frontiers in Genetics

ORIGINAL RESEARCH
published: 25 June 2019
doi: 10.3389/fgene.2019.00582

Identification of Novel Biomarkers for Drug Hypersensitivity After Sequencing of the Promoter Area in 16 Genes of the Vitamin D Pathway and the High-Affinity IgE Receptor

*Gemma Amo[1,2], Manuel Martí[1,2], Jesús M. García-Menaya[3,4], Concepción Cordobés[5,6], José A. Cornejo-García[7,8], Natalia Blanca-López[9,10], Gabriela Canto[9,10], Inmaculada Doña[11,12], Miguel Blanca[9,10], María José Torres[11,12], José A. G. Agúndez[1,2] and Elena García-Martín[1,2]**

[1] University Institute of Molecular Pathology Biomarkers, UEx, Cáceres, Spain, [2] ARADyAL Instituto de Salud Carlos III, Cáceres, Spain, [3] Allergy Service, Badajoz University Hospital, Badajoz, Spain, [4] ARADyAL Instituto de Salud Carlos III, Badajoz, Spain, [5] Allergy Service, Mérida Hospital, Badajoz, Spain, [6] ARADyAL Instituto de Salud Carlos III, Cáceres, Spain, [7] Research Laboratory, IBIMA, Regional University Hospital of Málaga, UMA, Málaga, Spain, [8] ARADyAL Instituto de Salud Carlos III, Cáceres, Spain, [9] Allergy Service, Infanta Leonor University Hospital, Madrid, Spain, [10] ARADyAL Instituto de Salud Carlos III, Madrid, Spain, [11] Allergy Unit, IBIMA, Regional University Hospital of Málaga, UMA, Málaga, Spain, [12] ARADyAL Instituto de Salud Carlos III, Málaga, Spain

OPEN ACCESS

Edited by:
Marcelo Rizzatti Luizon,
Federal University of Minas Gerais, Brazil

Reviewed by:
Ken Batai,
University of Arizona, United States
Meenal Gupta,
The University of Utah, United States

***Correspondence:**
Elena García-Martín
elenag@unex.es

Specialty section:
This article was submitted to Pharmacogenetics and Pharmacogenomics, a section of the journal Frontiers in Genetics

Received: 12 November 2018
Accepted: 04 June 2019
Published: 25 June 2019

Citation:
Amo G, Martí M, García-Menaya JM, Cordobés C, Cornejo-García JA, Blanca-López N, Canto G, Doña I, Blanca M, Torres MJ, Agúndez JAG and García-Martín E (2019) Identification of Novel Biomarkers for Drug Hypersensitivity After Sequencing of the Promoter Area in 16 Genes of the Vitamin D Pathway and the High-Affinity IgE Receptor. Front. Genet. 10:582. doi: 10.3389/fgene.2019.00582

The prevalence of allergic diseases and drug hypersensitivity reactions (DHRs) during recent years is increasing. Both, allergic diseases and DHRs seem to be related to an interplay between environmental factors and genetic susceptibility. In recent years, a large effort in the elucidation of the genetic mechanisms involved in these disorders has been made, mostly based on case-control studies, and typically focusing on isolated SNPs. These studies provide a limited amount of information, which now can be greatly expanded by the complete coverage that Next Generation Sequencing techniques offer. In this study, we analyzed the promoters of sixteen genes related to the Vitamin D pathway and the high-affinity IgE receptor, including *FCER1A, MS4A2, FCER1G, VDR, GC, CYP2R1, CYP27A1, CYP27B1, CYP24A1, RXRA, RXRB, RXRG, IL4, IL4R, IL13,* and *IL13RA1*. The study group was composed of patients with allergic rhinitis plus asthma (AR+A), patients with hypersensitivity to beta-lactams (BLs), to NSAIDs including selective hypersensitivity (SH) and cross-reactivity (CR), and healthy controls without antecedents of atopy or adverse drug reactions. We identified 148 gene variations, 43 of which were novel. Multinomial analyses revealed that three SNPs corresponding to the genes *FCER1G* (rs36233990 and rs2070901), and *GC* (rs3733359), displayed significant associations and, therefore, were selected for a combined dataset study in a cohort of 2,476 individuals. The strongest association was found with the promoter *FCER1G* rs36233990 SNP that alters a transcription factor binding site. This SNP was over-represented among AR+A patients and among patients with IgE-mediated diseases, as compared with control individuals or with the rest of patients in this study. Classification

models based on the above-mentioned SNPs were able to predict correct clinical group allocations in patients with DHRs, and patients with IgE-mediated DHRs. Our findings reveal gene promoter SNPs that are significant predictors of drug hypersensitivity, thus reinforcing the hypothesis of a genetic predisposition for these diseases.

Keywords: Next-Generation Sequencing (NGS), vitamin D, high-affinity IgE receptor (FCεRI), NSAIDs (non-steroidal anti-inflammatory drugs), beta-lactam antibiotic, drugs hypersensitivity reactions, allergic rhinitis, asthma

INTRODUCTION

The prevalence of atopy, allergic diseases, and drug hypersensitivity reactions (DHRs) is increasing worldwide. In Europe, studies have estimated a prevalence of 20–25% allergic diseases in adults, with many young people being unaware of their disease (Linneberg, 2011; Kruse and Vanijcharoenkarn, 2018), which means an important economic impact for healthcare (European Commission, 2008; Bouvy et al., 2015) reaching an amount from €55 to €151 billion per year in European Union, including indirect costs related to the absence or reduced productivity at work (Zuberbier et al., 2014; Kruse and Vanijcharoenkarn, 2018). Due to their complexity, it is difficult to understand the specific mechanisms and molecules involved in the development of these diseases or to establish a way to prevent or reduce them. Allergic rhinitis (AR) reduces the quality of life by affecting sleep, school, work productivity, and social life. AR is an immunoglobulin E (IgE) mediated inflammatory disease, which is associated with other inflammatory diseases such as asthma. It has been estimated that around 20% of people in the USA and Europe suffer from allergic rhinitis (Durham et al., 2012; Ozdoganoglu and Songu, 2012; Rondon et al., 2017). Taking this into consideration, AR has been classified as a major chronic respiratory disease (Brozek et al., 2017). Drug hypersensitivity reactions (DHR) account for, ∼3 to 6% of all hospital admissions. These reactions occur in 10 to 15% of hospitalized patients (Gomes and Demoly, 2005; Szczeklik and Nizankowska-Mogilnicka, 2009; Doña et al., 2014). Beta-lactam antibiotics (BLs) are the most common cause of DHRs mediated by specific immunological mechanisms (Antúnez et al., 2006; Doña et al., 2012, 2014) and, although the mechanisms of how the immune system recognizes these drugs are not fully determined, BLs are considered the classical model of this type of reactions (Blanca et al., 2009). Together with BLs, non-steroidal anti-inflammatory drugs (NSAIDs) are account for the vast majority of DHRs (Gomes et al., 2004; Messaad et al., 2004; Chen et al., 2012; Doña et al., 2012), but, in this case, these DHRs are not exclusively mediated by specific immunological mechanisms (selective hypersensitivity), involving a response to a single drug and good tolerance to other chemically unrelated NSAIDs (Canto et al., 2009; Cornejo-Garcia et al., 2009); but also by nonspecific immunological mechanisms (cross-reactions), which can be caused by more than one chemically unrelated NSAIDs (Agúndez et al., 2012; Kowalski et al., 2013).

Recent investigation proposes the vitamin D pathway among putative factors linked to allergic diseases, because of its important role in immune system (Veldman et al., 2000; Cantorna et al., 2015) and its direct relation with allergic diseases (Black and Scragg, 2005; Camargo et al., 2007; Benson et al., 2012; Suaini et al., 2015). There are many molecules involved in the vitamin D pathway: hydroxylases from CYP450 family, such as CYP27A1, CYP27B1, CYP2R1, and CYP24A1; the vitamin D binding protein (GC) that acts like a transporter, the vitamin D receptor (VDR), the retinoid receptor X (RXR) and interleukins which participate in downstream pathway (IL4 and IL13). In addition, there are other target molecules and signaling pathways, which could be involved in allergic mechanisms, such as the high-affinity IgE Receptor (FCεRI), which plays a key role in allergic reactions. This receptor is stimulated by IgE, triggering mast cells and basophils activation, and the consequent release of inflammatory mediators. In human mast cells and basophils, FCεRI consist of a heterotetramer composed by three subunits: FCεRIα, the ligand-binding subunit which is encoded by *FCER1A* gene; FCεRIβ, a signal-augmenting subunit encoded by *MS4A2*; and FCεRIγ, a signal-transducing subunit that is presented like a dimer and it is encoded by *FCER1G* (Kinet, 1999; Potaczek and Kabesch, 2012). Elevated levels of IgE have been detected in atopic conditions like allergic rhinitis, asthma, atopic dermatitis, anaphylaxia (Platts-Mills, 2001; Wallace et al., 2008) thus making FCεRI a plausible target molecule in the study of the mechanisms involved in the development and in the clinical presentation of allergy.

It could be hypothesized that variations related to expression and/or function in genes of the vitamin D signaling pathways or FCεRI might modify the risk of developing rhinitis or DHRs, and/or the presentation of clinical manifestations of these reactions. As a matter of fact, several studies demonstrated an association between different allergic diseases, including DHRs, and polymorphisms in these genes (Poon et al., 2004; Raby et al., 2004; Donfack et al., 2005; Bossé et al., 2009; Saadi et al., 2009; Pillai et al., 2011; Micheal et al., 2013; Berenguer et al., 2014; Amo et al., 2016a; Narozna et al., 2016). Several studies addressed the putative impact of exonic and intronic SNPs within the above-mentioned genes and the risk of allergic diseases and/or DHR (Wjst, 2005; Wjst et al., 2006; Battle et al., 2007; Arshad et al., 2008; Sadeghnejad et al., 2008; Weidinger et al., 2008; Black et al., 2009; Bossé et al., 2009; Ferreira et al., 2009; Knutsen et al., 2010; Li et al., 2010, 2012, 2014, 2016; Michel et al., 2010; Moffatt et al., 2010; Cooper et al., 2011; Joubert et al., 2011; Liu et al., 2011; Lu et al., 2011; Park et al., 2011; Paternoster et al., 2011; Pillai et al., 2011; Burkhardt et al., 2012; Choi et al., 2012; Granada et al., 2012; Lasky-Su et al., 2012; Ramasamy et al., 2012; Robinson et al., 2012; Zhou et al., 2012; Anderson et al., 2013; Hur et al., 2013; Ismail et al., 2013; Movahedi et al., 2013; Potaczek et al.,

2013; Sharma et al., 2014; Yang et al., 2014; Kumar et al., 2015; Papadopoulou et al., 2015; Pino-Yanes et al., 2015; Tian et al., 2015; Amo et al., 2016a,b; Han et al., 2016; Karaca et al., 2016; Narozna et al., 2016; Overton et al., 2016; Ådjers et al., 2017; Ashley et al., 2017; Park and Tantisira, 2017; Sun et al., 2017; Xu et al., 2017; Zhang et al., 2017; Zhao et al., 2017). However, there is little information about SNPs located in the promoters of these genes, which might have functional consequences.

In an attempt to identifying genetic susceptibility factors associated with allergy and/or DHRs, that may provide novel information to gain a better understanding of these pathologies, we carried out an exhaustive analysis of genetic variations situated in the promoter region of the mentioned genes by using Next Generation Sequencing (NGS) in patients with allergic rhinitis plus asthma (AR+A), BLs hypersensitivity, selective NSAIDs hypersensitivity (SH) and cross-reactions to NSAIDs (CR), as well as in healthy control individuals. The genes included in the study were *FCER1A*, *MS4A2*, *FCER1G*, *VDR*, *GC*, *CYP2R1*, *CYP27A1*, *CYP27B1*, *CYP24A1*, *RXRA*, *RXRB*, *RXRG*, *IL4*, *IL4R*, *IL13,* and *IL13RA1*. In addition, we also analyzed the interaction of genetic and non-genetic factors, such as age, gender, and antecedents of atopy, in the risk of developing these diseases.

PATIENTS AND METHODS

Study Population

A total cohort of 2,476 individuals participated in this study. All were Caucasian Spanish individuals. These included 406 healthy controls without antecedents of atopy or adverse drug reactions, 528 patients with AR+A, 561 individuals with BLs hypersensitivity, 668 patients with NSAIDs cross-reactivity (CR), and 313 selective hypersensitivity patients (SH) which were single-NSAIDs responders. Written consent for participation was obtained for all participants. Patients were recruited at Hospitals participating in the study. All the patients who were invited to participate in the study agreed to do so. Control individuals were selected among students and staff in the University and Hospitals participating in the study. Characteristics of the study groups are summarized in **Table 1**. The diagnosis was carried out as described elsewhere (García-Martín et al., 2007; Doña et al., 2011; Amo et al., 2016a; Lacombe-Barrios, 2018). The protocol for this study was in accordance with the Declaration of Helsinki and its subsequent revisions and was approved by the respective Ethics Committees of the participating Hospitals.

To get a further analysis of the sample, we put together some of the groups of patients which share a specific characteristic. Thus, we defined three new groups of study: "DHR group," were we included all the patients with DHR: namely, patients with hypersensitivity to BLs and NSAIDs (both, CR and SH); "DHR-IgE Group," which comprises selective hypersensitivity to BLs and SH; and "IgE Mediated Group," where we included all the IgE-mediated reactions (AR+A, BLs and SH).

Identification of Novel Variants Using NGS

A subset of participants were selected for this phase. A total cohort of 175 individuals participated in this NGS analysis. These included 22 healthy controls without antecedents of atopy or adverse drug reactions, 22 patients with AR+A, 43 individuals with BLs hypersensitivity, 41 patients with NSAIDs cross-reactivity (CR), and 46 selective hypersensitivity patients (SH) which were single-NSAIDs responders. Characteristics of the participants are summarized in **Table S1**. Genomic DNA was obtained from leukocytes and purified according to standard procedures. DNA samples were analyzed by NGS after specific enrichment based on the Haloplex design. Details of the areas sequenced are shown in **Table S2**. DNA was digested with restriction enzymes specific for this design (Haloplex, Agilent, Santa Clara, CA, USA), followed by hybridization with specific probes, DNA circularization and selection of the target areas, according to the protocol supplied. Sequencing was carried out in a MiSeq sequencer (Illumina, San Diego, CA, USA) using the pair end format. The coverage was always higher than that recommended by the manufacturer (23.7 Mb per sample). All variants identified had at least a 50X coverage and more than 95% of these had more than 100x coverage. The sequencing results were analyzed by using the application SureCall 4.0 (Agilent, Santa Clara, CA, USA), adapted to the analysis of enriched Haloplex sequences, and MiseqReporter V04 (Illumina, San Diego, CA). Sequence revision against human genome was carried out by using the Integrative Genomes Viewer (Broad Institute, Cambridge, MA, USA).

