

Multifactor-dimensionality reduction reveals interaction of important gene variants involved in allergy

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Summary

Elevated IgE levels in the atopic triad of asthma, allergic rhinitis and atopic dermatitis is a multifactorial condition whose genetic component involves interaction of several gene loci. One hundred and two matched pairs of allergic and nonallergic individuals were phenotyped for total serum IgE level using enzyme-linked immunosorbent assay (ELISA). Atopic status was defined by serum IgE concentration ≥ 100 IU mL⁻¹. SNPs genotyped include the *IL4* -590C>T (rs2243250), *FCER1B* E237G (rs569108), *CD14* -159C>T (rs2569190), *IL4RA* Q551R (rs1801275) and *ADRB2* R16G (rs1042713). Gene-gene interaction was analysed using multifactor-dimensionality reduction (MDR). Significant association between atopic allergy and the *IL4* -590C>T polymorphism was confirmed in three genetic models. Interaction among the 5 gene variants was validated by MDR. The five-locus model was chosen as the best to describe the interaction of the SNPs within the context of atopy. The strongest interaction was between *IL4* -590C>T and *IL4RA* Q551R and between *FCER1B* E237G and *ADRB2* R16G. The *IL4* variant also interacts synergistically with the *FCER1B* and *ADRB2* coding variants. *CD14* -159C>T, in general, interacts antagonistically with the rest of the SNPs. In

conclusion, a five-locus interaction exists among *IL4* -590C>T, *FCER1B* E237G, *CD14* -159C>T, *IL4RA* Q551R and *ADRB2* R16G in Filipino cases of atopic allergy.

Introduction

The triad of asthma, allergic rhinitis and atopic dermatitis is among the commonest causes of chronic illness (World Allergy Organization, 2013). In the Philippines, one in ten children has asthma and 32.5% of Filipino children aged 13–14 years old have allergic rhinitis and 7.1% have atopic dermatitis (The International Study of Asthma & Allergies in Childhood (ISAAC) Steering Committee, 1998). Among adults, recent prevalence rates display similar trend (Katelaris *et al.*, 2011; Abong *et al.*, 2012). The pathophysiology of these allergic disorders is complex and caused by largely uncharacterized interaction between genes and environmental factors. Atopy is marked by an abnormally high rate of IgE antibody production against common environmental allergens. Cross-linking of the mast cell-bound IgE upon exposure to the allergen leads to inflammatory reactions in the airways, nasal cavity and/or the skin that clinically manifest as symptoms of the allergy. Majority of the phenotypic symptoms of allergy have been defined by either binary response outcomes, that is physician's diagnosis and questionnaire, or 'intermediate' traits such as skin-prick test and serum total and specific IgE levels (Koppelman *et al.*, 1998). The genetic components of allergy, however, are still currently under study with the use of genomewide or candidate gene linkage analysis and association, interaction and functional studies.

Risk assessment with the use of a single candidate gene variant might not show the actual effect on the disease outcome given the multifactorial nature of chronic diseases. NonMendelian forms of inheritance, the ubiquitous characteristic of biomolecular communication and the inconsistent results of several single-variant, single-gene studies all support the idea that gene-gene interactions are common and can best describe the

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disease symptoms (Cordell, 2009). Several tools are being used to detect significant association of gene–gene interaction with the disease phenotype. Logistic and linear regression, and combinatorial partitioning method (CPM) are some of these statistical methods.

In this study, we utilized multifactor-dimensionality reduction (MDR) method to detect the possible interaction among 5 loci with atopic allergy in a matched, case–control study. The SNPs include IL4 -590C>T (rs2243250), FCER1B E237G (rs569108), CD14 -159C>T (rs2569190), IL4RA Q551R (rs1801275) and ADRB2 R16G (rs1042713). We selected the candidate genes and the variants at strategic points in the pathophysiology of allergy to better visualize the important role these genes play in atopy. Functions include (i) exposure to allergens where CD14 is involved in the innate immune system, (ii) polarization of T cells towards immediate hypersensitivity where IL4 and the IL4RA receptor are essential, (iii) the effector functions involving mast cells and basophils that capture allergen-specific IgE via FCER1B and (iv) the role of ADRB2 in bronchial hyper-responsiveness which has pharmacologic implications. We tested in this study the hypothesis of whether or not the gene variants interact among each other synergistically or antagonistically and whether particular allele combinations confer risk for atopy development.

