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A pathway-based association study reveals variants from Wnt signaling genes contributing to asthma susceptibility

(Short title: Wnt signaling genes in asthma susceptibility)

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Abstract

Background: Genetic susceptibility to asthma is currently linked to a handful of genes which have a limited ability to predict the overall disease risk, suggesting the existence

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of many other genes involved in disease development. Accumulated evidence from association studies in genes related by biological pathways could reveal novel asthma genes.

Objective: To reveal novel asthma susceptibility genes by means of a pathway-based association study.

Methods: Based on summary data from a previous a genome-wide association study (GWAS) of asthma, we first identified significant biological pathways using a gene-set enrichment analysis. We then mapped all tested single nucleotide polymorphisms (SNPs) on the genes contributing to significant pathways, and prioritized those with a disproportionate number of nominal significant associations for further studies. For those prioritized genes, association studies were performed for selected SNPs in independent case-control samples (n=1,765) using logistic regression models, and results were meta-analyzed with those from the GWAS.

Results: Two biological processes were significantly enriched: the cytokine-cytokine receptor interaction ($p=0.002$) and the Wnt signaling ($p=0.012$). From the 417 genes interacting in these two pathways, 10 showed an excess of nominal associations, including a known asthma susceptibility locus (encoding SMAD family member 3) and other novel candidate genes. From the latter, association studies of 14 selected SNPs evidenced replication in a locus near the frizzled class receptor 6 (*FZD6*) gene ($p=9.90 \times 10^{-4}$), which had a consistent direction of effects with the GWAS findings (meta-analyzed odds ratio=1.49; $p=5.87 \times 10^{-6}$), and was in high linkage disequilibrium with expression quantitative trait loci in lung tissues.

Conclusions and Clinical Relevance: This study revealed the importance of two biological pathways in asthma pathogenesis and identified a novel susceptibility locus near Wnt signaling genes.

Key words: airway remodeling, predisposition, tissue repair, Wnt/ β -catenin signaling.

Introduction

Asthma, a chronic and complex lung inflammatory disease [1], is developed as a consequence of the interaction of multiple environmental and genetic factors [2, 3]. Despite the large number of studies aimed to identify susceptibility loci [4, 5], genome-wide association studies (GWAS) of asthma have only evidenced a handful of them as firm asthma susceptibility genes [6-21]. Given this, it is unsurprising that the accumulation of risk variants in the genes identified so far only explain a small proportion of asthma heritability and have limited ability to predict the overall disease risk [11].

Most GWAS of asthma have focused on the single nucleotide polymorphisms (SNPs) surpassing strict genome-wide thresholds for significance. However, follow-up studies of SNPs showing suggestive associations can yield firm asthma susceptibility genes as well. That is the case of the gene encoding interleukin 6 receptor (*IL6R*) and 11q13.5 near the gene encoding leucine rich repeat containing 32 (*LRRC32*) [11], which were identified as novel asthma loci despite none of the assessed variants reached genome-wide significance in the GWAS discovery stage. It has been argued that a more appropriate way of recapitulating the complexity of disease susceptibility would be incorporating in the analyses the biological information of the interplay of genes within functional pathways [22]. After all, the proteins encoded by the genes act coordinately in sets within biological pathways or processes [23]. While this type of approach can provide mechanistic insights into the underlying biology and may contribute to identify novel asthma genes, none of the GWAS of asthma have integrated such a strategy in their analyses as an approach to identify new asthma genes.

We have previously performed a GWAS in Spanish individuals analyzing 6.5 million variants at individual level using a three-stage design in a total of 1,180 cases and 1,915 controls, allowing proposing the gene encoding the ADAM metallopeptidase with thrombospondin type 1 motif 9 (*ADAMTS9*) as a new asthma locus [24]. Here we hypothesize that a further integrated analysis of the GWAS results in the context of biological pathways, or gene sets, could identify novel asthma susceptibility genes.

