.0; Stata Corporation, College Station, TX) and SPSS (version 23.0; SPSS, Inc. Chicago, IL) software.

Results

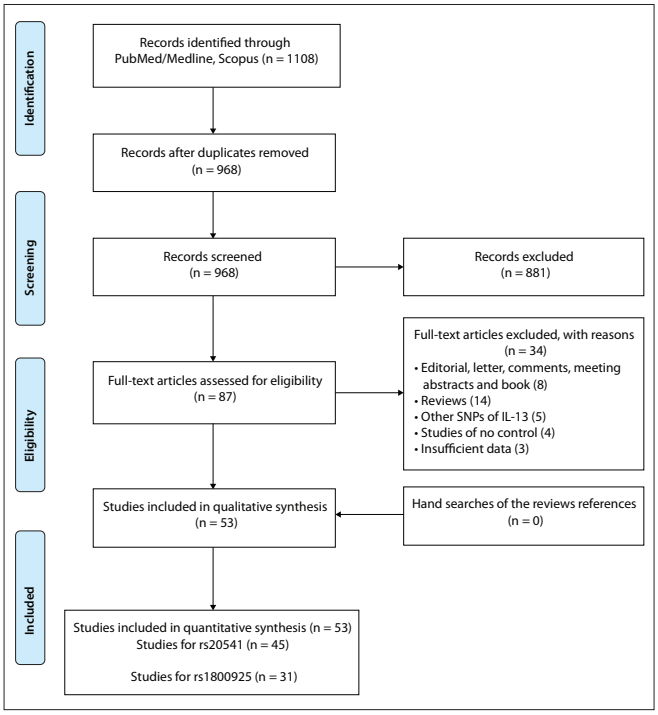
Study selection

The flow chart of implementing the inclusion/exclusion criteria and study selection process is summarized in the **figure 1**. A total of 1108 studies were retrieved from PubMed/Medline and Scopus databases according to the aforementioned key words and Mesh terms. Of them, 140 studies were duplicates and the remaining 968 studies were screened by title and abstract and full text. Finally, 53 publications met the inclusion criteria and were included for quantitative analysis. Of 53 eligible studies, 31 studies evaluated rs1800925 SNP and 45 studies evaluated rs20541 polymorphism. Among the included studies, some of them assessed one SNP (rs1800925 or rs20541) for two different ethnicities in one country and have reported the results in one paper; therefore, we considered them as two case-control studies in the quantitative analysis, but one article in the search strategy. All included studies were conducted between 1999 to 2018 and gained good methodological score, ranging from 5 to 8. Furthermore, case-control design was the most common type among the studies. **Table I** and **II** summarize the characteristics and genotype frequency of the included studies.

Meta-analysis of IL13 gene rs20541 SNP and the risk of asthma

Overall, 45 studies with 10572 cases and 11575 healthy controls were considered eligible and included for the pooled quantitative analysis (23-64). Among these 45 studies, 9 studies were in European countries, 26 studies in Asian countries, 8 studies in American countries, and one study in Africa and one in Oceania. The pooled OR for the association between rs20541 polymorphism and asthma risk revealed a significant correlation under all genotype models and highlighted this SNP as a predisposing factor for asthma: dominant model (OR = 1.18, 95% CI = 1.06-1.31, p < 0.001), recessive model (OR = 1.14, 95% CI = 1.03-1.27, p < 0.001), allelic model (OR = 1.16, 95% CI = 1.07-1.27, p < 0.001), AA *vs* GG model (OR = 1.17, 95% CI = 1.04-1.30, p < 0.001), and GA *vs* GG model (OR = 1.14, 95% CI = 1.02-1.26, p < 0.01; **figure 2**). The results of pooled ORs, heterogeneity tests and publication bias tests in different analysis models are shown in **table III**.

***Figure 1** - Flow diagram of study selection process.*



Subgroup meta-analysis of IL13 gene rs20541 SNP and the risk of asthma

We categorize studies into different subgroups on the basis of continent and ethnicity. The results of pooled ORs, heterogeneity tests and publication bias tests for different analysis models are shown in **table III**. The results of subgroup analysis of rs20541 polymorphism and the risk of asthma based on the continent showed different results. In details, there was no statistically association between rs20541 polymorphism and asthma risk in American population for all genetic models. Significant positive association was detected between rs20541 polymorphism and asthma risk in European population across dominant model (OR = 1.28, 95% CI = 1.06-1.53, p < 0.001), allelic model (OR = 1.17, 95% CI = 1.02-1.35, p = 0.02), and GA *vs* GG model (OR = 1.33, 95% CI = 1.14-1.56, p < 0.001), but not recessive and homozygote model. Furthermore, the results revealed a strong significant association between rs20541 polymorphism and asthma risk in Asian population under all genotype models except heterozygote model.

Table I *- Characteristics of studies included in meta-analysis of overall asthma.*

Study author	Year	Country	Continent	Ethnicity	Mean age Cases/Controls	Total cases / control	Genotyping method	Quality score
***IL13* (rs20541)**								
Hakonarson *et al.*	2001	Iceland	European	Caucasian	38 / NR	94 / 94	Ampli Taq Gold	5
Howard *et al.*	2001	Netherlands	European	Caucasian	52.1 / 51	152 / 120	TaqMan	6
Kauppi *et al.*	2001	Finland	European	Caucasian	NR / NR	163 / 132	Length-multiplexed single-base extension	6
Leung *et al.*	2001	China	Asian	East-Asian	10.3 / 11.4	157 / 54	RFLP-PCR	5
Xi *et al.*	2004	China	Asian	East-Asian	NR / NR	43 / 31	RFLP-PCR	5
Liu *et al.*	2004	China	Asian	East-Asian	NR / NR	100 / 100	RFLP-PCR	6
Donfack *et al.(i)*	2005	USA	American	Mixed	NR / NR	205 / 183	LAS	6
Donfack *et al.(ii)*	2005	USA	American	Mixed	NR / NR	126 / 205	LAS	6
Zhao *et al.*	2005	China	Asian	East-Asian	NR / NR	130 / 100	RFLP-PCR	6
Bernstein *et al.*	2005	USA	American	Mixed	NR / NR	62 / 79	RFLP-PCR	5
Wei *et al.*	2007	China	Asian	East-Asian	NR / NR	32 / 20	RFLP-PCR	5
Hosseini *et al.*	2007	Iran	Asian	Middle-East	34 ± 11 / 33 ± 9	30 / 50	RFLP-PCR	5
Battle *et al.*	2007	USA	American	Mixed	19.4 / 29.9	261 / 174	RFLP-PCR	7
Chan *et al.*	2008	China	Asian	East-Asian	10.4 / 11	273 / 141	RFLP-PCR	6
Kang *et al.*	2008	South Korea	Asian	East-Asian	9.71 ± 2.4 / 10.19 ± 2	374 / 229	RFLP-PCR	7
Kim *et al.*	2008	South Korea	Asian	East-Asian	9.13 ± 2.65 / 10.20 ± 2.68	709 / 227	RFLP-PCR	7
Black *et al.*	2009	UK	European	Caucasian	NR / NR	275 / 2462	Multiplex PCR	8
Daley *et al.*	2009	Australia	Oceania	Caucasian	NR / NR	644 / 750	Illumina Assay	8
Jiang *et al.*	2009	China	Asian	East-Asian	NR / NR	24 / 24	RFLP-PCR	5
Lianes *et al.*	2009	Spain	European	Caucasian	22.98 / 37.6	108 / 50	RFLP-PCR	5
Wang *et al.*	2009	Taiwan	Asian	East-Asian	7.82 ± 3.81 / 8.37 ± 2.45	446 / 505	TaqMan	8
Bottema *et al.*	2010	Dutch	European	Caucasian	31.8 ± 8.3 / 26.9 ± 5.4	114 / 89	Mass Array	5
Wu *et al.*	2010	China	Asian	East-Asian	8.8 / 9.2	252 / 227	RFLP-PCR	7
Yang *et al.*	2010	China	Asian	East-Asian	NR / NR	178 / 158	NR	6
Zdorova *et al.*	2010	Russia	European	Caucasian	NR / NR	283 / 227	MALDI-TOF mass spectrometry	7
Palikhe *et al.*	2010	Korea	Asian	East-Asian	42.29 ± 13.2 / 33.46 ± 14	463 / 430	SNAP shot	8
Undarmaa *et al.(i)*	2010	Japan	Asian	East-Asian	9.9 / 9.3	325 / 339	TaqMan	7
Undarmaa *et al.(ii)*	2010	Japan	Asian	East-Asian	45 / 43.2	367 / 676	TaqMan	8
Baye *et al.(i)*	2011	USA	American	Mixed	10.1 / 12	413 / 298	IGGAS	7
Baye *et al.(ii)*	2011	USA	American	Mixed	10.3 / 11.4	315 / 51	IGGAS	5
Yang *et al.*	2011	China	Asian	East-Asian	41.59 / 41.16	201 / 203	MALDI-TOF	6
Munoz *et al.*	2012	Mexico	American	Mixed	7.24 / 7.28	90 / 105	TaqMan	5
Liu *et al.*	2013	China	Asian	East-Asian	3-12 / 18.35	384 / 384	TaqMan	7
Shazia *et al.*	2013	Pakistan	Asian	South-Asian	NR / NR	214 / 120	RFLP-PCR	6

→

Study author	Year	Country	Continent	Ethnicity	Mean age Cases/Controls	Total cases / control	Genotyping method	Quality score
Berenguer *et al.*	2014	Portugal	European	Caucasian	NR / NR	98 / 105	TaqMan	6
Aguilar *et al.*	2014	Mexico	American	Mixed	10.8 ± 2.9 / NR	421 / 430	TaqMan	8
Ramphul *et al.*	2015	Mauritius	African	African	17.1 / 18.22	189 / 189	TaqMan	7
Davoodi *et al.*	2015	India	Asian	South-Asian	NR / NR	100 / 50	Mass Array	6
Hua *et al.*	2015	China	Asian	East-Asian	4.9 / 23.32	1000 / 1000	TaqMan	8
Resende *et al.*	2016	Poland	European	Caucasian	11.5 / 12.1	147 / 192	RFLP-PCR	6
Wan *et al.*	2016	China	Asian	East-Asian	39.32 / 56.06	103 / 125	TaqMan	5
Adjers *et al.*	2017	Singapore	Asian	Southeast -Asian	10.3 ± 8.2 / 10.0 ± 9.1	118 / 70	TaqMan	5
Alasandagutti *et al.*	2017	India	Asian	South-Asian	NR / NR	120 / 120	RFLP-PCR	5
Halwani *et al.*	2017	Saudi Arabia	Asian	Middle-East	NR / NR	232 / 228	Sanger-sequenced	6
Zhang *et al.*	2018	China	Asian	East-Asian	9.00 ± 2.78/ 8.21 ± 2.72	37 / 29	TaqMan	5
***IL13* (rs1800925)**								
Kraan *et al.*	1999	Netherlands	European	Caucasian	NR / NR	101 / 107	EMSA	5
Howard *et al.*	2001	Netherlands	European	Caucasian	52.1 / 51	171 / 119	TaqMan	6
Donfack *et al.(i)*	2005	USA	American	Mixed	NR / NR	126 / 205	LAS	6
Donfack *et al.(ii)*	2005	USA	American	Mixed	NR / NR	205 / 183	LAS	6
Moissidis *et al.*	2005	USA	American	Mixed	NR / NR	61 / 157	RFLP-PCR	5
Battle *et al.*	2007	USA	American	Mixed	24.5 /29.9	261 / 174	RFLP-PCR	7
Kang *et al.*	2008	South Korea	Asian	East-Asian	9.71 ± 2.42 / 10.19 ± 2.91	374 / 241	RFLP-PCR	7
Kim *et al.*	2008	South Korea	Asian	East-Asian	9.13 ± 2.65 / 10.20 ± 2.68	716 / 241	RFLP-PCR	7
Black *et al.*	2009	UK	European	Caucasian	NR / NR	263 / 2362	Multiplex PCR	8
Daley *et al.*	2009	Australia	Oceania	Caucasian	NR / NR	642 / 751	Illumina Assay	8
Wang *et al.*	2009	Taiwan	Asian	East-Asian	7.820 ± 3.81 / 8.37 ± 2.45	446 / 511	TaqMan	8
Lianes *et al.*	2009	Spain	European	Caucasian	22.9 /37.6	109 / 50	RFLP-PCR	5
Bottema *et al.*	2010	Netherlands	European	Caucasian	31.8 ± 8.3 / 26.9 ± 5.4	115 / 92	Mass ARRAY	5
Dewan *et al.*	2010	USA	American	Mixed	NR / NR	104 / 503	Affymetrix	7
Zdorova *et al.*	2010	Russia	European	Caucasian	NR / NR	283 / 227	MALDI-TOF	7
Undarmaa *et al.*	2010	Japan	Asian	East-Asian	9.9 / 9.3	325 / 336	TaqMan	7
Wu *et al.*	2010	China	Asian	East-Asian	8.8 / 9.2	252 / 227	PCR-RFLP	7
Baye *et al.(i)*	2011	USA	American	Mixed	NR / NR	413 / 298	IGGAS	7
Baye *et al.(ii)*	2011	USA	American	Mixed	NR / NR	315 / 51	IGGAS	5
Noguch *et al.*	2011	Japan	Asian	East-Asian	NR / NR	938 / 2376	GWAS	8
Yang *et al.*	2011	China	Asian	East-Asian	41.59 / 41.16	193 / 204	MALDI-TOF	6
Munoz *et al.*	2012	Mexico	American	Mixed	7.20 ± 0.9 / 7.5 ± 0.86	90 / 111	TaqMan	5
Liu *et al.*	2012	China	Asian	East-Asian	3.12 / 18.36	384 / 384	TaqMan	7
Dixit *et al.*	2014	India	Asian	South-Asian	6.29 ± 3.28 / 6.08 ± 3.22	275 / 275	RFLP-PCR	7
Li *et al.*	2014	China	Asian	East-Asian	8.4 ± 2.7 / 7.9 ± 3.2	491 / 505	TaqMan	8
Aguilar *et al.*	2014	Mexico	American	Mixed	10.8 ± 2.9 / NR	421 / 430	TaqMan	8

Study author	Year	Country	Continent	Ethnicity	Mean age Cases/Controls	Total cases / control	Genotyping method	Quality score
Li *et al.*	2015	China	Asian	East-Asian	5 / 5.06	652 / 752	SNaP Shot Assay	8
Ramphul *et al.*	2015	Mauritius	African	African	12.4 / 18.22	190 / 187	TaqMan	6
Hua *et al.*	2015	China	Asian	East-Asian	4.9 / 23.32	1000 / 1000	TaqMan	8
Wan *et al.*	2016	China	Asian	South-Asian	10.32 ± 8.23 / 10.09 ± 9.16	103 / 125	RFLP-PCR	5
Alasandagutti *et al.*	2017	India	Asian	South-Asian	34.1 / 34.1	120 / 120	RFLP-PCR	5

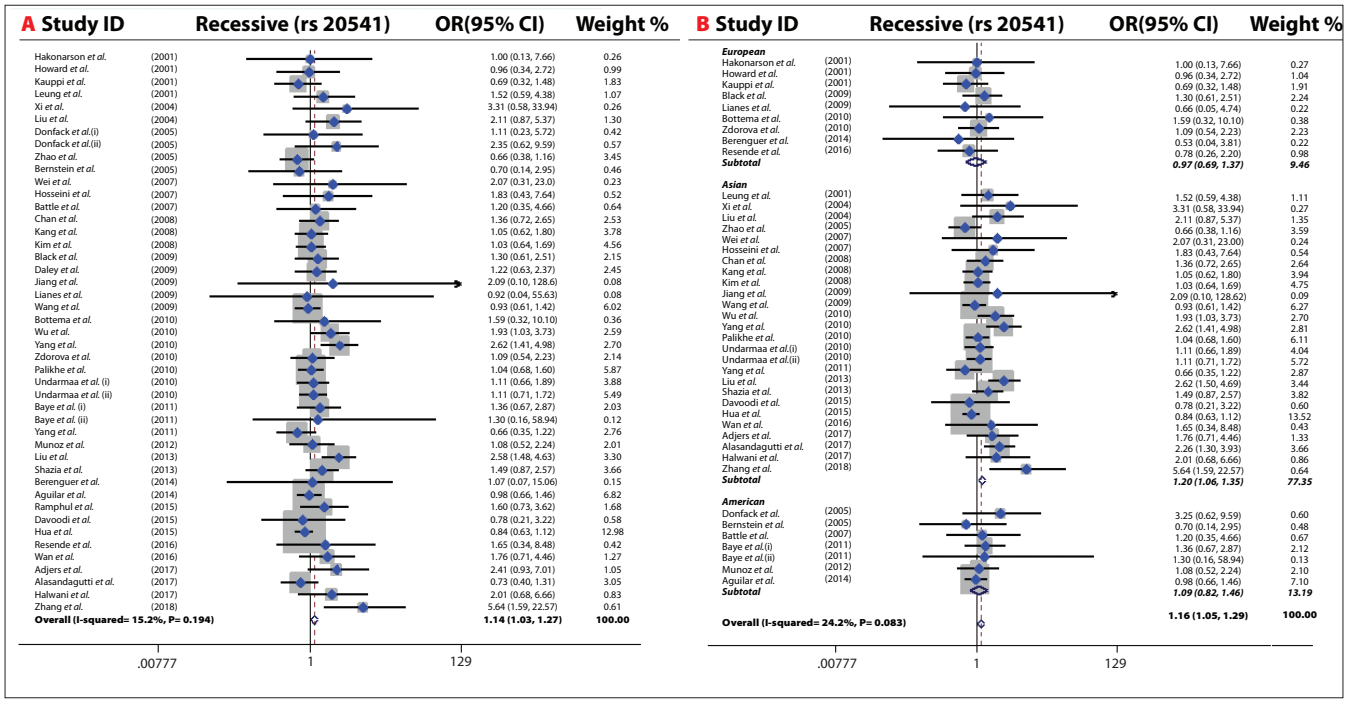
NR: not reported.

For more detailed information, we stratified the included studies based on ethnicity. According to this subgroup, people with mixed ethnicity which were mostly combination of Caucasians, Latins, and American-Africans were not affected by rs20541 polymorphism. However, there was significant association between rs20541 polymorphism and asthma risk in dominant, allelic and heterozygote models for Caucasian population. Eventually we categorized Asian populations to East-Asian, South-Asian and Middle-East. Interestingly, significant associations were observed in the South-Asian and Middle-East populations, but no significant association was observed between rs20541 polymorphism and asthma risk in East-Asian populations (**table III**).