Materials and methods

Study design, base and participants

Case–control method was used in the study. The appropriate sample size and power of the study were determined using web-based programs QUANTO v1.2 and Power for Association with Errors – PAWE-3D (Gordon *et al.*, 2005; Gauderman & Morrison, 2006). Blood samples from participants were taken at the University of Santo Tomas Hospital. The study design, conduct of sampling and experimental protocols were all approved by the Institutional Review Board of the hospital in accordance to the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (IRB Ref. No: IRB-AP081-D-MORP).

Participants were initially screened using standardized questionnaires of the International Study of Asthma and Allergy in Childhood (ISAAC) and the International Primary Care Airways Group (IPAG). To further qualify in the study, the following criteria were set: (i) naturally born Filipino, (ii) must be unrelated to any other individuals who have had already entered the study, (iii) was born and living in Luzon Island, (iv) aged 6–60 years old at the time of entry in the study, (v) with both parents also Filipinos and (vi) must not have any history of helminth infestation. Cases were conceptually defined in the study as individuals suffering from asthma, allergic rhinitis or atopic dermatitis, mild or severe, with total serum IgE level of ≥ 100 IU mL⁻¹ (Zimmerman, 1998; Shirakawa

et al., 1994; Laitinen *et al.*, 1997) and thus atopic. Controls, on the other hand, were defined as nonatopic individuals (total serum IgE level of <100 IU mL⁻¹) without any history of allergy and neither do have immediate relatives with it. Cases and controls were matched-paired with their age (± 2 years old), gender and geographic location of residence.

Clinical protocol and phenotyping

Ten millilitres of blood was extracted by venipuncture from all the participants. Five millilitres of the whole blood were centrifuged for 5 min at 5000 rpm to isolate the serum. Total serum IgE was quantitated using standard antibody sandwich ELISA protocol. The concentration was expressed in IU mL⁻¹ and was log-transformed to normalize the distribution.

Genotyping

Genomic DNA was extracted from the whole blood using the WIZARD® Genomic DNA Purification Kit (Promega, Madison, WI, USA). Polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) was used as previously described (Baldini *et al.*, 1999; Ikarashi *et al.*, 2004; Lee *et al.*, 2004; Isidoro-Garcia *et al.*, 2005) to amplify and analyse for IL4 -590C>T (*Ava*II enzyme), CD14 -159C>T (*Ava*II), IL4RA Q551R (*Msp*I) and ADRB2 R16G (*Bsr*DI). The PCR products were digested with 5 U of the respective enzyme at 37°C for 5 h. The digested DNA was separated on 3.5% agarose gel containing ethidium bromide at 60V for 1 h (Figure S1 in Supporting Information). FCER1B E237G was genotyped using allele-specific amplification or the amplification refractory mutation system (ARMS) as described previously (Hill & Cookson, 1996). The samples were randomly genotyped, and the profiles were read independently by two individuals. The samples were re-genotyped if there was any disagreement concerning the genotype.

Statistical analysis for single gene effects

Data were managed and analysed using the SPSS v11.5 (SPSS Inc., Chicago, IL, USA) and STATXACT (Cytel Inc., Cambridge, MA, USA) statistical packages and MS Excel (Microsoft Corp., Redmond, WA, USA). Hardy–Weinberg equilibrium was tested by Fisher's exact test χ^2 analysis for a 2 × 2 table. Differences and association between or among variables in all the inferential statistics were considered significant when *P*-value is <0.05 . Both parametric and nonparametric tests were done. To test for association, χ^2 , Fisher's, Fisher–Freeman–Halton, Armitage and odds ratio tests were performed.

