

Genetic variation in immune signaling genes differentially expressed in asthmatic lung tissues

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Background: Eight genes in the immune signaling pathway shown to be differentially expressed in asthmatic lung biopsy specimens in a previous microarray experiment were selected as candidate genes for asthma susceptibility.

Objective: We sought to perform an association study with these genes and asthma-related phenotypes in 3 independent Canadian familial asthma collections and 1 Australian asthma case-control study.

Methods: Tagging single nucleotide polymorphisms were selected by using the HapMap public database ($r^2 > 0.8$; minor allele frequency > 0.10) and genotyped with the Illumina platform. Family-based association and trend tests for asthma, atopy, airway hyperresponsiveness, and allergic asthma phenotypes were done in each sample, correcting for multiple testing.

Results: Uncorrected associations with polymorphisms within 7 genes were detected with 1 or more of the phenotypes in 1 or more of the 4 populations ($.001 < P < .05$). After correction, the 15-lipoxygenase (15-LO) associations with airway hyperresponsiveness and allergic asthma remained significant in 2 Canadian samples (corrected $P = .022$ and $.049$, respectively), and the association of the CD14 antigen with asthma remained significant in 1 Canadian sample (corrected $P = .042$). In both cases a protective effect of the minor alleles was observed.

Conclusion: Expression profiling studies are a useful way to identify candidate genes for asthma because this approach has

led to the first report of an association with 15-LO in 2 independent populations. Because 15-LO is involved in anti-inflammatory processes, further functional and clinical investigation of the role of this biologic pathway in asthma is warranted. (J Allergy Clin Immunol 2008;122:529-36.)

Key words: Asthma, microarrays expression profiling, candidate genes, genetic association study, tagging single nucleotide polymorphism, 15-lipoxygenase

Asthma is a chronic inflammatory disease of the airways associated with variable airflow obstruction and airway hyperresponsiveness (AHR).¹ Asthma is also recognized as a complex heritable disease, implying the interaction of numerous genetic and environmental factors.² Identification of the genes that influence asthma susceptibility and severity has been the subject of intense research over the last decade.^{3,4} This knowledge has significantly contributed to our understanding of the pathophysiology of asthma, but much work is needed to replicate reported associations, to characterize the functional effects of the genes already identified,⁵ and to identify new candidate genes for asthma.

Expression profiling studies in which transcript levels in tissues of affected and control subjects are compared allow the identification of genes related to a disease process.⁶ These genes, which might or might not have known function, can be selected as candidate genes in subsequent genetic association

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Abbreviations used

AHR:	Airway hyperresponsiveness
<i>ALOX15</i> :	Arachidonate 15-lipoxygenase gene
CAPPS:	Canadian Asthma Primary Prevention Study
<i>CXCL12</i> :	Chemokine (C-X-C motif) ligand 12 (stromal cell–derived factor 1) gene
FBAT:	Family-based Association Test
<i>IL2RB</i> :	IL-2 receptor β gene
<i>IL7R</i> :	IL-7 receptor gene
LD:	Linkage disequilibrium
15-LO:	15-Lipoxygenase
<i>NOS2A</i> :	Nitric oxide synthase 2A gene
SAGE:	Study of Asthma Genetics and Environment Cohort
<i>SFRP1</i> :	Secreted frizzled-related protein 1 gene
15(S)-HETE:	15(S)-hydroxyeicosatetraenoic acid
SLSJ:	Saguenay–Lac-Saint-Jean
SNP:	Single nucleotide polymorphism

studies on the basis of their differential expression in affected tissues.⁵ We used this approach in the selection of the *CX3CR1* gene, the fractalkine receptor, for a previous asthma association study⁷ because *CX3CR1* was underexpressed in bronchial biopsy specimens of asthmatic subjects compared with control subjects.⁸

CX3CR1 is related to immune signaling, and 8 other genes that we reported as differentially expressed in asthmatic bronchial biopsy specimens are also part of this biologic cluster.⁸ Three of these have been previously suggested as asthma genes (*CD14*, chemokine [C-X-C motif] ligand 12 [*CXCL12*], and nitric oxide synthase 2A [*NOS2A*]),⁴ but 5 have not been recognized as asthma genes or clearly implicated in asthma pathophysiology (arachidonate 15-lipoxygenase [*ALOX15*], *CD27*, IL-2 receptor β [*IL2RB*], IL-7 receptor [*IL7R*], and secreted frizzled-related protein 1 [*SFRP1*]; see Table I for a description of the genes). Because the approach used for *CX3CR1* allowed us to find a positive association that has been replicated in an independent cohort,⁷ we have applied a similar strategy to these 8 additional differentially expressed genes. We postulated that these genes represent asthma biomarkers, and if genetic variation explains the difference in gene expression, then we should observe an association between variants of these candidate genes and asthma and related phenotypes in a collection of asthmatic subjects. Specifically, we hypothesized that, based on their differential expression in asthmatic bronchial biopsy specimens⁸ and their biologic role in immunity, *ALOX15*, *CD14*, *CD27*, *CXCL12*, *IL2RB*, *IL7R*, *NOS2A*, and *SFRP1* represent candidate genes for asthma susceptibility.

The objectives of the present study are to report tests for association between asthma-related phenotypes and the 8 candidate genes in 4 independent asthmatic collections (differentiated by their recruitment, ascertainment of the subjects, and their genetic background) included in the Canadian AllerGen network (AllerGen NCE, Inc). Three of these are Canadian family studies, and the fourth is an Australian asthma case-control study. Our results demonstrated the usefulness of the expression profiling studies in the identification of asthma candidate genes because they allow us to report the first asthma genetic association for *ALOX15* in 2 independent Canadian population samples.

METHODS**Association study samples**

In the context of the AllerGen (Allergy, Genes, and Environment) NCE, Inc, Canadian network, 4 well-characterized samples of individuals affected with asthma and their family members, as well as case-control data, were combined as a resource to investigate the genetics of asthma and related phenotypes (see the complete description in this article's Methods section in the Online Repository at www.jacionline.org). Briefly, these include 3 Canadian independent studies: (1) the Saguenay–Lac-Saint-Jean (SLSJ) founder population family collection⁹⁻¹⁴; (2) the Canadian Asthma Primary Prevention Study (CAPPS) high-risk birth cohort¹⁵⁻¹⁷; and (3) the Study of Asthma Genes and the Environment Cohort (SAGE), a nested case-control sample from a population-based birth cohort. These 3 samples have been fully described in a recent article by Bégin et al.¹⁸ The fourth asthma sample is the Australian Busselton Health Study, which is a nested case-control sample selected from a population-based cohort that has a longitudinal design and a standardized phenotype classification.¹⁹⁻²³ Informed consent was approved by each center's research ethics board and collected for each study participant.

Phenotype definitions

Four dichotomous asthma related-phenotypes were analyzed. First was *asthma*, which was defined as doctor-diagnosed asthma in SLSJ, CAPPS, and SAGE and as a positive response to the question "Has a doctor ever told you that you had asthma, or bronchial asthma?" at either survey (1981 and 1994) in the Busselton cohort. Second was *atopy*, which was defined as at least 1 positive response (wheal diameter ≥ 3 mm at 10 minutes) on skin prick testing for any allergen tested. Third was *AHR*, which was assessed by means of methacholine challenge testing. A positive response was defined as a 20% decrease in FEV₁ (PC₂₀ or PD₂₀). For the SLSJ, CAPPS, and SAGE samples, AHR was defined as a PC₂₀ value of less than 8 mg/mL and for the Busselton cohort, it was defined as a PD₂₀ value of 3.9 μ mol or less. The fourth phenotype was *allergic asthma*, which was defined as being present in subjects given diagnoses of both asthma and atopy.