Meta-analysis of IL13 gene rs1800925 SNP and the risk of asthma

Overall, 31 studies containing 10139 cases and 13304 healthy controls were included to evaluate the association between *IL13* gene rs1800925 polymorphism and asthma risk (24, 29, 33, 35-38, 41-45, 47, 48, 50, 51, 54, 55, 59, 61, 65-71). Of which, 6 studies were in European countries, 14 studies were in Asian countries, 9 studies were in American countries, 1 study in Africa, and 1 in Oceania. The results of overall pooled analysis indicated no significant association between *IL13* gene rs1800925 polymorphism and asthma risk under four genotype models. However, a protective significant association was observed just in

Figure 2 - *Pooled odds OR and 95% confidence interval of individual studies and pooled data for the association IL-13 rs20541 polymorphism and the risk of asthma in overall populations and subgroup analysis for A: recessive model (overall population); B: recessive model (subgroup analysis).*



recessive model (OR = 0.85, 95% CI = 0.78-0.94, p < 0.001). **Figure 3** shows the overall analysis and subgroup analysis of *IL13* gene rs1800925 SNP in the dominant model.

Subgroup meta-analysis of IL13 gene rs1800925 SNP and the risk of asthma

Similar to rs20541, we stratified eligible studies based on the continent which studies were performed as well as the ethnicity. The analyses demonstrated that rs1800925 was not significantly associated with the risk of asthma in the American population. However, significant association was detected for European populations under dominant model (OR = 1.52, 95% CI = 1.24-1.86, p < 0.001), allelic model (OR = 1.40, 95% CI = 1.02-1.90, p = 0.03), and CT *vs* CC model (OR = 1.51, 95% CI = 1.23-1.87, p < 0.001), but not recessive and homozygote models. In addition, rs1800925 SNP was detected as a protective SNP in Asians for the TT *vs* CC (OR = 0.82, 95% CI = 0.69-0.97, p = 0.02) and the recessive models (OR = 0.81, 95% CI = 0.71-0.91, p < 0.001). Furthermore, ethnicity-specific subgroup indicated associations between rs1800925 and asthma risk in Caucasians (dominant model (OR = 1.37, 95% CI = 1.03-1.82, p = 0.03), TC *vs* CC model (OR = 1.33, 95% CI = 1.03-1.72, p = 0.03)), East-Asians (recessive model (OR = 0.82, 95% CI = 0.72-0.93, p < 0.001), TT *vs* CC model (OR = 0.79, 95% CI = 0.65-0.95, p = 0.01)), South-Asians (TC *vs* CC model (OR = 2.91, 95% CI = 1.17-7.21, p = 0.03)), but not countries with mixed ethnicity (**table III**).

Publication bias

Publication bias was estimated by using funnel plot, Begg's and Egger's tests. No evidence of publication bias was seen for overall population and subgroup analysis under all genetic models. Additionally, the shape of the funnel plot for the dominant model of rs1800925 and rs20541 SNPs appeared to be symmetrical, demonstrating that there was no significant publication bias (**figure 4**).

Sensitivity analysis

Sensitivity analysis was conducted after successive omission of each eligible study. The significance of the pooled ORs was not affected by any single study, indicating that our results were statistically robust (**figure 5**).

Meta-regression analyses

Meta-regression analyses were performed to explore potential sources of heterogeneity among included studies (**table IV**). The findings indicated that none of the expected heterogeneity parameters were the source of heterogeneity (**figure 6**).

Discussion

Up to now, numerous replication case-control investigations have tried to decipher the association of *IL13* gene polymorphisms and risk of asthma. That notwithstanding, these disperse investigation demonstrated incongruous reports. Diversity in the race of included populations, heterogenicity in the diagnostic criteria of the diseases, lack of statistical power, limited sample sizes, and the linkage disequilibrium (LD) between different genes or variations may be the underlying cause of such conflicting observations (72).

Figure 3 *- Pooled odds OR and 95% confidence interval of individual studies and pooled data for the association IL-13 rs1800925 polymorphism and the risk of asthma in overall populations and subgroup analysis for A: Dominant model (overall population); B: Dominant model (subgroup analysis).*

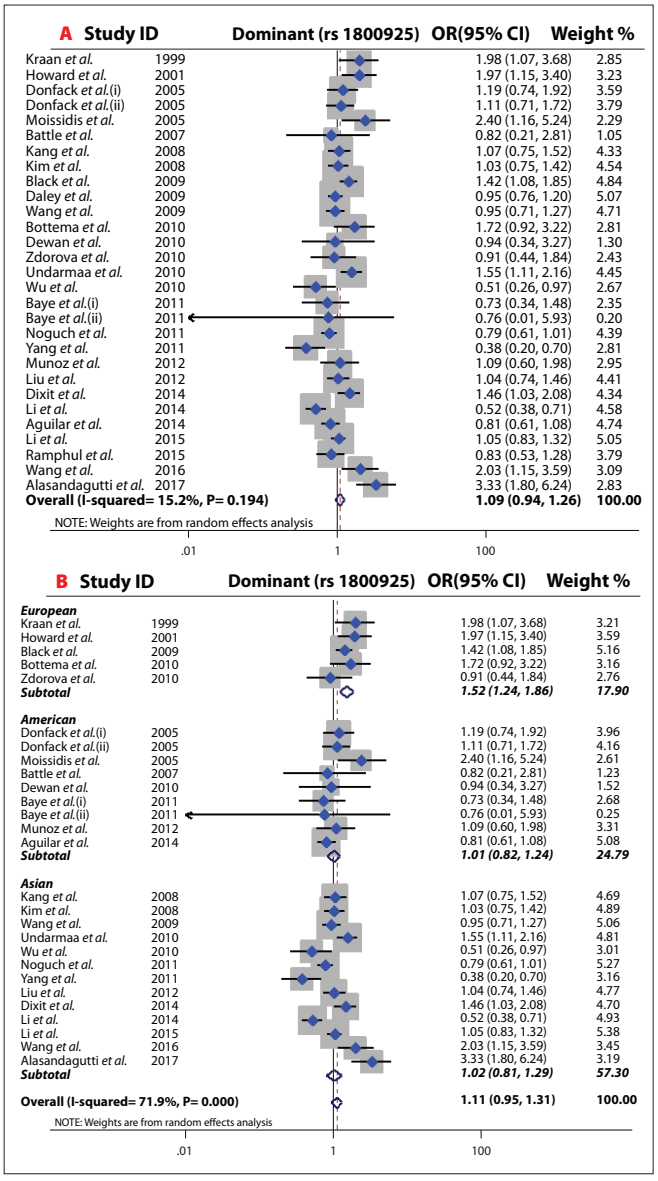


Table II *- Distribution of genotype and allele among asthma patients and controls.*

Study author	Asthma cases					Healthy control					P-HWE	MAF
	GG	GA	AA	G	A	GG	GA	AA	G	A		
***IL13* (rs20541)**												
Hakonarson *et al.*	66	25	3	157	31	64	27	3	155	33	0/94	0/175
Howard *et al.*	89	52	11	230	74	67	44	9	178	62	0/63	0/258
Kauppi *et al.*	64	82	17	210	116	62	51	19	175	89	0/11	0/337
Leung *et al.*	54	74	29	182	132	21	26	7	68	40	0/81	0/37
Xi *et al.*	10	25	8	45	41	16	13	2	45	17	0/76	0/274
Liu *et al.*	27	54	19	108	92	44	46	10	134	66	0/68	0/33
Donfack *et al.(i)*	132	68	5	332	78	126	53	4	305	61	0/56	0/166
Donfack *et al.(ii)*	78	41	7	197	55	127	73	5	327	83	0/14	0/202
Zhao *et al.*	18	60	52	96	164	8	42	50	58	142	0/84	0/71
Bernstein *et al.*	32	26	4	90	34	48	24	7	120	38	0/13	0/24
Wei *et al.*	18	8	6	44	20	15	3	2	33	7	0/03	0/175
Hosseini *et al.*	11	13	6	35	25	34	10	6	78	22	0/003	0/22
Battle *et al.*	171	81	9	423	99	117	52	5	286	62	0/78	0/178
Chan *et al.*	94	136	43	324	222	54	70	17	178	104	0/43	0/368
Kang *et al.*	160	166	48	486	262	101	100	28	302	156	0/67	0/34
Kim *et al.*	301	318	90	920	498	99	100	28	298	156	0/72	0/343
Black *et al.*	166	98	11	430	120	1729	657	76	4115	809	0/16	0/164
Daley *et al.*	426	196	22	1048	240	520	209	21	1249	251	0/99	0/167
Jiang *et al.*	20	2	2	42	6	18	5	1	41	7	0/42	0/145
Lianes *et al.*	68	38	2	174	42	41	8	1	90	10	0/43	0/1
Wang *et al.*	203	194	49	600	292	212	234	59	658	352	0/64	0/348
Bottema *et al.*	57	51	6	165	63	62	24	3	148	30	0/72	0/168
Wu *et al.*	105	111	36	321	183	125	84	18	334	120	0/46	0/264
Yang *et al.*	71	60	47	202	154	73	66	19	212	104	0/49	0/329
Zdorova *et al.*	144	116	23	404	162	125	85	17	335	119	0/62	0/262
Palikhe *et al.*	207	200	56	614	312	206	174	50	586	274	0/15	0/318
Undarmaa *et al.(i)*	145	144	36	434	216	156	149	34	461	217	0/85	0/32
Undarmaa *et al.(ii)*	166	162	39	494	240	322	289	65	933	419	0/98	0/309
Baye *et al.(i)*	230	157	26	617	209	183	101	14	467	129	0/98	0/216
Baye *et al.(ii)*	220	87	8	527	103	36	14	1	86	16	0/78	0/156
Yang *et al.*	105	73	23	283	119	90	80	33	260	146	0/03	0/359
Munoz *et al.*	17	52	21	86	94	17	65	23	99	111	0/01	0/528
Liu *et al.*	180	154	50	514	254	199	164	21	562	206	0/08	0/268
Shazia *et al.*	81	64	69	226	202	47	44	29	138	102	< 0.001	0/425
Berenguer *et al.*	69	27	2	165	31	71	32	2	174	36	0/45	0/171
Aguilar *et al.*	156	202	63	514	328	176	189	65	541	319	0/22	0/37
Ramphul *et al.*	93	76	20	262	116	83	93	13	259	119	0/05	0/314
Davoodi *et al.*	89	3	8	181	19	45	0	5	90	10	< 0.001	0/1

Study author	Asthma cases					Healthy control					P-HWE	MAF
	GG	GA	AA	G	A	GG	GA	AA	G	A		
Hua *et al.*	470	423	107	1363	637	390	486	124	1266	734	0/14	0/367
Resende *et al.*	92	50	5	234	60	136	52	4	324	60	0/7	0/156
Wan *et al.*	43	45	15	131	75	71	43	11	185	65	0/23	0/26
Adjers *et al.*	28	65	25	121	115	34	29	7	97	43	0/82	0/307
Alasandagutti *et al.*	67	19	34	153	87	43	35	42	121	119	< 0.001	0/495
Halwani *et al.*	141	79	12	361	103	175	47	6	397	59	0/19	0/129
Zhang *et al.*	7	10	20	24	50	11	13	5	35	23	0/73	0/396
Study author	**Asthma cases**					**Healthy control**					**P-HWE**	**MAF**
	CC	**CT**	**TT**	**C**	**T**	**CC**	**CT**	**TT**	**C**	**T**		
***IL13* (rs1800925)**												
Kraan *et al.*	57	31	13	145	57	77	28	2	182	32	0/76	0/149
Howard *et al.*	99	63	9	261	81	87	30	2	204	34	0/74	0/142
Donfack *et al.(i)*	72	42	12	186	66	126	71	8	323	87	0/6	0/212
Donfack *et al.(ii)*	69	100	36	238	172	66	85	32	217	149	0/6	0/407
Moissidis *et al.*	13	36	12	62	60	62	75	20	199	115	0/71	0/366
Battle *et al.*	9	81	171	99	423	5	52	117	62	286	0/78	0/821
Kang *et al.*	236	128	10	600	148	156	79	6	391	91	0/27	0/188
Kim *et al.*	455	236	25	1146	286	155	80	6	390	92	0/24	0/19
Black *et al.*	158	98	7	414	112	1609	673	80	3891	833	0/35	0/176
Daley *et al.*	425	195	22	1045	239	490	234	27	1214	288	0/88	0/191
Wang *et al.*	321	113	12	755	137	357	136	18	850	172	0/26	0/168
Lianes *et al.*	36	53	20	125	93	17	14	19	48	52	< 0.001	0/52
Bottema *et al.*	67	43	5	177	53	65	23	4	153	31	0/3	0/168
Dewan *et al.*	5	34	65	44	164	23	171	309	217	789	0/91	0/784
Zdorova *et al.*	23	116	144	162	404	17	85	125	119	335	0/62	0/737
Undarmaa *et al.*	186	119	20	491	159	227	98	11	552	120	0/91	0/178
Wu *et al.*	36	111	105	183	321	18	84	125	120	334	0/46	0/735
Baye *et al.(i)*	26	157	230	209	617	14	101	183	129	467	0/98	0/783
Baye *et al.(ii)*	8	87	220	103	527	1	14	36	16	86	0/78	0/843
Noguch *et al.*	113	438	387	664	1212	232	1033	1111	1497	3255	0/71	0/684
Yang *et al.*	144	43	6	331	55	148	50	6	346	62	0/48	0/151
Munoz *et al.*	45	34	11	124	56	58	46	7	162	60	0/59	0/27
Liu *et al.*	285	84	15	654	114	288	80	16	656	112	< 0.001	0/145
Dixit *et al.*	109	121	45	339	211	135	77	63	347	203	0	0/369
Li *et al.*	400	85	6	885	97	353	143	9	849	161	0/2	0/159
Aguilar *et al.*	202	177	42	581	261	185	189	56	559	301	0/48	0/35
Li *et al.*	450	187	15	1087	217	527	208	17	1262	242	0/5	0/16
Ramphul *et al.*	120	61	9	301	79	110	69	8	289	85	0/48	0/227
Hua *et al.*	683	282	35	1648	352	685	281	34	1651	349	0/43	0/174
Wan *et al.*	48	42	13	138	68	80	38	7	198	52	0/38	0/208
Alasandagutti *et al.*	67	41	12	175	65	97	12	11	206	34	0	0/141

P-HWE: P-value for Hardy-Weinberg equilibrium; MAF: minor allele frequency of control group.

Table III *- Main results of pooled ORs in meta-analysis of IL13 gene polymorphisms in asthma patients.*

Subgroup	Sample size		Test of association		Test of heterogeneity		Test of publication bias (Begg's test)		Test of publication bias (Egger's test)	
	Genetic model	Case/Control	OR	95% CI (P-value)	I^2 (%)	p	z	p	t	p
IL13 (rs20541)										
Overall	Dominant model		1.18	1.06 - 1.31 (< 0.001)	59.3	< 0.001	1.09	0.27	2.59	0.01
	Recessive model		1.14	1.03 - 1.27 (< 0.001)	15.1	0.19	1.25	0.21	2.04	0.04
	Allelic model	10572 / 11575	1.16	1.07 - 1.27 (< 0.001)	63.3	< 0.001	1.52	0.12	3.09	0.004
	AA *vs* GG		1.117	1.04 - 1.30 (< 0.001)	36.4	0.06	0.97	0.33	2.48	0.01
	GA *vs* GG		1.14	1.02 - 1.26 (0.01)	53.2	< 0.001	0.16	0.87	1.67	0.10
Subgroup by continent										
Asian	Dominant model		1.20	1.04 - 1.38 (0.01)	62	< 0.001	1.70	0.09	3.09	0.005
	Recessive model		1.20	1.06 - 1.35 (< 0.001)	47.9	< 0.001	1.92	0.05	2.47	0.01
	Allelic model	6441 / 5767	1.22	1.08 - 1.38 (< 0.001)	71.7	< 0.001	1.83	0.06	3.51	0.002
	AA *vs* GG		1.20	1.05 - 1.36 (0.007)	54.1	< 0.001	1.65	0.09	3.11	0.005
	vs		1.02	0.94 - 1.11 (0.65)	53.1	0.83	0.75	0.45	1.96	0.06
European	Dominant model		1.28	1.06 - 1.53 (< 0.001)	26.6	0.20	0	1	0.62	0.55
	Recessive model		0.97	0.69 - 1.37 (0.87)	0	0.96	- 1.25	0.21	- 0.66	0.53
	Allelic model	1463 / 3568	1.17	1.02 - 1.35 (0.02)	19.7	0.26	-0.83	0.40	- 0.77	0.46
	AA *vs* GG		1.09	0.77 - 1.56 (0.61)	0	0.96	- 1.25	0.21	- 0.92	0.39
	GA *vs* GG		1.33	1.14 - 1.56 (< 0.001)	27.7	0.19	0	1	- 0.41	0.69
American	Dominant model		1.15	0.97 - 1.35 (0.10)	0	0.93	- 1.35	0.17	- 1.11	0.31
	Recessive model		1.09	0.82 - 1.46 (0.55)	0	0.90	0.45	0.65	0.97	0.37
	Allelic model	1688 / 1342	1.10	0.97 - 1.25 (0.12)	0	0.93	0.15	0.88	- 0.44	0.67
	AA *vs* GG		1.17	0.85 - 1.61 (0.32)	0	0.94	0.45	0.65	0.54	0.61
	GA *vs* GG		1.14	0.96 - 1.35 (0.14)	0	0.83	- 0.45	0.65	- 0.86	0.43
Subgroup by Ethnicity										
Caucasian	Dominant model		1.25	1.11 - 1.42 (< 0.001)	22.8	0.23	0.27	0.78	- 0.07	0.94
	Recessive model		1.02	0.75 - 1.38 (0.89)	0	0.97	- 1.70	0.08	- 0.96	0.36
	Allelic model	2107/ 4318	1.17	1.04 - 1.31 (< 0.001)	11.2	0.34	- 0.63	0.53	- 0.47	0.65
	AA *vs* GG		1.13	0.83 - 1.54 (0.43)	0	0.97	- 1.52	0.12	- 1.16	0.28
	GA *vs* GG		1.27	1.12 - 1.45 (< 0.001)	26.4	0.20	0.45	0.65	0.21	0.84
Mixed	Dominant model		1.15	0.97 - 1.35 (0.10)	0	0.93	- 1.35	0.17	- 1.11	0.31
	Recessive model		1.09	0.82 - 1.46 (0.55)	0	0.90	0.45	0.65	0.97	0.37
	Allelic model	1688 / 1342	1.10	0.97 - 1.25 (0.12)	0	0.93	0.15	0.88	- 0.44	0.67
	AA *vs* GG		1.17	0.85 - 1.61 (0.32)	0	0.94	0.45	0.65	0.54	0.61
	GA vs GG		1.14	0.96 - 1.35 (0.14)	0	0.83	- 0.45	0.65	- 0.86	0.43
East-Asian	Dominant model		1.02	0.94 - 1.10 (0.69)	57.8	< 0.001	1.49	0.13	2.44	0.02
	Recessive model		1.13	0.99 - 1.29 (0.06)	51.7	< 0.001	2.21	0.2	2.70	0.01
	Allelic model	5642 / 5074	1.14	1 - 1.29 (0.04)	70.1	< 0.001	1.56	0.11	2.76	0.01
	AA *vs* GG		1.13	0.98 - 1.30 (0.09)	59.1	< 0.001	1.69	0.09	2.61	0.01
	GA *vs* GG		0.98	0.90 - 1.07 (0.71)	39.5	0.03	0.39	0.69	1.620	0.12
Middle-East	Dominant model		2.30	1.55 - 3.41 (< 0.001)	0	0.35	1	0.31	-	-