Statistical analysis for gene interactions

Multifactor-dimensionality reduction analysis was performed using MDR 3.0.2 software (Computational

Genetics Laboratory, Institute for Quantitative Biomedical Sciences, Dartmouth, New Hampshire, USA) as described previously (Ritchie *et al.*, 2001). Briefly, a 10-fold cross-validation was performed on the data set divided into ten equal sizes from which nine subsets served as training sets and 1 as the test set. The total number of combined factors used was (3^5) 343. The best factor combination was screened based on the minimum classification error in the training set. The process was repeated ten times. The final candidate model of gene–gene interaction was selected based on maximum cross-validation consistency (CVC) and maximum testing balanced accuracy. For risk assessment, the genotype combination was identified as high risk if the ratio of cases to controls exceeds the threshold; otherwise, the factor combination is of low risk.

Visualization of genetic model

Dendrogram and circle graph were used to display the selected model. The program was set to give hierarchical interaction entropy graphs based on information theory (Moore *et al.*, 2006). Individual and all possible pairing of SNPs received information percentage scores based on the risk for atopy. A positive percentage score was designated as synergistic interaction (red & orange in dendrogram), while score of 0 or below was designated redundant or antagonistic (green or blue). If paired percentage score is greater than individual score, the former was selected as better model to describe association with the risk of atopic allergy.

Results

Power analysis, demographics and phenotype

QUANTO v1.2 and PAWE-3D calculations for the study gave as high as 90% power that can detect variant allele frequency of at least 0.05 and genotype relative risk of ≥ 1.8 at 80% power. A total of 102 matched pairs were recruited in the study based on the minimum sample size of 100 pairs. The mean age of the participants was 25.99 ± 8.94 years with a range from 11 to 57 years. In the atopic-allergic case group, 43% have asthma, 35% have allergic rhinitis, and 22% have atopic dermatitis. A significant difference in total IgE was observed between atopic cases and matched control ($t = 27.649$, d.f. = 101, $P < 0.001$) (Fig. 1).

Association between atopy and the polymorphisms

Table 1 presents individual test for association of the SNP with atopic allergy. Among all the tests, only the *IL4* -590C>T gave significant association with the phenotype. This is true in both multiplicative and additive model with the latter being independent of Hardy–Weinberg equilibrium (Additive OR_{IT} : 3.558, 95% CI: 1.446–8.759, $P = 0.004861$; Multiplicative

OR_T : 2.060, 95% CI: 1.285–3.301, $P = 0.002$). The *IL4* receptor polymorphism Q551R gave marginal association with multiplicative model but not with the additive model.

MDR analysis

Table 2 summarizes the result of the multifactor-dimensionality reduction analysis performed for each SNP. The one-locus model for *IL4* -590C>T and the five-locus model gave the maximum cross-validation consistency of 10/10 and highest prediction accuracy of 71.43%. However, only the five-locus model is significant for prediction accuracy ($P = 0.0382$). Thus, the five-locus model which included all the SNPs in the study was regarded as the best model for interaction.

Interaction entropy model (Fig. 2a) shows how the five gene variants interact with each other. The strongest interaction existed between *IL4* -590C>T and *IL4RA* Q551R in an antagonistic manner. On the contrary, a strong synergistic interaction existed between *FCER1B* E237G and *ADRB2* R16G. These two SNPs exhibited a moderately strong, synergistic interaction with *IL4* -590C>T (Fig. 2b). The *IL4RA* Q551R, on the other hand, had a moderately strong antagonistic interaction with the *FCER1B* and *ADRB2* SNPs. The interaction of *CD14* -159C>T with the other four SNPs was not that strong with borderline percentage score for *IL4* and *ADRB2*, synergistic with *FCER1B*, and antagonistic with *IL4RA*.

Circle diagram (Fig. 2b) of information scores reveals that *IL4* -590C>T percentage score (3.26%) was the most important among the five gene variants in the study. *ADRB2* and *FCER1B* SNPs' paired score, and thus the interaction between the two, proved to be the more significant than individual contribution of each.