Genotyping

DNA samples were available for each studied sample. AllerGen TagSNP genotyping was performed with the Illumina BeadLab (<http://www.illumina.com>; Illumina, Inc, San Diego, Calif) platform, as described previously.²⁴ TagSNPs were selected from the International HapMap Project (<http://www.hapmap.org>) release 16c from among single nucleotide polymorphisms (SNPs) located in the vicinity of the candidate genes (if they were not located in neighboring genes) and included the flanking regions 10 kb upstream and 10 kb downstream of the first and the last exon, respectively. TagSNPs were selected in a manner similar to LD Select²⁵ by using an r^2 cutoff of 0.8. If equivalent TagSNPs were identified, those with the highest Illumina design score were kept. Only SNPs with a design score of greater than 0.4 were retained for the panel. Samples with call rates of less than 95% were excluded from the final dataset. Assays with poor cluster separation and with call rates of less than 90% were removed from the final dataset. All primers and conditions used are available on request to the corresponding author. For the 8 studied candidate genes, a panel of 77 TagSNPs was tested for association with asthma, atopy, AHR, and allergic asthma phenotypes in each sample. SNPs described in this report are cited by using their National Center for Biotechnology Information reference sequence numbers.

Statistical analyses

Allele distribution patterns and Mendelian errors for the 3 familial samples (SLSJ, CAPPS, and SAGE) were assessed by using Family-based Association Test (FBAT) software (version 1.7.2; Harvard University, Boston, Mass)²⁶⁻²⁸ under an additive genetic model. An empiric estimate of the variance has been used for the SLSJ sample.²⁶ FBAT results are reported for the minor alleles. For the Busselton cohort, association testing was performed with a general allelic likelihood ratio test, χ^2 test (1 df), as implemented in the UNPHASED program (Cambridge, United Kingdom [UK]).²⁹ This approach

TABLE I. Gene and TagSNP characteristics

Gene location*	Microarray†	Description‡	Function‡	TagSNP	Genomic features§
<i>ALOX15</i> 17p13.3 Gene ID: 246	4.19	Arachidonate 15-LO	Converts arachidonic acid to 15S-hydroperoxyeicosatetraenoic acid. Also acts on C-12 of arachidonate acid, as well as on linoleic acid.	rs1076039	Downstream 3'UTR
				rs4790210	Downstream 3'UTR
				rs916055	3'UTR
				rs743646	Exon 11 (T485T)
				rs7217186	Intron 7
				rs2664593	Upstream 5'UTR
<i>CD14</i> 5q31.1 Gene ID: 929	-2.76	CD14 molecule	Mediates the innate immune response to bacterial LPSs. Leads the NF-κB activation, cytokine secretion, and inflammatory response. Upregulates cell-surface adhesion molecules.	rs748694	Upstream 5'UTR
				rs778583	Downstream 3'UTR
				rs778584	Downstream 3'UTR
				rs4914	Exon 2 (L367L)
				rs2569190	Upstream 5'UTR
				rs5744455	Upstream 5'UTR
<i>CD27</i> 12p13 Gene ID: 939	-3.61	CD27 molecule	Member of the TNF receptor superfamily required for generation and long-term maintenance of T-cell immunity. Regulates B-cell activation and immunoglobulin synthesis. This receptor transduces signals that lead to the activation of NF-κB and MAPK8/JNK.	rs2569193	Upstream 5'UTR
				rs2364493	Upstream 5'UTR
				rs7970260	Upstream 5'UTR
				rs2250246	Upstream 5'UTR
				rs3136551	5'UTR
				rs25680	Exon 2 (T59A)
<i>CXCL12</i> 10q11.1 Gene ID: 6387	-2.39	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	Small cytokines that belongs to the intercrine family, members of which activate leukocytes and are often induced by proinflammatory stimuli, such as LPS, TNF, or IL-1.	rs2267966	Intron 2
				rs266105	Downstream 3'UTR
				rs185545	Downstream 3'UTR
				rs2839696	3'UTR
				rs197452	Intron 3
				rs266087	Intron 3
				rs2297630	Intron 3
				rs3780891	Intron 1
				rs1413519	Upstream 5'UTR
				rs2861442	Upstream 5'UTR
<i>IL2RB</i> 22q13.1 Gene ID: 3560	-3.00	IL-2 receptor β	Receptor for IL-2. This β-subunit is involved in receptor-mediated endocytosis and transduces the mitogenic signals of IL-2.	rs6593412	Upstream 3'UTR
				rs6000570	Upstream 3'UTR
				rs228934	Upstream 3'UTR
				rs228935	Upstream 3'UTR
				rs228937	Upstream 3'UTR
				rs3218339	Upstream 3'UTR
				rs228942	Exon 10 (E391D)
				rs84459	Intron 9
				rs228947	Intron 9
				rs3218322	Intron 9
				rs3218316	Intron 8
				rs3218315	Intron 8
				rs3218312	Intron 8

(Continued)

TABLE I. (Continued)

Gene location*	Microarray†	Description‡	Function‡	TagSNP	Genomic features§
<i>IL7R</i> 5p13 Gene ID: 3575	-2.41	IL-7 receptor	Receptor for IL-7. Requires the IL-2 receptor γ chain, which is a common chain shared by the receptors of various cytokines, including IL-2, IL-4, IL-7, IL-9, and IL-15.	rs228954	Intron 6
				rs3218292	Intron 6
				rs2281094	Intron 4
				rs1003694	Intron 3
				rs2235330	Intron 2
				rs3218258	Intron 1
				rs228979	Intron 1
				rs2016771	Upstream 5'UTR
				rs743776	Upstream 5'UTR
				rs6890853	Upstream 5'UTR
<i>NOS2A</i> 17q11.2-q12 Gene ID: 4843	7.25	Nitric oxide synthase 2A	Produces NO, which is a messenger molecule with diverse functions throughout the body. In macrophages NO mediates tumoricidal and bactericidal actions.	rs1353251	Intron 1
				rs1389832	Intron 1
				rs987106	Intron 6
				rs3194051	Exon 8 (V356I)
				rs9292618	Downstream 3'UTR
				rs4796017	Downstream 3'UTR
				rs8081248	Downstream 3'UTR
				rs2255929	Intron 23
				rs2297518	Exon 16 (L608S)
				rs1137933	Exon 10 (D385D)
<i>SFRP1</i> 8p12-p11.1 Gene ID: 6422	-2.16	Secreted frizzled-related protein 1	SFRPs function as modulators of Wnt signaling through direct interaction with Wnt proteins. They have a role in regulating cell growth and differentiation in specific cell types. SFRP1 decreases intracellular β -catenin levels.	rs3794764	Intron 5
				rs8072199	Intron 2
				rs3794766	Intron 2
				rs2779248	5'UTR
				rs2779252	Upstream 5'UTR
				rs2531860	Upstream 5'UTR
				rs3242	3'UTR
				rs1127379	3'UTR
				rs7013368	Intron 2
				rs11774662	Intron 2
				rs10098898	Intron 2
				rs10958672	Intron 2
				rs747417	Intron 2
				rs968427	Intron 1
				rs921142	Upstream 5'UTR
				rs4736965	Upstream 5'UTR

UTR, Untranslated region; NF, nuclear factor; MAPK, mitogen-activated protein kinase; JNK, c-jun N-terminal kinase; NO, nitric oxide.