Material and Methods

Pathway enrichment and gene prioritization

We used the association results obtained from 6,467,565 variants tested in the discovery stage of our GWAS of asthma, which were obtained by imputation with the 1000 Genomes Project (1KGP) data as reference. Demographic and clinical features of the samples used in the GWAS discovery stage are shown in Table 1 and are described elsewhere [24]. Briefly, 352 cases were derived from the Genetics Of Asthma study in the Spanish population (GOA), recruited in Respiratory Medicine and Allergy Departments [25], were aged ≥ 5 years, had an extensive clinical characterization of asthma using the Global Initiative for Asthma (GINA) guidelines, and were taking medications to control their asthma symptoms [1]. A total of 537 participants that self-reported absence of personal or familiar history of chronic diseases (infectious, cancerous, mental, cardiovascular or immunological) were used as controls. Genome-wide genotyping of the 889 samples was performed with the Axiom® Genome-Wide Human CEU 1 Array (Affymetrix, Santa Clara, CA) by the National Genotyping Center (CEGEN-PRB2-ISCI), Universidad de Santiago de Compostela Node, as described elsewhere [24].

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All variants reaching $p \leq 5 \times 10^{-3}$ in the above mentioned GWAS discovery stage were utilized as an input for i-GSEA4GWAS [26] to assess the gene set enrichment analysis (GSEA) over predefined canonical pathways. We assigned all the variants within the 100 kilobases (kb) flanking regions to the nearest gene, based on the fact that *cis*-acting expression quantitative trait loci (eQTLs) are most commonly located within such distance, up-stream and down-stream of the gene whose expression is affected [27]. The pathways identified as enriched in the data were considered significant if they had a false discovery rate (FDR) $\leq 5\%$. In order to prioritize genes for follow-up association studies, we downloaded all genes included on each significant pathway as listed in the Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.genome.jp/kegg>). We then calculated an enrichment score for each one by dividing the number of variants assigned to each gene that were nominally significantly associated with asthma by those that were independent. The latter was calculated using a stand-alone version of the SNPSpD software (<http://gump.qimr.edu.au/general/daleN/SNPSpD>) based on data from the 1KGP European populations. For each significant pathway, we then prioritized those genes identified as extreme outliers in the distribution of the enrichment scores, i.e. those genes resulting with an enrichment score > 3 standard deviations from the mean (Table S1).

Replication study

Study samples. To follow-up the prioritized SNPs, we performed an association study in a total of 1,824 independent individuals (555 cases and 1,269 controls) with at least two generations of ancestors born in Spain. Asthma cases were independent samples from the GOA study [25]. Controls were obtained from the Spanish National

DNA Biobank (<http://www.bancoadn.org>) and self-reported absence of personal or familiar history of allergic or pulmonary diseases (including asthma). The Ethics Committees of the participant centers approved the study and all participants or their parents (children under 18 years old) signed an informed consent.

SNP selection. To fit as much variants as possible in a single multiplex genotyping reaction, we selected at least one variant for each of the prioritized genes that were not associated with asthma in any previous GWAS. For each gene, we prioritized the variant(s) with the strongest association *p*-value in the GWAS discovery stage or SNPs in moderate-to-strong linkage disequilibrium with it (LD, $r^2 \geq 0.5$) (Table S2). When possible, we also included other SNPs in LD with the leading SNP within each gene to ensure that all prioritized gene were represented in the replication, in case any of the SNPs failed during genotyping.

Genotyping. Genotyping was performed with the MassARRAY® iPLEX Gold platform (Sequenom, San Diego, CA) at the National Genotyping Center (CeGen), Universidad de Santiago de Compostela Node. None of the variants was excluded for either the call rate <95% or for deviating from Hardy-Weinberg equilibrium (HWE) in the control samples after adjusting for the number of comparisons (threshold for significance was $0.05/14 = 3.6 \times 10^{-3}$; lowest *p*-value obtained=0.018) (Table S3).

Statistical analysis. Association testing for asthma was carried out with logistic regression models in PLINK v1.07 [28], providing the effects as odds ratios (OR) with 95% confidence intervals (CI). To estimate the overall effect size, a meta-analysis was performed between the summary data from the GWAS discovery and the replication study with METASOFT [29]. We used the Cochran's Q to evidence if the effects differed significantly between the two stages.