Seven genotype combinations (Fig. 3) resulted into significant high risk for atopic allergy. Interestingly, the combination that gave the highest risk (5-fold) was the haplotype heterozygote for the *CD14* -159 and

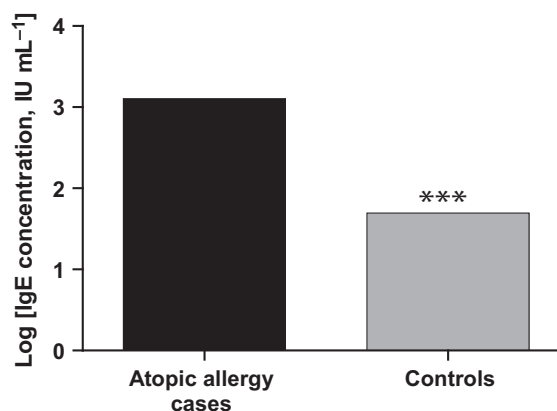


Figure 1. Total serum IgE levels of atopic cases and nonatopic controls recruited in the study. $N = 102$ per group. *** t -test $P < 0.001$.

Table 1. Genetic effects of individual SNPs in the study

Genotype frequencies				P-value			
				Additive	Dominant	Recessive	Multiplicative
IL4 -590C>T	CC	CT	TT				
Control	73 (71.6%)	23 (22.5%)	6 (5.9%)	0.004861	0.003	0.205	0.002
Cases	52 (51.0%)	39 (38.2%)	11 (10.8%)				
FCER1B E237G	EE	EG	GG				
Control	72 (70.5%)	28 (27.5%)	2 (2%)	0.1028	0.104	0.561	0.107
Cases	82 (80.4%)	19 (18.6%)	1 (1%)				
CD14 -159C>T	CC	CT	TT				
Control	48 (47.1%)	34 (33.3%)	20 (19.6%)	0.2755	0.203	0.607	0.224
Cases	39 (38.2%)	40 (39.3%)	23 (22.5%)				
IL4RA Q551R	QQ	QR	RR				
Control	86 (84.3%)	16 (15.7%)	0 (0%)	0.1214	0.083		0.055
Cases	76 (74.5%)	24 (23.5%)	2 (2%)				
ADRB2 R16G	RR	RG	GG				
Control	20 (19.6%)	70 (68.6%)	12 (11.8%)	0.4053	0.187	0.831	0.486
Cases	28 (27.5%)	61 (59.8%)	13 (12.7%)				

P-values are: Cochran–Armitage trend test (additive model), χ^2 (dominant, recessive, & multiplicative models).

Table 2. Multifactor-dimensionality reduction analysis of gene–gene interaction for atopy candidate SNPs

Number of loci	Best model for SNP combination	Training accuracy		Prediction accuracy		Odds ratio (95% CI)	Cross-validation consistency (CVC)
		%	P-value	%	P-value		
1	IL4 (-590C>T)	59.08	0.0112	71.43	0.0502	2.4204 (1.3559–4.3207)	10
2	IL4 (-590C>T), ADRB2 (R16G)	61.75	0.0015	66.67	0.1035	2.8417 (1.6098–5.0161)	8
3	IL4 (-590C>T), FCER1B (E237G), ADRB2 (R16G)	66.85	<0.0001	70.00	0.0679	4.349 (2.3968–7.8911)	9
4	IL4 (-590C>T), FCER1B (E237G), IL4RA (Q551R), ADRB2 (R16G)	70.11	<0.0001	60.00	0.136	5.7387 (3.0805–10.6904)	6
5	IL4 (-590C>T), FCER1B (E237G), CD14 (-159C>T), IL4RA (Q551R), ADRB2 (R16G)	73.22	<0.0001	71.43	0.0382	9.3903 (4.9232–17.9108)	10

Note: Shaded row is the five-locus model chosen to describe interaction of SNPs.

wild-type for the rest of the SNPs. This was followed by *CD14* TT, *IL4RA* QR, *ADRB2* RG, *IL4* CC and *FCER1B* EE haplotype with 3-fold risk. The haplotypes *IL4-ADRB2* and *IL4-CD14* heterozygotes followed with 2.3- and 2-fold risks, respectively. Then, the triple variants at *IL4*, *CD14* and *ADRB2* with risks from 1.1- to 2-fold.