*Gene location obtained from National Center for Biotechnology Information Gene (<http://www.ncbi.nlm.nih.gov>; freeze on August 25, 2007).

†Data with a *P* value of less than .000001 from the U95A microarray study performed by Laprise et al⁸ (2004) using RNA from bronchial biopsy specimens of control subjects (without asthma or atopy; *n* = 4) or allergic asthmatic subjects (*n* = 4). Mean fold change in logarithmic values is shown.

‡From public databases: National Center for Biotechnology Information (Gene, OMIM, PubMed); Genecards (<http://bioinformatics.weizmann.ac.il/cards/>); and Swiss-Prot (http://ca.expasy.org/sprot/sprot_t-top.html).

§Genomic features as described in HapMap (<http://www.hapmap.org>) and National Center for Biotechnology Information SNP databases.

TABLE II. Distribution of asthma, atopy, AHR, and allergic asthma phenotypes among the 4 studied samples

Phenotypes	SLSJ (n = 1186)*		CAPPS (n = 1253)		SAGE (n = 1247)		Busseton (n = 1524)	
	Probands (n = 209)†	Percentage	Children (n = 509)†	Percentage	Children (n = 684)†	Percentage	Cases‡	Percentage
Asthma	209/209	100	71/375	18.9	228/683	33.4	653/1422	45.9
Atopy	168/208	80.8	162/362	44.8	298/680	43.8	655/1264	51.8
AHR	158/178	88.8	254/343	74.1	342/624	54.8	222/1083	20.5
Allergic asthma	168/208	80.8	52/374	13.9	146/683	21.4	388/795	48.8

*Number of available genotypes for the study on each entire sample (probands plus family members for SLSJ, CAPPS, and SAGE and cases plus control subjects for Busseton).

†Number of probands or children presenting the phenotype on the total number of available phenotypes.

‡Number of subjects presenting the phenotype on the total number of available phenotypes. Individuals in each control groups might include cases from the other phenotype groups.

TABLE III. Significant FBAT results for TagSNPs of the studied candidate genes and asthma, atopy, AHR, and allergic asthma phenotypes under an additive genetic model in the SLSJ familial sample

Gene	TagSNP	Base change	MAF	Asthma			Atopy			AHR			Allergic asthma		
				N	Z	P value (corrected P value)*	N	Z	P value (corrected P value)	N	Z	P value (corrected P value)	N	Z	P value (corrected P value)
IL2RB	rs228979	C > A	0.27	—†	—	—	92	−2.10	.04 (1.00)	—	—	—	94	−2.29	.02 (0.92)
	rs2016771	G > T	0.34	128	2.26	.02 (.99)	—	—	—	—	—	—	—	—	—
NOS2A	rs3794766	G > A	0.26	—	—	—	—	—	—	—	—	—	109	−2.13	.03 (.80)
	rs2779248	A > G	0.45	—	—	—	—	—	—	—	—	—	111	−2.34	.02 (.47)

MAF, Minor allele frequency; N, number of families contributing the statistic; Z, Z score.

*The number of independent SNPs is estimated to be 14 for *IL2RB* and 8 for *NOS2A*. The number of independent phenotypes is estimated to be 3.†Dashes indicate that the test result is not significant ($P > .05$).

uses a standard retrospective case-control likelihood for case-control designs. Odds ratios were estimated by using the most common allele as the referent and are reported for each minor allele. For each gene and for each SNP within that gene, we applied a correction to the significance level that accounts for both the effective number of independent SNPs tested in that gene and the effective number of phenotypes tested. A Sidak correction was applied with respect to that number (independent SNPs $\times$ independent phenotypes). Because the TagSNPs are correlated, the effective number of independent SNPs was calculated by using the method proposed by Li and Ji³⁰ implemented in the Single Nucleotide Polymorphism Spectral Decomposition Web resource (<http://gump.qimr.edu.au/general/daledN/SNPSpD/>).³¹ We used a similar procedure, the Matrix Spectral Decomposition approach, to estimate the number of independent phenotypes ($n = 3$) in each sample. Hardy-Weinberg equilibrium P values were calculated using Haploview software (version 3.32; Broad Institute of MIT and Harvard University, Boston, Mass)³² for the SLSJ, CAPPS, and SAGE samples and by using a χ^2 test for the Busseton cohort, considering 1 df and a significance threshold of .001. The strength of linkage disequilibrium (LD) between pairs of SNPs was evaluated with Haploview software (version 3.32).³² LD plots for each gene in each population are presented in Figs E1 through E8 (available in this article's Online Repository at www.jacionline.org).

RESULTS

For the SLSJ sample, the probands had a male/female ratio of 1:1.2, a mean age of 18 years, and a mean PC₂₀ of 2.66 mg/mL. For the CAPPS sample, the children were 7 years old, the sex ratio was 1.05:1, and they had a mean PC₂₀ of 2.99 mg/mL. For the SAGE sample, the children were aged between 8 and 10 years, had a sex ratio of 1.26:1, and had a mean PC₂₀ of 2.72 mg/mL. Finally, for the Busseton cohort, the age range was from 17 to 91 years, the sex ratio was 1:1.35, and the mean PD₂₀ was 1.14 μ mol. The number of individuals genotyped and the phenotypic distribution in each studied sample are presented in Table II. Genotyping was achieved with a mean success rate of 99.9% for the

SLSJ, 99.6% for the CAPPS, 99.7% for the SAGE, and 99.7% for the Busseton samples. A total of 17 Mendelian errors were observed. Families with Mendelian errors were reset to zero for FBAT analyses. All SNPs were in Hardy-Weinberg equilibrium, and none of them showed more than 2 Mendelian errors.

The family-based association analyses for the SLSJ, CAPPS, and SAGE samples are presented in Tables III to V, respectively. Odds ratios for the Busseton cohort are presented in Table VI. For the sake of brevity, only results showing a P value less than the .05 threshold for 1 or more phenotypes are presented, with their respective corrected P values indicated in parentheses. The significance values for each population for all TagSNPs that generated significant associations are available in Tables E1 to E4 (available in this article's Online Repository at www.jacionline.org). After correction for multiple testing, only 4 associations remained significant. In the CAPPS sample, *ALOX15* rs7217186 showed a positive association for the AHR phenotype, suggesting a protective effect of the minor allele because it is undertransmitted to the hyperresponsive probands (corrected $P = .022$). In the SAGE sample, *ALOX15* showed a positive association for rs2664593 and allergic asthma. The undertransmission of the minor allele to the allergic asthmatic probands suggests a protective effect (corrected $P = .049$). In this sample, a protective association was also found for the *CD14* gene (corrected $P = .042$). For all other TagSNPs tested, no association remained significant after correcting for multiple testing, regardless of the studied phenotype in a given sample.

