

Letter to the Editor

Replication study of genetic variants associated with chronic rhinosinusitis and nasal polyposis*To the Editor:*

Chronic rhinosinusitis (CRS) is an inflammatory disorder of the sinuses with a large phenotypic variability. The mechanisms of the disease are largely unknown, but it is believed to result from a combination of environmental factors and the genetic background of affected individuals. CRS is subclassified into CRS with and without nasal polyposis (CRSwNP, CRSsNP), with further specific subgroups such as allergic fungal rhinosinusitis.¹ Previous studies of CRS have defined 53 single nucleotide polymorphisms (SNPs) as associated with the CRSwNP or CRSsNP phenotypes. A majority of these association studies (summarized in Table E1 in this article's Online Repository at www.jacionline.org) have been focused on candidate genes involved in innate immunity and inflammatory response, but 1 pooled genome-wide association study (pGWAS) has also been performed. Many of these studies have been based on fairly limited population sizes. Recently, the genetics of CRS has been thoroughly reviewed with respect to the involvement of different genes and molecular mechanisms.² The present study investigates the reproducibility of previous SNP associations with CRS with and without NP.

The patients and controls were Caucasians of European origin. All patients had a diagnosis of CRS supported by nasal endoscopy and computed tomography scan according to current guidelines.¹ The study population consisted of 613 patients with CRS (248 females and 365 males), with 275 patients having CRS with NP and 338 patients having CRS without NP, and a set of 1588 background population controls (1042 females and 546 males) from the publicly available Illumina iControl database. Description of patients and controls can be found in the Methods section (see this article's Online Repository at www.jacionline.org). All 53 SNPs previously reported as associated with CRS and NP were selected for analysis (see Table E1). After primer design and SNP genotyping on a Sequenom MassARRAY MALDI-TOF platform as previously described,³ a total of 43 SNPs produced high-quality data with a total genotyping rate of 99.8%. Statistical analyses were done by using R statistical software and PLINK v1.07. Genotype frequencies were calculated and tested for Hardy-Weinberg equilibrium in both cases and controls. Allele frequencies were investigated for association with CRS by using a χ^2 homogeneity test. Odds ratios (ORs) and 95% CIs were estimated by using the most common allele as the referent and are reported for each minor allele. False discovery rates were quantified by using the *Q* value introduced by Storey⁴ and calculated by using QVALUE (ver. 1.0). Further information about genotyping and statistical analyses can be found in the Methods section.

The primary CRS association test revealed a total of 7 SNPs with uncorrected *P* values of less than .05 (Table I; see Table E2 in this article's Online Repository at www.jacionline.org) in an allele frequency test of 613 CRS cases and 1588 controls. One of these had a *Q* value of 0.0054, and 4 had *Q* values of less than <0.1 (Table I). The rs2873551 SNP in the prolyl-tRNA

synthetase 2 (*PARS2*) gene was significantly associated with CRS even after a Bonferroni correction (*P* = .00022; OR = 0.77). This SNP has earlier been associated with CRS in a Canadian pGWAS of 173 cases and 130 controls (*P* = .00003; OR = 0.5)⁵ and was thus not initially identified in a hypothesis-driven study. *PARS2* is an activator of amino acids for protein synthesis through the formation of aminoacyl adenylates.⁶ Inhibition of the function of *PARS2* suppresses cell growth and may have implications for cell proliferation in connection to inflammation and innate immune response. The remaining 3 SNPs with *Q* values of 0.1 were reported in 3 different candidate gene studies (see Table E1). The rs1800469 SNP in the transforming growth factor B1 gene has earlier been associated with chronic obstructive pulmonary disease and rhinosinusitis in asthmatic patients.⁷ The rs1483757 SNP in the nitric oxide synthase 1 gene and the rs4657164 SNP in the nitric oxide synthase 1 adaptor protein gene both are also likely candidates for being associated with CRS because they are located in genes involved in asthma and allergic rhinitis (AR).⁸

In a separate test, the 613 cases in our study were subdivided into those with (275) and those without (338) NP and used to analyze a total of 43 SNPs for associations with CRSwNP (see Tables E1 and E2). The rs4504543 SNP in the acylglycerol hydrolase (*AOAH*) gene had an uncorrected *P* value of .022 (OR = 0.76). The *AOAH* gene is an *a priori* likely candidate to be involved in CRS with and without NP phenotypes because the *AOAH* protein degrades lipopolysaccharides and a dysfunctional *AOAH* protein may lead to decreased lipopolysaccharide degradation.⁹ The rs4504543 SNP has earlier been associated with the CRS phenotype in a pGWAS,⁵ and subsequently replicated in a Chinese population with CRS,⁹ in addition to being associated with asthma.¹⁰ However, our analysis lends weak statistical support for this association (*Q* = 0.62) despite this SNP having the lowest *P* value for association with CRSwNP.

The genetic variants tested for association with CRS in the present study have previously been associated with CRS, and in some cases also with asthma or AR. We therefore investigated asthma and AR as confounding factors for our results. The patients were subclassified on the basis of the presence or absence of either one or both of these phenotypes. To test for confounding, the analyses were then repeated for all combinations (see Table E3 in this article's Online Repository at www.jacionline.org). The SNP rs2873551 in *PARS2* showed a very consistent pattern in all tests, with respect to both the association test results and the minor allele frequency differences between the combinations. The remaining SNPs with *P* values of less than .05 in general showed the expected pattern of increasing *P* values due primarily to decreasing sample sizes. Thus, the presence of asthma and AR does not substantially influence the results.