Discussion

Atopic allergy was defined and delimited in the study by utilizing both binary response and quantitative trait definitions. The significantly high total serum IgE levels in individuals suffering from asthma, allergic rhinitis and atopic dermatitis make the conditions detrimental the moment of allergen exposure. Previously, we reported the possible role of the *IL4* -590C>T polymorphism in conferring atopic allergy in a Filipino population (De Guia & Ramos, 2010). The results gathered in this study showing that the -590T allele of *IL4* is a risk factor to the development of atopic allergy replicated the results in other populations (Rosenwasser *et al.*, 1995;

Noguchi *et al.*, 1998; Zhu *et al.*, 2000; Kabesch *et al.*, 2003). The multifactorial nature of allergy, however, makes it hard to make assumptions of risk from a single SNP of a single gene.

We then picked four other SNPs based on prior association data, positional and linkage studies, and functional and pharmacological criteria (Hattersley & McCarthy, 2005). All together, these candidate genes play major roles in the pathophysiology of atopic allergy (Fig. 4) from allergen exposure in the innate immune system (*CD14*) to polarization of T cells towards allergic response (*IL4* and *IL4RA*), and downstream in immune effector cells like mast cells and basophils that capture allergen-specific IgE (via *FCER1B*); and the pharmacologic target (*ADRB2*) of anti-asthma drug to relieve bronchospasm.

To assess the possible interaction among these candidate genes, multifactor-dimensionality reduction (MDR) analysis was used. Just like Cochran–Armitage trend test for genotype risk assessment in the additive model, MDR has the advantage of being model free and nonparametric in identifying gene–gene or

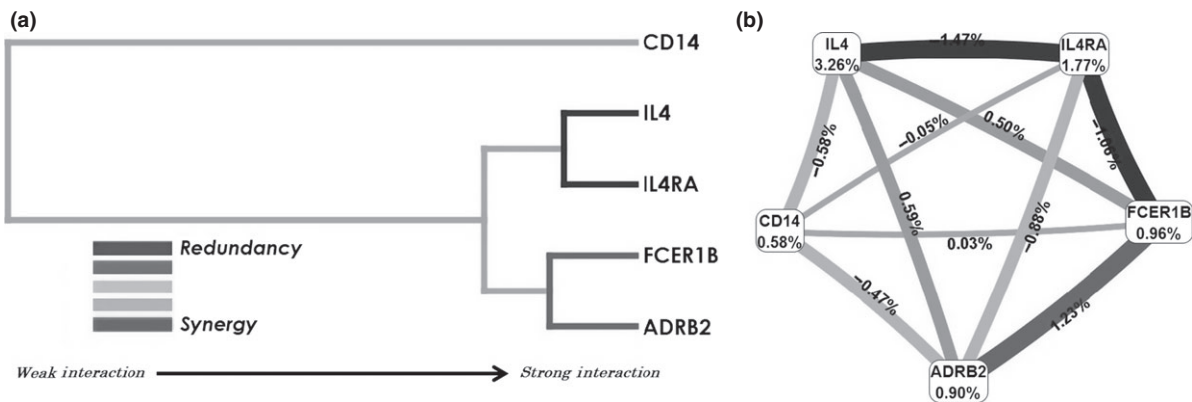


Figure 2. Interaction entropy and graphical model of gene-gene interaction for atopy candidate genes. (a) Dendrogram of the interaction showing SNPs that interact synergistically or antagonistically. (b) Circular graph of interaction showing positive (information gain or synergy) and negative (lack of gain or redundancy) percentage entropies.