→

Subgroup	Sample size		Test of association		Test of heterogeneity		Test of publication bias (Begg's test)		Test of publication bias (Egger's test)	
	Genetic model	Case/Control	OR	95% CI (P-value)	I^2 (%)	p	z	p	t	p
	Recessive model		1.94	0.79 - 4.74 (0.14)	0	0.92	- 1	0.31	-	-
	Allelic model	262 / 278	2.01	1.45 - 2.80 (< 0.001)	0	0.51	1	0.31	-	-
	AA *vs* GG		2.68	1.07 - 6.72 (0.03)	0	0.82	- 1	0.31	-	-
	GA *vs* GG		2.25	1.48 - 3.43 (< 0.001)	0.6	0.31	- 1	0.31	-	-
South-Asian	Dominant model		1.16	0.81 - 1.67 (0.40)	0	0.79	0.52	0.60	0.10	0.93
	Recessive model		1.71	1.18 - 2.49 (< 0.001)	19.8	0.28	- 0.52	0.60	- 1.06	0.48
	Allelic model	434 / 290	1.36	1.01 - 1.83 (0.04)	24.2	0.26	0.52	0.60	- 0.41	0.57
	AA *vs* GG		1.51	1 - 2.30 (0.51)	0	0.48	- 0.52	0.60	- 1.13	0.46
	GA *vs* GG		0.78	0.49 - 1.23 (0.28)	0	0.64	- 1	0.31	-	-
IL13 (rs1800925)										
Overall	Dominant model		1.09	0.94 - 1.26 (0.15)	70.3	< 0.001	0.54	0.58	0.95	0.35
	Recessive model		0.85	0.78 - 0.94 (< 0.001)	21.9	0.14	3.17	0.002	4.18	0.005
	Allelic model	10139/ 13304	1.04	0.95 - 1.15 (0.37)	70.1	< 0.001	2.48	0.01	3.03	0.005
	TT *vs* CC		0.90	0.79 - 1.03 (0.08)	29.8	0.06	2.21	0.02	3.47	0.003
	TC *vs* CC		1.11	0.98 - 1.27 (0.10)	61.9	< 0.001	0.68	0.49	0.98	0.33
Subgroup by continent										
Asian	Dominant model		1.02	0.81 - 1.29 (0.84)	81.6	< 0.001	0.73	0.46	0.46	0.65
	Recessive model		0.81	0.71 - 0.91 (< 0.001)	18.7	0.25	1.83	0.06	2.04	0.06
	Allelic model	5254/ 6251	1	0.84 - 1.18 (0.96)	81.2	< 0.001	0.61	0.54	1.48	0.16
	TT *vs* CC		0.82	0.69 - 0.97 (0.02)	50.9	0.01	1.46	0.14	1.66	0.12
	TC *vs* CC		1.06	0.84 -1.36 (0.61)	81.1	< 0.001	1.10	0.27	0.81	0.43
European	Dominant model		1.52	1.24 - 1.86 (< 0.001)	0	0.41	- 0.21	0.83	0.70	0.50
	Recessive model		0.93	0.67 - 1.28 (0.63)	42.5	0.13	2.71	0.002	5.21	0.001
	Allelic model	933 / 2907	1.40	1.02 - 1.90 (0.03)	70.9	< 0.001	1.88	0.06	2.44	0.04
	TT *vs* CC		1.16	0.70 - 1.90 (0.56)	42.7	0.13	0.21	0.83	1.44	0.19
	TC *vs* CC		1.51	1.23 - 1.87 (< 0.001)	0	0.74	- 0.42	0.67	0.66	0.53
American	Dominant model	1996 / 2112	1.01	0.82 - 1.24 (0.90)	10.1	0.35	- 0.98	0.32	0.31	0.77
	Recessive model		0.94	0.79 - 1.11 (0.46)	18.5	0.27	1.96	0.05	1.91	0.15
	Allelic model		1.01	0.87 - 1.18 (0.85)	39.6	0.10	0.98	0.32	1.53	0.22
	TT *vs* CC		0.99	0.75 - 1.32 (0.97)	34.9	0.13	1.96	0.05	3.01	0.05
	TC *vs* CC		0.98	0.81 - 1.19 (0.86)	0	0.65	- 0.98	0.32	- 0.07	0.94
Subgroup by Ethnicity										
Caucasian	Dominant model		1.37	1.03 - 1.82 (0.03)	61.5	0.02	- 0.19	0.85	1.37	0.24
	Recessive model		0.93	0.70 - 1.24 (0.62)	28.2	0.22	1.32	0.18	2.02	0.07
	Allelic model	1575 / 3658	1.28	0.99 - 1.65 (0.05)	72.9	< 0.001	1.32	0.18	2.30	0.08
	TT *vs* CC		1.06	0.72 - 1.57 (0.75)	31.2	0.20	2.07	0.03	3.04	0.04
	TC *vs* CC		1.33	1.03 - 1.72 (0.03)	48.9	0.08	- 0.94	0.34	1.13	0.32
Mixed	Dominant model		1.01	0.82 - 1.24 (0.90)	10.1	0.35	- 0.21	0.83	0.70	0.50
	Recessive model		0.94	0.79 - 1.11 (0.46)	18.5	0.27	2.71	0.002	5.21	0.001
	Allelic model	1996 / 2112	1.01	0.87 - 1.18 (0.85)	39.6	0.10	1.88	0.06	2.44	0.04
	TT *vs* CC		0.99	0.75 - 1.32 (0.97)	34.9	0.13	0.21	0.83	1.44	0.19

Subgroup	Sample size		Test of association		Test of heterogeneity		Test of publication bias (Begg's test)		Test of publication bias (Egger's test)	
	Genetic model	Case/Control	OR	95% CI (P-value)	I^2 (%)	p	z	p	t	p
	TC *vs* CC		0.98	0.81 - 1.19 (0.86)	0	0.65	- 0.42	0.67	0.66	0.53
East-Asian	Dominant model		0.92	0.73 - 1.14 (0.44)	77.6	< 0.001	0.08	0.93	- 0.40	0.69
	Recessive model		0.82	0.72 - 0.93 (< 0.001)	25.7	0.19	1.32	0.18	2.02	0.07
	Allelic model	4859 / 5856	0.94	0.79 - 1.12 (0.48)	79.9	< 0.001	- 0.08	0.93	0.81	0.43
	TT *vs* CC		0.79	0.65 - 0.95 (0.01)	55.6	0.01	1.17	0.24	1.37	0.20
	TC *vs* CC		0.93	0.75 - 1.14 (0.48)	72	< 0.001	- 0.08	0.93	- 0.35	0.73
South-Asian	Dominant model		2.12	0.95 - 4.73 (0.06)	80.5	0.02	1	0.31	-	-
	Recessive model		0.72	0.47 - 1.08 (0.11)	0	0.33	1	0.31	-	-
	Allelic model	395 / 395	1.50	0.72 - 3.12 (0.28)	86	< 0.001	1	0.31	-	-
	TT *vs* CC		0.99	0.64 - 1.53 (0.95)	7	0.30	1	0.31	-	-
	TC *vs* CC		2.91	1.17 - 7.21 (0.02)	77	0.03	1	0.31	-	-

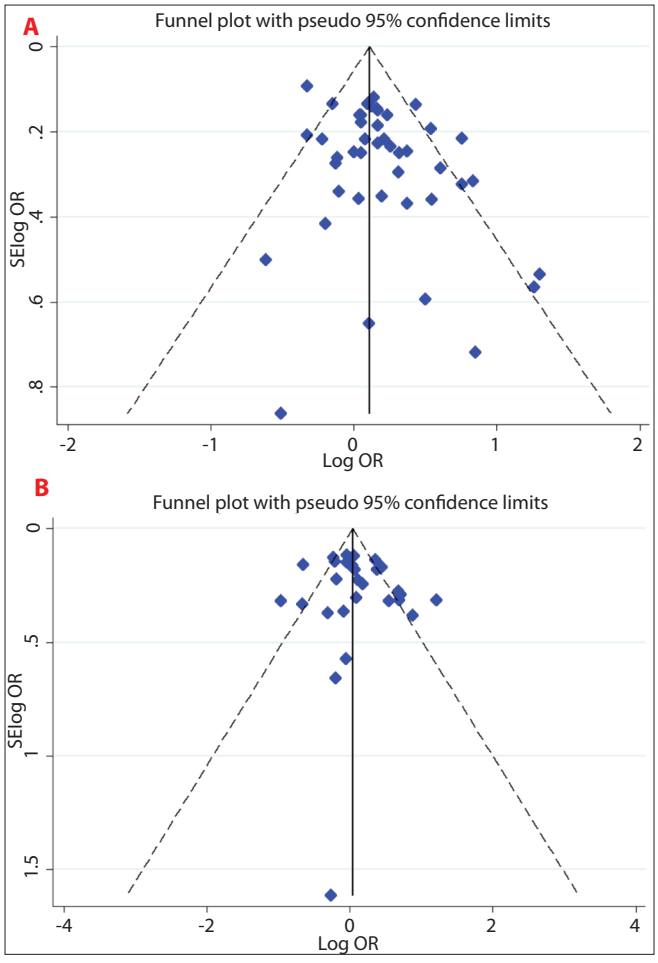


Figure 4 *- Begg's funnel plot for publication bias test in overall analysis. Each point represents a separate study for the indicated association. A: Dominant model (rs20541); B: Dominant model (rs1800925).*

Meta-analysis confers an appropriate technique to solve the issue of inconsistency through tackling the limitation of insufficient statistical power and small sample size present in the individual studies. As a consequence, in order to come up with a solution for the mentioned limitations with respect to *IL13* gene polymorphisms, the current most up-to-date meta-analysis was carried oud, in order to achieve a bona fide estimation of the association between *IL13* gene rs20541 and rs1800925 polymorphisms and the risk of asthma. Asthma is a chronic inflammatory disorder of the airways. The disease has been attributed with hyperactivity of Th2 responses of the immune system, in which the cytokines of the type 2 immunity, including IL-4, IL-5, and IL-13 promotes harmful events in the airways. IL-13 has been demonstrated to cause differentiation of goblet cells, bronchial hyperresponsiveness, fibroblast activation, and isotype switching of B cell antibody production to IgE (73). Targeting IL-13 in clinical trials has revealed an amelioration in the clinical presentations of asthma, suggesting the role of IL-13 in the pathogenesis of asthma (74). In addition, exogenous administration of IL-13 into airways in the animal models was shown to establish an inflammatory state by enhanced infiltration of eosinophils, and exacerbation of asthmatic responses, such airway hyperresponsiveness and contraction of the airway muscle (75). Moreover, blocking the IL-13 suppress the production of mucus (76).

Among the genes associated with asthma risk, *IL13* gene polymorphisms have been vastly surveyed. Functionally, *IL13* gene – 1112C/T (rs1800925) polymorphism was associated with upregulation IL-13 mRNA expression in the polarized Th2 cells as well as promoted release of this cytokine by mitogen-stimulated mononuclear cells (77). Additionally, the AA genotype of the *IL13* gene + 2044A/G (rs20541) polymorphism was reported to upregulate the IL-13 mRNA expression and reduce the affinity of IL-13 for binding to IL-13 receptor (78). As a result, these two SNPs in the *IL13* gene are involved in modulation of the cytokine level and contribution to the pathogenesis of asthma.

Figure 5 *- Sensitivity analysis in present meta-analysis investigates the individual influence of studies on pooled results. A: Dominant model (rs20541); B: Dominant model (rs1800925).*

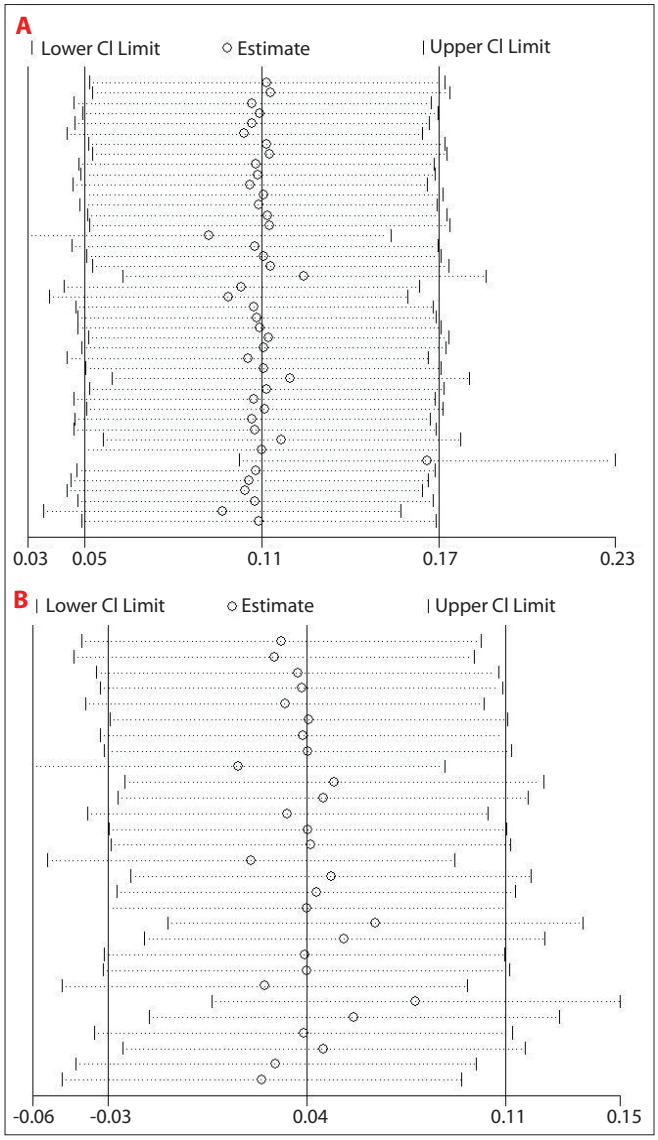
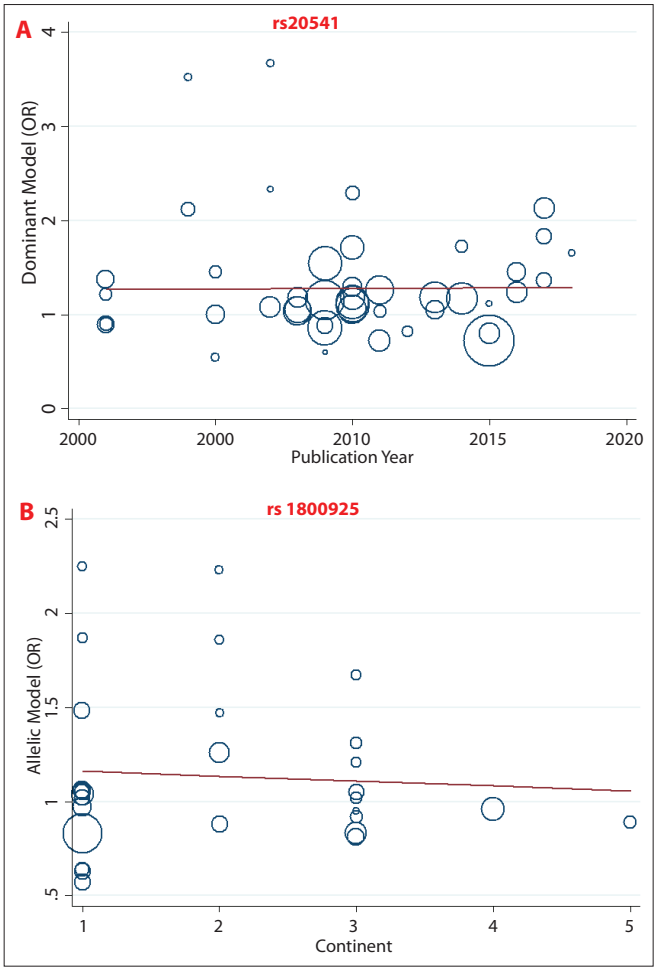


Figure 6 *- Meta-regression plots of the association between IL-13 gene polymorphism and risk of asthma. A: Dominant model based on publication year (rs20541); B: Allelic model based on continent (rs1800925).*



The latest meta-analysis by Mei *et al.* in 2017, by including 25 case-control studies, indicated that rs20541 polymorphism was associated in the overall analysis (OR = 1.49, 95% CI = 1.11-2.01) as well as in the Asian population in children (OR = 1.59, 95% CI = 1.05-2.40) (79). However, in the current meta-analysis, 45 case-control studies for rs20541 polymorphism, containing 10572 cases and 11575 healthy controls, were included. At the moment, this is the largest and most comprehensive meta-analysis of the *IL13* gene rs20541 polymorphism and asthma risk that considerably improved the sample size in comparison to the previous meta-analysis studies. In the pooled analysis, we observed than all of the genetic comparisons, including dominant (OR = 1.18), recessive (OR = 1.14), allelic (OR = 1.16), AA *vs* GG (OR = 1.17), and GA *vs* GG (OR = 1.14) models increased the susceptibility of asthma. In the subgroup analysis, it was detected that rs20541 polymorphism was not significantly associated with asthma risk in American population, while dominant (OR = 1.28), allelic (OR = 1.17), and GA *vs* GG (OR = 1.33) models increased asthma risk in Europeans. Additionally, except the heterozygote model, a strong significant association between rs20541 polymorphism and asthma risk in Asian population was detected. Additionally, the meta-analysis performed in 2011 (80) indicated that the allelic model of rs20541 was associated with an increased risk of asthma in Asians (OR 1.436, 95% CI = 1.101-1.873). To attain more precise conclusion, we per-