Combined Dataset Analyses

All patients and controls participated in this phase. Analyses were carried out by using TaqMan genotyping focused on the SNVs raised after multiple comparison analyses of the NGS phase (see the results section for further details). The SNPs were analyzed in triplicate, by using SNP TaqMan assays (Life Technologies S.A., Alcobendas, Madrid, Spain), and following the conditions specified by the manufacturer. Assay details are as follows: FCER1G-rs36233990, Custom TaqMan® Assay; FCER1G-rs2070901, (C__15867981_20); and GC-rs3733359, (C__25652813_40).

Statistical Analysis

The R package SNPasoc (Gonzalez et al., 2014) was used to calculate allele and genotypic frequencies, to determine the Hardy-Weinberg equilibrium using exact test (Wigginton, 2005) and to analyse differences between groups (González et al., 2007). The comparison between groups was performed with the Fisher's Exact Test (FET) and Likelihood Ratio Test (LRT) with an initial crude analysis followed by an adjusted analysis including gender as the categorical covariate when it was possible. False Discovery Rate (FDR) correction was used for the multiple comparison adjustments (Benjamini et al., 2001). The results were considered statistically significant when *P*-values were under 0.05. The association between SNPs and traits was estimated by odds ratio (OR) with a 95% confidence interval (CI) or by Relative Risk (RR) when the variation was not found in the control group. The Relative Risk was calculated by using EpiBasic, a tool for statistical analysis of tabular information, performing a stratified analysis, using the inverse variance ($1/SE^2$) as weigh. This tool was developed as a companion to a Danish textbook on epidemiology (Juul, 2012), and the

TABLE 1 | Characteristics of the participants.

	Healthy controls (n = 406)	Patients with AR+A (n = 528)	Patients with BLs hypersensitivity (n = 561)	Patients with NSAIDs cross-reactions (CR) (n = 668)	Patients with NSAIDs selective hypersensitivity (SH) (n = 313)
Women. n (%)	253 (62.3%)	292 (55.3%)	318 (56.7%)	390 (58.4%)	204 (65.2%)
Age + sd (range)	22.1 ± 4.7 (20–58)	32.4 ± 14.2 (14–79)	46.7 ± 14.5 (4–91)	41.8 ± 15.3 (5–92)	45.5 ± 16.0 (5–82)
Antecedents of atopy	0	100%	23.3%	20.8%	23.3%

spreedsheat could be download from the following link (Juul and Frydenberg, 2016): http://ph.medarbejdere.au.dk/undervisning-og-uddannelse/software/.

Association between each SNP and each clinical phenotype was assessed by using binary logistic regression. Then, predictive Models based on Multinomial Logistic Regression (MLR) (Agresti, 2003) were performed for SNPs showing association in the binary regression analyses by using SPSS (IBM SPSS Statistics for Windows, Version 22.0). The p-values associated with every SNP were calculated using the Chi-Square test. Each model has associated several pseudo-R^2 coefficients as indicators of the strength of the association between the response and the predictor variables. Cox and Snell is based on the loglikelihood for the model compared to the log likelihood for a baseline model and it has a theoretical maximum value of <1, even for a "perfect" model (Cox and Snell, 1989) and Nagelkerke is an adjusted version of the Cox & Snell R-square that adjusts the scale of the statistic to cover the full range from 0 to 1 (Nagelkerke, 1991). McFadden is another version, based on the log-likelihood kernels for the intercept-only model and the full estimated model. This is the pseudo-R^2 coefficient most frequently used and the correlation between variables is good when the values are comprised between 0.2 and 0.4, and better up to 0.4 (McFadden, 1974, 1977). The first model includes all the groups separately, that is, AR+A, BLs, CR and SH. Model 2 considered two groups of patients: AR+A and a group combining all DRH patients. Model 3 considered three groups of patients: AR+A, patients with Ige-Mediated DHR, and patients with DHR not related to IgE (that is, CR patients). For all models the control group was always the reference group. Coefficients were calculated by dropping samples with missing data in explanatory variables, which have been selected using stepwise regression method. The statistical power was calculated from variant allele frequencies with a genetic model analysing the frequency for carriers of the disease gene with a RR value = 2 ($p = 0.05$) for the genetic associations identified in the combined dataset model as described elsewhere (Pértegas Díaz and Pita Fernández, 2003). These values are shown in **Table S3**. The functional impact of the gene variants was analyzed by using TRANSFAC (Matys et al., 2003, 2006).

RESULTS

Identification of Novel Variants Using NGS

In this phase we identified 148 variations situated in the promoter region of genes related with vitamin D and FCεRI genes. The information about the variations, their frequency in the whole sample and the Hardy-Weinberg equilibrium values is summarized in **Table 2**. It is to be noted that 84 out of the 148 (56.7%) of the SNPs identified in this study were found in cases only and not in control individuals.

Among the 148 gene variations identified, 43 were novel. Within the 105 already described SNPs, 25 have not been described or studied earlier in European individuals, although they show marginal MAF in our study (only three SNPs show MAF above 0.010). Regarding known SNPs, the frequencies are concordant with the results previously described in the 1,000 Genomes public database (http://grch37.ensembl.org/index.html) for individuals with European descent for all the variations except for the rs4020369 SNP in the *GC* gene, where the described frequency for Europeans is equal to 0, but in our population it shows a MAF close to 0.040, that is in agreement with the global frequency described in 1,000 Genomes for overall individuals.

One hundred and three out of the 148 variants identified were at Hardy-Weinberg equilibrium (HWE) in the overall study population (see **Table S4**), which is to be expected given the high number of SNPs analyzed and the limited sample size in the NGS analyses. Within the 45 variants that were not in HWE, only 7 showed a MAF>0.050 in agreement with frequencies described in literature. We carried out binary logistic regression analyses excluding those variants with MAF<0.02 (see **Table 2**). Results of the regression analyses are shown in **Table S5**. Among the 44 variants we selected those with adjusted $P \leq 0.10$ for multinomial analyses. Therefore, 25 variants were included in the multinomial analysis as well as gender, antecedents of atopy and clinical group (Allergic rhinitis + Asthma; BLs, CR and SH). It is to be noted that some SNPs with a high significance after logistic binary regression analyses (See **Table S5**), such as rs1467664 (*RXRG*), rs3733359 (*GC*), rs2070874 (*IL4*), rs4303288, and rs4307775 (*VDR*) and rs2259735 (*CYP24A1)*, were not significant after multinomial analysis. The statistically significant variables raised after this analysis were three SNPs (*FCER1G* rs36233990, *FCER1G* rs2070901, and *GC* rs3733359), as well as antecedents of atopy.

Combined Dataset Analyses

The three SNPs mentioned above were analyzed in the whole study group. The *FCER1G* rs36233990 SNP was monomorphic in the control group, whereas heterozygous subjects were identified in all subgroups of patients and homozygous individuals were identified in the AR+A and CR groups. Statistically significant

TABLE 2 | SNPs with MAF ≥ 0.02 observed in the NGS study.

Gene	dbSNP	Chromosomal Location	Alleles	MAF	HWE	MAF 1,000 Genomes ALL individuals	MAF 1,000 Genomes European individuals	MAF genomAD ALL individuals	MAF genomAD European individuals
FCER1A	rs2427837	1:159258545	G/A	0.213 A	0.501	0.151 A	0.304 A	0.217 A	0.289 A
FCER1A	rs61828219	1:159258641	C/A	0.141 A	0.752	0.085 A	0.160 A	0.129 A	0.175 A
FCER1A	rs12135235	1:159259029	T/G	0.043 G	1.000	0.033 G	0.033 G	0.034 G	0.037 G
FCER1G	rs36233990	1:161184658	C/T	0.020 T	0.134	0.011 T	0.006 T	0.010 T	0.013 T
FCER1G	None	1:161184792	GTCTCAAAAA/G	0.023 G	1.000	–	–	–	–
FCER1G	None	1:161184793	TCTCAAAAA/T	0.026 T	1.000	–	–	–	–
FCER1G	None	1:161184794	CTCAAAAAA/C	0.052 C	1.000	–	–	–	–
FCER1G	None	1:161184795	TCAAAAA/T	0.121 T	0.474	–	–	–	–
FCER1G	None	1:161184796	C/A	0.020 A	0.000	–	–	–	–
FCER1G	rs11587213	1:161184875	A/G	0.171 G	0.284	0.146 G	0.179 G	0.142 G	0.171 G
FCER1G	rs41270847	1:161184976	G/A	0.023 A	1.000	0.007 A	0.020 A	0.010 A	0.015 A
FCER1G	rs2070901	1:161185058	G/T	0.276 T	0.328	0.391 T	0.269 T	0.314 T	0.248 T
RXRG	rs3753897	1:165414511	C/A	0.152 A	0.082	0.215 A	0.195 A	0.211 A	0.193 A
RXRG	rs1467664	1:165414933	T/C	0.178 C	0.073	0.203 C	0.142 C	0.181 C	0.138 C
GC	rs3733359	4:72649774	G/A	0.069 A	0.894	0.206 A	0.055 A	0.122 A	0.060 A
GC	rs76781122	4:72669661	C/A	0.034 A	1.000	0.013 A	0.034 A	0.019 A	0.028 A
GC	rs6843222	4:72669944	C/T	0.029 T	1.000	0.004 T	0.016 T	0.007 T	0.011 T
GC	rs35096193	4:72670093	C/A	0.236 A	0.059	0.165 A	0.284 A	0.203 A	0.272 A
GC	rs1565572	4:72670191	A/C	0.210 C	0.499	0.432 C	0.196 C	0.363 C	0.192 C
GC	rs4020369	4:72670448	G/A	0.037 A	1.000	0.033 A	0	0.037 A	0.008 A
IL13	None	5:131992098	G/C	0.023 C	0.000	–	–	–	–
IL13	rs1800925	5:131992809	C/T	0.223 T	0.001	0.255 T	0.178 T	0.270 T	0.226 T
IL13	rs2066960	5:131994435	C/A	0.082 A	1.000	0.199 A	0.115 A	0.176 A	0.125 A
IL13	rs1295687	5:131994462	G/C	0.072 C	0.045	–	–	0.131 C	0.064 C
IL4	rs2243250	5:132009154	C/T	0.141 T	0.752	0.470 T	0.168 T	0.371 T	0.176 T
IL4	rs2070874	5:132009710	C/T	0.132 T	0.507	0.401 T	0.168 T	0.279 T	0.148 T
RXRB	rs76929655	6:33169182	T/C	0.020 C	1.000	0.002 C	0.005 C	0.007 C	0.011 C
CYP2R1	rs12794714	11:14913575	G/A	0.445 A	0.647	0.349 A	0.447 A	0.406 A	0.433 A
MS4A2	rs573790	11:59855385	C/T	0.382 T	1.000	0.441 T	0.356 T	0.460 T	0.411 T
MS4A2	rs574700	11:59855483	C/T	0.032 T	1.000	0.124 T	0.039 T	0.084 T	0.018 T
MS4A2	rs1441585	11:59855711	T/C	0.032 C	1.000	0.112 C	0.038 C	–	–
MS4A2	rs1441586	11:59856028	T/C	0.414 C	0.755	0.460 C	0.456 C	0.425 C	0.418 C
VDR	rs117397914	12:48276613	A/G	0.031 G	0.150	0.009 G	0.018 G	0.011 G	0.016 G
VDR	rs11168293	12:48293716	G/T	0.284 T	0.712	0.166 T	0.321 T	0.282 T	0.355 T
VDR	rs4303288	12:48336619	A/C	0.467 C	0.035	0.404 A	0.397 A	0.402 A	0.389 A
VDR	rs4307775	12:48336623	C/G	0.139 G	0.000	0.171 G	0.209 G	0.185 G	0.249 G
IL4R	rs12927172	16:27325021	G/A	0.424 A	0.013	0.405 A	0.372 A	0.353 A	0.367 A
IL4R	rs12927543	16:27325023	A/G	0.109 G	0.125	0.082 G	0.088 G	0.066 G	0.080 G
CYP24A1	rs35873579	20:52788190	G/A	0.023 A	1.000	0.001 A	0.004 A	0.002 A	0.003 A
CYP24A1	rs36106327	20:52788294	C/A	0.026 A	0.100	0.007 A	0.020 A	0.010 A	0.016 A
CYP24A1	rs2259735	20:52788314	T/C	0.455 C	0.029	0.566 C	0.420 C	0.515 C	0.408 C
CYP24A1	rs2762943	20:52790786	G/T	0.095 T	0.652	0.034 T	0.085 T	0.037 T	0.081 T
CYP24A1	rs2585427	20:52790976	G/C	0.376 C	0.873	0.444 C	0.390 C	0.447 C	0.373 C
IL13RA1	rs6603441	X:117861321	T/G	0.356 G	0.000	0.452 G	0.315 G	0.407 G	0.322 G

-, not described; MAF, Minor Allele Frequency.

differences were identified for this SNP in all subgroups of patients (**Table 3**), although after FDR correction differences remained significant for the AR+A and BLs groups. The *FCER1G* rs2070901 SNP had a marginal trend toward higher frequency of the variant allele among CR patients, which was not statistically significant after FDR analysis. These two FCER1G SNPs are not

TABLE 3 | Combined dataset analyses.