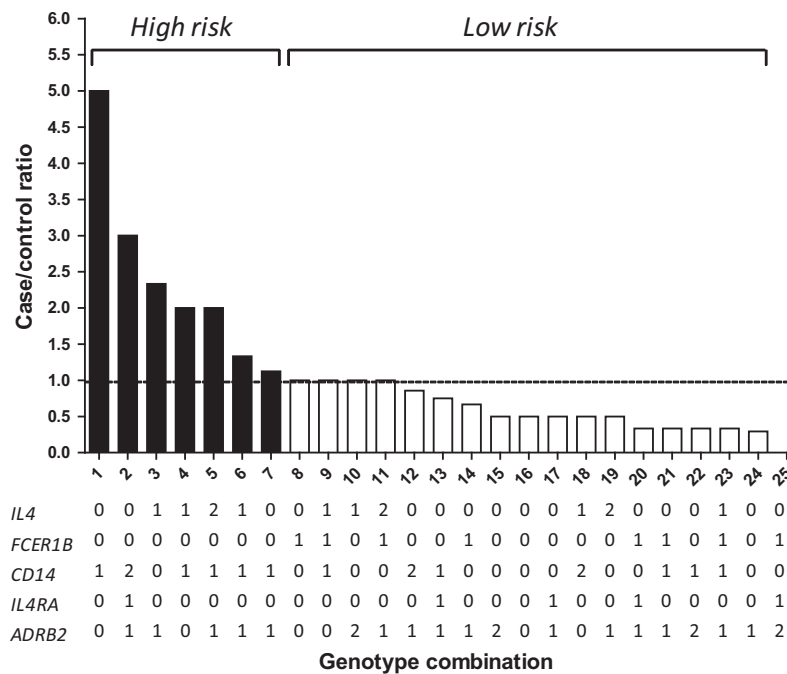


Figure 3. Five-locus gene-gene interaction between *IL4*, *FCER1B*, *CD14*, *IL4RA* and *ADRB2* for atopic allergy. Bar graph shows validation of high and low risk classification. Genotype coding are as follows: 0 – homozygous common allele, 1 – heterozygous and 2 – homozygous variant allele.

gene-environment interactions in a population (Ritchie *et al.*, 2003). Indeed, MDR was able to detect interaction of the *IL4* -590C>T polymorphism with other SNPs in the study. A strong interaction of this SNP with its receptor polymorphism *IL4RA* Q551R has been detected in this study. Reports on whether the *IL4* receptor Q551R polymorphism is a risk factor of allergy, however, have been inconsistent (Hackstein *et al.*, 1999; Kruse *et al.*, 1999). Here, we found that the interaction between *IL4* -590C>T and *IL4RA* Q551R was redundant or antagonistic. Considering that the *IL4* -590T allele was reported to alter tran-

scription of the cytokine towards maximum favouring increased IgE production (Cousins *et al.*, 1998), the *IL4RA* Q551R might be a protective rather than a risk factor.

A strong, significant synergistic interaction existed between the receptor polymorphisms *FCER1B* E237G and *ADRB2* R16G as shown by the high paired information percentage score versus the individual scores (Fig. 2b). No publication has reported the interaction between these two polymorphisms so far. Whether these two SNPs interact biologically remains to be investigated. Implications can be made based on