We used a background control population originating from the same geographic region as the patients, but without CRS classification or matching of age and gender. To validate the use of this control population, we performed a principal-component analysis to ensure that our results are not due to subpopulation structures. Using the 26 nonsignificant SNPs from the CRS association test, a completely random distribution of cases and

TABLE I. Replication of SNPs previously associated with CRS

Gene	SNP ID	Minor/major allele	MAF		P value	Q value	OR (95% CI)
			Cases (n = 613)	Controls (n = 1588)			
<i>PARS2</i>	rs2873551	C/T	0.34	0.40	.00022	0.0054	0.77 (0.70-0.84)
<i>TGFB1</i>	rs1800469	T/C	0.30	0.34	.0043	0.053	0.81 (0.74-0.89)
<i>NOS1</i>	rs1483757	G/A	0.39	0.44	.0095	0.079	0.84 (0.77-0.91)
<i>NOS1AP</i>	rs4657164	C/T	0.14	0.17	.015	0.092	0.79 (0.70-0.89)
<i>IL22RA1</i>	rs11579657	G/A	0.21	0.18	.022	0.11	1.21 (1.13-1.30)
<i>DCBLD2</i>	rs828618	A/G	0.32	0.29	.026	0.11	1.18 (1.10-1.25)
<i>ALOX5AP</i>	rs17238773	T/A	0.06	0.08	.039	0.14	0.75 (0.62-0.89)

ALOX5AP, Arachidonate 5-lipoxygenase-activating protein; *DCBLD2*, discoidin, CUB and LCCL domain containing 2; *IL22RA1*, interleukin 22 receptor, alpha 1; *MAF*, minor allele frequency; *NOS1*, nitric oxide synthase 1; *NOS1AP*, nitric oxide synthase 1 adaptor protein; *TGFB1*, transforming growth factor B1.

controls was seen (see Fig E1 in this article's Online Repository at www.jacionline.org). This result was corroborated by the use of the software STRUCTURE, which failed to detect a subpopulation structure (results not shown), and an estimate of the overall F_{ST} showed a low level (<0.005). Thus, subpopulation structure does not contribute to our results.

In this study, we replicated associations between genetic variants in 7 genes and the CRS phenotype. With P values of less than .01 and Q values of less than 0.1, the SNPs in *PARS2*, transforming growth factor B1, and *NOS1* are all good candidates for further analysis, in particular because they are also plausible candidates from a functional biological perspective.

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METHODS

Ethics statement

The study was carried out after approval by the Ethics Committee of Ghent University Hospital, Belgium, and written informed consent was obtained from each patient before inclusion in the study.

Study population (cases) and disease definitions

Nasal tissue, sera and EDTA blood samples were obtained from 613 consecutive patients (365 male and 248 female patients) who underwent routine surgical intervention unrelated to the study for the treatment of chronic rhinosinusitis with (n=275) or without (n=338) polyps (CRSwNP or CRSsNP) at the Ear, Nose, and Throat Department, University Hospital, Ghent, Belgium. The diagnosis of sinus disease was based on history, clinical examination, nasal endoscopy, and computed tomographic scanning of the sinuses. All patients were diagnosed to suffer either from CRSwNP or CRSsNP according to the criteria of the EPOS Guidelines 2007.^{E1} Patients were asked not to use oral or nasal corticosteroids or antibiotic treatment 4 weeks before the surgery. The atopic status of all patients was evaluated by skin prick tests to the most frequent inhalant allergens.^{E2} The diagnosis of asthma was confirmed by a chest physician according to the Global Initiative for Asthma 2006 guidelines based on symptoms and pulmonary function tests.^{E3}

Literature mining and SNP selection

The literature search was conducted 2012-08-29 on PubMed (www.ncbi.nlm.nih.gov/pubmed/) using the terms (“chronic rhinosinusitis” OR (nasal AND polyp*) OR “nasal polyposis”) AND (association OR case-control) AND (polymorphism OR SNP OR mutation) AND English [language]. Using this search algorithm, 21 articles were identified (Table E1) that together described 53 SNPs associated with CRS and/or NP. Reading of these 21 articles, revealed no additional studies of interest. Associations reported in studies with a focus on related phenotypes such as IgE levels and the severity of the CRS phenotype were not considered in this study.

Genotyping

Genomic DNA was extracted from blood collected in EDTA using QIAamp 96 DNA Blood kit (Qiagen, Hilden, Germany) and DNA concentrations were determined by fluorometry using PicoGreen (Molecular Probes, Invitrogen, Eugene, OR, USA). Assay design was attempted on all 53 SNPs using the MassARRAY Assay design ver.2 software (Sequenom Inc, San Diego, CA, USA). Primers were obtained for a total of 46 SNPs from Metabion GmbH (Martinsried, Germany).

Genotyping was performed on the Sequenom MassARRAY MALDI-TOF system as described in Nilsson et al.^{E4} Genotype data interpretation was performed on the Sequenom Massarray Analyzer 4.0 software (Sequenom Inc.). Three SNPs failed genotyping with the remaining 43 SNPs showing genotyping rates over 90%. After removal of low-quality DNA samples with a frequency of missing values above 5%, the final genotyping rate was 99.8% in 613 cases (248 female, 365 male).