Functional annotation of variants. To deduce the functional role of associated variants and LD proxies, we used the lung eQTL list supplied by Hao et al. [30] and the HaploReg v4 database, which integrates eQTL variant information and empirical data from their tissue-specific target genes from the Genotype-Tissue Expression (GTEx) resource, Geuvadis, and other recent studies [31].

Results

From the association results of near 6.5 million variants tested for association with asthma [24], we used a list of the top 34,876 variants reaching a $p \leq 5 \times 10^{-3}$ for GSEA. This analysis revealed a significant proportion of variants that mapped within or near 417 genes participating in two particular pathways: the cytokine-cytokine receptor interaction (hsa04060; $p=0.002$, FDR=0.01), and the Wnt signaling pathway (hsa04310; $p=0.012$, FDR=0.05), involving 265 and 152 genes, respectively. Alternative GSEA explorations using the top 30,000 or 60,000 variants did not change this observation (data not shown). Based on the distribution of the enrichment scores in the genes from the two significant pathways, we identified a total of ten genes as extreme outliers (Table S1): *BMPR2* (Bone morphogenetic protein receptor type II), *CCL28* (C-C motif chemokine ligand 28), *CCND3* (Cyclin D3), *CSF2* (Colony stimulating factor 2), *CSNK1A1L* (Casein kinase 1 alpha 1-like), *FZD3* (Frizzled class receptor 3), *FZD6* (Frizzled class receptor 6), *IL3* (Interleukin 3), *KITLG* (KIT ligand), and *SMAD3* (SMAD family member 3). As *SMAD3* is already known to be a firm asthma gene [9, 10], this gene was not analyzed in downsetram analyses. Variants assigned to *BMPR2* were not analyzed either, given that we have already analyzed this gene as part of our previous GWAS [24].

A total of seven loci were analyzed by genotyping 14 SNPs overall (Table S2) in follow-up association studies in independent samples. Note that, since the leading SNP was shared by *IL3* and *CSF2*, because of their proximity in the genome, they were considered as a single locus (thereafter referred to as *IL3-CSF2*). Out of the 1,824 independent individuals initially available, a genotyping completion rate <90% was obtained for 59 samples (5 cases and 54 controls) that were filtered out from the replication study. Therefore, follow-up analyses were finally performed in 550 cases and 1,215 controls. A demographic and clinical description of these samples is provided in Table 1. At this stage, all SNPs reached a minimum of 98.6% completion and none of them deviated significantly from HWE expectations in controls (Table S3), ensuring that the genotype data had high quality.

Association testing revealed six SNPs achieving nominal significance (Table 2). Of those, one SNP assigned to *CCL28* and the three assigned to *FZD6* provided evidence of replication by showing the same direction of effects as in the discovery stage. The rs6870783, which is located at 60 kb from *CCL28* and 50 kb away from *PAIP1* (encoding the poly(A) binding protein interacting protein 1), evidenced a protective effect for asthma susceptibility for its minor allele (G allele) in both the GWAS discovery (OR=0.94, 95%CI=0.91-0.98, $p=8.99 \times 10^{-4}$) and in the replication study (OR=0.85, 95%CI=0.73-0.98, $p=0.029$). However, despite the suggested role of *CCL28* as a chemokine for eosinophil recruitment to peribronchial regions of the lung [32], this finding was not significant in the context of the multiple tests performed (threshold for significance at $p < 0.05/14 = 0.0036$). The three SNPs assigned to *FZD6* have moderate ($r^2=0.51$) or strong LD ($r^2=0.99$) between each other and similar effect sizes, irrespective of the study. Therefore, we considered all three as a single hit with the leading SNP being rs3133805, which is located 60 kb away from *FZD6*, but only

within 10 kb from the genes encoding the collagen triple helix repeat-containing protein 1 (*CTHRC1*) and the solute carrier family 25 member 32 (*SLC25A32*). The minor allele of rs3133805 (G allele) was associated with risk for asthma susceptibility in both the GWAS discovery (OR=1.46, 95%CI=1.15-1.84, $p=1.80\times10^{-3}$) and in the replication study (OR=1.52, 95%CI=1.19-1.95, $p=9.90\times10^{-4}$), being significant in the context of the multiple tests done.