Table IV *- Meta-regression analyses of potential source of heterogeneity.*

Heterogeneity Factor		Coefficient	SE	T	P-value	95% CI	
IL13 **(rs20541)**						**UL**	**LL**
Publication Year	Dominant model	0.0011	0.018	0.06	0.95	- 0.035	0.037
	Recessive model	0.046	0.0270	1.72	0.093	- 0.0080	0.1012
	Allelic model	0.157	0.0134	1.17	0.24	- 0.0113	0.0427
	AA *vs* GG	0.0150	0.031	0.47	0.63	- 0.0489	0.0789
	GA *vs* GG	- 0.006	0.018	- 0.35	0.72	- 0.043	0.030
Continent	Dominant model	- 0.096	0.077	- 1.25	0.21	- 0.252	0.059
	Recessive model	- 0.128	0.122	- 1.05	0.299	- 0.375	0.118
	Allelic model	- 0.089	0.0580	- 1.55	0.13	- 0.206	0.027
	AA *vs* GG	- 0.180	0.136	- 1.32	0.19	- 0.456	0.095
	GA *vs* GG	- 0.056	0.076	- 0.73	0.46	- 0.211	0.098
Genotyping Methods	Dominant model	- 0.145	0.096	- 1.50	0.14	- 0.340	0.049
	Recessive model	0.002	0.151	0.02	0.98	- 0.302	0.307
	Allelic model	- 0.082	0.073	- 1.12	0.27	- 0.230	0.066
	AA *vs* GG	- 0.155	0.169	- 0.91	0.36	- 0.497	0.187
	GA *vs* GG	- 0.118	0.096	- 1.22	0.22	- 0.313	0.076
IL13 **(rs1800925)**							
Publication Year	Dominant model	- 0.016	0.028	- 0.58	0.56	- 0.074	0.041
	Recessive model	- 0.128	0.046	- 2.78	0.11	- 0.222	0.033
	Allelic model	- 0.030	0.019	- 1.58	0.12	- 0.070	0.009
	TT *vs* CC	- 0.190	0.058	- 3.24	0.23	- 0.311	0.069
	TC *vs* CC	0.020	0.036	0.55	0.58	- 0.055	0.095
Continent	Dominant model	- 0.058	0.104	- 0.56	0.58	- 0.272	0.156
	Recessive model	0.032	0.177	0.19	0.85	- 0.330	0.396
	Allelic model	- 0.026	0.072	- 0.37	0.71	- 0.174	0.121
	TT *vs* CC	0.031	0.251	0.13	0.90	- 0.485	0.548
	TC *vs* CC	- 0.118	0.136	- 0.87	0.39	- 0.397	0.160
Genotyping Methods	Dominant model	- 0.245	0.147	- 1.67	0.10	- 0.547	0.056
	Recessive model	0.084	0.267	0.32	0.75	- 0.465	0.634
	Allelic model	- 0.092	0.106	- 0.88	0.38	- 0.310	0.124
	TT *vs* CC	- 0.018	0.374	- 0.05	0.96	- 0.786	0.749
	TC *vs* CC	- 0.371	0.189	- 1.96	0.06	- 0.759	0.016

formed subgroup analysis based on the genetic stratification of the study subjects. It was divulged that rs20541 polymorphism was not a genetic risk factor for asthma in subjects with mixed ethnicity (mostly composed of Caucasians, Latins, and American-Africans). In addition, dominant, allelic and heterozygote models of rs20541 polymorphism were associated with asthma risk in Caucasians. Ultimately, we categorized Asian populations to East-Asian, South-Asian and Middle-East populations. Interestingly, significant associations were observed between rs20541 polymorphism and asthma risk in the South-Asian and Middle-East populations, but no significant association was observed in East-Asians. Interestingly, meta-regression analysis revealed that publication year, continent of the included patients and genotyping method were not source of heterogenicity in evaluating rs20541 polymorphism. Nonetheless, the diversity in the study design as well as genetic background of the populations between the different races/ethnicities/countries might be the possible reason for inconsistent results among the different populations.

The previous meta-analysis of *IL13* gene rs1800925 polymorphism in 2013, by including 22 studies (containing 5834 cases and 8110 controls), indicated that the dominant genetic model increased the risk of asthma (OR = 1.20, 95% CI = 1.08-1.34). Moreover, rs1800925 polymorphism was associated with increased asthma risk in Caucasians (OR = 1.30, 95% CI 1.09-1.55), but no significant association was found in Asians and African Americans (81). In addition, the meta-analysis in 2011 indicated that rs1800925 polymorphism was not associated with asthma risk in Asians (80). In the current meta-analysis, on the other hand, 31 studies, containing 10139 cases and 13304 healthy controls for rs1800925 polymorphisms were retrieved. The results of overall pooled analysis revealed no significant association between *IL13* gene rs1800925 polymorphism and asthma risk under different genotype models except for recessive model (OR = 0.86), which decreased asthma risk. In the subgroup analysis, it was revealed that rs1800925 was not significantly associated with the risk of asthma in Americans. Nonetheless, dominant (OR = 1.52), allelic (OR = 1.40), and CT *vs* CC (OR = 1.51) models were associated with increased susceptibility to asthma in the European populations. Furthermore, recessive and homozygote models decreased the risk of asthma in the Asians. Additionally, subgroup analysis based on the ethnicity-specific stratification supported the positive association of rs1800925 and asthma risk in Caucasians (dominant and TC *vs* CC) and South-Asian (just in TC *vs* CC). However, this association was negative in East-Asian (dominant and TT *vs* CC model). Based on the meta-regression analysis, it was found that publication year, continent of the included patients and genotyping method were not source of heterogenicity for rs1800925 polymorphism.

There are some limitations and caveats that needs to be mentioned. First, the analysis was performed according to crude estimation of *IL13* gene association with asthma susceptibility, regardless of the impression of confounders, such as age of the patients, age of diagnosis/time of follow-up, sex, smoking, asthma severity, and contribution of other genes in LD with *IL13*. Additionally, studies with diagnosis of atopy were excluded as this might be messed up with diagnosis of asthma. Second, we did not analyze several genes that could be helpful to understand the cytokine contribution in the pathogenesis of asthma. Third, there was a degree of heterogeneity during the overall analysis. From statistical perspective, this heterogeneity describes the variability between included studies and may originate from clinical or methodological heterogeneity, other unreported or unknown study characteristics, or by chance. Therefore, for finding any sources of heterogeneity and attenuating their effects, we conducted subgroup analysis and weighted meta-regression. Collectively, the results of meta-regression showed that none of the parameters, including publication year, continent of the study population, and genotyping methods were the expected source of heterogeneity. However, subgroup analysis was associated with a reduced heterogeneity in all groups and explained part of the observed heterogeneity. Furthermore, the other way of dealing with statistical heterogeneity performed in our analysis, was to incorporate "Random" term to account for it in a random-effects. Random effect model typically produces more conservative estimates of the significance of a result (a wider confidence interval). As it gives proportionately higher weights to smaller studies and lower weights to larger studies compared to the fixed effect analysis. Fourth, in the subgroup analysis, there was small sample size of many groups, leading to underpowered subgroup analyses. Moreover, there might be possible false-negative associations in the subgroup analysis due to unclarity of the actual number of cases/controls for each genetic model as well as no power assessment by each study. Fifth, this study was solely focused on the articles published in the English language which can be potential source of bias.

Conclusions

Considering all the facts, we performed the most up-to-date analysis of the *IL13* gene rs20541 and rs1800925 polymorphisms and asthma risk before September 2020. Our meta-analysis further validated some results of the last meta-analysis, while rejected some of them. In a whole insight, rs20541 polymorphism was detected as a potential risk factor for asthma in overall analysis, Asians (all models except GA *vs* GG), Europeans (all models except recessive and AA *vs* GG), Caucasians (all models except recessive and AA *vs* GG), East-Asians (just allelic model), Middle-East (all models except recessive and AA *vs* GG), and South-East (just in recessive and allelic models). Additionally, rs1800925 polymorphism showed statistically significant associated with asthma in the overall analysis (just in recessive model), Asians (in recessive and TT *vs* CC models), Europeans (all models except recessive and TT *vs* CC), Caucasians (just in dominant and TC *vs* CC), East-Asian (in dominant and TT *vs* CC model) and South-Asian (just in TC *vs* CC). Although previous met-analysis studies on small sample size indicated primitive associations of the IL13 gene polymorphisms with asthma susceptibility and regarding that our analysis large-

ly improved this limitation, we encourage further comprehensive analysis in the future after yielding more original data, particularly with respect to the role of IL13 polymorphisms in association with clinical presentations of asthmatic patients.

Contributors

MO, DI: originated the study and acquired data. BR, MM: performed statistical analysis, interpreted data and drafted the manuscript. SA, SF: revised the manuscript. All the authors read and approved the final version of the manuscript.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Acute urticaria in children: from pediatric Emergency Department to allergology consultation at a Central Hospital

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Key words

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Impact statement

Acute urticaria in children is caused by infections, food/drug hypersensitivity, physical triggers, insect bites and idiopathic causes. Physicians should provide appropriate aftercare instructions to patients with suspected allergy in order to provide a complete and careful diagnostic work-up.

Summary

Background. *Acute urticaria is a common condition in the pediatric Emergency Department (ED) and no data is available in Portugal.* ***Objective.*** *We aimed to characterize the prevalence, etiology and management of acute urticaria in children presenting at an ED of a Portuguese central hospital and report the follow-up investigation when drug or food allergy was suspected.* ***Methods.*** *Retrospective study of clinical records from children admitted to the ED with acute urticaria during one year period.* ***Results.*** *250 children were included, mean age of 7.4 ± 4.9 years (0-17 years). The most frequently suspected etiological factors were infections (22%), foods (12%), insect bites (9%) and drugs (8%), of which, upper respiratory tract infections, seafood and β-lactam antibiotics were the most frequent. In 44% of cases, the etiology of urticaria was not determined. After ED discharge, of the 50 patients with suggestive drug or food allergy, only 48% were sent to allergological workup and the allergy confirmed in 6 of them (2.4% of the 250 children).* ***Conclusions.*** *These data suggest that allergy is not the main trigger of acute urticaria in ED children, but when suspected, reference to an allergy department to complete allergological workup was insufficient.*

Introduction

Urticaria is a skin condition defined by the presence of wheals and/or angioedema (1). The diagnosis of this disorder is based on detailed clinical history and physical examination. By definition, acute urticaria lasts less than 6 weeks, is usually self-limiting and resolves typically within 30 minutes to 24 hours (1).

Acute urticaria in children can be caused by a wide variety of factors, such as infections, food or drug hypersensitivity, physical triggers, insect bites and idiopathic causes (2). It can be managed by the family physician, but this disease worries parents and children are frequently taken to the pediatric emergency department (ED). In a 2-year study, Ricci *et al.* reported 2.4% of children (aged 0-14 years) with urticaria referred to an Italian ED (1.1 accesses/day) (3). Kim *et al.* found that urticaria and angioedema were the most common cutaneous disease treated in children and adults in a Korean ED, during an 8-year period from 2003 to 2010 (4). In an Italian study, the prevalence of acute urticaria in children and adults ED in a 1-year period was 1.01% of the total ED visits, corresponding to 1.2 admissions per day (5). Although the

allergic cause is minor (3, 5), in case of suspicion an allergological evaluation is recommended.

There is a lack of childhood acute urticaria detailed information in Portugal, with no data available.

The aim of this study was to characterize the suspected aetiology and management of acute urticaria in children presenting to the ED of a portuguese central hospital covering an area of about 700,000 inhabitants. We also aim to analyse the follow-up investigation when drug or food allergy was suspected.

Materials and methods

Patient population

This retrospective study was conducted from January to December 2017. The database of pediatric patients aged less than 18 years presenting to the Centro Hospitalar Vila Nova de Gaia/Espinho ED was searched for "urticaria" (code 708) and subtypes (708.0 "allergic urticaria", 708.1 "idiopathic urticaria", 708.8 "other specified urticaria" and 708.9 "urticaria, unspecified") by International Classification of Diseases, Ninth Revision (ICD-9) codes.

Methods

Patient characteristics were collected from medical records and included age, gender, clinical manifestations, suspected trigger, personal allergic history, treatments and follow-up.

Children were divided into four age groups: infant (1 month to 1 year), preschool age (2–6 years), school age (7–12 years) and adolescent (13–17 years).

In addition to urticaria, the clinical presentation of children could include fever, respiratory tract symptoms (nasal obstruction, rhinorrhoea, sore throat, cough, dyspnoea and wheezing), gastrointestinal symptoms (nausea, vomiting, diarrhoea, constipation and abdominal pain), urinary tract symptoms (frequency, dysuria and pyuria), cardiovascular symptoms (tachycardia and palpitations) or others. Patients presented with anaphylaxis were excluded. Anaphylaxis was defined by the European Academy of Allergy and Clinical Immunology as "a severe, life-threatening generalized or systemic hypersensitivity reaction, which is characterized by being rapid in onset with life-threatening airway, breathing or circulatory problems, and is usually associated with skin and mucosal changes" (6).

The suspected etiological factors of acute urticaria were divided into 7 major categories based on the ED medical record: infections, drugs, foods, insect bites, contact allergens, physical agents and undetermined.

The personal allergic history of children included atopy, rhinitis, asthma, atopic dermatitis and food, drug and hymenoptera venom allergy. The term atopy as defined by World Allergy Organization "when individuals have an IgE sensitization as documented by IgE antibodies in serum or by a positive skin prick test" (7). Patients with chronic urticaria were excluded. The types of medical treatment and their methods of administration were recorded. The patients were discharged from the ED to home, a medical appointment or required hospitalization.

In an allergology consultation, a detailed clinical history was recorded, and additional data were collected from the patient's hospital and personal health records. Children with a clinical history compatible with drug or food allergy/hypersensitivity were proposed to continue the allergology evaluation, based on specific IgE determination, prick and intradermal skin testing for drugs, and prick and prick-to-prick skin tests for foods. Finally, a provocation test was performed if not contra-indicated and if all other investigations were inconclusive. If parents reported symptoms that were not consistent with allergy/hypersensitivity, or the child could tolerate the suspected food or drug, they did not undergo further assessment. Skin tests and provocation tests were considered positive if EAACI and AAAI criteria were met (8, 9). The study was approved by the local ethical committee.

Statistical analysis

Descriptive statistics were produced for each relevant variable. Categorical variables are presented as frequencies and percentages, and continuous variables as means and standard deviations. Normal distribution of variables was checked using skewness and kurtosis. Differences in the prevalence of the aetiologies were analysed among the four age groups by the χ^2 test. A P-value < 0.05 was regarded as statistically significant. Analysis was performed with the use of IBM® *SPSS*® Statistics version 24.

Results

Epidemiology, demographics and personal history

A total of 250 children with acute urticaria were included, which corresponds to 0.58% of the 43107 pediatric ED visits, between January and December 2017. There were 127 (50.8%) boys. The mean age was 7.4 ± 4.9 years, from neonate to 17 years. The majority of children were in the preschool-aged group (38.8%), followed by the school-aged (31.2%), adolescent (19.2%) and infant (10.8%) groups.

Considering personal allergic history, atopy was confirmed in 17 patients (6.8%). Rhinitis (10.8%) was the most prevalent disease, followed by asthma (10.4%) and atopic dermatitis (6.8%).

Clinical manifestations

Regarding clinical manifestations, 60% of reports had skin lesions only, and the remaining 40% had other clinical symptoms. Respiratory tract symptoms were the most commonly-associated symptoms (16.8%). Other included gastrointestinal symptoms (8%), fever (6.4%), cardiovascular symptoms (1.6%), urinary tract symptoms (0.4%) and other. Urticaria coexistent with angioedema was observed in 26 children (10.4%). Detailed demographic and clinical characteristics of the patients are summarized in **table I**.

Table I *- Demographic characteristics and clinical presentations of acute urticaria in children (n = 250).*

Sex	
Male	127 (50.8)
Female	123 (49.2)
Age, years	7.4 ± 4.9
Infant	27 (10.8)
Preschool age	97 (38.8)
School age	78 (31.2)
Adolescent	48 (19.2)
Personal allergic history	
Atopy	17 (6.8)
Rhinitis	27 (10.8)
Asthma	26 (10.4)
Atopic dermatitis	17 (6.8)
Food allergy	5 (2)
Drug allergy	1 (0.4)
Clinical presentations	
Only skin lesions	150 (60)
Respiratory tract symptoms	42 (16.8)
Gastrointestinal symptoms	20 (8)
Fever	16 (6.4)
Cardiovascular symptoms	4 (1.6)
Urinary tract symptoms	1 (0.4)
Other	37 (14.8)
Coexist with angioedema	26 (10.4)

Data are presented as n (%) or mean ± standard deviation.

Suspected aetiologies

Infections were the most common suspected etiological factor (22.0%), followed by foods (12.0%), insect bites (9.2%) and drugs (8.0%). Other suspected triggers were physical agents (4.0%) and contact allergens (0.8%). In 110 cases (44.0%), the cause of acute urticaria was not determined. Concerning the detailed aetiologies, upper respiratory tract infections were the most frequently documented infections associated with acute urticaria in children (13.2%). Other infectious causes included acute gastroenteritis (6.8%), skin infections (1.2%) and lower respiratory tract infections (0.8%). Foods were the second most common aetiology in our study with shrimp (2.4%) being the most common allergen. Egg (2%), milk (1.6%), fruits (1.2%), fish (1.2%), meat (1.2%) and peanut (0.8%) were the least common food-related allergens. Regarding insect bites, none was caused by hymenoptera insects. Of the drug-related causes, β-lactam antibiotics were the most common (6.0%). Analysis of aetiologies in different age groups showed that no determined etiology was more frequent in the preschool-aged group; and infections were more frequent in the preschool and school-aged groups than in the other groups. Suspected food allergy was more frequent in school-aged, followed by preschool-aged and adolescent groups. Suspected allergy to milk was only present in infants and preschool-aged groups. In the school-aged group, egg was the most suspected food trigger. Seafood, fish and peanut were more frequently suspected in the adolescent group. Drug-related aetiologies were higher in school-aged and adolescent groups. **Table II** describes all the suspected etiological factors. The prevalence of the various aetiologies did not differ significantly between gender groups ($p > 0.05$).

Treatment

The therapy most frequently prescribed in the ED was H1-antihistamine in 62.8%, followed by corticosteroids in 41.2%. Antihistamines in association with corticosteroids were prescribed in 98 cases (39.2%). In both therapies, the oral form was used more commonly than injection form. All antihistamines used were first-generation H1 antagonists. Of the 250 reported enrolments in this study, in 88 cases (35.2%), no therapy was established (**table III**). In addition, no one had received intramuscular epinephrine injections in ED.
The therapy at discharge was antihistamines only in 46.6% of cases, followed by antihistamines plus corticosteroids (35.3%). Intramuscular adrenalin injections were prescribed to 4 children (1.6%), and corticosteroids only to 2 children (0.8%). In 15.7% of cases, no treatment was prescribed (**table III**).

Discharge from ED

Of the 250 patients enrolled in this survey, 217 (86.8%) were discharged home, 32 (12.8%) to a medical appointment and 1 (0.4%) required hospitalization for intravenous fluid therapy associated to acute gastroenteritis.