DISCUSSION

This study reports association analyses of 8 candidate genes selected based on our previous microarray results⁷ in 4 independent asthma samples. Before correction for multiple testing,

TABLE IV. Significant FBAT results for TagSNPs of the studied candidate genes and asthma, atopy, AHR, and allergic asthma phenotypes under an additive genetic model in the CAPPS familial sample

Gene	TagSNP	Base change	MAF	Asthma			Atopy			AHR			Allergic asthma		
				N	Z	P value (corrected P value)*	N	Z	P value (corrected P value)	N	Z	P value (corrected P value)	N	Z	P value (corrected P value)
<i>ALOX15</i>	rs4790210	T > C	0.21	29	-2.61	.009 (.162)	61	-2.85	.004 (.079)	101	-2.06	.04 (.71)	22	-2.75	.006 (.109)
	rs916055	T > C	0.34	—†	—	—	—	—	—	129	-2.59	.01 (.172)	—	—	—
	rs743646	T > C	0.10	—	—	—	—	—	—	66	2.15	.03 (.57)	—	—	—
	rs7217186	A > G	0.47	43	-2.21	.03 (.48)	—	—	—	154	-3.24	.001 (.022)	—	—	—
	rs2664593	G > C	0.21	—	—	—	—	—	—	107	1.98	.05 (.87)	—	—	—
<i>CD14</i>	rs4914	G > C	0.11	—	—	—	—	—	—	65	2.02	.04 (.52)	—	—	—
<i>IL2RB</i>	rs228979	C > A	0.29	—	—	—	—	—	—	—	—	—	28	-2.27	.02 (1.00)
<i>NOS2A</i>	rs2297518	G > A	0.19	30	2.14	.03 (.78)	—	—	—	—	—	—	21	2.20	.03 (.67)
	rs2531860	C > T	0.15	—	—	—	—	—	—	—	—	—	18	-2.29	.02 (.52)

P values highlighted in bold and underlined indicate that they remain significant after correction for multiple testing.

MAF, Minor allele frequency; N, number of families contributing the statistic; Z, Z score.

*The number of independent SNPs is estimated to be 6 for *ALOX15*, 4 for *CD14*, 21 for *IL2RB*, and 8 for *NOS2A*. The number of independent phenotypes is estimated to be 3.

†Dashes indicate that the test result is not significant ($P > .05$).

TABLE V. Significant FBAT results for TagSNPs of the studied candidate genes and asthma, atopy, AHR, and allergic asthma phenotypes under an additive genetic model in the SAGE familial sample

Gene	TagSNP	Base change	MAF	Asthma			Atopy			AHR			Allergic asthma		
				N	Z	P value (corrected P value)*	N	Z	P value (corrected P value)	N	Z	P value (corrected P value)	N	Z	P value (corrected P value)
<i>ALOX15</i>	rs4790210	T > C	0.20	63	2.19	.03 (.51)	—	—	—	74	2.16	.03 (.56)	43	2.38	.02 (.31)
	rs2664593	G > C	0.23	71	-2.09	.04 (.67)	109	-2.23	.03 (.46)	84	-2.05	.04 (.73)	47	-2.99	.003 (.049)
<i>CD14</i>	rs4914	G > C	0.11	36	-2.92	.004 (.042)	—	—	—	—	—	—	—	—	—
<i>CXCL12</i>	rs2861442	A > G	0.26	—†	—	—	104	2.03	.04 (1.00)	—	—	—	—	—	—
<i>IL2RB</i>	rs6000570	G > A	0.47	—	—	—	139	1.96	.05 (1.00)	—	—	—	—	—	—
<i>NOS2A</i>	rs4796017	T > C	0.46	82	2.35	.02 (.45)	—	—	—	—	—	—	53	2.33	.02 (.48)
	rs2779252	G > T	0.06	—	—	—	—	—	—	27	-1.98	.05 (1.00)	—	—	—
<i>SFRP1</i>	rs1127379	T > C	0.44	—	—	—	127	-2.11	.03 (.63)	—	—	—	—	—	—
	rs7013368	C > T	0.34	—	—	—	119	-2.21	.03 (.49)	—	—	—	—	—	—
	rs11774662	T > C	0.32	90	-2.03	.04 (.77)	—	—	—	—	—	—	—	—	—
	rs968427	T > C	0.48	—	—	—	—	—	—	108	-2.06	.04 (.71)	—	—	—
	rs921142	T > C	0.42	—	—	—	—	—	—	106	-2.23	.03 (.46)	—	—	—

P values highlighted in bold and underlined indicate that they remain significant after correction for multiple testing.

MAF, Minor allele frequency; N, number of families contributing the statistic; Z, Z score.

*The number of independent SNPs is estimated to be 6 for *ALOX15*, 4 for *CD14*, 8 for *CXCL12*, 21 for *IL2RB*, 8 for *NOS2A*, and 6 for *SFRP1*. The number of independent phenotypes is estimated to be 3.

†Dashes indicate that the test result is not significant ($P > .05$).

this strategy identified 7 associations with asthma or related phenotypes in 1 or more of the samples studied. The genes that showed positive associations included *CD14*, *CXCL12*, and *NOS2A*, which are well known in asthma genetics,⁴ as well as novel genes, such as *ALOX15*, *CD27*, *IL2RB*, and *SFRP1*. However, we applied a correction of our P values to reduce the possibility of false-positive associations. Thus, without minimizing the potential effect of the 5 genes that did not survive correction, only the *ALOX15* and *CD14* associations remained significant. Taken together, these results demonstrate the usefulness, as well as the efficiency, of the microarray approach to identify genes relevant to asthma.

The *ALOX15* association remained significant in 2 of the 4 studied samples and is the first report of its association with asthma phenotypes, thus accentuating our interest in this gene and its biologic pathway. In the CAPPS sample the association found was for rs7217186 and AHR, and in the SAGE sample it

was for the rs2664593 and allergic asthma. In both cases a protective effect of the minor allele was suggested. The *ALOX15* gene, located on chromosome 17p13.3, encodes arachidonate 15-lipoxygenase (15-LO). This enzyme is expressed in several lung cell types, including human airway epithelial cells,³³ alveolar macrophages,^{34,35} and neutrophils,³⁶ and converts arachidonic acid into 15(S)-hydroxyeicosatetraenoic acid (15[S]-HETE), a major metabolite of the 15-LO pathway. Alterations in the 15-LO pathway have been suggested as potential contributors to the pathogenesis of asthma,^{34,37} but a protective anti-inflammatory role has also been proposed.

First, 15-LO could act through the uptake of soluble 15(S)-HETE into the cellular membrane phospholipids, contributing to a decrease in the pericellular concentration of this proinflammatory mediator.^{33,34} Second, 15-LO could have a protective effect through lipoxin A₄ biosynthesis (from 15[S]-HETE), which exerts diverse inhibitory mechanisms to protect against AHR and

TABLE VI. Significant odds ratios for TagSNPs of the studied candidate genes and asthma, atopy, AHR, and allergic asthma phenotypes in the Busselton case-control cohort

Gene	TagSNP	Base change	MAF	Asthma		Atopy		AHR		Allergic asthma	
				OR	P value (corrected P value)*	OR	P value (corrected P value)	OR	P value (corrected P value)	OR	P value (corrected P value)
<i>ALOX15</i>	rs7217186	A > G	0.46	—†	—	0.83	.026 (.39)	—	—	0.82	.05 (.73)
	rs2664593	G > C	0.22	—	—	1.25	.028 (.42)	—	—	—	—
<i>CD14</i>	rs778583	C > T	0.28	—	—	—	—	0.72	.008 (.071)	—	—
<i>CD27</i>	rs7970260	A > C	0.38	—	—	—	—	1.30	.02 (.27)	—	—
<i>IL2RB</i>	rs3136551	A > G	0.04	0.59	.008 (.113)	—	—	—	—	0.45	.004 (.056)
	rs3218312	A > G	0.23	—	—	—	—	0.76	.04 (1.00)	—	—
	rs228954	A > G	0.44	—	—	—	—	1.32	.01 (.46)	—	—
	rs2281094	C > T	0.27	—	—	—	—	1.31	.03 (1.00)	—	—
<i>NOS2A</i>	rs2531860	C > T	0.15	—	—	—	—	—	—	1.33	.05 (.95)

MAF, Minor allele frequency; OR, 95% CI odds ratio.