In a meta-analysis of the results from the GWAS discovery and the replication study, no heterogeneity was found across the two stages based on Cochran's Q (Table 2). A fixed-effects model evidenced a strong improvement of rs3133805 significance over that found in any of the two studies by separate (OR=1.49, $p=5.87\times10^{-6}$) (Table 2).

Finally, the functional empirical data available support that the leading SNP, rs3133805, is in high LD ($r^2>0.8$) with other nearby variants located in lung enhancer/promoter histone marks. The most prominent of those is rs3098228, which is positioned in a phylogenetically-conserved region in a *CTHRC1* intron and constitutes an eQTL in lung fibroblast primary cells. Additionally, the results by Hao et al. [30] support that another asthma associated variant of this locus (rs3133836; $r^2>0.5$ with rs3133805) is a lung eQTL by regulating the expression of the downstream gene *DCAF13* (Damage specific DNA binding protein 1 and cullin 4 associated factor 13).

Discussion

Previous studies have used pathway enrichment analysis to identify susceptibility genes either based on candidate gene studies (in asthma) [33] or GWAS data in other diseases [34]. However, to the best of our knowledge, this is the first study utilizing a GWAS-based pathway enrichment analysis to prioritize asthma susceptibility genes. In

order to reveal novel asthma genes, here we leveraged GWAS summary data from a previous study [24] to identify two significant biological pathways with ten genes accumulating a disproportionate number of the most significant GWAS findings, which included seven novel loci. Downstream analyses of selected SNPs from these novel regions in 1,765 independent samples evidenced three tightly linked SNPs near the *FZD6* region associated with asthma, supporting this region as a novel locus for asthma susceptibility. Out of these, the identification of *SMAD3* as one of the extreme outliers provided an internal validation of the prioritization method used, given that it has been consistently replicated in several studies, including the two largest GWAS meta-analyses in asthma published to date [9, 10]. Additionally, variants from the *IL3-CSF2* locus have been suggested to be associated with asthma susceptibility based on GWAS findings [35].

Despite the complexity and heterogeneity of asthma, it is well known that diverse cytokines, such as IL-3, IL-4, IL-5, IL-9, and IL-13, are the main drivers of asthma symptoms by recruiting and activating cells of the T helper 2 (Th2)-type inflammatory response (T cells, eosinophils, basophils, monocytes and macrophages). In agreement, the most significant pathway uncovered by our study was the cytokine-cytokine receptor interaction ($p=0.002$). This component of asthma is well supported by GWAS findings deposited in the GWAS Catalog of asthma-related traits to date (<https://www.ebi.ac.uk/gwas>), registering genes that participate in the regulation of cytokine production and secretion (including IL-5), cytokine-mediated signaling pathways, and macrophage activation.

Besides, multiple observations support that the interaction of these factors with structural cells of the airways, such as epithelial and smooth muscle cells, contributes not only to trigger epithelial stress and injury, but also to orchestrate and promote tissue

remodeling. Overall, this environment would help to perpetuate an altered epithelial tissue repair function leading to the chronic tissue remodeling and inflammation that is characteristic of asthma patients and is responsible for deterioration of lung function [36]. In this regard, a few firm asthma susceptibility genes such as *IL33* and *TSLP* [9, 10], encoding epithelial cell cytokines IL-33 and the thymic stromal lymphopoietin, respectively, as well as *SMAD3* and *IL2RB1* (encoding the interleukin-2 receptor beta chain-1), potentially involved in healing pathways [9, 10], supports the fundamental role of epithelia in the origin of asthma development. Here, we were able to identify for the first time among GWAS findings, a signal linking asthma susceptibility with the Wnt signaling pathway ($p=0.012$), which has been involved in myriad of processes, including the regulation of inflammation, fibroblast activation, cell differentiation, cell migration and adhesion, tissue homeostasis, and airway remodeling, among others [37]. Recently, this pathway was critically involved in airway remodeling in patients with asthma, based on observation in humans where increased sputum levels of WNT7A were observed in patients compared to healthy controls, and in animal models of chronic asthma, where silencing of β -catenin attenuated airway inflammation and remodeling (decreasing epithelial fibrosis, collagen accumulation, expression of TGF- β , and smooth muscle hyperplasia) [38].