Allergy evaluation

Among the 50 children whose ED doctors suspected they had a drug or food allergy, 24 (48.0%) were sent to an allergy department for further investigation. After a detailed anamnesis, 2 patients (8.3%) had already tolerated subsequent ingestion of suspected foods (1 milk, 1 egg). The remaining 22 children (91.7%) had a compatible clinical history of food or drug allergy and required further evaluation. Six (25%) refused the diagnostic procedures (3 amoxicillin, 2 shrimp, 1 nuts). Thus, 16 children (66.7%) agreed to proceed with diagnostic tests. Specific IgE (sIgE) and/or skin tests were carried out in all patients. Thirteen provocation tests were performed in 11 patients with the suspected trigger; the drugs tested were β-lactams in 7 patients (5 amoxicillin/clavulanic acid, 1 amoxicillin, 1 cefixime) and acetaminophen in 1 patient. Five provocation tests with foods were performed (1 shrimp, 1 nut, 1 fish, 1 milk and 1 egg) (**figure 1**). After complete evaluation, allergy was documented in 6 of 16 patients (37.5%), including 2 patients with positive sIgE (shrimp, amoxicillin); 2 with positive skin tests (amoxicillin, amoxicillin/clavulanic acid); 1 patient with positive sIgE, skin prick test and ImmunoCAP™ *ISAC* assay compatible with Lipid

Table II *- Detailed aetiologies causing acute urticaria in children (n = 250).*

	Infant (1 month to 1 year) (n = 27)	**Preschool age** (2-6 years) (n = 97)	**School age** (7-12 years) (n = 78)	**Adolescent** (13-17 years) (n = 48)	**Total**
Not determined	13 (5.2)	53 (21.2)	31 (12.4)	13 (5.2)	**110 (44.0)**
Infections	9 (3.6)	19 (7.6)	19 (7.6)	8 (3.2)	**55 (22.0)**
Upper respiratory tract infections	6 (2.4)	11 (4.4)	10 (4.0)	6 (2.4)	33 (13.2)
Acute gastroenteritis	3 (1.2)	6 (2.4)	6 (2.4)	2 (0.8)	17 (6.8)
Skin infections	-	2 (0.8)	1 (0.4)	-	3 (1.2)
Lower respiratory tract infections	-	-	2 (0.8)	-	2 (0.8)
Foods	2 (0.8)	9 (3.6)	11 (4.4)	8 (3.2)	**30 (12.0)**
Seafood (shrimp)	-	1 (0.4)	2 (0.8)	3 (1.2)	6 (2.4)
Egg	-	2 (0.8)	3 (1.2)	-	5 (2.0)
Milk	2 (0.8)	2 (0.8)	-	-	4 (1.6)
Fresh fruits	-	-	2 (0.8)	1 (0.4)	3 (1.2)
Fish	-	-	1 (0.4)	2 (0.8)	3 (1.2)
Meat	-	2 (0.8)	1 (0.4)	-	3 (1.2)
Peanut	-	-	-	2 (0.8)	2 (0.8)
Other	-	2 (0.8)	2 (0.8)	-	4 (1.6)
Insect bites	2 (0.8)	10 (4.0)	7 (2.8)	4 (1.6)	**23 (9.2)**
Drugs	1 (0.4)	5 (2.0)	7 (2.8)	7 (2.8)	**20 (8.0)**
β-lactam antibiotics	1 (0.4)	4 (1.6)	5 (2.0)	5 (2.0)	15 (6.0)
Acetaminophen	-	-	1 (0.4)	-	1 (0.4)
Other	-	1 (0.4)	1 (0.4)	2 (0.8)	4 (1.6)
Physical agents	-	1 (0.4)	2 (0.8)	7 (2.8)	**10 (4.0)**
Exercise	-	-	2 (0.8)	6 (2.4)	8 (3.2)
Heat	-	1 (0.4)	-	1 (0.4)	2 (0.8)
Contact allergens	-	-	1 (0.4)	1 (0.4)	**2 (0.8)**

Data are presented as n (%).

Transfer Protein syndrome; and one with positive provocation test (amoxicillin/clavulanic acid) (**figure 1**).

Overall, from the 24 evaluated patients, 6 (25%) refused the diagnostic procedures, 12 (50%) had a negative allergological work-up and could actually tolerate the suspected trigger, and 6 (25%) had confirmed allergy. In conclusion, in the total 250 urticaria ED episodes, 2.4% had allergy confirmation (**figure 1**).

Discussion

Acute urticaria is a common disease in the pediatric ED. Ricci *et al.* estimated that 2.4% of 33917 children referred to the emergency room were diagnosed with acute urticaria in a 2-year italian survey (3), but in our study only 0.6% of the emergency visits were due to acute urticaria episodes. Our explanation relies on codification system used on ED that can cause underdiagnosis. In our study, the prevalence of acute urticaria was higher in preschool-aged group (39%), which is consistent with the literature (2, 10), although other studies had found urticaria prevalence to be higher in children aged 0-24 months (28%), progressively decreasing thereafter (3).

Infections were the most common aetiologies (22%), being more frequent in the preschool and school-aged groups than in the other groups, with upper respiratory tract infections and acute gastroenteritis being the major infectious causes. This finding is compatible with those reported in previous studies (2, 3, 10–12), despite differences on age distribution. One study showed that infections as a cause of urticaria decreased as the age of children increased (2). In contrast, in a 1-year Italian

Figure 1 *- Study flow chart.*

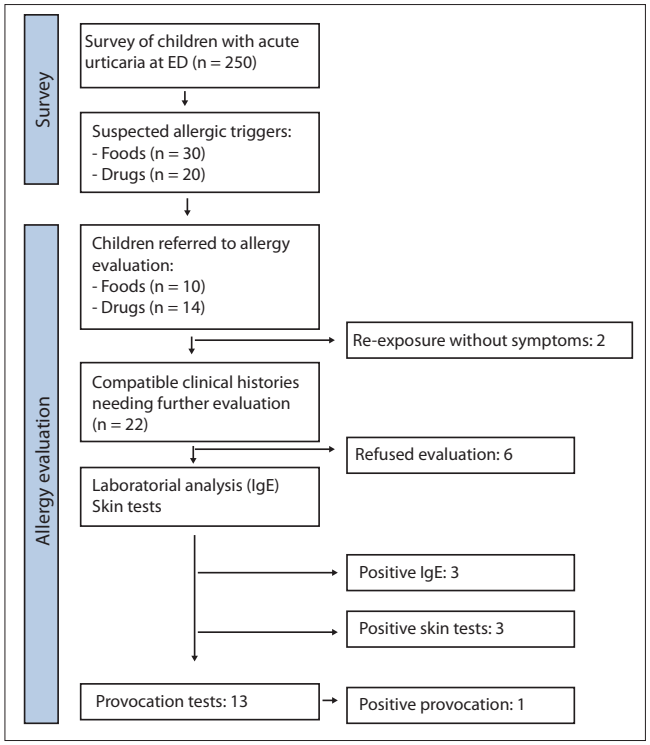

Table III *- Medical treatments of acute urticaria in children (n = 250).*

Treatments in ED	
Antihistamines	157 (62.8)
oral form	135 (54)
injection form	19 (7.6)
both	3 (1.2)
Corticosteroids	103 (41.2)
oral form	83 (33.2)
injection form	18 (7.2)
both	2 (0.8)
Antihistamines plus corticosteroids	98 (39.2)
Treatments at discharge	
Antihistamines only	117 (46.6)
Antihistamines plus corticosteroids	88 (35.3)
No treatment	39 (15.7)
Adrenalin	4 (1.6)
Corticosteroids only	2 (0.8)

Data are presented as n (%).

survey, infections were the cause of urticaria in less than 3% of the children, however the authors did not discriminate the age distribution of the children (5). As for foods, our results agree with previous reports (2, 10, 13), showing that foods were the second most common trigger, with shrimp and egg being the most frequently involved allergens. Suspected food allergy was more frequent in school-aged group (egg), followed by preschool-aged (egg, milk, meat) and adolescent (seafood, fish and peanut) groups. In infant group, the only suspected food trigger was milk. In contrast to other study that found that foods were more predominant with increasing age of children (2). One Italian study reported that food allergy showed two peaks of age prevalence: the first in children under 2 years (cow's milk or egg) and the second in those older than 5 years (nuts) (3). We reported very few cases due to peanuts, in contrast to other studies (2). In the opinion of the authors this is due to the fact that in Portugal most children do not eat nuts traditionally. A recent 10-year Portuguese anaphylaxis survey reported that in children nuts was the second most frequent cause of anaphylaxis due to foods, following milk (14). This founding showed that prevalence of nuts allergy is increasing in our country. Similar to other studies (3, 5), we found that in most cases (43.6%), the aetiology of acute urticaria in children could not be determined, mainly in the preschool-aged group. The differences between studies regarding the distribution of aetiologies of acute urticaria in each age group may be due to several causes: the inclusion criteria were different because of the use of different classification on ED; the included population had different age distribution; and regional differences regarding food consumption between the different countries, for example Portugal and Italy have similar food habits (Mediterranean diet), but different from Taiwan. Non-hymenoptera insect bites were the third most frequent aetiology, and we reported a higher prevalence (9.2%) when compared to other studies (2, 3, 13). The authors think that there may have been episodes of prurigo estrofulus that were misdiagnosed as urticaria. Although some studies have shown that drugs were an important cause of childhood urticaria (3, 5), in our survey they were only the fourth most common trigger (8%). Drug-related aetiologies were higher in school-aged and adolescent groups. In a Taiwan study, the adolescent group had more suspected drug allergies (2). Antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs) were the most frequent culprit drugs involved (2, 11, 12). However, in our study, only one patient had urticaria due to NSAIDs; with β-lactam antibiotics being the major drug-related aetiology (6%). These findings suggest that detailed medical history is extremely important in the study of children with acute urticaria, and the presence of infections in particular should be explored, especially those of the respiratory and gastrointestinal tracts. In addition, a possible relationship with food and drugs should always be evaluated.

The first level of acute urticaria treatment includes the use of non-sedating oral H1-antihistamine (1). In accordance with these guidelines, oral H1-antihistamines were administered to 55.2% of the children. Regarding treatments at discharge, H1-antihistamines were prescribed to 81.9% of the patients,

35.3% of which in association with a systemic corticosteroid. Similar results were found in other studies (2, 3, 13). Although adrenaline was not administered in the ED, it was prescribed to 4 patients at discharge, all of them with food as the suspected trigger. The authors can speculate that ED doctors suspected a possibly more serious future reaction, with criteria for anaphylaxis.

In our study, the majority of children (86.8%) were discharged home. Almost 13% were referred to a medical appointment for further investigation. Only 1 patient (0.4%) was hospitalized. In the Ricci *et al*. survey, 3.8% required hospitalization for either the disease or for serious associated infections.

Acute urticaria usually does not require a diagnostic workup, because the major cause is infection. Detailed history and physical examination are the most important steps towards establishing a diagnosis, identifying an underlying cause, and determining the need for further investigation. Allergological evaluation is recommended if there is a clinical history of allergy in order to confirm or exclude an allergic cause and identify the culprit drug, food or insect venom (1).

The results from the survey indicated that drug or food allergens were suspected triggers in 20% of acute urticarial cases. Contrary to our expectations, only 48% of them were referred to an allergy department for further investigation. Previous studies reported a prevalence of these suspected triggers between 17% to 36% (2, 3, 5, 10). However, these studies were not used to firmly demonstrate the allergy diagnosis. In our study, when a proper diagnostic work-up was carried out, allergy was excluded in most patients and diagnosed in only 6 of 24 cases (25%). Some studies reported that many children with adverse drug reactions are misdiagnosed as having drug allergy (15, 16).

However, identification of true drug hypersensitivity is uncommon, with 2 studies of more than 40 children with a history of drug allergy showing that more than 90% tolerate the drug after appropriate workup (15, 17). In line with this finding, Caubet and colleagues (18) were able to reproduce an urticarial reaction in only 6.8% of the 88 children presenting to the ED within 72 hours of ingesting b-lactams. As for foods, in a 16-year survey, only 1 out of 3 children had positive oral food challenges. Shrimps were the most common food involved, especially among children older than 3 years of age, followed by wheat, cow's milk and egg (19). In a birth cohort study, cow's milk allergy was suspected in 358 children and confirmed in 55, resulting in an overall incidence of challenge-proven cow's milk allergy of 0.54% (20).

The remaining 52% of patients that experienced a drug or food reaction resembling allergy, were catalogued as being allergic, without any further investigation. This leads to over-diagnosis of drug or food allergy/hypersensitivity that could contribute to an overrated avoidance measures in non-allergic children. However, underestimated allergy diagnosis could lead to an increased risk in truly allergic patients. Misdiagnosis has important undesirable consequences for the patients, but also a negative impact at socio-economic level.

There are some limitations in this study. Firstly, it was a retrospective study. Secondly, the usage of ICD-9 codes may lead to underdiagnosis or overdiagnosis of acute urticaria evaluated at ED. The exclusion of anaphylaxis is another limitation in this study, because the criteria used could lead to possible misdiagnosis, particularly in the presence of active infection. Lastly, aetiology could not be easily determined in children with acute urticaria who were prescribed antibiotics and NSAIDs during infection. In these cases, we always considered the drug as the suspected trigger, despite being the least likely.

Conclusions

In conclusion, children with acute urticaria were referred to the ED in 0.58% of the total pediatric ED visits and in most cases the aetiology was not determined. Upper respiratory tract infections were the most common etiological factor.

This study supports the opinion that allergy is not the main trigger of acute urticaria in children, with only 6 patients having a confirmed diagnosis of drug or food allergy, among the 50 patients with a suggestive clinical history. Most importantly, we found that in 52% of patients with suspected drug or food allergy, reference to an allergy department to complete allergological work-up was not performed. It is important that physicians practising emergency medicine provide appropriate aftercare instructions to patients with suspected allergy and refer these patients for allergological evaluation, in order to provide a complete and careful diagnostic work-up that is essential for a correct diagnosis. We reinforce the need of formation of doctors in pediatric ED concerning allergic diseases and the implementation of criteria for proper referral to allergology workup.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Immunoallergic disorders in the elderly

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Key words

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Summary

Although allergic diseases have become increasingly prevalent in the elderly, there are few data on this population. Through a retrospective analysis of the electronic medical records of patients aged 65 years and above followed in our Immunoallergology Unit, we aimed to characterize the immunoallergic diseases of the elderly. The most common disorders were respiratory diseases (n = 185; 50%), mucocutaneous diseases (n = 113; 31%), drug allergy (n = 31; 8%), food allergy (n = 9; 2%), and anaphylaxis (n = 9; 2%). Use of specific immunotherapy was residual (n = 2; 1%). There was an association between anaphylaxis and both, drug (p = 0.004) and food (p = 0.013) allergies. Non-allergic rhinitis and bronchial asthma were more frequent in females, and Angiotensin-converting-enzyme (ACE) inhibitors/ Angiotensin II receptor blockers (ARB) induced-angioedema in males. Recognizing the characteristics of immunoallergic diseases in the elderly and the specificities of this age group is paramount in providing these patients with the best possible care.

Impact statement

Immunoallergic diseases in the elderly have specificities that clinicians must be aware of in order to provide these patiens with the best care.

Introduction

In recent years, the average life expectancy of the population has been increasing, particularly in developed countries. The World Health Organization considers elderly people those over the age of 65 in developed countries, and those over the age of 60 living in developing countries (1). Thus, the elderly population has increased over the past few years. In Portugal, 20.1% of the population is 65 or older (2), 19.4% is the percentage in Europe (3). The diseases of this age group have become more frequent and complex, and allergic disorders (such as respiratory and mucocutaneous diseases, drug hypersensitivity, and food allergy), are no exception (4, 5). Although allergic conditions are often thought of as childhood disorders, they often persist into older age and can occasionally make its initial appearance in the elderly (6). Besides, clinical manifestations may be less straightforward than in younger age groups, hampering the recognition of the disease, and resulting in a more complicated differential diagnosis (6). As most studies regarding immunoallergic disorders do not include the elderly population, only limited data is available (6, 7) on its frequency and characteristics, making their management even more difficult.

Objective

We aimed to characterize the elderly population followed in our outpatient Immunoallergology (IAL) Unit regarding their immunoallergic diseases. With this study, we intended to focus our attention on this particular group of patients, with the ultimate goal of improving the care that is provided to them. Thus, we hope to contribute to a better understanding of the allergic diseases and their management in this age group with numerous specificities.

Methods

Patient selection

We performed a retrospective analysis of patients aged 65 years and older followed between 2009 and 2019 in the IAL outpatient Clinic at Centro Hospitalar Universitário de Lisboa Central (CHULC), in Portugal. Referral to our IAL Unit is done through the national Immunoallergology network. A total of 31,758 patients attended our Unit between 2009 and 2019, of which 1,721 (5.4%) were elderly. Based on an estimated rhinitis prevalence of 50%, in our population, with 5% error margin and a Confidence interval (CI) of 97%, we obtained a sample size of 370

patients. Randomization was performed through the Microsoft Excel® RAND function. Based on our previous experience, we estimated to lose 25-30% of patients because some of the clinical records from the period between 2009 and 2014 were not accessible in electronic format, as this represents the transition period from manual to electronic medical records in our Unit. Thereby, we included a total of 480 patients. Patients with incomplete (n = 1) or without filled electronic clinical records (n = 109) were excluded and thus, a total of 370 patients were analyzed.

Data collection and protection

Data was collected through the manual review of the electronic medical records. The study protocol was reviewed and approved by the local Ethics Committees.

Definitions

For the analysis of the immunoallergic diseases, we obtained the diagnosis registered by the assistant physician in the clinical files. Respiratory diseases, mucocutaneous diseases, drug allergy, food allergy, and other less frequent immunoallergic diseases were considered. Rhinitis, bronchial asthma, asthma-chronic obstructive pulmonary disease overlap (Asthma-COPD - overlap ACO), and Churg-Strauss syndrome were included in the respiratory diseases subgroup. In the mucocutaneous diseases subgroup we included angioedema without urticaria, urticaria with or without angioedema, contact dermatitis, and atopic dermatitis. The diagnosis of food allergy was made through the medical history, tests for sensitization, and in cases of doubt, oral food challenges. The diagnosis of drug hypersensitivity was based on the clinical history and, when appropriate, skin prick tests, intra-dermal tests, and drug provocation tests. All patients whose drug allergy was excluded underwent drug provocation tests.

Statistical analysis

The statistical analysis was made with IBM SPSS Statistics version 26 for Windows. The variables gender, age, immunoallergic diseases, and sensitization profile were analysed.

The normal distribution of continuous variables was tested using the Kolmogorov-Smirnov test and by visual analysis of the histogram. The non-parametric variables assessed were expressed as median and interquartile range. A comparison between the categorical variables was made using the chi-squared test or the Fisher's exact test with Bonferroni corrections, as appropriate. All statistical tests were bilateral and with a 5% significance level.