			Rhinits+Asthma (AR+A) vs. controls comparison values (*N* = 528 vs. 406)			Bls vs. Controls comparison values (*N* = 561 vs. 406)			CR vs. Controls comparison values (*N* = 668 vs. 406)			SH vs. Controls comparison values (*N* = 313 vs. 406)		
SNP ID	**Genotype**	**Control frequency**	**AR+A frequency**	**OR (95% CI) with covariates (1) (2)**	**P_{cov} (FDR) (2)**	**BLs frequency**	**OR (95% CI) with covariates (1) (2)**	**P_{cov} (FDR) (2)**	**CR Frequency**	**OR (95% CI) with covariates (1) (2)**	**P_{cov} (FDR) (2)**	**SH Frequency**	**OR (95% CI) with covariates (1) (2)**	**P_{cov} (FDR) (2)**
FCER1G-rs36233990	C/C	1	0.953	1.00	0.000 (0.000)	0.991	1.00	0.025 (0.041)	0.990	1.00	0.043 (0.071)	0.993	1.00	0.077 (0.231)
	C/T	0	0.045	1.76 (1.66–1.86)		0.009	1.67 (1.53–1.76)		0.008	*		0.007	*	
	T/T	0	0.002	*		–	–		0.002	*		0	–	
FCER1G-rs2070901	G/G	0.482	0.531	1.00	0.065 (0.097)	0.545	1.00	0.073 (0.073)	0.524	1.00	0.047 (0.071)	0.521	1.00	0.328 (0.492)
	G/T	0.453	0.381	0.75 (0.56–0.99)		0.380	0.73 (0.55–0.96)		0.382	0.77 (0.59–1.01)		0.399	0.81 (0.59–1.11)	
	T/T	0.065	0.088	1.18 (0.69–2.00)		0.075	1.00 (0.58–1.72)		0.094	1.32 (0.80–2.20)		0.080	1.13 (0.62–2.06)	
GC-rs3733359	G/G	0.874	0.892	1.00	0.646 (0.646)	0.910	1.00	0.027 (0.041)	0.901	1.00	0.212 (0.212)	0.886	1.00	0.632 (0.632)
	G/A	0.118	0.104	0.88 (0.57–1.36)		0.090	0.73 (0.47–1.13)		0.098	0.79 (0.52–1.22)		0.110	0.92 (0.57–1.49)	
	A/A	0.008	0.004	0.50 (0.08–3.03)		0	0.00 (0.00–)		0.002	0.22 (0.02–2.10)		0.003	0.37 (0.04–3.63)	

Comparison between groups of patients and controls. (1) Relative Risk was calculated when the reference group (control) shows only non-mutated frequency. (2) OR and P-value were adjusted for gender. (*) OR adjusted by gender cannot be calculated due to lack of cases.

at linkage disequilibrium, being the D' value = 0.3415 and the *r* value = 0.0504. The minor allele frequency for the *GC* rs3733359 SNP was lower in the BLs group as compared to that of healthy individuals. This comparison remained statistically significant after FDR analysis.

The patients were grouped according to the underlying mechanism of the reaction. The first group was composed of all patients with drug hypersensitivity (DHR group), that is, (BLs + CR + SH). The second group of patients was composed of all patients with IgE-mediated drug hypersensitivity (D-IgE) (BLs + SH). The third group of patients was composed of all patients with IgE-mediated reactions (BLs + SH + AR+A), which were compared vs. healthy controls (**Table 4**). The two *FCER1G* SNPs displayed statistically significant differences in DHR and IgE-mediated reactions as compared to control individuals, although the only difference that remained significant after FDR correction was that of the SNP FCER1G rs2070901 in patients with IgE-mediated diseases (**Table 4**).

Classification Models

We built models including the three SNPs with significant associations in the combined dataset analyses phase, as well as the antecedents of atopy and gender, and the pseudo R-square values for each model (those which provide the best classification of each patient in its correct group) are shown in **Table 5**. All the models selected the *FCER1G* rs36233990 as a good variable. The variable "Antecedents of atopy" was also selected, although this was expected because none of the control individuals had antecedents of atopy. Model 1 was made by including the three SNPs with a significant *P*-value and all the clinical groups separately, as compared to control subjects. Model 2 included all patients with DHR compared to control individuals, and model 3 included all patients with IgE-mediated diseases vs. control individuals. The classifications per group are shown in **Table 6**. It is to be noted the high percentage of correct allocations using the three SNPs only (that is, without considering antecedents of atopy and gender). For comparison, we show in **Table 6** the results of the same models including covariables such as antecedents of atopy, age and gender. The fact that antecedents of atopy predicted 100% of AR+A patients has little value because control individuals had no antecedents. Age and gender, however, are not good predictors either (**Table 6**). Therefore, these covariables did not improve the predictive capacity of the models based on the SNPs.

DISCUSSION

Genetic variation is a major cause of interindividual differences in the susceptibility to a number of disorders. In this regard, a huge number of genetic association studies related to allergic disorders and drug hypersensitivity events have been carried out. Most of these studies have a case-control design interrogating only a few polymorphisms, typically, a few SNPs located within the coding region. The use of NGS techniques allows for a complete coverage of large areas thus revealing novel SNPs or analysing SNPs that are not included in most studies. In a previous NGS study in the promoter area of the genes encoding the COX-1 and COX-2 enzymes, we identified several novel SNPs. More

TABLE 4 | Combined dataset analyses.

SNP ID	Genotype	Control Freq.	DHR vs. Control comparison values (*N* = 1,542 vs. 406)			DHR-IGE- vs. Control comparison values (*N* = 874 vs. 406)			All IGE-Mediated vs. Control comparison values (*N* = 1402 vs. 406)		
			Drugs (BLs. CR. SH) Freq	OR (95% CI) with covariates (1) (2)	P_{cov} (FDR) (2)	Drugs IgE (BLs. SH) Freq.	OR (95% CI) with covariates (1) (2)	P_{cov} (FDR) (2)	IgE Mediated (AR+A. SH. BLs) Freq.	OR (95% CI) with covariates (1) (2)	P_{cov} (FDR) (2)
FCER1G-rs36233990	C/C	1	0.991	1.00	0.047 (0.071)	0.992	1.00	0.025 (0.075)	0.977	1.00	0.001 (0.003)
	C/T	0	0.008	1.26 (1.23–1.29)		0.008	1.43 (1.38–1.49)		0.022	1.28 (1.25–1.31)	
	T/T	0	0.001	*		0	–		0.001	*	
FCER1G-rs2070901	G/G	0.482	0.531	1.00	0.044 (0.071)	0.536	1.00	0.089 (0.094)	0.534	1.00	0.047 (0.071)
	G/T	0.453	0.385	0.77 (0.61–0.97)		0.387	0.76 (0.59–0.98)		0.385	0.76 (0.60–0.96)	
	T/T	0.065	0.084	1.17 (0.74–1.87)		0.077	1.06 (0.64–1.74)		0.081	1.11 (0.69–1.77)	
GC-rs3733359	G/G	0.874	0.901	1.00	0.076 (0.076)	0.902	1.00	0.094 (0.094)	0.898	1.00	0.181 (0.181)
	G/A	0.118	0.097	0.80 (0.55–1.15)		0.097	0.80 (0.54–1.18)		0.100	0.83 (0.57–1.20)	
	A/A	0.008	0.001	0.17 (0.03–1.00)		0.001	0.14 (0.01–1.32)		0.002	0.26 (0.05–1.30)	

Comparisons depending on the mechanism. (1) Relative Risk was calculated when the reference group (control) shows only non-mutated frequency. (2) OR and P-value were adjusted for gender. (*) OR adjusted by gender cannot be calculated due to lack of cases.

TABLE 5 | Statistically significant variables in each model and goodness-of-fit measures.

Variables	Model 1: Single groups model (*p*-value)*	Model 2: DHR Model (BLs, CR, SH)/Rhinitis+ asthma/Control) (*p*-value)*	Model 3: IgE Mediated Drugs Model (BLs, SH)/Rhinitis+ asthma/CR/Control) (*p*-value)*
FCER1G rs36233990	0.001	0.000	0.000
FCER1G rs2070901	0.520	0.169	0.337
GC rs3733359	0.810	0.517	0.766
Antecedents of atopy	0.030	0.000	0.000
Gender	0.000	0.106	0.137
Pseudo R-square			
Cox and Snell	0.522	0.520	0.521
Nagelkerke	0.546	0.606	0.557
McFadden	0.237	0.376	0.268

**The overall effectiveness of the model was assessed by using the Chi-square statistic.*

TABLE 6 | Prediction models.

Observed (clinical classification)	Predicted (Group allocation according to the model) SNPs only (%) correct	Predicted (Group allocation according to the model) SNPs + Antecedents of atopy + gender+ age (%) correct
AR+A ($n = 481$)	4.1	98.2
BLs ($n = 230$)	49.0	7.0
CR ($n = 553$)	53.4	68.6
SH ($n = 300$)	0	12.3
DHR ($n = 1083$)	99.0	81.8
DHR IgE-Mediated ($n = 530$)	99.2	49.6
All IgE-Mediated ($n = 1,326$)	99.9	79.6

than 70 SNPs modified transcription factor binding sites, either by disrupting existing sequences or by creating new binding sites (Agundez et al., 2014).

The present study is aimed to analyse the promoter areas of 16 genes related to allergic diseases and drug hypersensitivity reactions (DHRs). We have focused on the promoter gene region due to its crucial role in transcriptional activity and expression of the gene, as it has been observed for the SNPs located in the promoter of *FCER1A* (Potaczek et al., 2009), or *IL13* (Cameron et al., 2006; Kiesler et al., 2009; Li et al., 2014). The rationale for the selection of the 16 genes included in this study is based on putative mechanisms involved this type of reactions. FCERI plays an essential role in IgE-mediated mechanisms and variants in *FCERI* genes have been previously described as genetic factors related to asthma (Cui et al., 2003; Kim et al., 2006; Palikhe et al., 2008; Joubert et al., 2011; Ramphul et al., 2014; Yang et al., 2014), allergy (Hasegawa et al., 2003; de Guia et al., 2015; Liao et al., 2015; Amo et al., 2016a,b), and food sensitization (Liu et al., 2011; Hong and Wang, 2012). It has been also described that some variants in genes involved in the vitamin D pathway are related with asthma (Poon et al., 2004; Raby et al., 2004; Wjst, 2005; Wjst et al., 2006; Bossé et al., 2009; Saadi et al., 2009; Li et al., 2011; Pillai et al., 2011; Maalmi et al., 2013; Leung et al., 2015; Hutchinson et al., 2017), especially variations in genes regulated by vitamin D, such as *IL4* and its receptor (Burchard et al., 1999; Donfack et al., 2005; Ober and Hoffjan, 2006; Battle et al., 2007; Michel et al., 2010; Baye et al., 2011; Hesselmar et al., 2012; Liu et al., 2012; Micheal et al., 2013; Nie et al., 2013; Zhu et al., 2013; Al-Muhsen et al., 2014; Berenguer et al., 2014; Klaassen et al., 2015; Zhang et al., 2015; Narozna et al., 2016) and *IL13* (Black et al., 2009; Bottema et al., 2010; Palikhe et al., 2010; Cui et al., 2012; Accordini et al., 2016; Xu et al., 2017), which are also related to IgE (Marsh et al., 1994; Kabesch et al., 2006). According to previous research, the mechanisms involved in cross-reactions and selective ones are different, (Doña et al., 2012; Ayuso et al., 2013; Torres et al., 2014; Nissen et al., 2015; Amo et al., 2016a) and previously published results show that some variations, either related to *FCERI* or to vitamin D, are strongly associated with IgE-mediated pathologies, like rs12135235 in *FCER1A*, rs144205117 in *CYP2R1*, rs1467664 in *RXRG* or rs4303288 in *VDR*. Association between the rs2070874 in *IL4* and atopy and hypersensitivity, has been described in previously published works (Burchard et al., 1999; Donfack et al., 2005; Kabesch et al., 2006; Ober and Hoffjan, 2006; Kim et al., 2010; Madore and Laprise, 2010; Baye et al., 2011; Lu et al., 2011; Liu et al., 2012; Andiappan et al., 2013; Hsu et al., 2013; Micheal et al., 2013; Movahedi et al., 2013; Zhu et al., 2013; Berenguer et al., 2014; Caniatti et al., 2014; Li et al., 2014; de Guia et al., 2015; Klaassen et al., 2015; Zhang et al., 2015; Hua et al., 2016; Narozna et al., 2016).

Although binary logistic regression analyses pointed to six SNPs corresponding to *RXRG*, *GC*, *IL4*, *VDR*, and *CYP24A1* (see the Results section), statistical significance for these SNPs was not supported after multinomial analysis, except for the *GC* SNP. By turn, two additional *FCER1G* SNPs, as well as the *GC* SNP, were statistically significant after multinomial analyses. It is important to note that the major findings obtained in the present study are novel, since only one of the three SNPs that remained after the multinomial analysis have been previously related with atopy or drug hypersensitivity. Among these, one of the *FCER1G* SNPs is novel and hence have not been studied before, and the other one has been related with food sensitization (Liu et al., 2011) and has been previously studied in patients with selective hypersensitivity to NSAIDs and allergic rhinitis without significant association (Amo et al., 2016a,b). After the NGS and combined dataset analyses phases, prediction models revealed that one of these SNPs, designated as *FCER1G* rs36233990 was correct in all models and it allowed an excellent prediction for patients with DHR, IgE-mediated DHR and all IgE-mediated diseases analyzed. It should be taken into consideration that the significant *p*-values observed for rs36233990 in case-control association analyses might be inflated because this SNP was not observed in controls. However, this is a commonly observed SNP in European populations,

which underscores the need for large control sets. This is a limitation in this study. The rs36233990 variation is located in a regulatory region where multiple transcription factor binding sites exist. The variant allele T triggers the appearance of E2F-3:Prrxl1 complex and GKLF (KLF4). On the other hand, the variant allele T leads the disappearance of a binding site for the transcription factor p300. Our own previous findings supported a role of *FCER* gene variations in patients with AR+A, but not in patients with SH (Park et al., 2011; Amo et al., 2016a) which are consistent with those raised in this study. The minor allele of rs2070901 in *FCERIG* triggers the disappearance of a transcription factor binding site for ELK-1: OC-2. The *GC* variation designated as rs3733359 is located in a splice region for transcripts 2 and 3 of GC, and in the 5' untranslated region for transcripts 1 and X1. This variant has been previously related to immune and other disorders (Jung et al., 2011; Wang et al., 2015; Xie et al., 2018). Our findings regarding the *GC* polymorphism support the hypothesis of a relevant role of vitamin D in allergy (Hall and Agrawal, 2017; Tian and Cheng, 2017.

In summary, our findings show that the analysis of the gene promoters is useful for the identification of genetic biomarkers of risk for DHRs and AR+A. Models using these gene variations allow a high degree of prediction, that is, correct group allocations (**Table 6**) based on these SNPs only. It should be kept in mind that the variant allele frequencies for these SNPs are relatively low, that is, the frequency of carriers of the risk variants is relatively low, specially for the most significant SNP *FCER1G* rs36233990 (<2% of patients). The frequencies of carriers for other two SNPs are 47 and 10.5% for *FCERIG* rs2070901 and *GC* rs3733359, respectively. Therefore, the presence of these gene variations cannot explain, by itself, the development of most cases of DHR. However, the SNPs raised in this study, point to mechanisms involved in DHR and add novel information that can be used as a proof of mechanism.

AUTHOR CONTRIBUTIONS

EG-M and JA contributed conception and design of the study. JG-M, CC, JC-G, NB-L, GC, ID, MB, and MT recruited and characterized patients. MM performed the statistical analysis. GA wrote the first draft of the manuscript. MM, EG-M, and JA wrote sections of the manuscript. All authors contributed to manuscript critical revision with important intellectual contribution, read, and approved the submitted version.