Illumina control population

The control population was selected from the publicly available Illumina iControl database (<http://www.illumina.com/science/icontrolldb.ilmn>). Using the iControl DB Client individuals were selected based on chip (Hap550 v3) and population (Caucasian). This resulted in a set of 1593 controls. Out of the 43 SNPs successfully genotyped in the CRS cases, a set of 26 SNPs were directly available in the database. An additional seven SNPs were selected as proxies as they were in strong linkage disequilibrium ($r^2 > 0.8$) with the genotyped SNPs: rs16829209, rs10169916, rs17245204, rs2241714, rs1864414, rs4251425 and rs5029936 as proxies for rs16829225, rs16944, rs17238773, rs1800469, rs3780894, rs4251431 and rs5029938, respectively. After quality control allowing < 10% missing data for markers and < 5% missing data for individuals the dataset contained data for 33 markers and 1588 individuals with a genotyping completion rate of 99.8%.

Statistical analysis

Statistical analysis was performed using PLINK ver. 1.07 and R program ver. 2.10.1 software. Calculations of linkage disequilibrium used Haploview ver. 4.2 software and genotype data from HapMap (build 28; CEU individuals). All *P*-values were initially reported without correction for multiple testing. False discovery rates were then quantified using the *Q*-value introduced by Storey^{E5} and calculated using QVALUE (ver. 1.0). To investigate the potential effect of confounding factors the patients were sub-classified into those with allergic rhinitis (+AR) and those without (–AR). In total there were 193 +AR and 420 –AR patients. Subsequently allele frequency association tests were made separately for both groups. The same procedure was made for patients with asthma (+AS) and without (–AS). In total there were 147 +AS and 466 –AS patients. In a second step the data was cross-classified with respect to AR and AS resulting in four groups, i.e. +AR+AS (85 patients), +AR–AS (108 patients), –AR+AS (62 patients) and –AR–AS (358 patients). The latter four groups were primarily investigated with respect to consistency of the minor allele frequencies. Results are shown in Table E3. To validate the use of the background control population we performed a principal component analysis using R software PRCOMP. This analysis used 26 non-significant SNPs from the CRS association test. An analysis of population structure was also made using the software STRUCTURE.^{E6}

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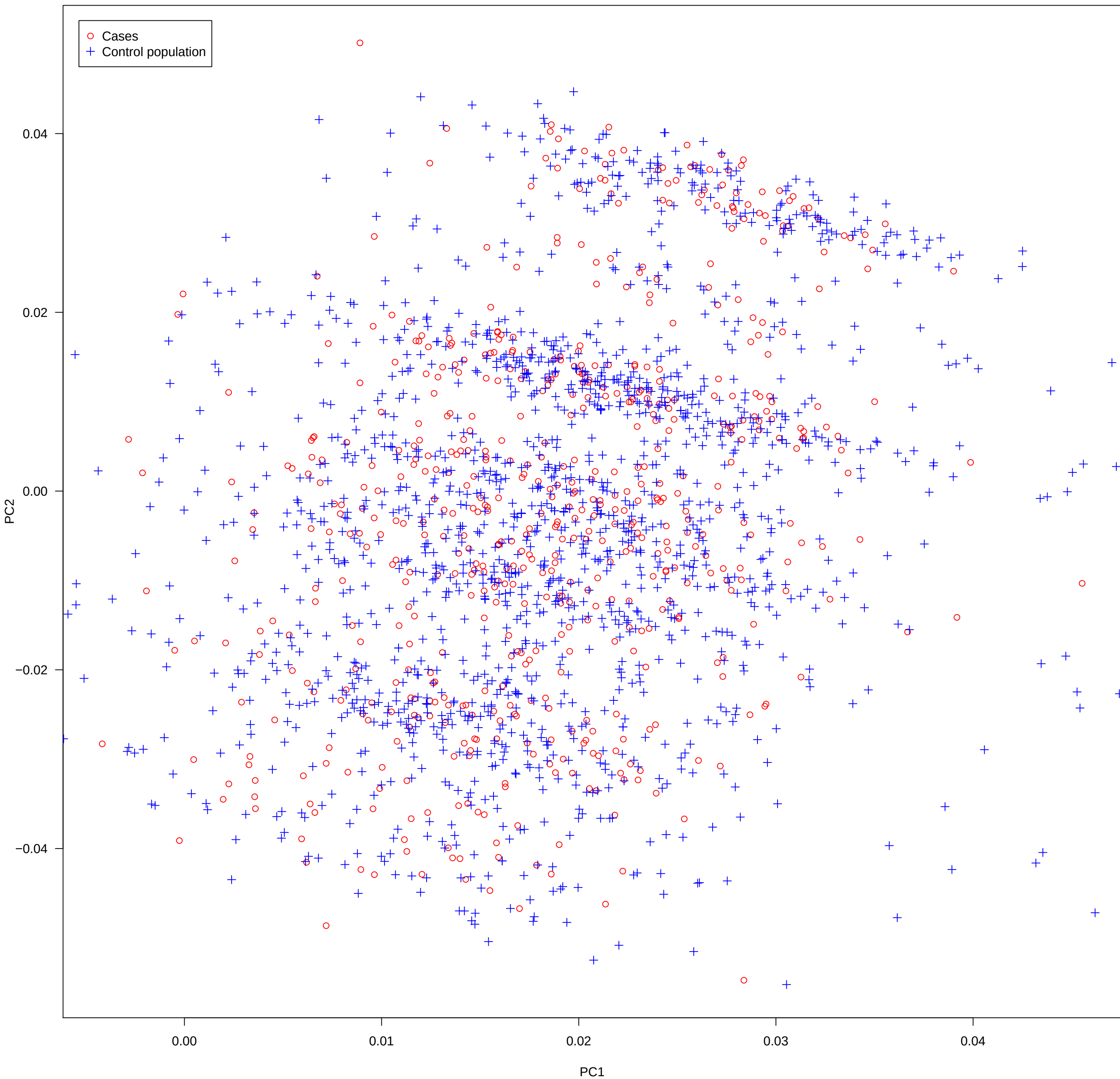


Figure E1. Principal component analysis of 613 cases (red), 1588 controls (blue) and 26 non-significant SNPs. The first principal component (PC1) has been plotted against the second principal component (PC2). The analysis shows no signs of population structure as the case individuals are evenly spread amongst the control individuals.