Results

Characterization of population

The median (P25-P75) age of the patients in our sample was 75 (71-81) years. There was a predominance of the female gender (n = 261; 71%). For most immunoallergic diseases, there was no statistically significant difference in gender distribution; there were, however, some exceptions, namely non-allergic rhinitis and bronchial asthma, more frequent among females, and ACE (Angiotensin-converting-enzyme) inhibitors/ARB (Angiotensin II receptor blockers) induced-angioedema, more common in males (**table I**). The frequency of different groups of immunoallergic disorders is shown in **figure 1**. The most common diseases were respiratory diseases (n = 185; 50%), mucocutaneous diseases (n = 113; 31%), drug allergy (n = 31; 8%), food allergy (n = 9; 2%), and anaphylaxis (n = 9; 2%). Immunoallergic diseases were excluded in 16% (n = 61) of patients.

Respiratory diseases

Respiratory diseases were the most common disorders affecting these elderly patients (n = 185; 50%), with rhinitis (n = 169; 46%) and bronchial asthma (n = 73; 20%) being the most frequent diagnoses. The concomitant diagnosis of rhinitis and bronchial asthma was present in 31% (n = 58) of these individuals. The sensitization profile for aeroallergens was assessed in 162 patients (88%), through skin prick tests and/or specific serum Immunoglobulin E (IgE): 57% (n = 92) were sensitized to aeroallergens, as shown in **table II**. Sensitization to a single aeroallergen group was found in 68% (n = 63): either house dust mites (n = 32; 51%) or pollen (n = 31; 49%). All sensitized patients to pet or mold allergens, were also sensitized to other allergen groups.

Specific immunotherapy to inhalant allergens was being administered to only one patient in our sample. This patient had allergic rhinitis and was taking subcutaneous immunotherapy for grass pollen.

Considering the patients with rhinitis, the sensitization profile was obtained in 93 % (n = 157), with 58% (n = 91) classified as allergic rhinitis. Among the 66 patients with non-allergic rhinitis (42%), senile rhinitis was diagnosed in 15% (n = 10). Concomitant diagnosis of sinusitis was found in 7% (n = 11) of rhinitis patients, of which 64% (n = 7) also had nasal polyposis. Nasal surgery was

Figure 1 *- Most common immunoallergic disorders (n = 370).*

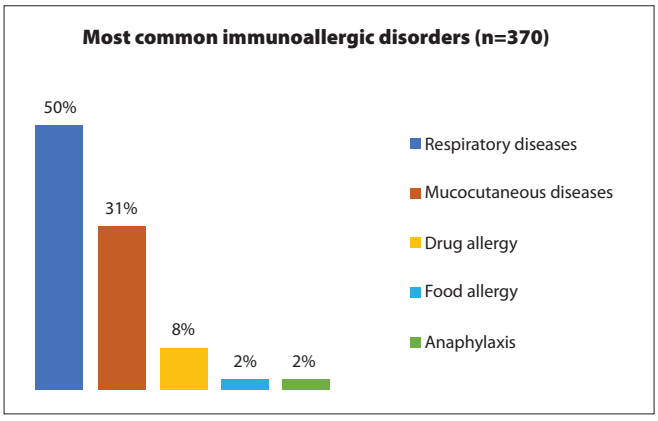

Table I *- Gender distribution of the main immunoallergic disorders (n = 370).*

Disorder	Female n (%)	Male n (%)	P-value*
Rhinitis	**131 (50)**	**38 (35)**	**0.007**
Allergic	68 (26)	23 (21)	0.313
Non-allergic	54 (21)	12 (11)	0.027
Sensitization not assessed	9 (3)	3 (3)	
Bronchial asthma	**61 (23)**	**12 (11)**	**0.006**
Allergic	32 (12)	6 (6)	0.051
Non-allergic	17 (7)	5 (5)	0.475
Sensitization not assessed	12 (5)	1 (1)	
Acute urticaria	**9 (3)**	**3 (3)**	**1.000**
Chronic urticaria	**26 (10)**	**8 (7)**	**0.426**
Spontaneous	20 (8)	8 (7)	0.915
Inducible	6 (2)	0 (0)	0.186
Contact dermatitis	**11 (4)**	**1 (1)**	**0.193**
Angioedema	**30 (11)**	**25 (23)**	**0.005**
Idiopathic	18 (7)	11 (10)	0.297
ACE/ARB	11 (4)	13 (12)	0.006
C1 deficiency	1 (0)	1 (1)	0.503
Drug allergy	**55 (22)**[#]	**23 (21)**	**0.995**
Confirmed	22 (8)	9 (8)	0.816
Excluded	27 (10)	12 (11)	0.889
Evaluation not completed	9 (3)	2 (2)	0.519
Food allergy	**4 (2)**	**5 (5)**	**0.131**
Hymenoptera venom allergy	1 (0)	1 (1)	0.503
Anaphylaxis	**7 (3)**	**2 (2)**	**1.000**
Total of patients	**261 (71)**	**109 (29)**	

ACE: angiotensin-converting-enzyme; ARB: angiotensin II receptor blockers. *Level of significance < 0.05. [#]In three female patients, a hypersensitivity reaction was confirmed to one drug and excluded to another one.

performed in 57% (n = 4) of the patients with rhinosinusitis and nasal polyposis, and the remaining (n = 3; 43%) were managed with medical therapy, namely topical nasal glucocorticoids.

In what concerns asthma, most of the patients in our sample had disease onset after the age of 40 (n = 39; 76%). Asthma onset before the age of 40 was recorded in 12 cases (24%). Information on the age of asthma onset was missing from the electronic medical records of the remaining 22 patients. The sensitization profile of the patients with bronchial asthma was evaluated in 82% (n = 60), and sensitization to aeroallergens was found in 63% of them (n = 38). Allergic asthma was more common among patients with disease onset before the age of 40 (80%; n = 8 of 10 patients with skin prick tests or specific serum IgE) than among patients with asthma onset after the age of 40 (52%; n = 16 of 31 patients with skin prick tests or specific serum IgE). ACO was diagnosed in 8% (n = 6). None of these patients were managed with biological therapies. Other respiratory diseases were rare. Churg-Strauss syndrome was diagnosed in one patient (0.3%), well controlled with daily oral 5 mg of equivalent prednisolone; another one (0.3%) had nonsteroidal anti-inflammatory drugs (NSAID)-exacerbated respiratory disease.

Table II *- Characterization of the sensitization profile of aeroallergens in patients with respiratory disorders (n = 162).*

Aeroallergen sensitization	Total n (%)
House dust mite	**59 (36)**
Pollen	**55 (34)**
Wall pellitory	27 (17)
Grass	26 (16)
Olive tree	21 (13)
Weed mix	19 (12)
Plane tree	14 (9)
Birch tree	1 (1)
Pet epithelium	**13 (8)**
Cat dander	11 (7)
Dog dander	7 (4)
Mold	**3 (2)**
Negative	**70 (43)**
Total of patients	**162**

Weed mix extract contains: plantago, artemisia, salsola, and chenopodium.

Mucocutaneous diseases

Mucocutaneous disorders were the second most frequent subgroup of diseases (n = 113; 31%), with the most common being angioedema without urticaria (n = 55; 15%), urticaria (n = 46; 12%), and contact dermatitis (n = 12; 3%).

The aetiology of angioedema without urticaria, could not be identified in 53% (n = 29) of the affected patients, thus classified as having idiopathic angioedema. In 42% (n = 23), it was attributed to angiotensin-converting-enzyme inhibitors and in 2% (n = 1) to angiotensin II receptor blockers. From the subgroup of patients with ACE inhibitors-induced angioedema, an ARB was tried as alternative in 39% (n = 9), all with tolerance. C1 inhibitor deficiency was found in 4% (n = 2) of the patients with angioedema; a haematological neoplasm was diagnosed in one of them (acquired deficiency) and the other one did not complete the investigation.

Table III *- Characterization of the patients with a completed drug allergy evaluation (n = 70).*

Drug	Suspected n (%)	Excluded n (%)	Confirmed n (%)
Analgesics	**17 (24)**	**3 (8)**	**14 (45)**
NSAIDs	11 (65)	0 (0)	11 (79)
Metamizole	2 (12)	0 (0)	2 (14)
Paracetamol	4 (24)	3 (100)	1 (7)
Antibiotics	**33 (47)**	**25 (64)**	**8 (26)**
Beta-lactam	26 (79)	20 (80)	6 (75)
Macrolides	5 (15)	4 (16)	1 (13)
Sulphonamides	1 (3)	0 (0)	1 (13)
Metronidazole	1 (3)	1 (4)	0 (0)
Cardiovascular	**7 (10)**	**5 (13)**	**2 (6)**
Antiplatelet agents	1 (14)	0 (0)	1 (50)
Gliptins	1 (14)	0 (0)	1 (50)
ACE inhibitors/ARB	2 (29)	2 (40)	0 (0)
Beta-blockers	1 (14)	1 (20)	0 (0)
Calcium channel blockers	1 (14)	1 (20)	0 (0)
Amiodarone	1 (14)	1 (20)	0 (0)
Anaesthetics	**6 (9)**	**5 (13)**	**1 (3)**
Local	5 (83)	4 (80)	1 (100)
General	1 (17)	1 (20)	0 (0%)
Others	**16 (23)**	**8 (21)**	**8 (26)**
Allopurinol	6 (38)	1 (13)	5 (63)
Iodinated contrast agents	5 (31)	4 (50)	1 (13)
Inhaled budesonide	1 (6)	0 (0)	1 (13)
Mydriatic ocular drugs	1 (6)	0 (0)	1 (13)
B12 vitamin	1 (6)	1 (13)	0 (0)
Prednisolone	1 (6)	1 (13)	0 (0)
Iodopovidone	1 (6)	1 (13)	0 (0)
Total of patients	**70 (100)**	**39 (56)**	**31 (44)**

Only two patients with suspected ACE inhibitors/ARB hypersensitivity were included in this table, both with a maculopapular rash, since bradykinin-mediated symptoms were not considered hereby. Some patients were evaluated for more than one suspected drug hypersensitivity. ACE: angiotensin-converting-enzyme; ARB: angiotensin II receptor blockers; NSAIDs: nonsteroidal anti-inflammatory drugs.

Urticaria was diagnosed in 46 patients, most of them with chronic urticaria (n = 34; 74%). Chronic urticaria was classified as spontaneous in 82% (n = 28) of the cases and as inducible in the remaining (n = 6; 18%). Patients with chronic spontaneous urticaria were mostly managed with oral H1 antihistamine agents, taken once a day (n = 18; 64%), twice a day (n = 8; 29%), or four times a day (n = 2; 7%). Because of lack of response to antihistamines, one patient received treatment with cyclosporin, which had to be stopped after one year due to haematological toxicity. This patient was then treated with oral H1 antihistamine agents taken four times a day and mirtazapine, with a good response and no adverse effects. Treatment with omalizumab had not been started in any patient in our sample. Among the patients with inducible urticaria, dermographism was diagnosed

Table IV *- Characterization of the sensitization profile of patients with food allergy (n = 9).*

Food allergen sensitization	Total n (%)
Crustaceans	6 (67%)
Nuts	3 (33%)
Fresh fruits	2 (22%)
Fish	2 (22%)
Molluscs	2 (22%)
Legumes	1 (11%)
Total of patients	9

in 83% (n = 5), and cold-induced urticaria in 17% (n = 1); all of them were well controlled with oral H1 antihistamine agents taken twice a day (n = 3; 50%), three times a day (n = 1; 17%), or as needed (n = 2; 33%). Acute urticaria was diagnosed in 26% (n = 12) of urticaria patients, being idiopathic and self-limited in all cases. Twelve patients (3%) had a diagnosis of contact dermatitis. During the etiological investigation, epicutaneous tests were carried out. Several agents were identified, with nickel (n = 4; 33%) and caines (n = 4; 33%) being the most common ones. In our sample no patient was diagnosed with atopic dermatitis.

Drug allergy

Investigation of suspected drug hypersensitivity was carried out in 21% (n = 78) of patients (**table III**). The diagnosis was excluded in 50% (n = 39), 14% (n = 11) did not complete the investigation, and the remaining 40% (n = 31) were diagnosed with drug allergy. Three patients (4%) were studied for more than one drug allergy. Mild mucocutaneous symptoms were the most frequent clinical manifestation in patients with confirmed drug allergy (n = 24; 77%). In 16% of patients (n = 5), anaphylaxis was the clinical presentation (local anaesthetic, iodinated contrast agent, inhaled budesonide, NSAID, and gliptin) and one patient (3%) had a severe cutaneous adverse reaction, namely drug reaction with eosinophilia and systemic symptoms (DRESS) due to allopurinol. In the patients who did not complete the investigation (n = 11; 14%) and in those in whom drug allergy was excluded (n = 39; 50%), mild mucocutaneous symptoms were the most frequent manifestation (n = 8; 73% and n = 26; 67%, respectively). In both groups, unspecific symptoms were the second most frequent clinical presentation (n = 3; 27% and n = 10; 26%, respectively) and no severe reactions were registered.

Food allergy

Food allergy was diagnosed in 2% (n = 9) of the patients (**table IV**). The most implicated food group was the crustaceans (n = 6; 67%); a concomitant sensitization to house dust mite was found in four of these patients. The two patients with allergy to fresh fruits also had allergy to nuts, with sensitization to lipid transfer protein, both with a concomitant sensitization to pollens. There was no diagnosis of milk, eggs, cereals, or profilins allergy. Most patients presented mild mucocutaneous reactions (n = 6; 67%). However, there were 3 cases of anaphylaxis (33%), all due to nuts.

Other less frequent immunoallergic diseases

Hymenoptera venom allergy was confirmed in 2 patients (1%). Both presented with anaphylactic reaction, one after a bee sting and the other one after a wasp sting. Specific immunotherapy was administered only to the former since the latter refused it. Hymenoptera venom allergy was excluded in another patient whose symptoms were attributed to a toxic reaction after being stung by a swarm of about 200 bees. Common variable immunodeficiency was diagnosed in one patient (0.3%), controlled with regular administration of subcutaneous immunoglobulin. There was no record of latex allergy in this sample.

Associations between immunoallergic diseases

Allergic rhinitis had a statistically significant association with allergic bronchial asthma ($p < 0.001$) and food allergy ($p = 0.012$). Non-allergic rhinitis had a statistically significant association with non-allergic bronchial asthma ($p < 0.001$).

Confirmed drug allergy ($p = 0.004$) and food allergy ($p = 0.013$) were significantly associated with anaphylaxis.

No additional statistically significant associations were found between the other analyzed disorders.

Discussion

In our outpatient Unit, the elderly population accounted for a minority of the patients observed. However, we expect that an increasing number of older people will be referred to allergy clinics as the current younger allergic cohorts age and are joined by those developing allergies in later life (7). More targeted studies are needed to understand if this expected increase is only due to the ageing of the general population, or if immunoallergic diseases are also becoming more prevalent and recognized in the elderly. Respiratory diseases were the most common immunoallergic disorders affecting our sample, with rhinitis and bronchial asthma being the most frequent. In line with previously published data, we found sensitization to aeroallergens in 57% of these patients, with a clear predominance of house dust mite and pollen allergens (4, 8-10). Pollen sensitization varies worldwide depending on species prevalence in each region. In our sample, similarly to the available data in our country in younger ages, the most commonly involved pollen allergens were wall pellitory, grass, and olive tree (10). However, while grass pollen represents the most common pollen sensitization in the general

population (10), sensitization to wall pellitory and grass pollen were equally common in our elderly sample. Most patients were sensitized only to a single group of aeroallergens – either house dust mites or pollens – and less common sensitizations, like pet epithelia or molds, were only found in polysensitized patients.

Bronchial asthma was one of the more common disorders, alerting to the importance of defining strategies to deal with the particular problems presented by these patients: difficulty with therapy administration due to their less dexterity, reduced self-management capability associated with less disease awareness, and frequent cognitive impairment (11). In congruence with previous studies (12, 13), while asthma diagnosed before the age of 40 was more often allergic, late-onset asthma was more often non-allergic. Moreover, whereas atopic disease usually begins in childhood and early adulthood, non-allergic asthma may be related to cumulative exposure to irritants such as occupational exposures and smoking and thereby becomes evident only later in life (13).

In our sample, some patients were diagnosed with ACO in concordance with its greater prevalence in older patients (14). A substantial percentage of elderly patients had a simultaneous diagnosis of allergic rhinitis and allergic bronchial asthma, with a statistically significant association between these diseases, similarly to what is found in younger patients (15). Non-allergic rhinitis was also a common diagnosis, a result we could expect since it is a frequent condition in this age (4). We have also found a significant association between non-allergic rhinitis and non-allergic asthma, in line with previously published data (16). The diagnosis of local allergic rhinitis was not taken into account because nasal provocation tests were not implemented in the routine diagnostic approach. Thus, we admit that some patients may have been wrongly diagnosed with non-allergic rhinitis, which is one of the limitations of our study.

Angioedema without urticaria was the most common mucocutaneous disorder. The majority of patients was diagnosed with idiopathic angioedema, which is in line with published data in younger cohorts (17). ACE inhibitors/ARB-induced angioedema represented the second most common cause of angioedema without urticaria in our sample, with a higher frequency compared to other published studies (17, 18). Most patients had this clinical presentation in response to an ACE inhibitor, and a considerable percentage tolerated an ARB as an alternative drug. This is a relevant finding given the well-established benefits of this drug class in cardiovascular and renal diseases, when compared to the available alternatives, namely calcium channel blockers. More studies are needed to support our results.

Similarly, to other published studies (6), urticaria, especially chronic spontaneous urticaria, was quite common and well managed with oral H1 antihistamine agents. The patient who remained uncontrolled despite treatment with oral H1 antihistamine agents taken four times a day, and thus treated with cyclosporin, was diagnosed in 2014. By that time, the available guidelines advocated the treatment either with cyclosporin or omalizumab in patients who did not respond to oral antihistamines (19). In this patient, the choice of mirtazapine after cyclosporin discontinuation was based on the presence of depressive manifestations and on published reports of its efficacy in several skin disorders, including chronic urticaria (20, 21). It had a good efficacy and tolerance showing that, in some cases, alternative drugs for the treatment of urticaria failing to respond to H1 antihistamine agents might be useful and more cost-effective than the therapy advocated by the guidelines. This fact can be especially relevant in the context of limited financial resources for health care. As for idiopathic angioedema, it is particularly important in the elderly to exclude other disorders that may be the cause of chronic urticaria, before assuming a diagnosis of chronic spontaneous urticaria (6, 22).

Contact dermatitis was not very frequent in our sample and, in line with another published study in elderly patients, the most implicated allergens were nickel and caines (23). No patient was diagnosed with atopic dermatitis, as expected from its lower prevalence compared to younger patients (6).