FUNDING

This work was supported in part by Grants PI15/00303, PI15/00726, PI17/01593, PI18/00540 and ARADyAL RD16/0006/0001, RD16/0006/0004 and RD16/0006/0024 from Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Spain, IB16170 and GR18145 from Junta de Extremadura, Spain. Financed in part with FEDER funds from the European Union.

ACKNOWLEDGMENTS

JC-G is a researcher from the Miguel Servet Program (Ref CP14/00034), and ID from the Juan Rodés Program (Ref JR15/0036), both from the Carlos III National Health Institute, Spanish Ministry of Economy and Competitiveness).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2019.00582/full#supplementary-material

REFERENCES

Ådjers, K., Luukkainen, A., Pekkanen, J., Hurme, M., and Huhtala, H., Renkonen, R., et al. (2017). Self-reported allergic rhinitis and/or allergic conjunctivitis associate with IL13 rs20541 polymorphism in finnish adult asthma patients. *Int. Arch. Allergy Immunol*. 172, 123–128. doi: 10.1159/000456009

Accordini, S., Calciano, L., Bombieri, C., Malerba, G., Belpinati, F., Lo Presti, A. R., et al. (2016). An interleukin 13 polymorphism is associated with symptom severity in adult subjects with ever asthma. *PLoS ONE* 11:e0151292. doi: 10.1371/journal.pone.0151292

Agresti, A. (2003). Logit models for multinomial responses. *Categ Data Anal*. doi: 10.1002/0471249688.ch7

Agúndez, J. A., Ayuso, P., Cornejo-García, J. A., Blanca, M., Torres, M. J., Doña, I., et al. (2012). The diamine oxidase gene is associated with hypersensitivity response to non-steroidal anti-inflammatory drugs. *PLoS ONE* 7:e47571. doi: 10.1371/journal.pone.0047571

Agundez, J. A., Gonzalez-Alvarez, D. L., Vega-Rodriguez, M. A., Botello, E., and Garcia-Martin, E. (2014). Gene variants and haplotypes modifying transcription factor binding sites in the human cyclooxygenase 1 and 2 (PTGS1 and PTGS2) genes. *Curr. Drug Metab*. 15, 182–195. doi: 10.2174/1389200215020140327180336

Al-Muhsen, S., Vazquez-Tello, A., Alzaabi, A., Al-Hajjaj, M. S., Al-Jahdali, H. H., and Halwani, R. (2014). IL-4 receptor alpha single-nucleotide polymorphisms rs1805010 and rs1801275 are associated with increased risk of asthma in a Saudi Arabian population. *Ann. Thorac. Med*. 9, 81–86. doi: 10.4103/1817-1737.128849

Amo, G., Cornejo-García, J. A., García-Menaya, J. M., Cordobes, C., Torres, M. J., Esguevillas, G., et al. (2016b). FCERI and histamine metabolism gene variability in selective responders to NSAIDS. *Front. Pharmacol*. 7:353. doi: 10.3389/fphar.2016.00353

Amo, G., García-Menaya, J., Campo, P., Cordobés, C., Plaza Serón, M. C., Ayuso, P., et al. (2016a). A Nonsynonymous FCER1B SNP is associated with risk of developing allergic rhinitis and with IgE levels. *Sci. Rep*. 6:19724. doi: 10.1038/srep19724

Anderson, W. H., Koshy, B. T., Huang, L., Mosteller, M., Stinnett, S. W., Condreay, L. D., et al. (2013). Genetic analysis of asthma exacerbations. *Ann. Allergy Asthma Immunol*. 110, 416–422.e2. doi: 10.1016/j.anai.2013.04.002

Andiappan, A. K., Nilsson, D., Halldén, C., Yun, W. D., Säll, T., Cardell, L. O., et al. (2013). Investigating highly replicated asthma genes as candidate genes for allergic rhinitis. *BMC Med. Genet*. 14:51. doi: 10.1186/1471-2350-14-51

Antúnez, C., Martín, E., Cornejo-García, J. A., Blanca-Lopez, N. R., Pena, R., Mayorga, C., et al. (2006). Immediate hypersensitivity reactions to penicillins and other betalactams. *Curr. Pharm. Des*. 12, 3327–3333. doi: 10.2174/138161206778194042

Arshad, S. H., Karmaus, W., Kurukulaaratchy, R., Sadeghnejad, A., Huebner, M., and Ewart, S. (2008). Polymorphisms in the interleukin 13 and GATA

binding protein 3 genes and the development of eczema during childhood. *Br. J. Dermatol*. 158, 1315–1322. doi: 10.1111/j.1365-2133.2008.08565.x

Ashley, S. E., Tan, H. T., Peters, R., Allen, K. J., Vuillermin, P., Dharmage, S. C., et al. (2017). Genetic variation at the Th2 immune gene IL13 is associated with IgE-mediated paediatric food allergy. *Clin. Exp. Allergy* 47, 1032–1037. doi: 10.1111/cea.12942

Ayuso, P., Blanca-López, N., Doña, I., Torres, M. J., Guéant-Rodríguez, R. M., Canto, G., et al. (2013). Advanced phenotyping in hypersensitivity drug reactions to NSAIDs. *Clin. Exp. Allergy* 43, 1097–1109. doi: 10.1111/cea.12140

Battle, N. C., Choudhry, S., Tsai, H. J., Eng, C., Kumar, G., Beckman, K. B., et al. (2007). Ethnicity-specific gene-gene interaction between IL-13 and IL-4ralpha among African Americans with asthma. *Am. J. Respir. Crit. Care Med*. 175, 881–887. doi: 10.1164/rccm.200607-992OC

Baye, T. M., Butsch Kovacic, M., Biagini Myers, J. M., Martin, L. J., Lindsey, M., Patterson, T. L., et al. (2011). Differences in candidate gene association between european ancestry and african american asthmatic children. *PLoS ONE* 6:e16522. doi: 10.1371/journal.pone.0016522

Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N., and Golani, I. (2001). Controlling the false discovery rate in behavior genetics research. *Behav. Brain Res*. 125, 279–284. doi: 10.1016/S0166-4328(01)00297-2

Benson, A. A., Toh, J. A., Vernon, N., and Jariwala, S. P. (2012). The role of vitamin D in the immunopathogenesis of allergic skin diseases. *Allergy* 67, 296–301. doi: 10.1111/j.1398-9995.2011.02755.x

Berenguer, A. G., Fernandes, A. T., Oliveira, S., Rodrigues, M., Ornelas, P., Romeira, D., et al. (2014). Genetic polymorphisms and asthma: findings from a case-control study in the Madeira island population. *Biol. Res*. 47:40. doi: 10.1186/0717-6287-47-40

Black, P. N., and Scragg, R. (2005). Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. *Chest* 128, 3792–3798. doi: 10.1378/chest.128.6.3792

Black, S., Teixeira, A. S., Loh, A. X., Vinall, L., Holloway, J. W., Hardy, R., et al. (2009). Contribution of functional variation in the IL13 gene to allergy, hay fever and asthma in the NSHD longitudinal 1946 birth cohort. *Allergy* 64, 1172–1178. doi: 10.1111/j.1398-9995.2009.01988.x

Blanca, M., Romano, A., Torres, M. J., Férnandez, J., Mayorga, C., Rodriguez, J., et al. (2009). Update on the evaluation of hypersensitivity reactions to betalactams. *Allergy* 64, 183–193. doi: 10.1111/j.1398-9995.2008.01924.x

Bossé, Y., Lemire, M., Poon, A. H., Daley, D., He, J. Q., Sandford, A., et al. (2009). Asthma and genes encoding components of the vitamin D pathway. *Respir. Res*. 10:98. doi: 10.1186/1465-9921-10-98

Bottema, R. W., Nolte, I. M., Howard, T. D., Koppelman, G. H., Dubois, A. E., de Meer, G., et al. (2010). Interleukin 13 and interleukin 4 receptor-alpha polymorphisms in rhinitis and asthma. *Int. Arch. Allergy Immunol*. 153, 259–267. doi: 10.1159/000314366

Bouvy, J. C., De Bruin, M. L., and Koopmanschap, M. A. (2015). Epidemiology of adverse drug reactions in europe: a review of recent observational studies. *Drug Saf*. 38, 437–453. doi: 10.1007/s40264-015-0281-0

Brozek, J. L., Bousquet, J., Agache, I., Agarwal, A., Bachert, C., Bosnic-Anticevich, S., et al. (2017). Allergic rhinitis and its impact on asthma (ARIA) guidelines-2016 revision. *J. Allergy Clin. Immunol*. 140, 950–958. doi: 10.1016/j.jaci.2017.03.050

Burchard, E. G., Silverman, E. K., Rosenwasser, L. J., Borish, L., Yandava, C., Pillari, A., et al. (1999). Association between a sequence variant in the IL-4 gene promoter and FEV(1) in asthma. *Am. J. Respir. Crit. Care Med*. 160, 919–922. doi: 10.1164/ajrccm.160.3.9812024

Burkhardt, J., Kirsten, H., Wolfram, G., Quente, E., and Ahnert, P. (2012). Differential allelic expression of IL13 and CSF2 genes associated with asthma. *Genet. Mol. Biol*. 35, 567–574. doi: 10.1590/S1415-47572012005000055

Camargo, C. A., Rifas-Shiman, S. L., Litonjua, A. A., Rich-Edwards, J. W., Weiss, S. T., Gold, D. R., et al. (2007). Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. *Am. J. Clin Nutr*. 85, 788–795. doi: 10.1093/ajcn/85.3.788

Cameron, L., Webster, R. B., Strempel, J. M., Kiesler, P., Kabesch, M., Ramachandran, H., et al. (2006). Th2 cell-selective enhancement of human IL13 transcription by IL13–1112C>T, a polymorphism associated with allergic inflammation. *J. Immunol*. 177, 8633–8642. doi: 10.4049/jimmunol.177.12.8633

Caniatti, M. C., Marchioro, A. A., Guilherme, A. L., and Tsuneto, L. T. (2014). Association of cytokines in individuals sensitive and insensitive to dust mites in a brazilian population. *PLoS ONE* 9:e107921. doi: 10.1371/journal.pone.0107921

Canto, M. G., Andreu, I., Fernandez, J., and Blanca, M. (2009). Selective immediate hypersensitivity reactions to NSAIDs. *Curr. Opin. Allergy Clin. Immunol*. 9, 293–297. doi: 10.1097/ACI.0b013e32832db943

Cantorna, M. T., Snyder, L., Lin, Y. D., and Yang, L. (2015). Vitamin D and 1,25(OH)2D regulation of T cells. *Nutrients* 7, 3011–3021. doi: 10.3390/nu7043011

Chen, C. J., Cheng, C. F., Lin, H. Y., Hung, S. P., Chen, W. C., and Lin, M. S. (2012). A comprehensive 4-year survey of adverse drug reactions using a network-based hospital system. *J. Clin. Pharm. Ther*. 37, 647–651. doi: 10.1111/j.1365-2710.2012.01359.x

Choi, W. A., Kang, M. J., Kim, Y. J., Seo, J. H., Kim, H. Y., Kwon, J. W., et al. (2012). Gene-gene interactions between candidate gene polymorphisms are associated with total IgE levels in Korean children with asthma. *J. Asthma*. 49, 243–252. doi: 10.3109/02770903.2012.660294

Cooper, J. D., Smyth, D. J., Walker, N. M., Stevens, H., Burren, O. S., Wallace, C., et al. (2011). Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. *Diabetes* 60, 1624–1631. doi: 10.2337/db10-1656

Cornejo-Garcia, J. A., Blanca-López, N., Doña, I., Andreu, I., Agúndez, J. A., Carballo, M., et al. (2009). Hypersensitivity reactions to non-steroidal anti-inflammatory drugs. *Curr. Drug Metab*. 10, 971–980. doi: 10.2174/138920009790711841

Cox, D. R., and Snell, E. J. (1989). *Analysis of Binary Data, 2nd Edn*. London: Taylor & Francis.

Cui, L., Jia, J., Ma, C. F., Li, S. Y., Wang, Y. P., Guo, X. M., et al. (2012). IL-13 polymorphisms contribute to the risk of asthma: a meta-analysis. *Clin. Biochem*. 45, 285–288. doi: 10.1016/j.clinbiochem.2011.12.012

Cui, T. P., Wang, L., Xie, J. G., Hu, L. H., and Wu, J. M. (2003). Study on the high-affinity IgE receptor beta gene polymorphism and its association with the susceptibility to allergic asthma in Han nationality of Hubei province. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 20, 307–310.

de Guia, R. M., Echavez, M. D., Gaw, E. L., Gomez, M. R., Lopez, K. A., Mendoza, R. C., et al. (2015). Multifactor-dimensionality reduction reveals interaction of important gene variants involved in allergy. *Int. J. Immunogenet*. 42, 182–189. doi: 10.1111/iji.12200

Doña, I., Barrionuevo, E., Blanca-Lopez, N., Torres, M. J., Fernandez, T. D., Mayorga, C., et al. (2014). Trends in hypersensitivity drug reactions: more drugs, more response patterns, more heterogeneity. *J. Invest. Allergol. Clin. Immunol*. 24, 143–153) quiz 1 following 153.

Doña, I., Blanca-López, N., Cornejo-García, J. A., Torres, M. J., Laguna, J. J., Fernández, J., et al. (2011). Characteristics of subjects experiencing hypersensitivity to non-steroidal anti-inflammatory drugs: patterns of response. *Clin. Exp. Allergy* 41, 86–95. doi: 10.1111/j.1365-2222.2010.03651.x

Doña, I., Blanca-López, N., Torres, M. J., García-Campos, J., García-Núñez, I., Gómez, F., et al. (2012). Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. *J. Invest. Allergol. Clin. Immunol*. 22, 363–371.

Donfack, J., Schneider, D. H., Tan, Z., Kurz, T., Dubchak, I., Frazer, K. A., et al. (2005). Variation in conserved non-coding sequences on chromosome 5q and susceptibility to asthma and atopy. *Respir. Res*. 6:145. doi: 10.1186/1465-9921-6-145

Durham, S. R., Emminger, W., Kapp, A., de Monchy, J. G., Rak, S., Scadding, G. K., et al. (2012). SQ-standardized sublingual grass immunotherapy: confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. *J. Allergy Clin. Immunol*. 129, 717–725.e5. doi: 10.1016/j.jaci.2011.12.973

European Commission (2008). *Proposal for a Regulation Amending, as Regards Pharmacovigilance of Medicinal Products for Human Use*. Regulation (EC) No 726/2004. Impact assessment. Available online at: http://ec.europa.eu/health/files/pharmacos/pharmpacks_12_2008/pha.