The three statistically significant SNPs from this study were assigned in the GSEA to *FZD6*, which encodes a receptor for Wnt signaling proteins that functions as a negative regulator of the canonical Wnt/ β -catenin signaling cascade. Interestingly, another receptor from the frizzled class, FZD8, has been involved in tissue repair and remodeling mechanisms by promoting profibrotic signaling according to evidence from primary human lung fibroblasts and a rodent model of fibrosis [39]. Despite that, with the current evidence is not possible to disentangle if any of these SNPs has a functional

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role and, if any, which gene is being affected. This is because there are two other genes in their vicinity, namely *CTHRC1* and *SLC25A32*. While *SLC25A32* encodes a mitochondrial folate carrier [40] with no clear links with asthma or immune conditions, *CTHRC1* does not belong to the Wnt signaling pathway according to KEGG, but it interacts with many Wnt proteins (2, 4, 7A and 11) and has been involved in idiopathic pulmonary fibrosis [41]. Besides, according to the Expression Atlas (<https://www.ebi.ac.uk/gxa/home>), *CTHRC1* is moderately expressed in the adult lungs and participates in the frizzled binding, interacting directly with *FZD6*, and also acts as a negative regulator of collagen matrix deposition. Because of this, and the fact that the leading SNP (rs3133805) is in high LD with a likely functional variant in the lungs within a *CTHRC1* intron, its potential role in asthma susceptibility deserves further investigations.

Given that genetic risks may vary among ethnic groups, the main strength of this study is that all samples included were of Spanish descent, a population with sizeable African contributions that is underrepresented in most previous GWAS in asthma to date [24]. Besides, asthma and atopy were diagnosed by a clinical characterization among cases, and not based on questionnaires. In addition, the discovery study was based on the imputation with 1KGP data that, with a few exceptions, constitutes the most comprehensive catalogue of genetic variation utilized so far in any GWAS of asthma, therefore improving the power to detect novel asthma genes. Furthermore, the two study samples showed evidence of replication with the same direction of effects in both stages for three variants near *FZD6*, which adds support its role in asthma susceptibility. Finally, while the immune component of asthma is prevalent even at the genetic level, because this study was focused on predefined gene relationships in biological processes and not on individual genes, we were able to evidence for the first

time a genetic link between the Wnt pathway and asthma susceptibility, providing a novel potential therapeutic target for this disease.

We acknowledge some limitations in our study. By one hand, because associations have focused on common variants (>5% frequency), the potential role of less frequent variants was not investigated. Furthermore, it is likely that the GSEA itself have revealed only a fraction of the biological pathways involved in asthma, as a consequence of the limited sample size used in the GWAS discovery. Besides, we have only followed up the most significant SNP or a few proxies in the prioritized genes for this study, reducing our power to detect more significant findings among the prioritized ones. With respect to the controls, these were not clinically characterized for asthma or atopic sensitization although they all self-reported absence of any chronic condition that require continuous treatment. While using uncharacterized controls leads to a reduction in power, the consequences of having unrecognized cases among the controls are modest for a trait such as asthma [42, 43]. Even in such a scenario, we were still able to detect a sound and replicable association, bolstering the validity of our results. On the patient side, asthma is a complex phenotype where clinical clusters have been described based on the atopic status and onset of the disease [21]. Most of our cases also have atopy, as it is pervasive in most current genetic association studies with asthma being conducted. Genetic studies support that atopic-related traits and asthma are different expressions of the same phenotype [9, 44-46]. Similarly, firm support of major genetic differences between allergic and non-allergic asthma and between childhood and adult asthma is lacking, except for the larger effects found for *ORMDL3* gene in early onset cases [9]. Because of that, the association detected might be due to either asthma or atopy or both. However, we found no dependence of effects for the variants near *FZD6* with the age at onset, given that effects replicated and were consistent between the