TABLE E1. Genes and SNPs associated with chronic rhinosinusitis (CRS) and nasal polyposis (NP) in candidate gene and pooled GWAS studies. A total of 53 unique SNPs are listed as significant ($P < 0.05$). **A.** SNPs associated with CRS. **B.** SNPs associated with NP. **C.** Replicated CRS signals. **D.** Replicated NP signals

SNP-information*						Population					
Gene	SNP ID	Chromosome	Position	Minor/major allele	Location	Population	Size (Ca; Co)	MAF (Ca;Co)	P-value	OR	Reference
A. CRS associated											
TP73	rs3765731	1	3611892	A/G	Intron	Canadian	206; 196	0.27; 0.36	0.008	0.7	E24
IL22RA1	rs10794665	1	24446128	A/G	Near gene 3'	Canadian	206; 196	0.37; 0.46	0.01	0.7	E18
IL22RA1	rs7418238	1	24463417	G/A	Intron	Canadian	206; 196	0.16; 0.10	0.03	1.6	E18
IL22RA1	rs10751768	1	24466259	C/T	Intron	Canadian	206; 196	0.35; 0.46	0.004	0.6	E18
IL22RA1	rs4648936	1	24468461	G/A	Intron	Canadian	206; 196	0.37; 0.25	0.001	1.7	E18
IL22RA1	rs4292900	1	24469814	T/C	Flanking 5'UTR	Canadian	206; 196	0.37; 0.25	0.0006	1.8	E18
IL22RA1	rs16829225	1	24470393	T/C	Near gene 5'	Canadian	206; 196	0.19; 0.11	0.001	2.0	E18
IL22RA1	rs3936073	1	24472154	T/A	Unknown	Canadian	206; 196	0.14; 0.20	0.03	0.6	E18
IL22RA1	rs11579657	1	24476886	G/A	Unknown	Canadian	206; 196	0.20; 0.27	0.02	0.7	E18
PARS2	rs2873551	1	55217907	C/T	UTR 3'	Canadian	173; 130	0.34; 0.51	0.00003	0.5	E25
NOS1AP	rs4657164	1	162160920	C/T	Intron	Canadian	206; 196	0.20; 0.13	0.02	1.7	E22
IL1RL1	rs13431828	2	102954653	T/C	UTR 5'	Canadian	206; 196	0.10; 0.17	0.008	0.6	E21
IL1RL1	rs2160203	2	102960824	C/T	UTR 3'	Canadian	206; 196	0.19; 0.26	0.03	0.7	E21
IL1RL1	rs10208293	2	102966310	A/G	Intron	Canadian	206; 196	0.24; 0.31	0.03	0.7	E21
IL1RL1	rs4988957	2	102968075	C/T	Coding synonymous	Canadian	206; 196	0.32; 0.40	0.03	0.7	E21
IL1RL1	rs10204137	2	102968212	G/A	Coding nonsynonymous	Canadian	206; 196	0.32; 0.40	0.04	0.7	E21
IL1A	rs2048874	2	113523967	T/C	Near gene 5'	Canadian	206; 196	0.10; 0.16	0.01	0.6	E19
IL1A	rs17561	2	113537223	T/G	Coding nonsynonymous	Canadian	206; 196	0.31; 0.24	0.02	1.5	E19
IL1A	rs2856838	2	113539972	T/C	Intron	Canadian	206; 196	0.35; 0.46	0.003	0.6	E19
IL1A	rs1800587	2	113542960	T/C	UTR 5'	Canadian	206; 196	0.32; 0.24	0.02	1.5	E19
TRIP12	rs1035833	2	230709198	C/T	Intron	Canadian	173; 130	0.21; 0.34	0.0002	0.5	E25
RYBP	rs4532099	3	72315956	A/G	UTR 5'	Chinese	638; 315	0.14; 0.06	0.000002	2.6	E7
LAMA2	rs2571584	6	129803485	G/A	Intron	Canadian	173; 130	0.20; 0.37	0.000006	0.4	E25
TNFAIP3	rs3757173	6	138190154	C/T	Intron	Canadian	206; 196	0.08; 0.12	0.04	1.7	E17
TNFAIP3	rs5029938	6	138195633	T/C	Intron	Canadian	206; 196	0.05; 0.09	0.02	2.0	E17
AOAH	rs4504543	7	36682895	C/T	Intron	Chinese	638; 315	0.13; 0.21	0.0001	0.6	E7
AOAH	rs4504543	7	36682895	C/T	Intron	Canadian	173; 130	0.26; 0.40	0.0003	0.5	E25
LAMB1	rs4727695	7	107614003	G/A	Intron	Canadian	173; 130	0.06; 0.16	0.00009	0.4	E25
MET1	rs38850	7	116337649	A/G	Intron	Canadian	206; 196	0.37; 0.26	0.004	1.7	E20
MSRA	rs7001821	8	10129773	T/C	Intron	Canadian	173; 130	0.44; 0.30	0.0003	1.9	E25
KIAA1456	rs11779957	8	12874915	G/T	Intron	Canadian	173; 130	0.31; 0.17	0.0002	2.1	E25
MUSK	rs10817091	9	113527226	A/G	Intron	Canadian	173; 130	0.37; 0.52	0.0002	0.5	E25
ALOX5	rs3780894	10	45874660	G/A	Intron	Canadian	206; 200	0.20; 0.14	0.02	1.5	E23
IRAK-4	rs1461567	12	44164689	T/C	Intron	Chinese	638; 315	0.48; 0.54	0.02	0.8	E7
IRAK-4	rs3794262	12	44165430	A/T	Intron	Chinese	638; 315	0.14; 0.18	0.03	0.7	E7
IRAK-4	rs4251580	12	44174766	T/C	Intron	Canadian	206; 196	0.13; 0.20	0.02	-	E27
IRAK-4	rs1141168 (rs4251559)	12	44182706	G/A	Unknown	Chinese	638; 315	0.45; 0.51	0.02	0.8	E7
NAV3	rs1726427	12	78534233	C/T	Intron	Canadian	173; 130	0.38; 0.23	0.00007	2.1	E25
NOS1	rs1483757	12	117761540	G/A	Intron	Canadian	206; 196	0.39; 0.51	0.002	0.6	E22
NOS1	rs9658281	12	117767578	A/G	Intron	Canadian	206; 196	0.27; 0.35	0.01	0.7	E22
ALOX5AP	rs17238773 (rs17612127)	13	31319751	T/A	Unknown	Canadian	206; 200	0.10; 0.06	0.05	1.7	E23
TGFB1	rs1800469	19	41860296	T/C	Promoter	Korean	203; 324	0.51; 0.48	0.001	-	E16
CACNA1I	rs3788568	22	40011273	G/T	Intron	Canadian	173; 130	0.31; 0.47	0.0001	0.5	E25
CYSLTR1	rs321090	X	77559987	G/A	Intron	Canadian	206; 200	0.20; 0.28	0.01	0.7	E23
B. NP associated											
IL1A	rs17561	2	113537223	T/G	Coding nonsynonymous	Turkish	82; 106	0.44; 0.18	0.001	5.8	E8