Ferreira, M. A., Zhao, Z. Z., Thomsen, S. F., James, M., Evans, D. M., Postmus, P. E., et al. (2009). Association and interaction analyses of eight genes under asthma linkage peaks. *Allergy* 64, 1623–1628. doi: 10.1111/j.1398-9995.2009.02091.x

García-Martín, E., García-Menaya, J., Sánchez, B., Martínez, C., Rosendo, R., and Agúndez, J. A. (2007). Polymorphisms of histamine-metabolizing enzymes and

clinical manifestations of asthma and allergic rhinitis. *Clin. Exp. Allergy* 37, 1175–1182. doi: 10.1111/j.1365-2222.2007.02769.x

Gomes, E., Cardoso, M. F., Praça, F., Gomes, L., Mariño, E., and Demoly, P. (2004). Self-reported drug allergy in a general adult Portuguese population. *Clin. Exp. Allergy* 34, 1597–1601. doi: 10.1111/j.1365-2222.2004.02070.x

Gomes, E. R., and Demoly, P. (2005). Epidemiology of hypersensitivity drug reactions. *Curr. Opin. Allergy Clin. Immunol.* 5, 309–316. doi: 10.1097/01.all.0000173785.81024.33

González, J. R., Armengol, L., Solé, X., Guinó, E., Mercader, J. M., Estivill, X., et al. (2007). SNPassoc: an R package to perform whole genome association studies. *Bioinformatics* 23, 644–645. doi: 10.1093/bioinformatics/btm025

Gonzalez, J. R., Lluis-Armengol, E. G., Sole, X., and Moreno, V. (2014). *SNPs-Based Whole Genome Association Studies*. Available online at: https://CRAN.R-project.org/package=SNPassoc

Granada, M, Wilk, JB, Tuzova, M., Strachan, D. P., Weidinger, S., Albrecht, E., et al. (2012). A genome wide Association study of plasma total IgE concentration in the framingham heart study. *J. Allergy Clin. Immunol.* 129, 840–845.e21. doi: 10.1016/j.jaci.2011.09.029

Hall, S. C., and Agrawal, D. K. (2017). Vitamin D and bronchial asthma: an overview of the last five years. *Clin. Ther.* 39, 917–929. doi: 10.1016/j.clinthera.2017.04.002

Han, J. C., Du, J., Zhang, Y. J., Qi, G. B., Li, H. B., Zhang, Y. J., et al. (2016). Vitamin D receptor polymorphisms may contribute to asthma risk. *J. Asthma* 53, 790–800. doi: 10.3109/02770903.2016.1158267

Hasegawa, M., Nishiyama, C., Nishiyama, M., Akizawa, Y., Mitsuishi, K., Ito, T., et al. (2003). A novel−66T/C polymorphism in Fc epsilon RI alpha-chain promoter affecting the transcription activity: possible relationship to allergic diseases. *J. Immunol.* 171, 1927–1933. doi: 10.4049/jimmunol.171.4.1927

Hesselmar, B., Enelund, A. C., Eriksson, B., Padyukov, L., Hanson, L. Å., and Aberg, N. (2012). The heterogeneity of asthma phenotypes in children and young adults. *J. Allergy* 2012:163089. doi: 10.1155/2012/163089

Hong, X., and Wang, X. (2012). Early life precursors, epigenetics, and the development of food allergy. *Semin. Immunopathol.* 34, 655–669. doi: 10.1007/s00281-012-0323-y

Hsu, J., Avila, P. C., Kern, R. C., Hayes, M. G., Schleimer, R. P., and Pinto, J. M. (2013). Genetics of chronic rhinosinusitis: state of the field and directions forward. *J. Allergy Clin. Immunol.* 131, 977–993.e5. doi: 10.1016/j.jaci.2013.01.028

Hua, L., Zuo, X. B., Bao, Y. X., Liu, Q. H., Li, J. Y., Lv, J., et al. (2016). Four-locus gene interaction between IL13, IL4, FCER1B, and ADRB2 for asthma in Chinese Han children. *Pediatr. Pulmonol.* 51, 364–371. doi: 10.1002/ppul.23322

Hur, G. Y., Ye, Y. M., Koh, D. H., Kim, S. H., and Park, H. S. (2013). IL-4 receptor α polymorphisms may be a susceptible factor for work-related respiratory symptoms in bakery workers. *Allergy Asthma Immunol Res.* 5, 371–376. doi: 10.4168/aair.2013.5.6.371

Hutchinson, K, Kerley, CP, Faul, J., Greally, P., Coghlan, D., Louw, M., et al. (2017). Vitamin D receptor variants and uncontrolled asthma. *Eur. Ann. Allergy Clin. Immunol.* 50:108–116. doi: 10.23822/EurAnnACI.1764-1489.46

Ismail, M. F., Elnady, H. G., and Fouda, E. M. (2013). Genetic variants in vitamin D pathway in Egyptian asthmatic children: a pilot study. *Hum. Immunol.* 74, 1659–1664. doi: 10.1016/j.humimm.2013.08.284

Joubert, B. R., Reif, D. M., Edwards, S. W., Leiner, K. A., Hudgens, E. E., Egeghy, P., et al. (2011). Evaluation of genetic susceptibility to childhood allergy and asthma in an African American urban population. *BMC Med. Genet.* 12:25. doi: 10.1186/1471-2350-12-25

Jung, K. H., Kim, T. H., Sheen, D. H., Lim, M. K., Lee, S. K., Kim, J. Y., et al. (2011). Associations of vitamin d binding protein gene polymorphisms with the development of peripheral arthritis and uveitis in ankylosing spondylitis. *J. Rheumatol.* 38, 2224–2229. doi: 10.3899/jrheum.101244

Juul, S. (2012). *Epidemiologi Og Evidens. 2nd Edn*. København: Munksgaard.

Juul, S., and Frydenberg, M. (2016). EpiBasic. Available online at: http://ph.medarbejdere.au.dk/undervisning-og-uddannelse/software/ (accessed February 06, 2019).

Kabesch, M., Schedel, M., Carr, D., Woitsch, B., Fritzsch, C., Weiland, S. K., et al. (2006). IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. *J. Allergy Clin. Immunol.* 117, 269–274. doi: 10.1016/j.jaci.2005.10.024

Karaca, S., Civelek, E., Karaca, M., Sahiner, U. M., Ozgul, R. K., Kocabas, C. N., et al. (2016). Allergy-specific phenome-wide association study for immunogenes in turkish children. *Sci. Rep.* 6:33152. doi: 10.1038/srep 33152

Kiesler, P., Shakya, A., Tantin, D., and Vercelli, D. (2009). An allergy-associated polymorphism in a novel regulatory element enhances IL13 expression. *Hum. Mol. Genet.* 18, 4513–4520. doi: 10.1093/hmg/ddp411

Kim, B. S., Park, S. M., Uhm, T. G., Kang, J. H., Park, J. S., Jang, A. S., et al. (2010). Effect of single nucleotide polymorphisms within the interleukin-4 promoter on aspirin intolerance in asthmatics and interleukin-4 promoter activity. *Pharmacogenet. Genomics* 20, 748–758. doi: 10.1097/FPC.0b013e3283402155

Kim, S. H., Bae, J. S., Holloway, J. W., Lee, J. T., Suh, C. H., Nahm, D. H., et al. (2006). A polymorphism of MS4A2 (- 109T > C) encoding the beta-chain of the high-affinity immunoglobulin E receptor (FcepsilonR1beta) is associated with a susceptibility to aspirin-intolerant asthma. *Clin. Exp. Allergy* 36, 877–883. doi: 10.1111/j.1365-2222.2006.02443.x

Kinet, J. P. (1999). The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. *Annu. Rev. Immunol.* 17, 931–972. doi: 10.1146/annurev.immunol.17.1.931

Klaassen, E. M., Penders, J., Jöbsis, Q., van de Kant, K. D., Thijs, C., Mommers, M., et al. (2015). An ADAM33 polymorphism associates with progression of preschool wheeze into childhood asthma: a prospective case-control study with replication in a birth cohort study. *PLoS ONE* 10:e0119349. doi: 10.1371/journal.pone.0119349

Knutsen, A. P., Vijay, H. M., Kariuki, B., Santiago, L. A., Graff, R., Wofford, J. D., et al. (2010). Association of IL-4RA single nucleotide polymorphisms, HLA-DR and HLA-DQ in children with Alternaria-sensitive moderate-severe asthma. *Clin. Mol. Allergy* 8:5. doi: 10.1186/1476-7961-8-5

Kowalski, M. L., Asero, R., Bavbek, S., Blanca, M., Blanca-Lopez, N., Bochenek, G., et al. (2013). Classification and practical approach to the diagnosis and management of hypersensitivity to nonsteroidal anti-inflammatory drugs. *Allergy* 68, 1219–1232. doi: 10.1111/all.12260

Kruse, R. L., and Vanijcharoenkarn, K. (2018). Drug repurposing to treat asthma and allergic disorders: progress and prospects. *Allergy* 73, 313–322. doi: 10.1111/all.13305

Kumar, A., Das, S., Agrawal, A., Mukhopadhyay, I., and Ghosh, B. (2015). Genetic association of key Th1/Th2 pathway candidate genes, IRF2, IL6, IFNGR2, STAT4 and IL4RA, with atopic asthma in the Indian population. *J. Hum. Genet.* 60, 443–448. doi: 10.1038/jhg.2015.45

Lacombe-Barrios, J, Gomez, F, Perez, N, et al. (2018). Accurate of the diagnosis of allergy reactions at the Emergency Department. *J. Invest. Allergol. Clin. Immunol.* doi: 10.18176/jiaci.0313. [Epub ahead of print].

Lasky-Su, J., Lange, N., Brehm, J. M., Damask, A., Soto-Quiros, M., Avila, L., et al. (2012). Genome-wide association analysis of circulating vitamin D levels in children with asthma. *Hum. Genet.* 131, 1495–1505. doi: 10.1007/s00439-012-1185-z

Leung, T. F., Wang, S. S., Tang, M. F., Kong, A. P., Sy, H. Y., Hon, K. L., et al. (2015). Childhood asthma and spirometric indices are associated with polymorphic markers of two vitamin D 25-hydroxylase genes. *Pediatr. Allergy Immunol.* 26, 375–382. doi: 10.1111/pai.12392

Li, F., Jiang, L., Willis-Owen, S. A., Zhang, Y., and Gao, J. (2011). Vitamin D binding protein variants associate with asthma susceptibility in the Chinese han population. *BMC Med. Genet.* 12:103. doi: 10.1186/1471-2350-12-103

Li, J., Lin, L. H., Wang, J., Peng, X., Dai, H. R., Xiao, H., et al. (2014). Interleukin-4 and interleukin-13 pathway genetics affect disease susceptibility, serum immunoglobulin E levels, and gene expression in asthma. *Ann. Allergy Asthma Immunol.* 113, 173–179.e1. doi: 10.1016/j.anai.2014.05.004

Li, T., Ren, Z., Deng, Y., Wang, Y., and Zhou, H. (2016). Lack of association between RAD50-IL13 polymorphisms and pediatric asthma susceptibility in Northeastern Han Chinese. *J. Asthma* 53, 114–118. doi: 10.3109/02770903.2015.1067322

Li, X., Ampleford, E. J., Howard, T. D., Moore, W. C., Torgerson, D. G., Li, H., et al. (2012). Genome-wide association studies of asthma indicate opposite immunopathogenesis direction from autoimmune diseases. *J. Allergy Clin. Immunol.* 130, 861–8.e7. doi: 10.1016/j.jaci.2012.04.041

Li, X., Howard, T. D., Zheng, S. L., Haselkorn, T., Peters, S. P., Meyers, D. A., et al. (2010). Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. *J. Allergy Clin. Immunol*. 125, 328–335.e11. doi: 10.1016/j.jaci.2009.11.018

Liao, E. C., Chang, C. Y., Hsieh, C. W., Yu, S. J., Yin, S. C., and Tsai, J. J. (2015). An exploratory pilot study of genetic marker for ige-mediated allergic diseases with expressions of fcepsilonr1alpha and cepsilon. *Int. J. Mol. Sci*. 16, 9504–9519. doi: 10.3390/ijms16059504

Linneberg, A. (2011). The increase in allergy and extended challenges. *Allergy* 66 (Suppl. 9), 1–3. doi: 10.1111/j.1398-9995.2011.02619.x

Liu, S., Li, T., and Liu, J. (2012). Interleukin-4 rs2243250 polymorphism is associated with asthma among Caucasians and related to atopic asthma. *Cytokine* 59, 364–369. doi: 10.1016/j.cyto.2012.05.006

Liu, X., Wang, G., Hong, X., Wang, D., Tsai, H. J., Zhang, S., et al. (2011). Gene-vitamin D interactions on food sensitization: a prospective birth cohort study. *Allergy* 66, 1442–1448. doi: 10.1111/j.1398-9995.2011.02681.x

Lu, M. P., Chen, R. X., Wang, M. L., Zhu, X. J., Zhu, L. P., Yin, M., et al. (2011). Association Study on IL4, IL13 and IL4RA polymorphisms in mite-sensitized persistent allergic rhinitis in a chinese population. *PLoS ONE* 6:e27363. doi: 10.1371/journal.pone.0027363

Maalmi, H., Sassi, F. H., Berraies, A., Ammar, J., Hamzaoui, K., and Hamzaoui, A. (2013). Association of vitamin D receptor gene polymorphisms with susceptibility to asthma in Tunisian children: a case control study. *Hum. Immunol*. 74, 234–240. doi: 10.1016/j.humimm.2012.11.005

Madore, A. M., and Laprise, C. (2010). Immunological and genetic aspects of asthma and allergy. *J. Asthma Allergy* 3, 107–121. doi: 10.2147/JAA.S8970

Marsh, D. G., Neely, J. D., Breazeale, D. R., Ghosh, B., Freidhoff, L. R., Ehrlich-Kautzky, E., et al. (1994). Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 264, 1152–1156. doi: 10.1126/science.8178175

Matys, V., Fricke, E., Geffers, R., Gössling, E., Haubrock, M., Hehl, R., et al. (2003). TRANSFAC: transcriptional regulation, from patterns to profiles. *Nucl. Acids Res*. 31, 374–378. doi: 10.1093/nar/gkg108

Matys, V., Kel-Margoulis, O. V., Fricke, E., Liebich, I., Land, S., Barre-Dirrie, A., et al. (2006). TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. *Nucl. Acids Res*. 34:D108–D110. doi: 10.1093/nar/gkj143

McFadden, D. (1974). "Conditional logit: analysis of qualitative choice behavior," in *Frontiers in Ecnometrics*, ed P. Zarembka (New York, NY: Academic Press), 105–142.

McFadden, D. (1977). *Quantitative Methods for Analyzing Travel Behaviour of Individuals: Some Recent Developments*. Cowles Foundation Discussion Papers 474, Cowles Foundation for Research in Economics, Yale University.