discovery (accumulating cases with earlier onset) and the replication stages. Lastly, although we recognize that medication use could be an important clinical variable to factor in for association testing, pharmacogenetic interaction analyses were not performed in this study given the reduced sample size involved in each treatment stratum. A sufficiently large study would be needed to explore this possibility.

In conclusion, besides the Th2-type inflammatory response, our data suggest a role of the Wnt signaling pathway genes in asthma susceptibility, providing support to the central role of epithelia in asthma development. Association studies in selected genes from these two key components of asthma allowed identifying a novel susceptibility locus near *FZD6*. Therefore, independent studies like this will help to identify novel asthma genes that, despite adding minor components of heritability, will serve the purpose of enlarging the asthma gene list and will contribute to improve our knowledge of the mechanisms underlying disease susceptibility.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Supporting information

Table S1. Genes included in the pathways that showed a significant enrichment of association signals with asthma susceptibility.

Table S2. Loci analyzed in this study and the leading SNP for each according to the GWAS discovery data.

Table S3. Quality control evaluation of the SNPs assessed in this study.

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Table 1. Relevant demographic and clinical features of study samples analyzed in association studies

Variable	Discovery sample (n=889)			Replication sample (n=1,765)		
	Cases (n=352)	Controls (n=537)	<i>p</i> -value	Cases (n=550)	Controls (n=1,215)	<i>p</i> -value
Gender (male, %)	53.4	51.2	0.566	36.7	60.5	<0.001
Age, median (IQR, years)	30 (20-40)	54 (46-52)	<0.001	34 (24-44)	40 (32-48)	<0.001
Smoking habits (ever smokers, %)	23.5	NA	NA	27.7	47.4	<0.001
Clinical features of asthma cases:						
Total IgE levels (IU/mL)	188 (81.8-349.5)	NA	NA	218 (103.0-556.5)	NA	NA
Age at diagnosis (years)	9 (5-17)	NA	NA	29 (17-42)	NA	NA
FEV ₁ (% predicted)	87 (76-97)	NA	NA	87 (70-100)	NA	NA
SPT (% positive)	98.0	NA	NA	92.8	NA	NA
Specific IgE (% positive)	89.1	NA	NA	85.5	NA	NA
Allergic sensitization ^a (%):	75.0	NA	NA	87.9	NA	NA
Dust mites	72.7	NA	NA	77.1	NA	NA
Pollens	14.2	NA	NA	47.5	NA	NA
Animal epithelia	32.1	NA	NA	39.4	NA	NA
Fungi	3.7	NA	NA	9.2	NA	NA
Cockroaches	1.4	NA	NA	1.2	NA	NA

	Food	11.6	NA	NA	10.2	NA	NA
	Latex	3.6	NA	NA	2.2	NA	NA
Treatment:							
	Inhaled corticosteroids (%)	77.0	NA	NA	75.8	NA	NA
	Oral corticosteroids (%)	5.9	NA	NA	4.9	NA	NA
	Long-acting β 2-agonists (%)	59.8	NA	NA	45.7	NA	NA
	Short acting β 2 agonists (%)	61.7	NA	NA	79.3	NA	NA
	Body mass index (kg/m ²)	24.5 (20.9-28.4)	NA	NA	24.5 (21.1-27.7)	NA	NA

^aPositive allergic sensitization was defined by positive skin prick test (a wheal with a diameter 3 mm greater than the saline control) to at least one common allergen, including dust mite, animal epithelia, pollen, fungi, food, latex and others, or by specific serum IgE levels >0.35 UI mL⁻¹.