*The number of independent SNPs is estimated to be 5 for *ALOX15*, 3 for *CD14*, 5 for *CD27*, 14 for *IL2RB*, and 7 for *NOS2A*. The number of independent phenotypes is estimated to be 3.†Dashes indicate that the test result is not significant ($P > .05$).

asthmatic inflammation.^{35,38} Finally, 15-LO could act through the inhibition of the chemotactic mediator leukotriene B₄, which modulates neutrophilic trafficking and infiltration.^{36,39} Thus the protective association we report for *ALOX15* rs2664593 and rs7217186 with respect to allergic asthma and AHR could be explained by these anti-inflammatory roles of the 15-LO pathway. Obviously, this hypothesis remains speculative because further functional investigations of the *ALOX15* gene in inflammation are needed to delineate its role in the pathophysiology of asthma.

The variability in association patterns among the 4 studies might reflect the differences in their respective designs,¹⁸ as well as genetic and environmental heterogeneity. Indeed, the recruitment of the subjects differed between the studies: the SLSJ probands were recruited at a mean age of 18 years; the CAPPS subjects were recruited prenatally based on a family history of asthma, allergy, or both; the SAGE subjects were ascertained as part of a population-based sample; and the Busselton cohort is a study of adult asthmatic subjects. Because the natural history of asthma shows considerable variability implying complex pathologic mechanisms,^{40,41} the variable recruitment strategies could have resulted in subjects who are at different disease stages or actual phenotypic subsets (eg, childhood transient vs persistent asthma). Because different genes might predispose to these different phenotypes, this could be a cause of heterogeneous results. Another potential source of different association patterns is variable LD patterns for the studied SNPs across the populations.⁴² The SLSJ sample is recognized as a founder population,^{11,12,43-46} whereas for the CAPPS, SAGE, and Busselton studies, individuals come from admixed Canadian or Australian populations. These populations present variable *ALOX15* LD patterns, such that a polymorphism might be in LD with a nearby disease allele in the CAPPS or SAGE samples but not in the SLSJ or Busselton studies. A last possible factor that could explain, in part, the different associations found for *ALOX15* is population-specific gene-gene and gene-environment interactions.⁴² This possibility is also likely to explain the unique *CD14* association found in the SAGE sample. In fact, a literature review reports *CD14* as a classical example of gene-environment interactions for asthma and atopy phenotypes, and to date, numerous positive and

negative associations have been reported across diverse populations.⁴⁷

In conclusion, we believe that hypothesis-driven candidate gene selection from microarray experiments is a methodological approach that improves the likelihood of finding positive and novel asthma associations within a limited number of tests performed. We also think that studying numerous independent asthma samples has the advantage of being able to document the biologic relevance of candidate genes across diverse populations living in different environments. Based on the results presented here, we believe that *ALOX15* represents an interesting potential candidate for further study.

We thank the participants in each asthma study presented in this report.

Key messages

- This study demonstrates the efficiency of the microarrays in the identification of asthma candidate genes.
- The findings suggest a novel asthma-protective role for the gene encoding 15-LO.
- Because 15-LO is involved in anti-inflammatory processes, functional and clinical investigation of its role in asthma is warranted.

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METHODS

The SLSJ familial sample

The SLSJ region of northeastern Quebec, Canada, is inhabited by 287,000 individuals who descend predominantly from approximately 2500 founders originating from France who settled in Quebec in the 17th century.^{E1} The population grew at a high rate with little admixture over 12 to 14 generations and is an example of a young founder population.^{E2} Recruitment occurred through media advertisement (newspaper and radio) and from the Chicoutimi Hospital's specialized clinics (pediatric and respiratory). To be included, probands were required to fulfill at least 2 of the following criteria: (1) a minimum of 3 clinic visits for acute asthma within 1 year; (2) 2 or more asthma-related hospital admissions within 1 year; or (3) steroid dependency, as defined by either 6 months of oral or 1 year of inhaled corticosteroid use. Families were included in the study if parents were available for phenotypic assessment, if at least 1 parent was unaffected, and if all 4 grandparents were of French-Canadian origin. The family participation rate was about 60%. A total of 223 independent families with family size ranging from 3 to 17 and the number of affected family members ranging from 1 to 10 were analyzed.

The affection status of the SLSJ subjects was determined by means of clinical evaluation and the completion of a standard respiratory questionnaire that was modified to include questions about asthma and atopy severity; family history of asthma, atopy, or both; age of onset; and presence of other respiratory diseases.^{E3} In 41 cases the age of onset described by parents was less than 2 years; because of the uncertainty of this information, we used a default class of 2 years. Measurements of expiratory flow were done with a Morgan spirometer (Morgan Spiro 232; PK Morgan Ltd), according to the American Thoracic Society's recommendations.^{E4} Bronchodilator response was measured as the increase in FEV₁ at 15 minutes after a 200-mg dose of salbutamol was inhaled. Peak expiratory flow rates were measured in the morning and evening over a period of 2 weeks by using a mini-Wright peak flowmeter (Armstrong Medical, Lincolnshire, Ill). The best of 3 repeated measurements was recorded on a diary card. Skin prick tests were performed for 24 common airborne allergens, which were divided into 6 main categories: (1) cat, dog, horse, cow, and feathers; (2) dust; (3) *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; (4) grass, weed, ambrosia, timothy, and ryegrass; (5) tree mix, birch, maple, oak, and elm; and (6) *Cladosporium* species, *Hormodendrum* species, *Alternaria alternata*, *Alternaria tenuis*, *Aspergillus* species, and *Penicillium* species. Serum IgE levels were measured with enzyme immunoassay (QuantiCLONE total IgE kit; Kallestad Diagnostics, Chaska, Minn). Methacholine-based bronchoprovocation tests were performed for participants 12 years and older, according to the method described by Juniper et al.^{E5} The dose of methacholine that resulted in a 20% decrease in FEV₁ was recorded as the PC₂₀. All the tests were performed by the investigators for every participant (except for bronchoprovocation) at the University of Montreal Community Genomic Medicine Centre in Chicoutimi, Canada. Medical charts were also reviewed for any previous function tests and medication. AHR was defined as a PC₂₀ of less than 8 mg/mL. If PC₂₀ results were unavailable, AHR was defined as either an increase in the postbronchodilator FEV₁ of greater than 15% from baseline or a variation of the morning-evening peak expiratory flow rate of greater than 12% over a 2-week period. Participants were defined as asthmatic if (1) they had a reported history of asthma (validated by a physician) or (2) they had asthma-related symptoms and a positive PC₂₀ result. Subjects were deemed atopic if they had at least 1 positive response (wheal diameter ≥ 3 mm at 10 minutes) on skin prick testing. The Chicoutimi University Hospital Ethics Committee approved the study, and subjects provided informed consent.