Drug allergy is particularly important in this age group considering its polypharmacy and, therefore, the higher probability of developing a drug allergy (24). On the other hand, polypharmacy may make the identification of the culprit drug more difficult and complex, especially when it is an essential drug which cannot be easily withdrawn. In our study, the most implicated drugs were NSAIDs, beta-lactam antibiotics, and allopurinol, in agreement with other published data (6, 24). Although mild mucocutaneous symptoms were the most common presentation, anaphylaxis had a considerable frequency in our sample. Moreover, we found a statistically significant association between confirmed drug allergy and anaphylaxis. It is crucial to perform an early diagnosis and a correct approach to anaphylaxis cases, minimizing new episodes that could have drastic consequences in a high-risk population for a fatal outcome (25, 26).

It is important to underline that we were able to exclude drug allergy in half of the patients with suspected drug hypersensitivity. The most often suspected and excluded drugs were beta-lactam and macrolide antibiotics, local anaesthetics, and iodinated contrast agents. Exclusion of drug allergy has a huge importance as it allows these elderly patients to receive the most effective and less toxic drug and, in the case of antibiotic agents, contributes to improve the resistance profile of many bacterial microorganisms (27).

Food allergy had a relatively low frequency in this aged population, mimicking other published studies (6). The most implicated allergens were crustaceans and nuts. Although mild mucocutaneous symptoms were the most common clinical presentation, we found a statistically significant association between food allergy and anaphylaxis, which underlines the critical importance of making a correct diagnosis to avoid a second episode that could be fatal in such a fragile population (26). A significant association between food allergy and allergic rhinitis was also

found. This association may be due to cross-reactivity between house dust mites and crustaceans (house-dust mite-crustaceans syndrome) or pollens and vegetable foods (pollen-food allergy syndrome) (28). As expected, there were no cases of allergy to milk or egg, the most common food allergies in paediatric patients and with a natural tendency for resolution (29, 30).

Only two patients were receiving specific immunotherapy (one to grass pollen and one to bee venom), in agreement with the previously published data referring to low use of this resource in the elderly for reasons of efficacy and safety (31, 32). However, recent studies (31) support the use of specific immunotherapy in this population. We expect that its application in the elderly may increase in the near future, with beneficial effects on symptom control and quality of life.

For most immunoallergic disorders, we did not find any statistically significant differences between genders. However, the frequency of non-allergic rhinitis and bronchial asthma was higher in females, as previously published (33). ACE inhibitors/ARB-induced angioedema was more common in males, in line with previously reported data (18) and against what was found in younger ages (17, 34).

Limitations

Some limitations can be identified in our study. Because this constitutes a single-centre study, the results should not be generalized. Being a retrospective study, the established associations between disorders could not be definitive since many confounding factors could not be controlled. Moreover, the analyzed data were obtained from electronic medical records, and possibly resulted from subjective interpretation from each patient's assistant physician. As mentioned earlier, the diagnosis of local allergic rhinitis was not considered and, therefore, some misdiagnosis of non-allergic rhinitis was possibly made. Hypersensitivity to chemotherapy drugs was not observed in our series because the approach to those patients is usually performed at the Chemotherapy Day Unit and not at the Outpatient Unit.

Conclusions

The most frequent immunoallergic disorders of the elderly patients evaluated in our IAL outpatient Unit are respiratory diseases, namely allergic and non-allergic rhinitis and bronchial asthma. Patients with respiratory allergies are mainly sensitized to house dust mites or pollen. Mucocutaneous diseases are also common, especially angioedema, both idiopathic and ACE inhibitors/ARB induced, and chronic spontaneous urticaria. Drug allergy represents an important diagnosis in a polymedicated population. We were able to exclude it, however, in half of the cases. Food allergy is less common. We found an association between anaphylaxis and both drug and food allergies.

In summary, it is crucial to consider the specificities of this population, whose referral to the IAL consultation will likely increase in the upcoming years. Particular attention is required if we are to provide them with the best quality care and, ultimately, improve their quality of life. More tailored studies targeting this population group are needed to support our results.

Fundings

None.

Conflict of interests

The authors declare that they have no conflict of interests.

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Predictor of buckwheat allergy in children based on challenge test results: a retrospective observational study in Japan

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Key words

Buckwheat; food allergy; oral food challenge; predictor; specific-total IgE ratio.

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Impact statement

Assessment of BW-sIgE/total IgE ratio may be more useful predictor of BW OFC results than BW-sIgE

Summary

Background. *Buckwheat (BW) is a major food allergen and one of the leading causes of food-induced anaphylaxis in Japan. The standard method of diagnosing food allergy is the oral food challenge (OFC). The BW-specific IgE (BW-sIgE) value is used to assess BW allergy but its utility is limited.* ***Aim.*** *The aim of the present study was to identify factors with predictive value for the diagnosis of BW allergy using the OFC.* ***Methods.*** *We evaluated 37 patients who were classified into the positive or negative group according to their OFC results.* ***Results.*** *Ten patients (27.0%) showed objective or persistent, moderate, subjective symptoms during the OFC. The positive group had a significantly higher BW-sIgE/total IgE ratio than the negative group ($p < 0.001$), but the total IgE ($p = 0.139$) and BW-sIgE ($p = 0.130$) did not differ significantly. Receiver operator characteristic (ROC) analysis showed that the BW-sIgE/total IgE ratio had a larger area under the curve (AUC: 0.885) than BW-sIgE (AUC: 0.667). The statistically optimal cut-off was 0.0058 for the BW-sIgE/total IgE ratio, which corresponded to a clinical sensitivity and specificity of 90.0% and 81.5%, respectively.* ***Conclusions.*** *BW-sIgE/total IgE ratio may be more useful predictor of BW OFC results than BW-sIgE.*

Introduction

Buckwheat (*Fagopyrum esculentum*; BW) is a member of family *Polygonaceae* and is commonly consumed worldwide. Examples of BW-based foods include soba (Japanese noodles), guksu (Korean noodles), memilmuk (Korean jelly), groat porridge (Asia, Eastern Europe), pizzoccheri (Italian pasta), polenta taragna (combined with maize), several forms of pancake-blinis in Russia, galettes in Brittany and pofferjes in Netherlands (1, 2). Thanks to the recent trend of avoiding gluten, BW is becoming increasingly popular in the West among individuals with celiac disease (2).

Buckwheat (BW) is a major food allergen and one of the leading causes of food-induced anaphylaxis in Japan and Korea (3, 4). However, several studies in Europe and Australia have also reported sensitization and allergy to BW (5, 6). The standard method of diagnosing any food allergy is the oral food challenge (OFC). However, when BW allergy is suspected, food avoidance without the OFC is frequently prescribed to avoid the risk of anaphylaxis (7).

The BW-specific immunoglobulin E (BW-sIgE) value is used to assess for BW allergy, but its diagnostic accuracy is controversial (7). We herein hypothesized that the BW-specific IgE/total IgE ratio would be useful for diagnosing BW allergy as it is for al-

lergies to other food items, including peanut and tree nut (8). The present study evaluated the diagnostic performance of the BW-sIgE/total IgE ratio in light of BW OFC results.

Materials and methods

Patient selection

The records of patients who underwent an OFC between April 2017 and January 2020 at Tokyo Metropolitan Children's Medical Center were retrospectively reviewed. The patients were classified into a positive or negative group by their OFC results. Patients with a negative OFC result at a total intake < 80 g and those who did not undergo an OFC with a total intake ≥ 80 g were excluded. The present study was conducted in accordance with the principles of the Declaration of Helsinki and the ethical guidelines of Japan and was approved by the Ethics Committee at Tokyo Metropolitan Children's Medical Center [H2019b-181].

OFC

To assess for the presence of BW allergy, an OFC was performed for the patients with suspected BW allergy history and in those who were positive for BW-sIgE but had no history of BW ingestion or a history of consuming only small quantities of the item. The OFC was performed at admission in accordance with the Japanese Food Allergy guidelines (9). The BW dosage in the form of boiled noodles was determined by each physician. The cumulative dosage was divided into a low-dose category (< 80 g containing approximately 3000 mg of BW protein) and a non-low dose category (≥ 80 g). Eighty grams of boiled BW noodle is the highest total challenge dosage recommended by the Japanese Food Allergy guidelines as a total challenge dose (9). The OFC was performed by a physician. In accordance with the Japanese Food Allergy guidelines, an OFC was considered positive if objective symptoms or persistent, moderate, subjective symptoms were observed (9). The severity of the symptoms appearing during an OFC were classified in accordance with the criteria described in the afore-mentioned guidelines (9). The definition of anaphylaxis was based on the diagnostic criteria of the World Allergy Organization Anaphylaxis Guidelines (10). At symptom onset, the patients were treated in accordance with the methods recommended by the European Academy of Allergy and Clinical Immunology's (EAACI) food allergy and anaphylaxis guidelines (11). Written informed consent was obtained from the patients' guardians before each OFC.

Laboratory data

Blood samples before the OFC were analyzed. Total IgE and BW-specific IgE (BW-sIgE) levels were assessed using ImmunoCAP (Thermo Fisher Scientific/Phadia, Uppsala, Sweden), which can detect levels as low as 0.1kU$_A$/L. If the BW-sIgE value was less than 0.1 kU$_A$/L, the measurement was treated as equivalent to 0.05 kU$_A$/L as previously described (12).

Outcome measures

The patient characteristics, including sex, age at OFC, history of immediate-type reactions to BW, anaphylaxis due to BW, and allergic complications at the time of the OFC, were reviewed. The present study's primary aim was to evaluate the diagnostic performance of the BW-sIgE value and the BW-sIgE/total IgE ratio for BW allergy. The secondary objective was to evaluate the difference in baseline characteristics between the positive and negative groups.

Statistical analysis

Patient age, sex, symptoms during OFC, treatments administered, and laboratory data, including total IgE, BW-sIgE, and the BW-sIgE/total IgE ratio, were employed as variables in the analysis. Univariate analysis of the groups was conducted using the Mann-Whitney U test or Fisher's exact test. $P < 0.05$ was considered to indicate statistical significance. Receiver operating characteristic (ROC) curves were generated for the BW-sIgE value and the BW-sIgE/total IgE ratio. The diagnostic performance of the variables was evaluated using the area under the curve (AUC). All statistical analyses were performed using IBM SPSS statistics Version 26.0 (IBM Corp., Armonk, NY, USA).

Results

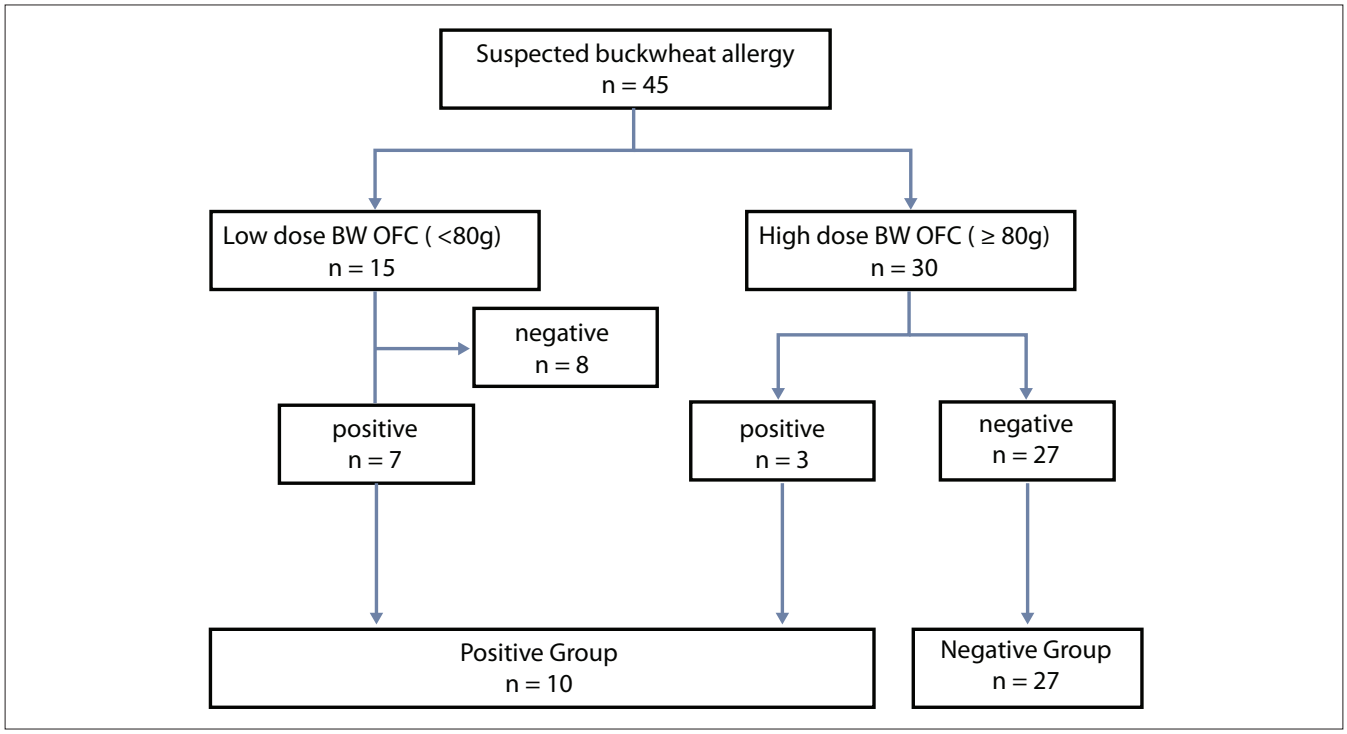
Patient characteristics

Figure 1 shows the OFC results. Thirty-seven patients were enrolled in the final analysis. **Table I** summarizes the patient characteristics. Twenty-five patients (67.6%) were male. The median age at diagnosis was 89 months (range: 46-173 months). Twenty-two patients (59.6%) had never eaten BW with fear of an allergic reaction caused by BW because they were BW-sIgE positive or had concomitant food allergies. Nine patients (24.3%) had a history of eating buckwheat without an allergic reaction, but they had only eaten a very small quantity. Six patients (16.2%) had a suspected history of immediate-type reaction to BW, and two of them experienced anaphylaxis due to BW. Almost all the patients (97.3%) had a history of allergy to foods other than BW. **Table II** lists the causative foods in the concomitant cases of non-BW food allergies. The most common causative food was egg. Fourteen patients (37.8%) had atopic dermatitis, and seven patients (18.9%) had bronchial asthma.

OFC results, induced symptoms, and treatments

Ten of the 37 (27.0%) patients were classified as positive. **Table III** shows the symptoms and treatments of the positive group during the OFC. In the positive group, eight, one, and one patient presented with mild, moderate, and severe symptoms, respectively. The most common symptom during the OFC was gastrointestinal symptoms, which were observed in seven patients. Antihistamine, the most frequently prescribed treatment, was administered

Figure 1 *- Results of BW OFC.*



BW: buckwheat; OFC: oral food challenge.

Table I *- Patient characteristics.*

Characteristics	Total (n = 37)	Positive (n = 10)	Negative (n = 27)	P-value
Male, n (%)	25 (67.6)	9 (90.0)	16 (59.2)	0.119
Age, mean (range), months	89 (46-173)	94 (66-138)	89 (46-173)	0.489
Atopic dermatitis, n (%)	14 (37.8)	5 (50.0)	9 (33.3)	0.454
Bronchial asthma, n (%)	7 (18.9)	0 (0.0)	7 (25.9)	0.155
Food allergy other than BW, n (%)	36 (97.3)	9 (90.0)	27 (100)	0.270
History of immediate reaction to BW, n (%)	6 (16.2)	4 (60.0)	2 (7.4)	0.035
History of anaphylaxis due to BW, n (%)	2 (5.4)	2 (20.0)	0 (0.0)	0.068
Total loading dose of BW (g), median (range)	150 (0.5-195)	10 (0.5-195)	180 (81.0-195)	< 0.001
Total IgE, median (range), IU/mL	1200 (6.18-5000)	492 (6.18-2310)	1380 (16.5-5000)	0.139
BW-sIgE, median (range), kU_A/L	4.73 (0.05-49.8)	9.38 (0.1-49.8)	3.58 (0.05-35.3)	0.130
BW-sIgE/Total IgE ratio, median (range)	0.00431 (0.00027-0.06199)	0.02089 (0.00088-0.06199)	0.00362 (0.00027-0.00948)	< 0.001

IgE: immunoglobulin E; BW: buckwheat.

Table II *- The summarize of causative food in 36 patients with concomitant food allergies other than BW.*

Causative food	Total number
Egg	28
Cow's Milk	15
Wheat	4
Peanuts	3
Shrimp	3
Kiwi	3
Soybean	2
Walnut	2
Cashew	2
Almond	1
Sesame	1
Peach	1
Crab	1
Octopus	1

Table III *- Symptoms, severity, and treatments during BW OFC in positive group.*

	Positive group (n = 10), n (%)
Symptoms	
Skin	3 (30)
Gastrointestinal	7 (70)
Respiratory	3 (30)
Nervous	2 (20)
Cardiovascular	0 (0)
Anaphylaxis	2 (20)
Severity	
Mild	5 (50)
Moderate	4 (40)
Severe	1 (10)
Persistent moderate subjective symptoms	3 (30)
Abdominal pain	3 (30)
Treatment	
Antihistamine	4 (40)
Steroid	1 (10)
Drip infusion	1 (10)
β stimulant inhalation	2 (20)
Adrenaline (intramuscular)	0 (0)

OFC: oral food challenge.

to four patients. Two patients demonstrated anaphylaxis but none of the patients received intramuscular adrenaline because the skin and respiratory symptoms in those two patients did not appear simultaneously and were therefore treated at different times with antihistamine and β stimulant inhalation.

Comparison of OFC results between the positive and negative groups

As shown in **table I**, patients in the positive group more frequently had a history of immediate-type reactions to BW ($p = 0.035$). The serum BW-sIgE value did not differ significantly between the groups ($p = 0.116$), but the BW-sIgE/total IgE ratio was significantly higher in the positive group ($p < 0.001$).