Messaad, D., Sahla, H., Benahmed, S., Godard, P., Bousquet, J., and Demoly, P. (2004). Drug provocation tests in patients with a history suggesting an immediate drug hypersensitivity reaction. *Ann. Intern. Med*. 140, 1001–1006. doi: 10.7326/0003-4819-140-12-200406150-00009

Micheal, S., Minhas, K., Ishaque, M., Ahmed, F., and Ahmed, A. (2013). IL-4 gene polymorphisms and their association with atopic asthma and allergic rhinitis in Pakistani patients. *J. Invest. Allergol. Clin. Immunol*. 23, 107–111.

Michel, S., Liang, L., Depner, M., Klopp, N., Ruether, A., Kumar, A., et al. (2010). Unifying candidate gene and GWAS approaches in asthma. *PLoS ONE* 5:e13894. doi: 10.1371/journal.pone.0013894

Moffatt, M. F., Gut, I. G., Demenais, F., Strachan, D. P., Bouzigon, E., Heath, S., et al. (2010). A large-scale, consortium-based genomewide association study of asthma. *N. Engl. J. Med*. 363, 1211–1221. doi: 10.1056/NEJMoa0906312

Movahedi, M., Amirzargar, A. A., Nasiri, R., Hirbod-Mobarakeh, A., Farhadi, E., Tavakol, M., et al. (2013). Gene polymorphisms of Interleukin-4 in allergic rhinitis and its association with clinical phenotypes. *Am. J. Otolaryngol*. 34, 676–681. doi: 10.1016/j.amjoto.2013.05.002

Nagelkerke, NJD. (1991). A note on a general definition of the coefficient of determination. *Biometrika* 78, 691–692. doi: 10.1093/biomet/78.3.691

Narozna, B., Hoffmann, A., Sobkowiak, P., Schoneich, N., Breborowicz, A., and Szczepankiewicz, A. (2016). Polymorphisms in the interleukin 4, interleukin 4 receptor and interleukin 13 genes and allergic phenotype: a case control study. *Adv. Med. Sci*. 61, 40–45. doi: 10.1016/j.advms.2015.07.003

Nie, W., Zang, Y., Chen, J., and Xiu, Q. (2013). Association between Interleukin-4 Receptor α Chain (IL4RA) I50V and Q551R Polymorphisms and asthma risk: an update meta-analysis. *PLoS ONE* 8:e69120. doi: 10.1371/journal.pone.0069120

Nissen, C. V., Bindslev-Jensen, C., and Mortz, C. G. (2015). Hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs): classification of a Danish patient cohort according to EAACI/ENDA guidelines. *Clin. Transl. Allergy* 5:10. doi: 10.1186/s13601-015-0052-0

Ober, C., and Hoffjan, S. (2006). Asthma genetics the long and winding road to gene discovery. *Genes Immun*. 7:6364284. doi: 10.1038/sj.gene.6364284

Overton, N. L., Denning, D. W., Bowyer, P., and Simpson, A. (2016). Genetic susceptibility to allergic bronchopulmonary aspergillosis in asthma: a genetic association study. *Allergy Asthma Clin. Immunol*. 12:47. doi: 10.1186/s13223-016-0152-y

Ozdoganoglu, T., and Songu, M. (2012). The burden of allergic rhinitis and asthma. *Ther. Adv. Respir. Dis*. 6, 11–23. doi: 10.1177/1753465811431975

Palikhe, N. S., Kim, S. H., Cho, B. Y., Choi, G. S., Kim, J. H., Ye, Y. M., et al. (2010). IL-13 Gene Polymorphisms are Associated With Rhinosinusitis and Eosinophilic Inflammation in Aspirin Intolerant Asthma. *Allergy Asthma Immunol Res*. 2, 134–140. doi: 10.4168/aair.2010.2.2.134

Palikhe, N. S., Kim, S. H., Cho, B. Y., Ye, Y. M., Hur, G. Y., and Park, H. S. (2008). Association of three sets of high-affinity IgE receptor (FcepsilonR1) polymorphisms with aspirin-intolerant asthma. *Respir. Med*. 102, 1132–1139. doi: 10.1016/j.rmed.2008.03.017

Papadopoulou, A., Kouis, P., Middleton, N., Kolokotroni, O., Karpathios, T., Nicolaidou, P., et al. (2015). Association of vitamin D receptor gene polymorphisms and vitamin D levels with asthma and atopy in Cypriot adolescents: a case-control study. *Multidiscip. Respir. Med*. 10:26. doi: 10.1186/s40248-015-0025-0

Park, H. W., and Tantisira, K. G. (2017). Genetic signatures of asthma exacerbation. *Allergy Asthma Immunol. Res*. 9, 191–199. doi: 10.4168/aair.2017.9.3.191

Park, K. Y., Park, M. K., Kim, E. J., Lee, M. K., and Seo, S. J. (2011). FCεRI gene promoter polymorphisms and total IgE levels in susceptibility to atopic dermatitis in korea. *J. Korean Med. Sci*. 26, 870–874. doi: 10.3346/jkms.2011.26.7.870

Paternoster, L., Standl, M., Chen, C. M., Ramasamy, A., Bønnelykke, K., Duijts, L., et al. (2011). Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. *Nat. Genet*. 44, 187–192. doi: 10.1038/ng.1017

Pértegas Díaz, S., and Pita Fernández, S. (2003). Cálculo del poder estadístico de un estudio. *Cad Atención Primaria*. 10, 59–63.

Pillai, D. K., Iqbal, S. F., Benton, A. S., Lerner, J., Wiles, A., Foerster, M., et al. (2011). Associations between genetic variants in vitamin D metabolism and asthma characteristics in young African Americans: a pilot study. *J. Invest. Med*. 59, 938–946. doi: 10.2310/JIM.0b013e318220df41

Pino-Yanes, M., Gignoux, C. R., Galanter, J. M., Levin, A. M., Campbell, C. D., Eng, C., et al. (2015). Genome-wide association study and admixture mapping reveal new loci associated with total IgE levels in Latinos. *J. Allergy Clin. Immunol*. 135, 1502–1510. doi: 10.1016/j.jaci.2014.10.033

Platts-Mills, T. A. (2001). The role of immunoglobulin E in allergy and asthma. *Am. J. Respir. Crit. Care Med*. 164:S1–5. doi: 10.1164/ajrccm.164.supplement_1.2103024

Poon, A. H., Laprise, C., Lemire, M., Montpetit, A., Sinnett, D., Schurr, E., et al. (2004). Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. *Am. J. Respir. Crit. Care Med*. 170, 967–973. doi: 10.1164/rccm.200403-412OC

Potaczek, D. P., and Kabesch, M. (2012). Current concepts of IgE regulation and impact of genetic determinants. *Clin. Exp. Allergy* 42, 852–871. doi: 10.1111/j.1365-2222.2011.03953.x

Potaczek, D. P., Maeda, K., Wang, Q. H., Nakano, N., Kanada, S., Stepien, E., et al. (2009). FcepsilonRIalpha gene−18483A>C polymorphism affects transcriptional activity through YY1 binding. *Immunogenetics* 61, 649–655. doi: 10.1007/s00251-009-0391-x

Potaczek, D. P., Michel, S., Sharma, V., Zeilinger, S., Vogelberg, C., von Berg, A., et al. (2013). Different FCER1A polymorphisms influence IgE levels in asthmatics and non-asthmatics. *Pediatr. Allergy Immunol*. 24, 441–449. doi: 10.1111/pai.12083

Raby, B. A., Lazarus, R., Silverman, E. K., Lake, S., Lange, C., Wjst, M., et al. (2004). Association of vitamin D receptor gene polymorphisms with

childhood and adult asthma. *Am. J. Respir. Crit. Care Med*. 170, 1057–1065. doi: 10.1164/rccm.200404-447OC

Ramasamy, A., Kuokkanen, M., Vedantam, S., Gajdos, Z. K., Couto Alves, A., Lyon, H. N., et al. (2012). Genome-wide association studies of asthma in population-based cohorts confirm known and suggested loci and identify an additional association near HLA. *PLoS ONE* 7:e44008. doi: 10.1371/journal.pone.0044008

Ramphul, K., Lv, J., Hua, L., Liu, Q. H., Fang, D. Z., Ji, R. X., et al. (2014). Single nucleotide polymorphisms predisposing to asthma in children of Mauritian Indian and Chinese Han ethnicity. *Brazi. J. Med. Biol. Res*. 47, 394–397. doi: 10.1590/1414-431X20143751

Robinson, C. L., Baumann, L. M., Gilman, R. H., Romero, K., Combe, J. M., Cabrera, L., et al. (2012). The Peru Urban versus Rural Asthma (PURA) Study: methods and baseline quality control data from a cross-sectional investigation into the prevalence, severity, genetics, immunology and environmental factors affecting asthma in adolescence in Peru. *BMJ Open* 2:e000421. doi: 10.1136/bmjopen-2011-000421

Rondon, C, Blanca-Lopez, N, Campo, P., Mayorga, C., Jurado-Escobar, R., Torres, M. J., et al. (2017). Specific immunotherapy in local allergic rhinitis: A randomized, double-blind placebo-controlled trial with Phleum pratense subcutaneous allergen immunotherapy. *Allergy* 73, 905–915. doi: 10.1111/all.13350

Saadi, A., Gao, G., Li, H., Wei, C., Gong, Y., and Liu, Q. (2009). Association study between vitamin D receptor gene polymorphisms and asthma in the chinese han population: a case-control study. *BMC Med. Genet*. 10:71. doi: 10.1186/1471-2350-10-71

Sadeghnejad, A., Karmaus, W., Arshad, S. H., Kurukulaaratchy, R., Huebner, M., and Ewart, S. (2008). IL13 gene polymorphisms modify the effect of exposure to tobacco smoke on persistent wheeze and asthma in childhood, a longitudinal study. *Respir. Res*. 9:2. doi: 10.1186/1465-9921-9-2

Sharma, V., Michel, S., Gaertner, V., Franke, A., Vogelberg, C., von Berg, A., et al. (2014). A role of FCER1A and FCER2 polymorphisms in IgE regulation. *Allergy* 69, 231–236. doi: 10.1111/all.12336

Suaini, N. H., Zhang, Y., Vuillermin, P. J., Allen, K. J., and Harrison, L. C. (2015). Immune modulation by vitamin D and its relevance to food allergy. *Nutrients* 7, 6088–6108. doi: 10.3390/nu7085271

Sun, L., Xue, W., Li, J., Zhou, Z., and Han, W. (2017). Palm dermatoglyphs and interleukin-4 receptor polymorphisms in asthma. *Biomed. Rep*. 6, 21–26. doi: 10.3892/br.2016.803

Szczeklik, A., and Nizankowska-Mogilnicka, E. S. M. (2009). *Middleton's Allergy Principles and Practice*, eds N. Adkinson, W. Busse, B. Bochner, S. Holgate, and R. Lemanske (London: Mosby Inc; Elsevier), 1227–1243.

Tian, H. Q., Chen, X. Y., Lu, Y., Lu, W. M., Wang, M. L., Zhao, H. L., et al. (2015). Association of VDR and CYP2R1 Polymorphisms with Mite-Sensitized Persistent Allergic Rhinitis in a Chinese Population. *PLoS ONE* 10:e0133162. doi: 10.1371/journal.pone.0133162

Tian, H. Q., and Cheng, L. (2017). The role of vitamin D in allergic rhinitis. *Asia Pac. Allergy* 7, 65–73. doi: 10.5415/apallergy.2017.7.2.65

Torres, M. J., Mayorga, C., Blanca-López, N., and Blanca, M. (2014). Hypersensitivity reactions to beta-lactams. *EXS* 104, 165–184. doi: 10.1007/978-3-0348-0726-5_11

Veldman, C. M., Cantorna, M. T., and DeLuca, H. F. (2000). Expression of 1,25-Dihydroxyvitamin D3 receptor in the immune system. *Arch. Biochem. Biophys*. 374, 334–338. doi: 10.1006/abbi.1999.1605

Wallace, D. V., Dykewicz, M. S., Bernstein, D. I., Blessing-Moore, J., Cox, L., Khan, D. A., et al. (2008). The diagnosis and management of rhinitis: an updated practice parameter. *J. Allergy Clin. Immunol*. 122 (Suppl. 2), S1–84. doi: 10.1016/j.jaci.2008.06.003

Wang, Y., Wang, O., Li, W., Ma, L., Ping, F., Chen, L., et al. (2015). Variants in vitamin d binding protein gene are associated with gestational diabetes mellitus. *Medicine* 94:e1693. doi: 10.1097/MD.0000000000001693

Weidinger, S., Gieger, C., Rodriguez, E., Baurecht, H., Mempel, M., Klopp, N., et al. (2008). Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. *PLoS Genet*. 4:e1000166. doi: 10.1371/journal.pgen.1000166

Wigginton, JE, Cutler, DJ, and Abecasis, GR. (2005). A note on exact tests of hardy-weinberg equilibrium. *Am. J. Hum. Genet*. 76, 887–893. doi: 10.1086/429864

Wjst, M. (2005). Variants in the vitamin D receptor gene and asthma. *BMC Genet*. 6:2. doi: 10.1186/1471-2156-6-2

Wjst, M., Altmüller, J., Faus-Kessler, T., Braig, C., Bahnweg, M., and André, E. (2006). Asthma families show transmission disequilibrium of gene variants in the vitamin D metabolism and signalling pathway. *Respir. Res*. 7:60. doi: 10.1186/1465-9921-7-60

Xie, C. N., Yue, M., Huang, P., Tian, T., Fan, H. Z., Wu, M. P., et al. (2018). Vitamin D binding protein polymorphisms influence susceptibility to hepatitis C virus infection in a high-risk Chinese population. *Gene* 679, 405–411. doi: 10.1016/j.gene.2018.09.021

Xu, Y., Li, J., Ding, Z., Li, J., Li, B., Yu, Z., et al. (2017). Association between IL-13 +1923C/T polymorphism and asthma risk: a meta-analysis based on 26 case-control studies. *Biosci. Rep*. 37, 1–13. doi: 10.1042/BSR20160505

Yang, H. J., Zheng, L., Zhang, X. F., Yang, M., and Huang, X. (2014). Association of the MS4A2 gene promoter C-109T or the 7th exon E237G polymorphisms with asthma risk: a meta-analysis. *Clin. Biochem*. 47, 605–611. doi: 10.1016/j.clinbiochem.2014.01.022

Zhang, S., Li, Y., and Liu, Y. (2015). Interleukin-4−589C/T polymorphism is associated with increased pediatric asthma risk: a meta-analysis. *Inflammation* 38, 1207–1212. doi: 10.1007/s10753-014-0086-9

Zhang, Y., Wang, Z., and Ma, T. (2017). Associations of Genetic Polymorphisms relevant to metabolic pathway of vitamin d3 with development and prognosis of childhood bronchial asthma. *DNA Cell Biol*. 36, 682–692. doi: 10.1089/dna.2017.3730

Zhao, D. D., Yu, D. D., Ren, Q. Q., Dong, B., Zhao, F., and Sun, Y. H. (2017). Association of vitamin D receptor gene polymorphisms with susceptibility to childhood asthma: a meta-analysis. *Pediatr. Pulmonol*. 52, 423–429. doi: 10.1002/ppul.23548

Zhou, J., Zhou, Y., Lin, L. H., Wang, J., Peng, X., Li, J., et al. (2012). Association of polymorphisms in the promoter region of FCER1A gene with atopic dermatitis, chronic uticaria, asthma, and serum immunoglobulin E levels in a Han Chinese population. *Hum. Immunol*. 73, 301–305. doi: 10.1016/j.humimm.2011.12.001

Zhu, N., Gong, Y., Chen, X. D., Zhang, J., Long, F., He, J., et al. (2013). Association between the polymorphisms of interleukin-4, the interleukin-4 receptor gene and asthma. *Chin. Med. J*. 126, 2943–2951.