The CAPPS

The CAPPS was initiated in 1995 to assess the effectiveness of a multifaceted intervention program applied in the first 12 months of life on the primary prevention of asthma and other atopic disorders in high-risk infants.^{E6} Infants at high risk for asthma and other allergic disorders were identified, and their mothers were recruited during their third trimester of pregnancy. Infants at high risk were defined as those who had a parent with asthma or 2 first-degree relatives with allergies or atopic disorders. The study had 2 recruitment centers in Canada (Vancouver and Winnipeg). The

diagnosis of asthma at the 7-year time point^{E7} was made by a pediatric allergist in each center without knowledge of the group allocation status of the children and who did not provide health care services to the families. The physician conducted a structured interview with parents by using a standardized form to record symptoms and physical findings. Spirometry and methacholine challenge testing were performed after obtaining parental consent. The diagnoses of asthma and other atopic disorders were clinical decisions made by the pediatric allergists without knowledge of the results of the questionnaire, allergy skin tests, spirometry, or methacholine challenge tests. Atopy was defined as at least 1 positive response (wheal diameter ≥ 3 mm at 10 minutes) on skin prick tests. Allergy skin tests were performed with the epicutaneous method by using a prick lancet (Hollister-Stier, Omega Laboratories LTD, Montreal, Quebec, Canada) with the following allergens (Hollister-Stier, Omega Laboratories LTD): house dust mite (*D pteronyssinus* and *D farinae*), cat, dog, cockroach, *Alternaria* species, *Cladosporium* species, tree, grass and weed (ragweed) pollens, cow's milk, egg white, wheat, soy, and peanut. Histamine (1 mg/mL) was used as the positive control, and saline was used as the negative control. The largest wheal diameter and its perpendicular were measured at 15 minutes after testing. A mean wheal diameter of 3 mm or greater than that elicited by the negative control was considered a positive reaction. Atopy was defined as a positive skin test reaction to 1 or more of these common allergens.

Methacholine (Methapharm, Inc, Brantford, Ontario, Canada) challenge testing was carried out according to the protocol of Cockcroft et al.^{E8} Two children who had FEV₁ values of less than 70% of predicted value were excluded from methacholine testing, but both of them had a postbronchodilator change in FEV₁ of 12% or greater, and they were considered to have AHR. The methacholine PC₂₀ value was determined. The Ethics Committees of the University of British Columbia and the University of Manitoba approved the study, and parents provided written informed consent for participation.

The SAGE

The SAGE is derived from a population-based cohort of 16,320 children born in the province of Manitoba, Canada, between January 1, 1995, and December 31, 1995. A survey on child health and home environment exposures was mailed to the parents of this birth cohort in 2002. After parent response to the mailed survey and approval to be contacted, a subset of children was invited to join the study at age 8 to 10 years. This included all children with parent-declared asthma and a comparable number of children without asthma who were randomly selected after stratification of the received surveys by urban/rural location and family income. All recruited children underwent clinical assessment for asthma by a pediatric allergist (AB) on the basis of the Canadian Asthma Consensus Guidelines. To aid in the diagnosis, a standardized history was used, including questions on cough, wheeze, shortness of breath, response to current medications (ie, bronchodilators and corticosteroids), and the presence of other allergic conditions (eg, allergic rhinitis, atopic dermatitis, and food allergies). Physical examination included examination for chest symptoms (hyperinflation, wheeze, prolonged expiration, and decreased breath sounds). Hospitalization and medical visits for breathing difficulty in the past year were also noted. All findings were recorded and a diagnosis of asthma in the last 12 months was made. In total, 723 study trios (child and both parents) were recruited, 247 of which have a child affected with asthma. A child affected with asthma was defined as follows: pediatric allergist diagnosis of asthma based on history and physical examination. AHR was assessed in all children after a methacholine challenge test (Methapharm, Inc, Brantford, Ontario Canada) carried out according to the protocol of Cockcroft et al.^{E8} The children with FEV₁ values of less than 70% of predicted value had reversibility testing with an aerosol bronchodilator. Reversibility was defined as an improvement in FEV₁ by greater than 12% after inhalation of a β_2 -adrenergic agonist. Bronchoprovocation was not attempted for these children. Atopic status was determined for all children; skin prick tests to common relevant allergens were performed (tree pollen mix, weed pollen mix, ragweed [separate], grass pollen mix, *Alternaria* species, *Cladosporium* species, *Penicillium* species, house dust mites [*D pteronyssinus* and *D farinae*], cockroach, cat, dog, feathers, and peanut), and atopy was defined as at least 1 positive response (wheal diameter ≥ 3 mm at 10 minutes). For

children in Winnipeg and the immediate surroundings, the assessment was carried out in the investigator's laboratories in the Manitoba Institute of Child Health. For children in remote and northern communities, a team travelled to the communities to assess children in and surrounding those communities. The study was approved by the Ethics Committee of the University of Manitoba, and parents provided written informed consent for participation.

The Busselton Health Study population (Busselton cohort)

Residents of the town of Busselton in the southwest of Western Australia have been involved in a series of health surveys since 1966. The population is predominantly of European origin. In the present nested case-control study, all subjects ($n = 1599$, of which 1549 were sent for genotyping [679 cases and 870 control subjects]) who attended any of 6 cross-sectional surveys from 1996 to 1981 and the follow-up survey in 1994 and who had available DNA were included. Subjects were considered to have asthma if they answered yes to the question "Has your doctor ever told you that you had asthma/bronchial asthma?" at any survey. All other subjects were considered control subjects. The panel includes a sample of allergic and nonallergic individuals, as determined based on skin prick tests to common allergens. Methacholine challenge tests using a modification of the Yan method^{E9} were performed on all participants of the 1994 follow-up survey. The early dose-response slope with PD₂₀ was determined.

Pairwise LD pattern

The strength of LD between pairs of SNPs in each gene has been measured as D' ^{E10} by using Haploview software (3.32 version).^{E11} Figs E1 to E8 present these LD patterns for each studied gene in each studied sample.

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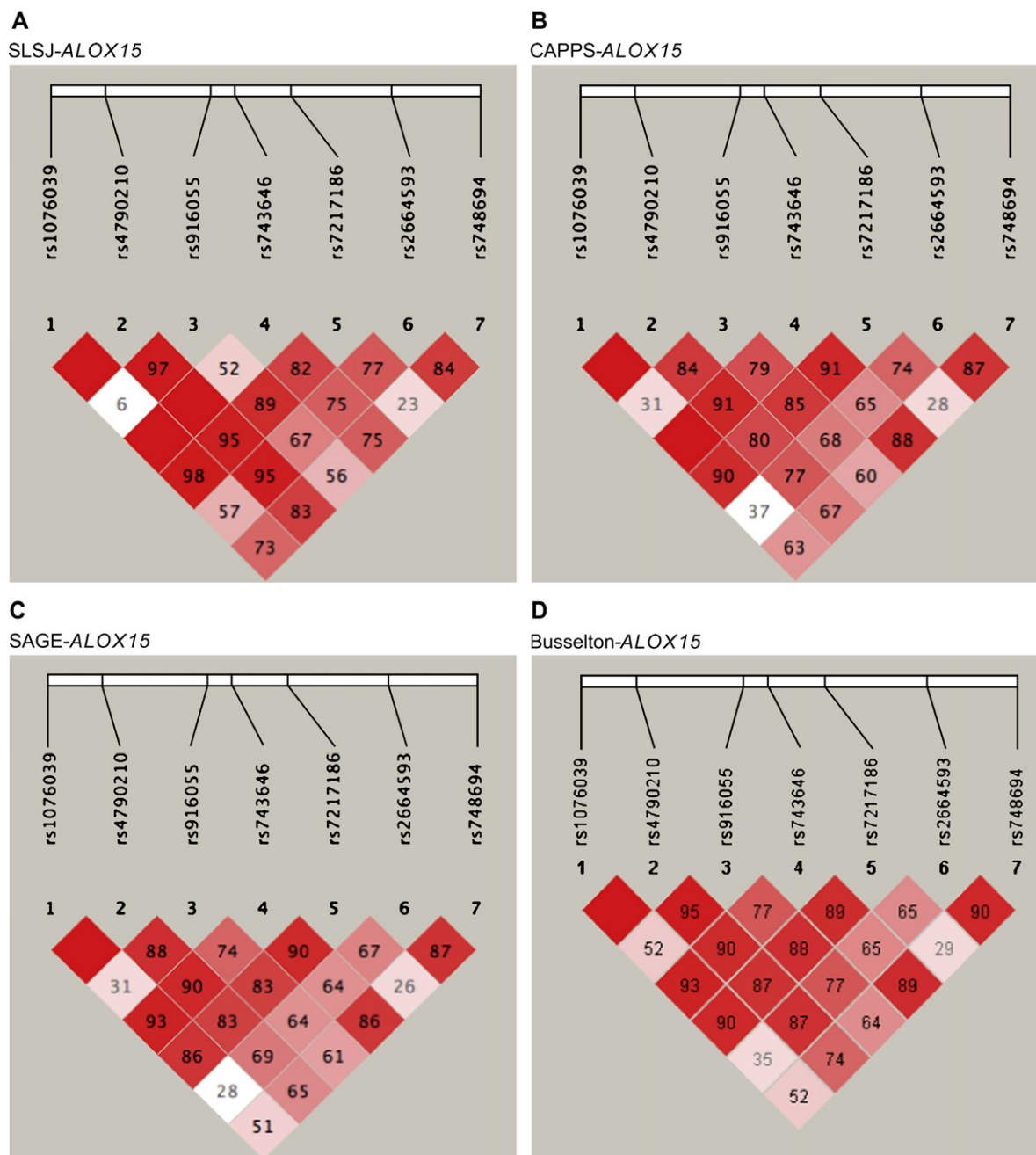