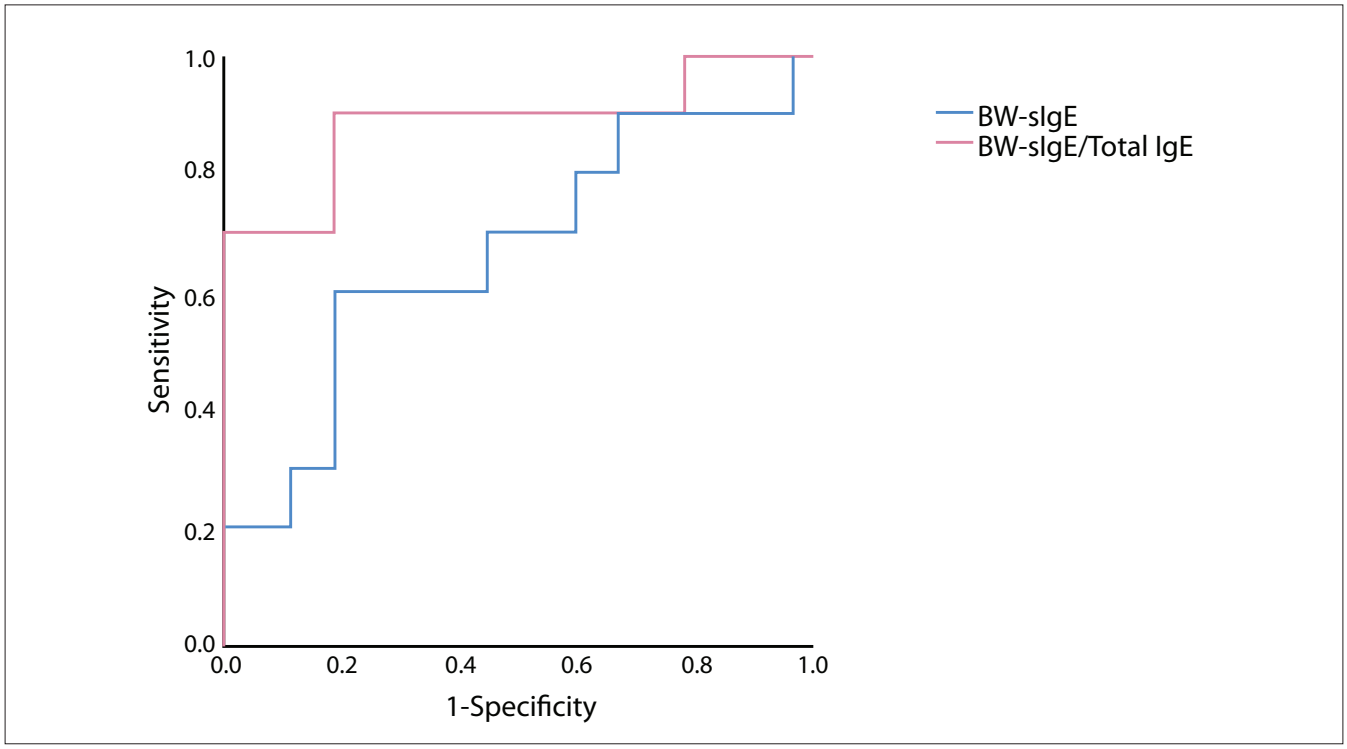
Receiver operator characteristic (ROC) analysis (**figure 2**) demonstrated that the area under the curve (AUC) for the BW-sIgE value and BW-sIgE/total IgE ratio in the positive group was 0.664 and 0.888, respectively (**table IV**). The statistically optimal cut-off value for the BW-sIgE/total IgE ratio was 0.0058, corresponding to a clinical sensitivity and specificity of 90.9% and 81.5%, respectively.

Discussion

To the best of our knowledge, the present study is the first to demonstrate that the BW-sIgE/total IgE ratio may have a higher predictive value for BW allergy than BW-sIgE. The allergen-specific IgE/total IgE ratio is a biomarker that can be easily calculated. Its utility has been assessed for many food items, such as milk, egg, wheat, peanut, and tree nut (8, 13). The utility of the specific IgE/total IgE ratio is thought to derive from the probability that the surface density of IgE antibody molecules on mast cells and basophils with specificity for the same allergen, which are capable of inducing mediator release after an allergen encounter, increases in proportion to the increase in the ratio of a particular IgE antibody specificity. The ratio may more accurately reflect the specific binding capacity on the surface of mast cells and basophils and therefore by extension the probability of allergen cross-linking and subsequent activation. This reduces the false-positive test rate that may be due to non-associated IgE and confounding immune markers not represented on testing (8).

In the largest retrospective study of BW OFC ever done, 44 of 419 (10.5%) pediatric patients with suspected BW allergy tested positive; of these 54.5% presented anaphylaxis, and 22.7% presented with a severe reaction (14). In our study, an anaphylactic reaction occurred in two of ten patients in the positive group, of whom one presented with a severe reaction. On the other hand, 6-10% of patients who tested positive on an OFC with milk,

Figure 2 *- Receiver operating characteristic curve for positive oral food challenge results.*



BW-sIgE: buckwheat-specific immunoglobulin E; Total IgE: total immunoglobulin E.

Table IV *- Diagnostic performance of BW-sIgE and BW-sIgE/total IgE.*

	BW-sIgE	BW-sIgE/Total IgE
AUC	0.667	0.885
95% CI	0.455-0.878	0.735-1.000
Optimal cut-off value	4.52 kU_A/L	0.0058
Sensitivity, %	70.0	90.0
Specificity, %	55.6	81.5

BW-sIgE: buckwheat-specific immunoglobulin E; Total IgE: total immunoglobulin E.

eggs, wheat, and soy presented with severe symptoms (15). The incidence of anaphylactic reactions during BW OFC with a severity equal to or greater than that induced by other food items suggests the need for careful caution when performing the test. Knowing the risk factors of an allergic reaction prior to performing a BW OFC may therefore be desirable. The present study demonstrated not only that the presence of a high BW-sIgE/total IgE ratio, but also a history of allergy to BW were associated with positivity on a BW OFC, in line with the findings of a previous study (14). These risk factors were also found in a retrospective study of 93 children who underwent a wheat OFC (16).

Although previous studies reported the skin prick test (SPT) and Fag e 3-sIgE, one of the components of BW, as useful predictors of OFC results (17, 18), these were not assessed in the present study because SPT using BW is not part of routine clinical practice at the study institution, and Fag e 3-sIgE was unable to be clinically assessed. However, the allergen-specific IgE/total IgE ratio is easy to determine on the basis of blood test results alone and may therefore be extremely useful in daily clinical practice. Although diagnostic performance of the BW-sIgE/total IgE ratio was not sufficient to obviate the need for an OFC, it was superior to BW-sIgE alone in predicting the risks and results of BW OFC when combined with an assessment of the patient's medical history.

The present study had a number of limitations. First, it was retrospective, and the OFC conditions (dosage, interval, and frequency of loading) were decided by individual physicians, thus possibly introducing variations affecting the OFC results. Second, we were unable to ascertain whether patients with negative results on a low dose (< 80 g) BW OFC would be able to

tolerate a dosage ≥ 80 g partly because the patients' parents were unwilling to consent to the test out of fear of a severe allergic reaction, *etc*. These patients were excluded from the analysis despite the possibility of introducing a selection bias because OFC results were thought to be highly dose-dependent. Third, the number of patients analyzed was too small to allow any definitive conclusions to be drawn. However, this fact may conversely be a strength in that statistically significant results were able to be obtained for the diagnostic performance of the BW-sIgE/total IgE ratio in spite of the small sample size. Forth, the OFC were not double-blind placebo-controlled food challenges (DBPCFC). Since subjective symptoms, such as stomachache, occurred in patients with a positive OFC result, DBPCFC would theoretically be appropriate for diagnosing the allergy. However, the OFC is time-consuming when performed multiple times, making DBPCFC for BW difficult to perform in practice.

Conclusions

The findings of the present study suggest that the BW-sIgE/total IgE ratio may be a more useful predictor of BW OFC results than the BW-sIgE value.

Contributors

NK: wrote the manuscript. NK, KY, EK, MN: designed the study. NK, KY, KH, SY: conducted the OFC. KY, EK, MN: reviewed the manuscript for critical content. All the authors have read and approved the final version of the manuscript.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Nonsteroidal anti-inflammatory drugs hypersensitivity in chronic spontaneous urticaria in the light of its pathogenesis

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Key words

Chronic urticaria; nonsteroidal anti-inflammatory drugs; drug allergy; omalizumab; autoimmunity.

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Impact statement

In chronic urticaria NSAID hypersensitivity is independent on the pathogenesis of the skin disease.

Summary

Up to 15% of patients with chronic spontaneous urticaria (CSU) experience severe exacerbations of their baseline cutaneous disease after taking nonsteroidal anti-inflammatory drugs that inhibit cycloxygenase-1 (COX-1) enzyme. These subjects are defined as having a NECD (NSAID-exacerbated cutaneous disease). The way NSAID hypersensitivity correlates with the different pathogenic mechanisms of CSU has not been investigated so far. 235 adults with severe CSU submitted to omalizumab treatment were studied. A rapid omalizumab response was considered as a marker of auto-allergic (Type I) CSU whereas patients showing a slow response or not responding at all were regarded as having a type IIb autoimmune disease. At the first visit medical history of tolerance to aspirin and/or other COX-1 inhibiting NSAID was ascertained. Duration of disease, atopic status, thyroid autoimmunity, CRP, D-dimer plasma levels, and total IgE were assessed appropriately. 23 (10%) were hypersensitive to NSAID. Patients with or without did not differ in any of the variable considered, and a similar proportion in the two groups showed type I or type IIb CSU. The study suggests that in CSU hypersensitivity to NSAID represents a phenomenon that is independent on the pathogenesis of the underlying skin disease.

Introduction

Chronic spontaneous urticaria (CSU) is a disease characterized by the recurrent occurrence of short-lived wheals with or without angioedema for more than 6 weeks (1). Its prevalence may reach 1% of the general population. Although it may appear at any age, it occurs more frequently in adults, affects more women than men, and may show a familiar pattern (2). In recent years, the pathogenic mechanisms underlying this disease have been partially elucidated, particularly after the introduction of anti-IgE mAb (omalizumab) as treatment. We currently recognize three main subsets of CSU characterized by: a) type I autoimmunity (characterized by an IgE-mediated autoimmune mechanism), b) type IIb autoimmunity (characterized by an IgG-mediated autoimmune mechanism targeting the high affinity IgE receptor or the cell membrane bound IgE itself) or c) hitherto unclear mechanisms. The main markers of the former subset are an elevation of total IgE serum levels and a rapid and frequently complete response to omalizumab. In contrast, patients with type IIb CSU frequently show an association with thyroid autoimmunity (3). Type I and type IIb CSU may overlap in some patients (4).

Nonsteroidal anti-inflammatory drugs (NSAID) are the most frequently consumed drugs worldwide, and one of the most frequent causes of drug-induced allergic reactions as well. A large number of studies have eventually led to classify NSAID-induced hypersensitivity reactions into distinct categories (5), one of which specifically addresses the association

of NSAID hypersensitivity with CSU. Patients showing such specific pattern are defined as having NECD (NSAID-exacerbated cutaneous disease); in practice, these CSU patients show a dramatic worsening of the disease after taking NSAID that inhibit Cyclooxygenase 1 (COX-1) enzyme. These subjects generally tolerate selective COX-2 inhibitors (*e.g.*, coxibs or paracetamol). NSAID hypersensitivity may precede by years the onset of frank CSU (6). It has been estimated that up to 30% of CSU patients may have a NECD (7) although this largely depends on the clinical activity of the disease (8). The way NSAID hypersensitivity is associated with CSU in the light of the currently known pathogenic mechanisms has not been investigated so far and represents the issue of the present study.

Patients and methods

Two-hundred-thirty-five patients (M/F ratio: 75/160; mean age 49.8 years, range 7-89) with severe CSU unresponsive to second-generation antihistamines at any dosage were studied. Following the indications by the Italian regulatory agency (AIFA), omalizumab at a fixed dose of 300 mg/month was prescribed to all patients; the treatment was given for at least three months after which responders could pursue the monthly treatment further, whereas non-responders had to stop it. The reason why the present study included only subjects with severe CSU is that omalizumab response (either rapid/complete or slow/incomplete or absent) represents a good and reliable clinical criterion to discriminate between patients with type I or type IIb autoimmune disease, respectively. Omalizumab response was classified as immediate if UAS7 dropped by at least 80% one month after the first administration, delayed if the clinical response was appreciable within 3 months after the first administration, or absent if no clinical change was appreciable one month after the third administration.

At the first visit, all the participants were thoroughly interviewed about their tolerance to aspirin and/or other COX-1 inhibiting NSAID. Following the current guidelines, patients reporting an unequivocal exacerbation of their disease within two hours after taking at least one COX-1 inhibitor were considered as having a NECD. In most cases, patients showed Emergency Room reports regarding such adverse reactions. All patients with NECT underwent oral challenges with drugs exerting little or no COX-1 inhibition including paracetamol, opiates and coxibs as previously described (9); alternative drugs were tolerated in all cases. Duration of disease was recorded, and all patients were assessed for atopic status by skin testing with a complete panel of commercial extracts of seasonal and perennial aeroallergens (Lofarma, Milan, Italy). Thyroid peroxidase IgG autoantibodies, CRP, D-dimer plasma levels, and total IgE were measured. Patients gave an informed written consent to the use of their clinical data in anonymous form. The Internal review board of the Clinic approved the study. Since the study was observational and based only on routine analyses, a formal approval by an external Ethical Committee was not requested. Means were compared by two-tailed Student's t test. Proportions were compared by a χ^2 test with Yates' correction. Probability values less than 5% were considered statistically significant.

Table I *- Clinical features of CSU patients with and without NECD.*

	Total	NECD	NSAID-tolerant	p
No	235	23	212	
Mean Age	49.8	46.1	50.3	NS
Sex (M/F)	75/160	8/15	67/145	NS
Disease duration (months)	51.8	79.8	48.7	NS
Elevated CRP	41 (17%)	7 (30.4%)	34 (16.0%)	NS
Thyroid autoimmunity	53 (23%)	7 (30.4%)	46 (21.7%)	NS
Elevated D-dimer	94 (40%)	13 (56.5%)	81 (38.2%)	NS
Atopic status	68 (29%)	8 (34.7%)	60 (28.3%)	NS
Elevated IgE	84/180 (47%)	12/17 (70.6%)	72/163 (44.1%)	NS
Early response OMA	162 (69%)	19 (82.6%)	143 (67.4%)	NS
Late response OMA	40 (17%)	4 (17.4%)	36 (16.9%)	NS
No response OMA	34 (14%)	1 (4.3%)	33 (15.6%)	NS

Results

Results are summarized in **table I**. The study population characteristics were similar to those found in other studies of CSU: about one fourth of patients showed thyroid autoimmunity, and about one third were atopic. D-dimer plasma levels were elevated in 40% of the population, which is not surprising as these were patients with severe CSU. Altogether, based on rapid omalizumab response 69% of patients were considered as having an autoallergic pathogenic mechanism underlying their skin disease.
Twenty-tree out of 235 (10%) patients had a documented history of severe exacerbations of their underlying cutaneous disease after taking COX-1 inhibiting NSAID. Patients tolerant and not tolerant to COX-1 inhibitors did not show statistical differences in any of the parameters considered, although the latter showed a longer disease duration and a higher prevalence of elevated CRP, thyroid autoimmunity, elevated D-dimer, and elevated total IgE. Similar proportions in the two study groups showed a rapid response, a slow response, or a non-response to omalizumab.

Discussion

This study group, albeit including only patients with severe CSU, was representative of the general population of subjects with CSU as it showed a prevalence of NSAID hypersensitivity of 10% (8, 10). The recent introduction of the mAb omalizumab for the treatment of recalcitrant CSU led to a tremendous acceleration of our understanding of the etiopathogenesis of this disease. We now know that CSU is in most cases an autoimmune disorder characterized by two distinct mechanisms: one mediated by IgG and one mediated by IgE. Although NSAID hypersensitivity may parallel the activity of the underlying skin disease (8), the present study suggests that it represents an independent event as it occurs equally in rapid, slow, or non-omalizumab responders as well as in patients with elevated or normal IgE levels, with/without thyroid autoimmunity, or with/without atopic diseases. Both NECD and NERD (NSAID-exacerbated respiratory disease) are characterized by specific, similar defects in the metabolism of arachidonic acid (8).

Conclusions

This study shows that such defects are not associated with a specific pathogenic subset of the underlying skin disorder.

Fundings

None.

Conflict of interests

The author declares that he has no conflict of interests.

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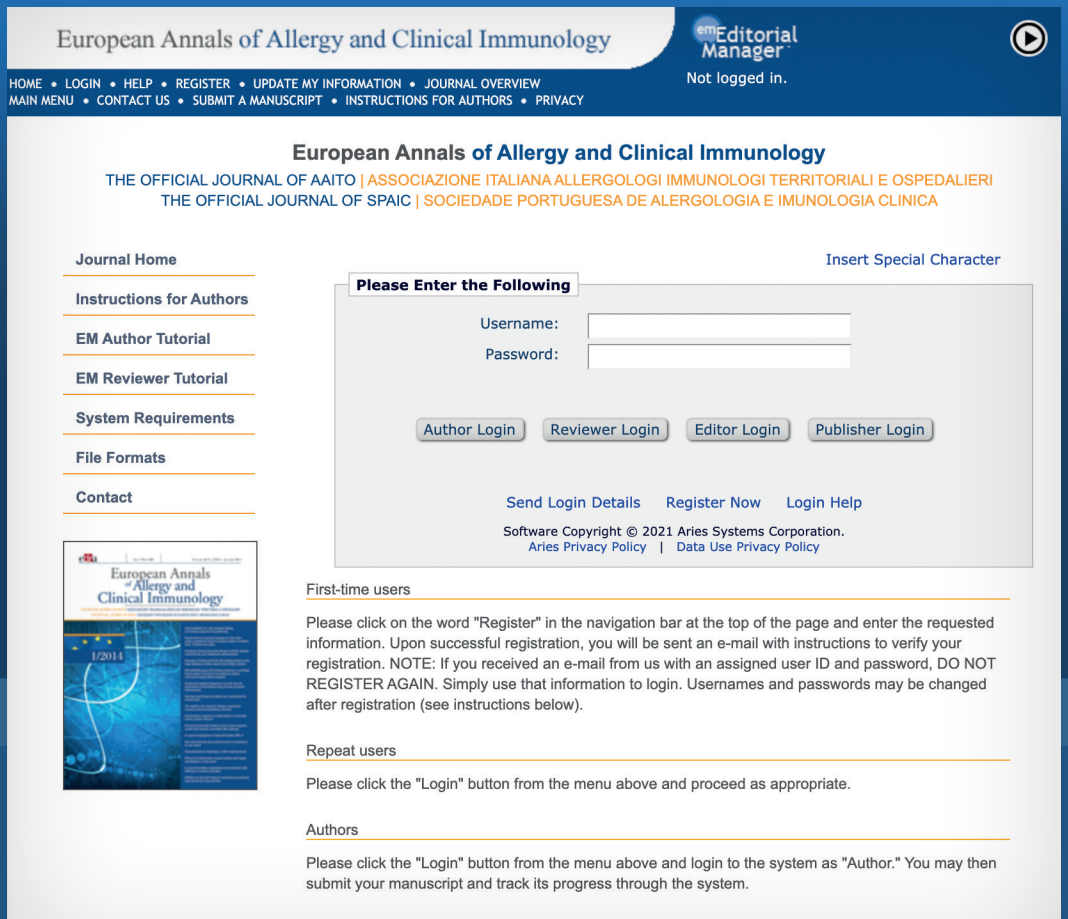

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