For continuous variables, the median and interquartile ranges (IQR) are displayed and Mann-Whitney U-test *p*-values are presented for the comparison of cases versus controls.

For categorical variables, the proportion of subjects in each category is shown and χ^2 -test *p*-values are presented for the comparison of cases versus controls.

NA, not available.

Table 2. Summary of association results for the 14 SNPs followed up in this study

SNP	Chr	Position ^a	Gene	A1	A2	GWAS discovery ^b (352 Cases: 537 Controls)			Replication study (550 Cases: 1,215 Controls)			Meta-analysis ^c (902 Cases: 1,752 Controls)		
						Freq. ^c	OR	<i>p</i> -value	Freq. ^c	OR	<i>p</i> -value	OR	<i>p</i> -value	Cochran's <i>p</i> -value
rs6870783	5	43472630	<i>CCL28</i>	G	C	0.405	0.94	8.99x10⁻⁴	0.427	0.85	0.029	0.94	1.90x10⁻⁴	0.172
rs715285	5	131485383	<i>IL3-CSF2</i>	G	A	0.468	0.98	2.84x10 ⁻²	0.471	0.91	0.186	0.98	0.020	0.278
rs2025951	6	41827193	<i>CCND3</i>	A	G	0.499	0.95	2.12x10 ⁻²	0.478	0.99	0.882	0.96	0.024	0.616
rs7739064	6	41834037	<i>CCND3</i>	C	A	0.502	1.01	2.05x10 ⁻²	0.489	1.02	0.779	1.01	0.020	0.842
rs1536703	6	41852755	<i>CCND3</i>	G	A	0.461	0.92	5.64x10 ⁻³	0.439	1.02	0.841	0.93	0.013	0.213
rs7833751	8	28362792	<i>FZD3</i>	T	G	0.403	1.05	5.22x10 ⁻³	0.435	0.92	0.238	1.05	0.016	0.066
rs3133835	8	104363126	<i>FZD6</i>	A	G	0.063	1.44	7.48x10⁻³	0.058	1.49	4.30x10⁻³	1.47	9.24x10⁻⁵	0.886
rs3133836	8	104363338	<i>FZD6</i>	C	T	0.063	1.45	7.48x10⁻³	0.057	1.51	3.10x10⁻³	1.48	6.80x10⁻⁵	0.851
rs3133805	8	104405021	<i>FZD6</i>	G	T	0.060	1.46	1.80x10⁻³	0.068	1.52	9.90x10⁻⁴	1.49	5.87x10⁻⁶	0.802
rs4284478	12	88907563	<i>KITLG</i>	A	G	0.251	0.89	2.30x10 ⁻⁴	0.212	1.26	6.53x10 ⁻³	1.05 ^d	0.776 ^d	1.33x10 ⁻⁴
rs1022034	12	88918743	<i>KITLG</i>	T	G	0.253	0.89	2.28x10 ⁻⁴	0.221	1.23	0.015	1.03 ^d	0.837 ^d	3.33x10 ⁻⁴
rs1937757	13	37676259	<i>CSNK1A1L</i>	C	T	0.397	0.99	1.63x10 ⁻²	0.401	1.07	0.398	0.99	0.017	0.383
rs1336754	13	37677995	<i>CSNK1A1L</i>	G	T	0.398	0.99	1.75x10 ⁻²	0.399	1.07	0.361	0.99	0.020	0.295
rs11147629	13	37688839	<i>CSNK1A1L</i>	C	T	0.416	1.02	2.44x10 ⁻²	0.415	1.09	0.246	1.02	0.017	0.392

^aPositions are shown in hg19 coordinates.

^bSummary data extracted from our previous study [24].

^cA1 allele frequency in controls.

^dResults from a random effect meta-analysis are shown as heterogeneity was found among studies based on Cochran's Q test (*p*-value<.05). The others results are based on a fixed effect meta-analysis because not heterogeneity was found.

Chr: chromosome; A1: allele 1 for which OR is reported; A2: allele 2, alternative allele.

In bold, SNPs with evidence of replication in the two studies.