FIG E1. LD pattern for *ALOX15*. **A**, *ALOX15* LD pattern in the SLSJ familial sample. **B**, *ALOX15* LD pattern in the CAPPS cohort. **C**, *ALOX15* LD pattern in the SAGE cohort. **D**, *ALOX15* LD pattern in the Busselton cohort. The location of each tested TagSNP along the chromosome is indicated in the upper part of the figure. The number in each diamond indicates the magnitude of LD (D') as a percentage between respective pairs of SNPs. The strength of LD is depicted by progression of color: the color moves from red to white as D' runs from 1 to 0.

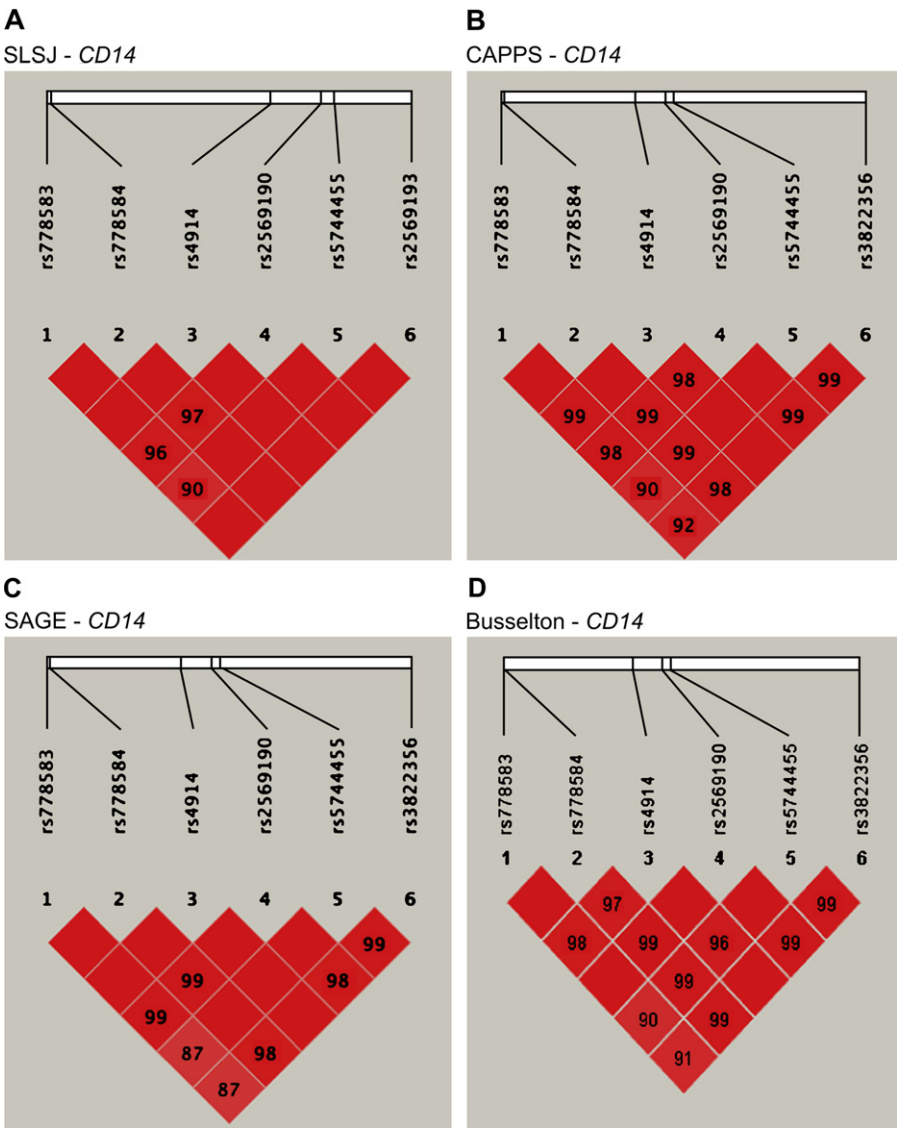


FIG E2. LD pattern for *CD14*. **A**, *CD14* LD pattern in the SLSJ familial sample. **B**, *CD14* LD pattern in the CAPPS cohort. **C**, *CD14* LD pattern in the SAGE cohort. **D**, *CD14* LD pattern in the Busseton cohort. The location of each tested TagSNP along the chromosome is indicated in the upper part of the figure. The number in each diamond indicates the magnitude of LD (D') as a percentage between respective pairs of SNPs. The strength of LD is depicted by progression of color: the color moves from red to white as D' runs from 1 to 0.