<i>IL1A</i>	rs17561	2	113537223	T/G	Coding nonsynonymous	Finnish	35; 208	0.20; 0.34	0.005	-	E9
<i>IL1B</i>	rs16944	2	113594867	A/G	Promoter	Turkish	82; 106	0.38; 0.46	0.02	0.4	E8
<i>RYBP</i>	rs4532099	3	72315956	A/G	UTR 5'	Chinese	306; 315	0.15; 0.06	3E-07	2.8	E7
<i>DCBLD2</i>	rs828618	3	98541633	A/G	Intron	Korean	158; 309	0.38; 0.44	0.05	0.7	E26
<i>TNFA</i>	rs1800629	6	31543031	A/G	Promoter	Turkish	82; 106	0.15; 0.18	0.001	3.2	E8
<i>TNFA</i>	rs1800629	6	31543031	A/G	Promoter	Turkish	97; 95	0.19; 0.06	0.02	3.7	E13
<i>TNFA</i>	rs1800629	6	31543031	A/G	Promoter	American	179; 153	-	0.01	1.9	E15
<i>TNFA</i>	rs361525	6	31543101	A/G	Promoter	Turkish	82; 106	0.25; 0.21	0.05	1.0	E8
<i>AOAH</i>	rs4504543	7	36682895	C/T	Intron	Chinese	332; 315	0.07; 0.21	8E-11	0.3	E7
<i>IL33</i>	rs3939286	9	6210099	A/G	Unknown	Belgian	273; 415	0.32; 0.23	0.0004	1.5	E10
<i>IRAK-4</i>	rs4251431	12	44154690	T/G	Intron	Chinese	306; 315	0.07; 0.11	0.01	0.6	E7
<i>IL10</i>	rs1800870	17	19552477	T/C	Promoter	Korean	173; 260	0.10; 0.05	0.007	2.1	E14
<i>CACNG6</i>	rs192808	19	54510717	T/C	Intron	Korean	102; 429	0.10; 0.04	0.0004	2.9	E11
<i>MMP9</i>	rs3918242	20	44635976	T/C	Promoter	Chinese	203; 730	0.15; 0.10	0.02	1.6	E12
<i>MMP9</i>	rs17577 (rs2274756)	20	44643111	A/G	Coding nonsynonymous	Chinese	203; 730	0.16; 0.11	0.03	-	E12
C. Replicated CRS signals											
<i>AOAH</i>	rs4504543	7	36682895	C/T	Intron	Chinese	638; 315	0.13; 0.21	0.0001	0.6	E7
<i>AOAH</i>	rs4504543	7	36682895	C/T	Intron	Canadian	173; 130	0.26; 0.40	0.0003	0.5	E25
D. Replicated NP signals											
<i>IL1A</i>	rs17561	2	113537223	T/G	Coding nonsynonymous	Turkish	82; 106	0.44; 0.18	0.001	5.8	E8
<i>IL1A</i>	rs17561	2	113537223	T/G	Coding nonsynonymous	Finnish	35; 208	0.20; 0.34	0.005	-	E9
<i>TNFA</i>	rs1800629	6	31543031	A/G	Promoter	Turkish	82; 106	0.15; 0.18	0.001	3.2	E8
<i>TNFA</i>	rs1800629	6	31543031	A/G	Promoter	Turkish	97; 95	0.19; 0.06	0.02	3.7	E13
<i>TNFA</i>	rs1800629	6	31543031	A/G	Promoter	American	179; 153	-	0.01	1.9	E15

*Information of genes, SNPs and their positions are taken from NCBI build 37.3

Ca, cases.