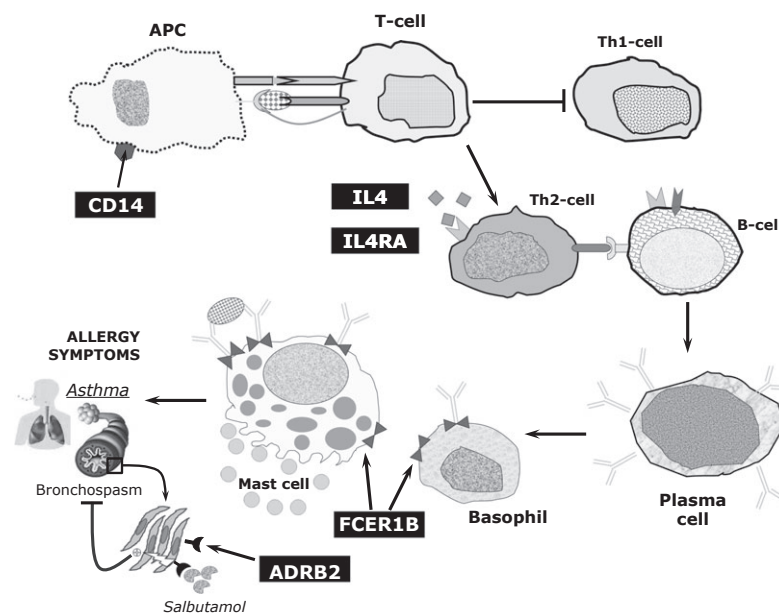


Figure 4. The pathophysiology of atopic allergy highlighting the candidate genes. Abbreviations: *ADRB2*, β_2 -Adrenergic receptor; APC, antigen-presenting cell; *CD14*, cluster of differentiation antigen 14; *FCER1B*, Fc ϵ receptor R1B – the high-affinity IgE receptor; *IL4*, interleukin-4; *IL4RA*, interleukin 4 receptor A.

results of association and biological studies reporting the effects by which these two coding variants can affect the expression of the receptor itself. Both coding variants have been reported also to be associated with airway hyper-responsiveness in asthma (Hill & Cookson, 1996; Hall *et al.*, 2006). The *IL4* -590 SNP interacts with these two coding variants in synergistic fashion although not as strong as between the two coding variants. Biologically, the interaction among the three could be possible as atopy brought by the *IL4* promoter polymorphism could translate to further exacerbation of allergy symptoms if the high-affinity IgE receptor and adrenergic receptor coding variants favour the same phenotype (Shirakawa *et al.*, 1996; Llanes *et al.*, 2009). The *IL4RA* variant interacts weakly and redundantly with the other receptor coding variants. This is possibly due to the stronger effect exerted by *IL4* -590 variant to the *FCER1B* and *ADRB2* SNPs.

The monocyte-differentiation antigen *CD14* is a coreceptor commonly linked to sensitization of the innate immune system via interaction with bacterial endotoxins or lipopolysaccharides (Gupta *et al.*, 1996). *CD14* has been reported to play an important role in the so-called 'endotoxin switch' of the 'hygiene hypothesis' stating that diminished exposure to childhood infections and overly clean environment has resulted in a population predisposed to allergies (Ober & Thompson, 2005). The *CD14* -159C>T promoter polymorphism has been shown to be associated with increased *CD14* expression probably due to the defunct binding of important transcription factors in this region (LeVan *et al.*, 2001). In this study, with the exception of

FCER1B, this promoter SNP interacts with the other variants in antagonistic manner. Although the interaction is weak, this supports the possible protective role of the variant against atopic allergy.

In conclusion, our findings support the multifactorial nature of atopic allergy involving the interactions of several key genes to bring about the expression of the phenotype. The strategic locations of the candidate genes selected in this study further depicts the important role of the immune response starting from the innate immunity down to effector cells of the adaptive immune system. Furthermore, the results gathered can provide insights for future strategies on allergy medications and management.

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Conflict of interest

None declared.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1. Genotyping for the five gene variants in the study. (a) *IL4* -590 C>T *Ava*II RFLP 223 bp – CC; 21, 202, 223 bp – CT; 21, 202 bp – TT, (b) *FCER1B* E237G ARMS-PCR: 446 bp – Control band; 280 bp – wt sequence; 238 bp – 237G (c) *CD14* -159C>T *Ava*II RFLP: 497 bp – C allele; 114, 353 bp – T allele, (d) *IL4RA* Q551R *Msp*I RFLP: 212 bp – 551Q; 155 bp – 551R, and (e) *ADRB2* R16G *Bsr*DI RFLP: 14, 56, 131 bp – 16R; 23, 108 bp – 16G.

Supplementary Figure 1