Zuberbier, T., Lötvall, J., Simoens, S., Subramanian, S. V., and Church, M. K. (2014). Economic burden of inadequate management of allergic diseases in the European Union: a GA(2) LEN review. *Allergy* 69, 1275–1279. doi: 10.1111/all.12470

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Amo, Martí, García-Menaya, Cordobés, Cornejo-García, Blanca-López, Canto, Doña, Blanca, Torres, Agúndez and García-Martín. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

SUPPLEMENTAL MATERIAL

Table S1. Characteristics of the participants in the NGS phase.

	Healthy controls (n=22)	Patients with AR+A (n=22)	Patients with BLs hypersensitivity (n=43)	Patients with NSAIDs cross-reactions (CR) (n=41)	Patients with NSAIDs selective hypersensitivity (SH) (n=46)
Women. n (%)	14 (63.6%)	8 (36.4%)	22 (51.2%)	26 (63.4%)	29 (63.0%)
Age+sd (range)	23.9±8.8 (20-54)	28.9±12.3 (14-58)	43.5±14.8 (18-77)	43.2±15.7 (5-77)	45.3±14.2 (20-79)
Antecedents of atopy	0	22 (100%)	11 (25.6%)	14 (34.1%)	9 (19.6%)

Table S2. Details of the sequenced areas.

Gene-(protein coding transcript)	Coordinate: Origin	Coordinate: End
FCER1A-001	chr1:159258504	chr1:159259543
FCER1A-002	chr1:159272096	chr1:159272209
FCER1G-001, 002	chr1:161184111	chr1:161185160
RXRG-001	chr1:165414080	chr1:165415430
CYP27A1-001	chr2:219645905	chr2:219647160
GC-001,004, 007	chr4:72649678	chr4:72650079
GC-006	chr4:72669643	chr4:72670664
IL13-001	chr5:131991955	chr5:131995163
IL4-001, 002	chr5:132008742	chr5:132009877
RXRB-001, 002, 201, 202	chr6:33168019	chr6:33169254
RXRA-002	chr9:137217900	chr9:137218505
CYP2R1-001	chr11:14913527	chr11:14914751
MS4A2-001, 003	chr11:59855238	chr11:59856294
VDR-008	chr12:48276476	chr12:48276719
VDR-002,004,006, 007, 201, 202	chr12:48298340	chr12:48299414
VDR-001	chr12:48336470	chr12:48337000
CYP27B1-001	chr12:58160630	chr12:58161825
IL4R_001, 004, 006, 007, 009, 202, 203	chr16:27324989	chr16:27325562
CYP24A1-006	chr20:52788116	chr20:52788492
CYP24A1-003, 004	chr20:52789861	chr20:52791119
IL13RA1-001, 002	chrx:117860601	chrx:117861689

The coordinates for sequencing correspond to the GRCh37 assembly of the human genome.

Table S3. Statistical power for the genetic associations identified in this study.

	Patients with AR+A (Two-tailed)	Patients with BLs (Two-tailed)	Patients with NSAIDs cross-reactions (Two-tailed)	Patients with NSAIDs selective hypersensitivity (Two-tailed)	Patients with DHR (Two-tailed)	Patients with DHR-IGE (Two-tailed)	All IGE-mediated (Two-tailed)
FCER1G rs36233990	40.9%	43.0%	54.3%	32.3%	54.9%	47.6%	53.0 %
FCER1G rs2070901	>99%	>99%	>99%	>99%	>99%	>99%	>99%
GC rs3733359	97.5%	98.1%	98.8%	91.7%	>99%	>99%	>99%

TABLE S4. SNPs observed in the NGS study.

Gene	dbSNP	Chromosomal Location	Alleles	MAF	HWE	MAF 1000 Genomes ALL individuals	MAF 1000 Genomes European individuals	MAF genomAD ALL individuals	MAF genomAD European individuals
FCER1A	rs185716663	1:159258536	C/G	0.009 G	1.000	0.003 G	0.008 G	0.007 G	0.010 G
FCER1A	rs2427837	1:159258545	G/A	0.213 A	0.501	0.151 A	0.304 A	0.217 A	0.289 A
FCER1A	rs61828219	1:159258641	C/A	0.141 A	0.752	0.085 A	0.160 A	0.129 A	0.175 A
FCER1A	rs556527481	1:159258709	G/A	0.003 A	1.000	0.0002 A	0	0.0005 A	0.0005 A
FCER1A	rs190737507	1:159258798	A/G	0.003 G	1.000	0.001 G	0.003 G	0.002 G	0.003 G
FCER1A	rs12135235	1:159259029	T/G	0.043 G	1.000	0.033 G	0.033 G	0.034 G	0.037 G
FCER1A	None	1:159259290	A/C	0.003 C	1.000	-	-	-	-
FCER1A	rs746917868	1:159259461	A/C	0.003 C	1.000	-	-	0.001 C	0.0004 C
FCER1A	rs76362697	1:159259492	A/G	0.017 G	1.000	0.009 G	0.026 G	0.014 G	0.020 G
FCER1A	rs983088525	1:159272158	G/T	0.003 T	1.000	-	-	0.000004 T	0
FCER1A	rs200728340	1:159272209	G/A	0.003 A	1.000	-	-	0.00004 A	0.00005 A
FCER1G	rs77823804	1:161184213	T/G	0.006 G	1.000	0.002 G	0.006 G	0.002 G	0.003 G
FCER1G	rs567742742	1:161184367	G/A	0.003 A	1.000	-	-	0.0006 A	0.001 A
FCER1G	rs36233990	1:161184658	C/T	0.020 T	0.134	0.011 T	0.006 T	0.010 T	0.013 T
FCER1G	rs750727758	1:161184669	G/T	0.006 T	0.003	-	-	-	-
FCER1G	rs150957027	1:161184763	C/T	0.003 T	1.000	0.002 T	0	0.001 T	0.0001 T
FCER1G	None	1:161184792	GTCTCAAAAA/G	0.023 G	1.000	-	-	-	-
FCER1G	None	1:161184793	TCTCAAAAA/T	0.026 T	1.000	-	-	-	-
FCER1G	None	1:161184794	CTCAAAAAA/C	0.052 C	1.000	-	-	-	-
FCER1G	None	1:161184795	TCAAAAA/T	0.121 T	0.474	-	-	-	-
FCER1G	None	1:161184796	C/A	0.020 A	0.000	-	-	-	-
FCER1G	rs11587213	1:161184875	A/G	0.171 G	0.284	0.146 G	0.179 G	0.142 G	0.171 G
FCER1G	None	1:161184964	C/T	0.003 T	1.000	-	-	-	-
FCER1G	rs41270847	1:161184976	G/A	0.023 A	1.000	0.007 A	0.020 A	0.010 A	0.015 A
FCER1G	rs183879788	1:161185037	G/A	0.006 A	1.000	0.001 A	0.002 A	0.0007 A	0.001 A
FCER1G	rs2070901	1:161185058	G/T	0.276 T	0.328	0.391 T	0.269 T	0.314 T	0.248 T
RXRG	rs61800591	1:165414180	C/T	0.011 T	1.000	0.004 T	0.018 T	0.014 T	0.021 T
RXRG	rs57637440	1:165414267	C/A	0.003 A	1.000	0.008 A	0	0.008 A	0.00006 A
RXRG	None	1:165414386	ATGC/A	0.006 A	1.000	-	-	-	-
RXRG	rs3753897	1:165414511	C/A	0.152 A	0.082	0.215 A	0.195 A	0.211 A	0.193 A
RXRG	rs114530659	1:165414855	A/C	0.003 C	1.000	0.037 C	0.003 C	0.025 C	0.005 C
RXRG	rs1467664	1:165414933	T/C	0.178 C	0.073	0.203 C	0.142 C	0.181 C	0.138 C
RXRG	None	1:165415140	G/C	0.003 C	1.000	-	-	-	-
RXRG	None	1:165415222	A/G	0.003 G	1.000	-	-	-	-
CYP27A1	rs147477169	2:219646129	T/C	0.009 C	1.000	0.003 C	0.011 C	0.011 C	0.015 C
CYP27A1	rs114345604	2:219646470	T/C	0.006 C	1.000	0.014 C	0	0.013 C	0.0002 C
CYP27A1	rs182545847	2:219646536	T/C	0.006 C	1.000	0.0002 C	0	0.005 C	0.007 C
CYP27A1	None	2:219646547	AC/A	0.017 A	0.000	-	-	-	-
CYP27A1	rs778193906	2:219646962	C/T	0.006 T	0.003	-	-	0.000007 A	0
CYP27A1	rs757653354	2:219646967	G/T	0.006 T	0.003	-	-	0.000007 A	0
GC	rs3733359	4:72649774	G/A	0.069 A	0.894	0.206 A	0.055 A	0.122 A	0.060 A

Gene	dbSNP	Chromosomal Location	Alleles	MAF	HWE	MAF 1000 Genomes ALL individuals	MAF 1000 Genomes European individuals	MAF genomAD ALL individuals	MAF genomAD European individuals
GC	rs76781122	4:72669661	C/A	0.034 A	1.000	0.013 A	0.034 A	0.019 A	0.028 A
GC	rs6843222	4:72669944	C/T	0.029 T	1.000	0.004 T	0.016 T	0.007 T	0.011 T
GC	rs1565571	4:72670025	C/T	0.011 T	0.000	0.002 T	0	0.002 T	0
GC	rs35096193	4:72670093	C/A	0.236 A	0.059	0.165 A	0.284 A	0.203 A	0.272 A
GC	rs1565572	4:72670191	A/C	0.210 C	0.499	0.432 C	0.196 C	0.363 C	0.192 C
GC	rs2365085	4:72670432	T/A	0.003 A	1.000	0.033 A	0.001 A	0.038 A	0.009 A
GC	rs113387725	4:72670436	G/A	0.003 A	1.000	0.155 A	0.059 A	0.099 A	0.057 A
GC	rs4020369	4:72670448	G/A	0.037 A	1.000	0.033 A	0	0.037 A	0.008 A
IL13	rs186788419	5:131991999	G/C	0.014 C	0.000	-	-	0.0005 C	0.0007 C
IL13	rs544266451	5:131992049	T/C	0.011 C	0.000	-	-	-	-
IL13	None	5:131992053	C/T	0.006 T	0.003	-	-	-	-
IL13	None	5:131992056	G/A	0.011 A	0.000	-	-	-	-
IL13	None	5:131992060	A/C	0.011 C	0.003	-	-	-	-
IL13	None	5:131992064	C/T	0.006 T	0.003	-	-	-	-
IL13	None	5:131992065	T/C	0.006 C	0.003	-	-	-	-
IL13	None	5:131992067	C/A	0.006 A	0.003	-	-	-	-
IL13	rs1010937360	5:131992068	C/T	0.006 T	0.003	-	-	-	-
IL13	None	5:131992073	G/C	0.006 C	0.003	-	-	-	-
IL13	None	5:131992080	C/T	0.006 T	0.003	-	-	-	-
IL13	None	5:131992098	G/C	0.023 C	0.000	-	-	-	-
IL13	None	5:131992103	G/C	0.006 C	0.003	-	-	-	-
IL13	None	5:131992115	G/A	0.011 A	0.000	-	-	-	-
IL13	None	5:131992117	A/C	0.006 C	0.003	-	-	-	-
IL13	None	5:131992118	G/A	0.006 A	0.003	-	-	-	-
IL13	None	5:131992120	T/C	0.014 C	0.000	-	-	-	-
IL13	None	5:131992126	T/C	0.011 C	0.000	-	-	-	-
IL13	None	5:131992127	T/C	0.006 C	0.003	-	-	-	-
IL13	None	5:131992129	T/C	0.006 C	0.003	-	-	-	-
IL13	None	5:131992197	C/T	0.006 T	0.003	-	-	-	-
IL13	None	5:131992229	C/T	0.011 T	0.000	-	-	-	-
IL13	None	5:131992241	T/C	0.011 C	0.000	-	-	-	-
IL13	None	5:131992243	C/A	0.011 A	0.000	-	-	-	-
IL13	rs770116349	5:131992295	C/T	0.003 T	1.000	-	-	0.00006 T	0.0001 T
IL13	None	5:131992807	A/C	0.006 C	0.003	-	-	-	-
IL13	rs1800925	5:131992809	C/T	0.223 T	0.001	0.255 T	0.178 T	0.270 T	0.226 T
IL13	rs2066960	5:131994435	C/A	0.082 A	1.000	0.199 A	0.115 A	0.176 A	0.125 A
IL13	rs528784778	5:131994444	G/A	0.003 A	1.000	0.0002 A	0.001 A	-	-
IL13	rs1295687	5:131994462	G/C	0.072 C	0.045	-	-	0.131 C	0.064 C
IL13	rs2069744	5:131994669	C/T	0.014 T	1.000	0.124 T	0.010 T	0.091 T	0.004 T
IL13	rs574210453	5:131994954	A/G	0.009 G	1.000	0.0004 G	0.001 G	0.0002 G	0
IL13	rs533616846	5:131994965	G/T	0.006 T	1.000	0.0004 T	0.001 T	0.0001 T	0.0001 T
IL13	rs2069746	5:131995079	C/T	0.003 T	1.000	0.013 T	0	0.003 T	0.0001 T
IL4	rs145217073	5:132008886	G/C	0.006 C	1.000	0	0.001 C	0.002 C	0.002 C
IL4	rs752517771	5:132009033	G/C	0.006 C	0.003	-	-	-	-
IL4	rs2243250	5:132009154	C/T	0.141 T	0.752	0.470 T	0.168 T	0.371 T	0.176 T
IL4	rs894692486	5:132009374	T/C	0.003 C	1.000	-	-	0.00003 C	0.00006 C
IL4	rs539499377	5:132009579	T/A	0.003 A	1.000	0.0002 A	0	-	-
IL4	rs17772853	5:132009599	C/T	0.006 T	0.003	0.010 T	0.011 T	0.008 T	0.004 T
IL4	rs2070874	5:132009710	C/T	0.132 T	0.507	0.401 T	0.168 T	0.279 T	0.148 T
IL4	rs2243251	5:132009787	A/G	0.009 G	1.000	0.059 G	0.002 G	0.017 G	0.0007 G