Co, controls.

MAF, minor allele frequency.

OR, odds ratio.

TABLE E2. Complete allele association test results for: **A**, chronic rhinosinusitis (CRS) using 613 cases and 1588 controls and **B**, nasal polyposis (NP) using 275 cases with NP and 338 without NP

A. CRS cases vs iControl controls							
Gene	SNP ID	Minor/major allele	MAF		χ^2	P-value	OR (95% CI)
			Cases	Controls			
<i>PARS2</i>	rs2873551	C/T	0.34	0.40	13.7	0.00022	0.77 (0.70-0.84)
<i>TGFB1</i>	rs1800469	T/C	0.30	0.34	8.16	0.0043	0.81 (0.74-0.89)
<i>NOS1</i>	rs1483757	G/A	0.39	0.44	6.72	0.0095	0.84 (0.77-0.91)
<i>NOS1AP</i>	rs4657164	C/T	0.14	0.17	5.94	0.015	0.79 (0.70-0.89)
<i>IL22RA1</i>	rs11579657	G/A	0.21	0.18	5.27	0.022	1.21 (1.13-1.30)
<i>DCBLD2</i>	rs828618	A/G	0.32	0.29	4.92	0.026	1.18 (1.10-1.25)
<i>ALOX5AP</i>	rs17238773	T/A	0.06	0.08	4.24	0.039	0.75 (0.62-0.89)
<i>AOAH</i>	rs4504543	C/T	0.37	0.35	2.68	0.10	1.12 (1.05-1.19)
<i>IL1RL1</i>	rs13431828	T/C	0.14	0.16	2.68	0.10	0.86 (0.76-0.95)
<i>MMP9</i>	rs17577	A/G	0.13	0.14	1.83	0.18	0.87 (0.77-0.97)
<i>IRAK-4</i>	rs4251431	T/G	0.10	0.08	1.65	0.20	1.16 (1.04-1.28)
<i>MSRA</i>	rs7001821	T/C	0.37	0.40	1.60	0.21	0.92 (0.85-0.99)
<i>ALOX5</i>	rs3780894*	G/A	0.16	0.15	1.60	0.21	1.12 (1.03-1.22)
<i>NOS1</i>	rs9658281	A/G	0.27	0.29	1.51	0.22	0.91 (0.84-0.99)
<i>IL22RA1</i>	rs4292900	T/C	0.31	0.33	1.21	0.27	0.92 (0.85-1.00)
<i>TNFAIP3</i>	rs3757173	C/T	0.09	0.10	1.20	0.27	0.88 (0.76-1.00)
<i>IL1RL1</i>	rs10204137*	G/A	0.37	0.39	0.841	0.36	0.94 (0.87-1.01)
<i>IL1A</i>	rs17561	T/G	0.29	0.30	0.539	0.46	0.95 (0.87-1.02)
<i>IL1A</i>	rs2048874	T/C	0.13	0.13	0.407	0.52	1.07 (0.97-1.16)
<i>CACNA1I</i>	rs3788568	G/T	0.36	0.37	0.317	0.57	0.96 (0.89-1.03)
<i>IL22RA1</i>	rs10794665	A/G	0.39	0.40	0.270	0.60	0.96 (0.90-1.03)
<i>LAMB1</i>	rs4727695	G/A	0.08	0.09	0.253	0.62	0.94 (0.82-1.06)
<i>IL1B</i>	rs16944	A/G	0.34	0.33	0.227	0.63	1.03 (0.96-1.11)
<i>MUSK</i>	rs10817091	A/G	0.47	0.46	0.223	0.64	1.03 (0.96-1.10)
<i>TP73</i>	rs3765731	A/G	0.31	0.30	0.192	0.66	1.03 (0.96-1.11)
<i>TNFAIP3</i>	rs5029938	T/C	0.06	0.06	0.150	0.70	0.95 (0.80-1.09)
<i>IL22RA1</i>	rs16829225	T/C	0.17	0.18	0.145	0.70	0.97 (0.88-1.06)
<i>IL22RA1</i>	rs10751768	C/T	0.38	0.37	0.119	0.73	1.02 (0.95-1.09)
<i>LAMA2</i>	rs2571584	G/A	0.26	0.26	0.0902	0.76	0.98 (0.90-1.05)
<i>TRIP12</i>	rs1035833	C/T	0.29	0.29	0.0242	0.88	0.99 (0.91-1.06)
<i>IRAK-4</i>	rs4251580	T/C	0.14	0.14	0.0134	0.91	0.99 (0.89-1.09)
<i>NAV3</i>	rs1726427	C/T	0.32	0.31	0.0112	0.92	1.01 (0.94-1.08)
<i>IL22RA1</i>	rs7418238	G/A	0.12	0.12	0.00136	0.97	1.00 (0.89-1.10)

MAF, minor allele frequency.

OR, odds ratio.

CI, confidence interval.

*Hardy-Weinberg test $P < 0.05$.

B.		CRS cases with NP vs without NP					
Gene	SNP ID	Minor/major allele	MAF		χ^2	P-value	OR (95% CI)
			with NP	without NP			
AOAH	rs4504543	C/T	0.34	0.40	5.41	0.020	0.76 (0.64-0.88)
ALOX5	rs3780894	G/A	0.19	0.14	3.96	0.047	1.36 (1.20-1.51)
IL1RL1	rs10204137	G/A	0.34	0.39	3.00	0.083	0.81 (0.69-0.93)
IL1A	rs2048874	T/C	0.15	0.12	2.75	0.097	1.32 (1.15-1.49)
IL1RL1	rs13431828	T/C	0.12	0.16	2.74	0.098	0.76 (0.59-0.93)
IRAK-4	rs1141168	G/A	0.45	0.50	2.68	0.10	0.83 (0.71-0.94)
NOS1AP	rs4657164	C/T	0.13	0.16	2.53	0.11	0.77 (0.60-0.93)
IL1RL1	rs10208293	A/G	0.26	0.30	2.51	0.11	0.82 (0.69-0.94)
IL1RL1	rs4988957	C/T	0.34	0.39	2.48	0.12	0.83 (0.71-0.95)
IL1A	rs1800587	T/C	0.26	0.30	2.17	0.14	0.83 (0.70-0.96)
IL1A	rs17561	T/G	0.27	0.30	1.77	0.18	0.84 (0.72-0.97)
CACNA1I	rs3788568	G/T	0.38	0.35	1.28	0.26	1.14 (1.02-1.26)
IRAK-4	rs4251580	T/C	0.15	0.13	1.20	0.27	1.20 (1.03-1.37)
LAMA2	rs2571584*	G/A	0.24	0.27	1.19	0.27	0.87 (0.73-1.00)
IL1A	rs2856838	T/C	0.44	0.41	1.16	0.28	1.13 (1.02-1.25)
IRAK-4	rs4251431	T/G	0.09	0.10	0.799	0.37	0.84 (0.64-1.04)
MET1	rs38850	A/G	0.28	0.30	0.744	0.39	0.90 (0.77-1.02)
TNFAIP3	rs3757173	C/T	0.08	0.09	0.709	0.40	0.84 (0.63-1.05)
NAV3	rs1726427	C/T	0.33	0.31	0.538	0.46	1.09 (0.97-1.22)
DCBLD2	rs828618	A/G	0.31	0.33	0.509	0.48	0.92 (0.79-1.04)
IL1B	rs16944	A/G	0.35	0.33	0.465	0.50	1.09 (0.96-1.21)
IRAK-4	rs3794262	A/T	0.10	0.12	0.441	0.51	0.88 (0.70-1.07)
LAMB1	rs4727695	G/A	0.09	0.08	0.334	0.56	1.13 (0.92-1.33)
IL22RA1	rs10751768	C/T	0.39	0.37	0.316	0.57	1.07 (0.95-1.19)
ALOX5AP	rs17238773	T/A	0.06	0.06	0.298	0.58	1.14 (0.90-1.38)
MUSK	rs10817091	A/G	0.48	0.46	0.280	0.60	1.06 (0.95-1.18)
PARS2	rs2873551	C/T	0.33	0.34	0.217	0.64	0.94 (0.82-1.07)
MSRA	rs7001821	T/C	0.37	0.38	0.216	0.64	0.95 (0.83-1.07)
TGFB1	rs1800469	T/C	0.30	0.29	0.167	0.68	1.05 (0.93-1.18)
NOS1	rs9658281	A/G	0.26	0.27	0.167	0.68	0.95 (0.82-1.08)
IL22RA1	rs7418238	G/A	0.12	0.12	0.132	0.72	1.07 (0.89-1.24)
IL22RA1	rs16829225	T/C	0.17	0.17	0.0854	0.77	0.96 (0.80-1.11)
IL22RA1	rs11579657	G/A	0.21	0.21	0.0580	0.81	1.03 (0.89-1.18)
CACNG6	rs192808	T/C	0.32	0.32	0.0301	0.86	0.98 (0.86-1.10)
TNFAIP3	rs5029938	T/C	0.06	0.06	0.0218	0.88	1.04 (0.79-1.28)
IRAK-4	rs1461567	T/C	0.27	0.27	0.0188	0.90	1.02 (0.89-1.15)
TP73	rs3765731	A/G	0.31	0.30	0.0139	0.91	1.01 (0.89-1.14)
IL22RA1	rs4648936*	G/A	0.32	0.31	0.0107	0.92	1.01 (0.89-1.14)
IL22RA1	rs4292900*	T/C	0.32	0.31	0.0107	0.92	1.01 (0.89-1.14)
NOS1	rs1483757	G/A	0.39	0.39	0.0103	0.92	1.01 (0.89-1.13)
TRIP12	rs1035833	C/T	0.29	0.29	0.00658	0.94	1.01 (0.88-1.14)
MMP9	rs17577	A/G	0.13	0.13	0.00645	0.94	1.01 (0.84-1.19)
IL22RA1	rs10794665	A/G	0.39	0.39	0.00178	0.97	1.00 (0.89-1.12)

MAF, minor allele frequency.

OR, odds ratio.

CI, confidence interval.

*Hardy-Weinberg test $P < 0.05$.