Gene	dbSNP	Chromosomal Location	Alleles	MAF	HWE	MAF 1000 Genomes ALL individuals	MAF 1000 Genomes European individuals	MAF genomAD ALL individuals	MAF genomAD European individuals
RXRB	rs376711309	6:33168304	G/T	0.003 T	1.000	0.0002 T	0.001 T	0.0005 T	0.0008 T
RXRB	rs577415071	6:33168901	T/C	0.003 C	1.000	0.0002 C	0.001 C	0.0004 C	0.0008 C
RXRB	rs76929655	6:33169182	T/C	0.020 C	1.000	0.002 C	0.005 C	0.007 C	0.011 C
RXRB	rs772036010	6:33169223	T/G	0.006 G	0.003	-	-	0.000004 C	0
CYP2R1	rs12794714	11:14913575	G/A	0.445 A	0.647	0.349 A	0.447 A	0.406 A	0.433 A
CYP2R1	rs187639972	11:14913900	G/C	0.003 C	1.000	0.001 C	0.003 C	0.001 C	0.002 C
CYP2R1	None	11:14914082	A/C	0.006 C	0.003	-	-	-	-
CYP2R1	rs144205117	11:14914601	C/A	0.012 A	1.000	0.030 A	0.010 A	0.008 A	0.009 A
MS4A2	rs573790	11:59855385	C/T	0.382 T	1.000	0.441 T	0.356 T	0.460 T	0.411 T
MS4A2	rs574700	11:59855483	C/T	0.032 T	1.000	0.124 T	0.039 T	0.084 T	0.018 T
MS4A2	rs756668901	11:59855532	T/G	0.003 G	1.000	-	-	0.0002 G	0.0004 G
MS4A2	rs780370785	11:59855542	T/C	0.003 C	1.000	-	-	0.0001 C	0.0001 C
MS4A2	rs1441585	11:59855711	T/C	0.032 C	1.000	0.112 C	0.038 C	-	-
MS4A2	None	11:59855918	T/C	0.003 C	1.000	-	-	-	-
MS4A2	rs73479087	11:59855960	A/G	0.003 G	1.000	0.015 G	0	0.011 G	0.0004 G
MS4A2	rs1441586	11:59856028	T/C	0.414 C	0.755	0.460 C	0.456 C	0.425 C	0.418 C
MS4A2	rs920168033	11:59856086	T/C	0.003 C	1.000	-	-	-	-
MS4A2	rs140124027	11:59856240	T/G	0.003 G	1.000	-	-	0.0002 G	0.0003 G
VDR	rs117397914	12:48276613	A/G	0.031 G	0.150	0.009 G	0.018 G	0.011 G	0.016 G
VDR	rs11168293	12:48293716	G/T	0.284 T	0.712	0.166 T	0.321 T	0.282 T	0.355 T
VDR	None	12:48293730	G/A	0.003 A	1.000	-	-	-	-
VDR	None	12:48298652	C/A	0.003 A	1.000	-	-	-	-
VDR	rs144185611	12:48299279	G/T	0.003 T	1.000	0.001 T	0.005 T	0.003 T	0.002 T
VDR	None	12:48336499	T/A	0.003 A	1.000	-	-	-	-
VDR	rs4303288	12:48336619	A/C	0.467 C	0.035	0.404 A	0.397 A	0.402 A	0.389 A
VDR	rs4307775	12:48336623	C/G	0.139 G	0.000	0.171 G	0.209 G	0.185 G	0.249 G
VDR	rs138142537	12:48336736	A/C	0.006 C	1.000	0.007 C	0.015 C	0.008 C	0.012 C
CYP27B1	None	12:58160838	G/C	0.003 C	1.000	-	-	-	-
CYP27B1	rs529566631	12:58161026	A/G	0.003 G	1.000	-	-	0.001 G	0.001 G
CYP27B1	rs117864436	12:58161242	C/T	0.011 T	1.000	0.002 T	0.006 T	0.005 T	0.009 T
CYP27B1	rs148627073	12:58161599	G/A	0.014 A	0.029	0.004 A	0.015 A	0.003 A	0.005 A
CYP27B1	None	12:58161746	G/A	0.006 A	0.003	-	-	-	-
IL4R	rs12927172	16:27325021	G/A	0.424 A	0.013	0.405 A	0.372 A	0.353 A	0.367 A
IL4R	rs12927543	16:27325023	A/G	0.109 G	0.125	0.082 G	0.088 G	0.066 G	0.080 G
IL4R	rs55919742	16:27325028	G/A	0.006 A	1.000	0.013 A	0.002 A	0.011 A	0.001 A
CYP24A1	rs35873579	20:52788190	G/A	0.023 A	1.000	0.001 A	0.004 A	0.002 A	0.003 A
CYP24A1	rs36106327	20:52788294	C/A	0.026 A	0.100	0.007 A	0.020 A	0.010 A	0.016 A
CYP24A1	rs2259735	20:52788314	T/C	0.455 C	0.029	0.566 C	0.420 C	0.515 C	0.408 C
CYP24A1	rs17219315	20:52788446	A/G	0.014 G	1.000	0.009 G	0.018 G	0.013 G	0.019 G
CYP24A1	rs59262760	20:52789417	C/T	0.006 T	1.000	0.066 T	0	0.022 T	0.002 T
CYP24A1	None	20:52789466	CCTT/C	0.006 C	1.000	-	-	-	-
CYP24A1	rs61755338	20:52789885	A/C	0.014 C	1.000	0.009 C	0.018 C	0.016 C	0.022 C
CYP24A1	rs61749689	20:52790005	C/A	0.017 A	1.000	0.008 A	0.018 A	0.016 A	0.023 A
CYP24A1	rs73913755	20:52790194	G/A	0.006 A	1.000	0.050 A	0	0.046 A	0.001 A
CYP24A1	rs144737632	20:52790218	A/G	0.017 G	1.000	0.008 G	0.018 G	0.012 G	0.019 G
CYP24A1	None	20:52790469	GTC/G	0.006 G	1.000	-	-	-	-
CYP24A1	None	20:52790470	TC/T	0.011 T	1.000	-	-	-	-
CYP24A1	rs73913757	20:52790518	C/T	0.006 T	1.000	0.049 T	0	0.044 T	0.001 T
CYP24A1	rs536169079	20:52790704	G/C	0.014 C	1.000	0.008 C	0.018 C	0.012 C	0.019 C
CYP24A1	rs373075992	20:52790754	C/T	0.014 T	1.000	0.008 T	0.018 T	0.012 T	0.019 T

Gene	dbSNP	Chromosomal Location	Alleles	MAF	HWE	MAF 1000 Genomes ALL individuals	MAF 1000 Genomes European individuals	MAF genomAD ALL individuals	MAF genomAD European individuals
CYP24A1	rs2762943	20:52790786	G/T	0.095 T	0.652	0.034 T	0.085 T	0.037 T	0.081 T
CYP24A1	rs2585427	20:52790976	G/C	0.376 C	0.873	0.444 C	0.390 C	0.447 C	0.373 C
CYP24A1	rs187147516	20:52790984	C/A	0.014 A	1.000	0.010 A	0.024 A	0.015 A	0.023 A
IL13RA1	rs141993027	X:117860913	T/C	0.003 C	1.000	0.001 C	0	0.001 C	0
IL13RA1	rs187367085	X:117861106	G/A	0.003 A	1.000	0.001 A	0.003 A	0.0006 A	0.0004 A
IL13RA1	rs6603441	X:117861321	T/G	0.356 G	0.000	0.452 G	0.315 G	0.407 G	0.322 G
IL13RA1	rs555458472	X:117861629	C/T	0.006 T	0.003	-	-	0.00004 T	0

-: not described; MAF: Minor Allele Frequency.

Table S5. Adjusted *P*-values after logistic binary regression analyses of SNPs with MAF ≥ 0.02 observed in the NGS study.

SNP	Chromosomal location	R+A vs controls	BLs vs Controls	CR vs Controls	SH vs Controls	Drugs-Controls	Drugs IgE-Controls	IgE med-Controls	R+A vs rest of patients	BLs vs rest of patients	CR vs rest of patients	SH vs rest of patients
rs2427837	1:159258545	0.240	0.121	0.464	0.278	0.289	0.228	0.213	0.568	0.200	0.311	0.857
rs61828219	1:159258641	0.238	0.780	0.294	0.865	0.749	0.885	0.764	0.437	0.787	0.413	0.370
rs12135235	1:159259029	0.045	0.043	0.101	0.071	0.049	0.042	0.034	0.677	0.685	0.552	0.800
rs36233990	1:161184658	0.144	0.075	0.037	0.070	0.014	0.024	0.015	0.514	0.894	0.182	0.822
None	1:161184792	0.335	0.951	0.293	0.130	0.857	0.545	0.431	0.123	0.959	0.012	0.021
None	1:161184793	0.335	0.051	0.185	0.374	0.117	0.112	0.134	0.538	0.096	0.883	0.215
None	1:161184794	0.147	0.080	0.003	0.072	0.018	0.060	0.071	0.192	0.512	0.022	0.444
None	1:161184795	0.238	0.338	0.198	0.009	0.037	0.041	0.035	0.809	0.039	0.336	0.009
None	1:161184796	0.383	0.153	-	-	0.411	0.320	0.485	0.141	0.251	0.285	0.236
rs11587213	1:161184875	0.169	0.340	0.880	0.514	0.557	0.328	0.224	0.355	0.745	0.183	0.982
rs41270847	1:161184976	1.000	0.817	0.447	0.124	0.988	0.488	0.547	0.959	0.983	0.085	0.022
rs2070901	1:161185058	0.626	0.226	0.315	0.056	0.103	0.074	0.097	0.801	0.593	0.666	0.284
rs3753897	1:165414511	0.792	0.312	0.645	0.188	0.296	0.209	0.273	0.317	0.650	0.551	0.357
rs1467664	1:165414933	0.007	0.178	0.256	0.026	0.105	0.098	0.053	0.023	0.017	0.484	0.365
rs3733359	4:72649774	0.571	0.318	0.342	0.092	0.224	0.170	0.174	0.725	0.529	0.106	0.008
rs76781122	4:72669661	1.000	0.922	0.523	0.435	0.623	0.693	0.744	0.626	0.399	0.579	0.432
rs6843222	4:72669944	0.425	0.507	0.226	0.690	0.436	0.588	0.520	0.613	0.636	0.382	0.524
rs35096193	4:72670093	0.626	0.393	0.405	0.820	0.514	0.586	0.637	0.341	0.626	0.412	0.530
rs1565572	4:72670191	0.724	0.815	0.508	0.708	0.683	0.794	0.761	0.988	0.785	0.684	0.519
rs4020369	4:72670448	-	0.197	0.018	0.042	0.035	0.068	0.094	0.062	0.290	0.157	0.608
None	5:131992098	0.335	0.197	-	0.371	0.327	0.241	0.229	0.626	0.360	0.113	0.830
rs1800925	5:131992809	0.210	0.335	0.712	0.489	0.454	0.363	0.334	0.238	0.744	0.667	0.431
rs2066960	5:131994435	0.336	0.903	0.612	0.270	0.699	0.492	0.753	0.144	0.724	0.302	0.207
rs1295687	5:131994462	0.095	0.444	0.392	0.554	0.559	0.432	0.436	0.085	0.342	0.944	0.249
rs2243250	5:132009154	0.036	0.114	0.022	0.329	0.110	0.276	0.181	0.266	0.250	0.209	0.123
rs2070874	5:132009710	0.019	0.024	0.008	0.159	0.032	0.091	0.060	0.443	0.277	0.271	0.185
rs76929655	6:33169182	0.335	0.197	0.179	0.203	0.170	0.170	0.169	0.857	0.979	0.870	0.974
rs12794714	11:14913575	0.685	0.424	0.729	0.742	0.767	0.715	0.775	0.610	0.153	0.727	0.910
rs573790	11:59855385	0.071	0.089	0.038	0.225	0.094	0.146	0.107	0.265	0.743	0.134	0.830
rs574700	11:59855483	0.081	0.552	0.802	0.949	0.699	0.690	0.512	0.102	0.639	0.614	0.316
rs1441585	11:59855711	0.081	0.552	0.802	0.949	0.699	0.690	0.512	0.102	0.639	0.614	0.316
rs1441586	11:59856028	0.260	0.487	0.279	0.881	0.544	0.678	0.594	0.496	0.891	0.250	0.507
rs117397914	12:48276613	0.147	0.946	0.447	0.670	0.807	0.782	0.781	0.319	0.724	0.268	0.318
rs11168293	12:48293716	0.535	0.573	0.146	0.047	0.155	0.208	0.184	0.891	0.131	0.807	0.109

rs4303288	12:48336619	0.288	0.011	0.726	0.884	0.298	0.193	0.211	0.284	0.002	0.414	0.016
rs4307775	12:48336623	0.014	0.001	0.002	0.000	0.000	0.000	0.000	0.994	0.635	0.639	0.925
rs12927172	16:27325021	0.490	0.831	0.906	0.171	0.572	0.403	0.716	0.113	0.970	0.616	0.077
rs12927543	16:27325023	0.394	0.931	0.895	0.869	0.862	0.890	0.811	0.181	0.957	0.960	0.761
rs35873579	20:52788190	0.425	0.743	0.953	0.976	0.908	0.864	0.919	0.145	0.957	0.960	0.761
rs36106327	20:52788294	0.097	0.237	0.095	0.130	0.079	0.166	0.096	0.041	0.394	0.394	0.019
rs2259735	20:52788314	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.567	0.625	0.630	0.308
rs2762943	20:52790786	0.453	0.547	0.716	0.647	0.647	0.556	0.633	0.492	0.05	0.471	0.897
rs2585427	20:52790976	0.287	0.856	0.595	0.562	0.622	0.651	0.526	0.420	0.702	0.762	0.975
rs6603441	X:117861321	0.251	0.295	0.054	0.318	0.124	0.272	0.190	0.451	0.354	0.311	0.781