TABLE E3. Association test results and allele frequency differences for subgroups with and without allergic rhinitis (AR) and asthma (AS)

Gene	SNP	Total	P-value				ΔMAF				Coef. Var.
			+AR	-AR	+AS	-AS	I	II	III	IV	
PARS2	rs2873551	0.00022	0.010	0.0025	0.025	0.0013	-0.06	-0.08	-0.08	-0.06	0.16
TGFB1	rs1800469	0.0043	0.030	0.027	0.30	0.0044	-0.08	-0.03	0.04	-0.05	1.7
NOS1	rs1483757	0.0095	0.061	0.038	0.21	0.015	-0.05	-0.06	-0.03	-0.05	0.26
NOS1AP	rs4657164	0.015	0.57	0.0067	0.13	0.037	-0.05	0.03	0	-0.04	2.5
IL22RA1	rs11579657	0.022	0.051	0.093	0.45	0.019	0.02	0.06	0.01	0.03	0.72
DCBLD2	rs828618	0.026	0.97	0.0054	0.83	0.012	-0.02	0.01	0.03	0.05	1.7
ALOX5AP	rs17238773	0.039	0.10	0.13	0.22	0.073	-0.03	-0.02	-0.02	-0.02	0.22
IL1RL1	rs13431828	0.10	0.26	0.18	0.69	0.082	0	-0.04	-0.01	-0.02	0.98
AOAH	rs4504543	0.10	0.81	0.026	0.27	0.17	0.02	-0.04	0.04	0.04	2.5
MMP9	rs17577	0.18	0.34	0.27	0.85	0.087	-0.03	-0.01	0.05	-0.02	14
ALOX5	rs3780894	0.20	0.051	0.72	0.41	0.28	0.04	0.04	-0.01	0.01	1.2
IRAK-4	rs4251431	0.21	0.36	0.030	0.77	0.17	0.01	-0.03	0	0.03	10
MSRA	rs7001821	0.21	0.80	0.15	0.58	0.073	0.01	-0.02	0.02	-0.04	3.7
NOS1	rs9658281	0.22	0.22	0.44	0.89	0.17	-0.03	-0.04	0.02	-0.02	1.5
TNFAIP3	rs3757173	0.27	0.043	0.92	0.41	0.39	-0.04	-0.04	0	-0.01	0.92
IL22RA1	rs4292900	0.27	0.25	0.51	0.30	0.44	-0.04	-0.02	-0.02	-0.01	0.56
IL1RL1	rs10204137	0.36	0.87	0.29	0.80	0.34	-0.03	0	0.01	-0.03	1.6
IL1A	rs17561	0.46	0.14	1.0	0.86	0.44	-0.02	-0.05	0.01	0	1.8
IL1A	rs2048874	0.52	0.017	0.46	0.68	0.59	0.02	0.06	-0.01	-0.01	2.2
IL22RA1	rs10794665	0.57	0.96	0.49	0.89	0.58	0	-0.01	-0.02	-0.02	0.77
MUSK	rs10817091	0.60	0.32	0.22	0.49	0.84	-0.01	-0.03	0.07	0.02	3.5
IL1B	rs16944	0.62	0.30	0.96	0.28	0.99	0.06	0	-0.01	0	2.6
CACNA1I	rs3788568	0.63	0.53	0.75	0.72	0.39	0.02	-0.04	0.01	0	11
LAMB1	rs4727695	0.64	0.77	0.41	0.71	0.43	0	0.01	0.01	-0.01	3.8
IL22RA1	rs10751768	0.66	0.90	0.60	0.99	0.68	-0.01	0.01	0.02	0.01	1.7
IL22RA1	rs16829225	0.70	0.49	0.97	0.80	0.56	0	-0.03	0.01	-0.01	2.3
TP73	rs3765731	0.70	0.19	0.77	0.92	0.57	0.01	0.05	-0.03	0	4.4
TNFAIP3	rs5029938	0.73	0.14	0.66	0.94	0.62	-0.01	-0.02	0.02	0	6.8
LAMA2	rs2571584	0.76	0.75	0.86	0.63	0.92	0	-0.01	-0.03	0	1.4
TRIP12	rs1035833	0.88	0.21	0.31	0.59	0.93	0.03	0.03	-0.07	-0.01	9.5
NAV3	rs1726427	0.91	0.16	0.45	0.77	0.98	0	0.07	0.03	-0.02	2.0
IRAK-4	rs4251580	0.92	0.20	0.33	0.44	0.59	0.03	0.01	-0.01	-0.02	8.9
IL22RA1	rs7418238	0.97	0.51	0.71	0.072	0.39	-0.04	0.01	-0.02	0.01	2.4

P-total: P-values in the association test between all the 613 cases and 1588 controls (from Table E2).

P-value +AR: P-values in the association test between 193 cases with AR and 1588 controls.

P-value -AR: P-values in the association test between 420 cases without AR and 1588 controls.

P-value +AS: P-values in the association test between 147 cases with AS and 1588 controls.

P-value -AS: P-values in the association test between 466 cases without AS and 1588 controls.

ΔMAF I: Difference in minor allele frequency between 85 cases with both AR and AS and the controls.

ΔMAF II: Difference in minor allele frequency between 108 cases with AR and without AS and the controls.

ΔMAF III: Difference in minor allele frequency between 62 cases without AR and with AS and the controls.

ΔMAF IV: Difference in minor allele frequency between 358 cases without both AR and AS and the controls.

Coef. Var.: Coefficient of variation, standard deviation/mean, for the ΔMAF values.

P-values in bold in the allele tests show $P < 0.05$.

ΔMAF values in bold show SNPs with consistent ΔMAF for all 4 tests.