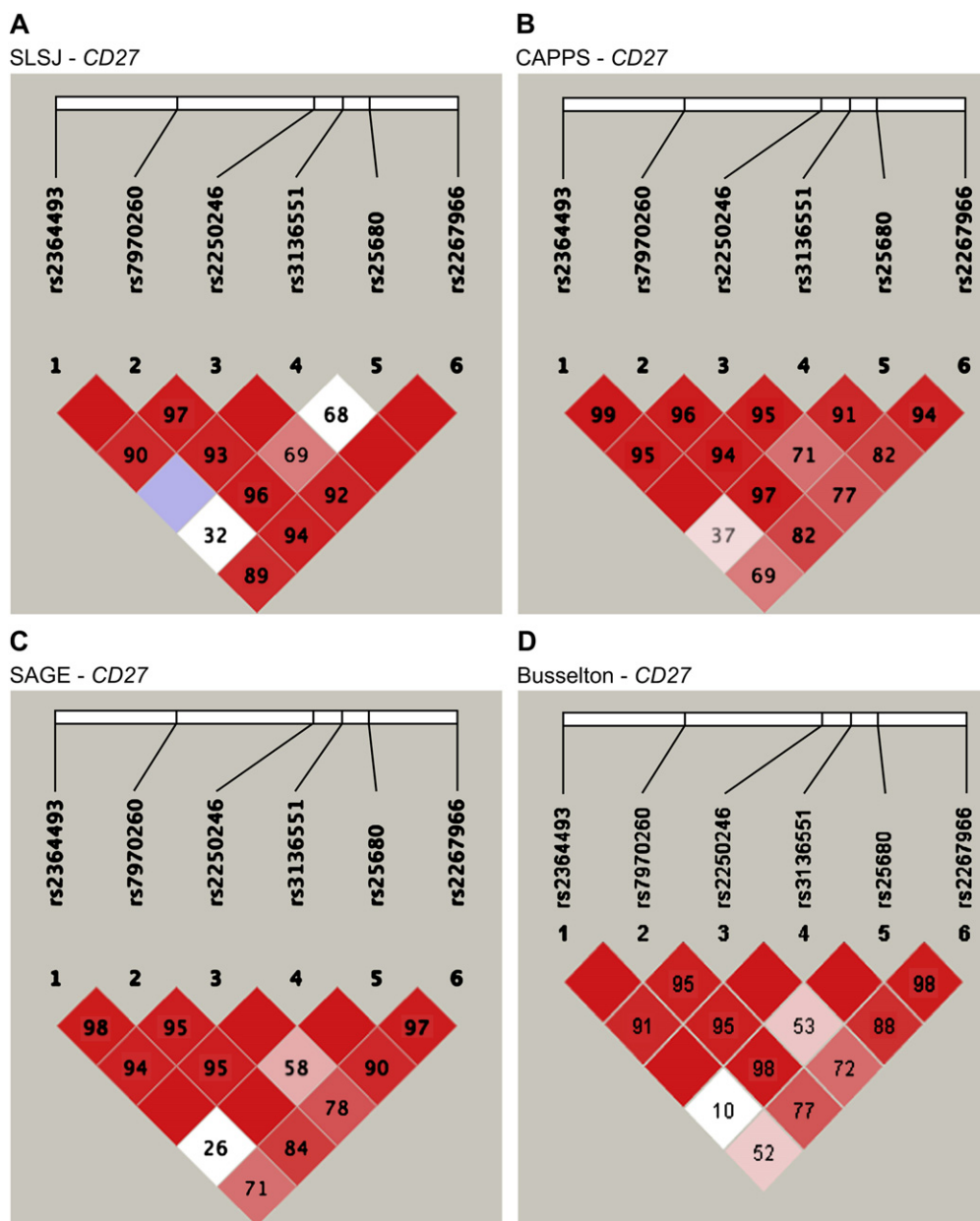


FIG E3. LD pattern for *CD27*. **A**, *CD27* LD pattern in the SLSJ familial sample. **B**, *CD27* LD pattern in the CAPPS cohort. **C**, *CD27* LD pattern in the SAGE cohort. **D**, *CD27* LD pattern in the Busselton cohort. The location of each tested TagSNP along the chromosome is indicated in the upper part of the figure. The number in each diamond indicates the magnitude of LD (D') as a percentage between respective pairs of SNPs. The strength of LD is depicted by progression of color: the color moves from red to white as D' runs from 1 to 0.

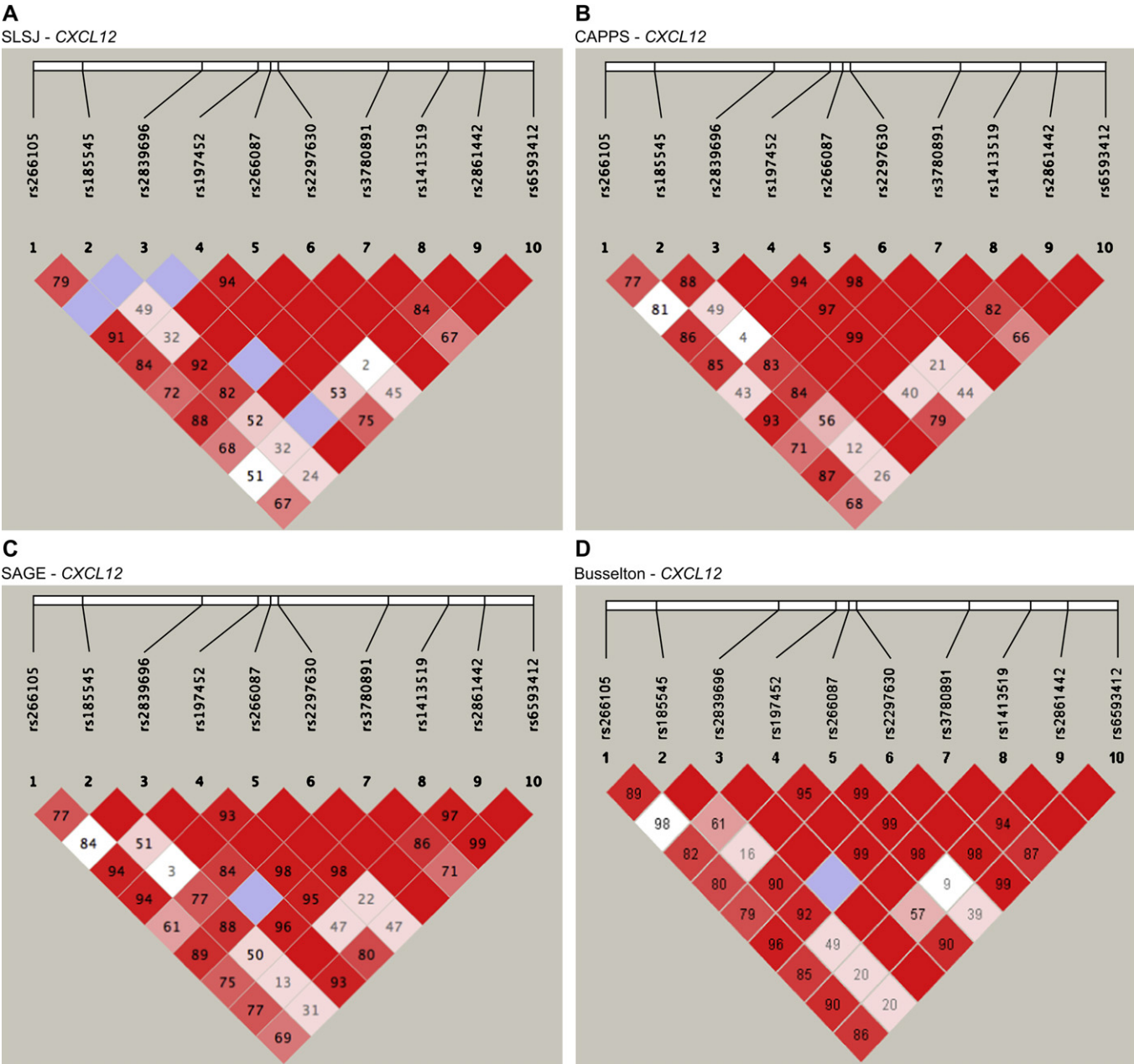


FIG E4. LD pattern for *CXCL12*. **A**, *CXCL12* LD pattern in the SLSJ familial sample. **B**, *CXCL12* LD pattern in the CAPPS cohort. **C**, *CXCL12* LD pattern in the SAGE cohort. **D**, *CXCL12* LD pattern in the Busselton cohort. The location of each tested TagSNP along the chromosome is indicated in the upper part of the figure. The number in each diamond indicates the magnitude of LD (D') as a percentage between respective pairs of SNPs. The strength of LD is depicted by progression of color: the color moves from red to white as D' runs from 1 to 0.

A

SLSJ - *IL2RB*

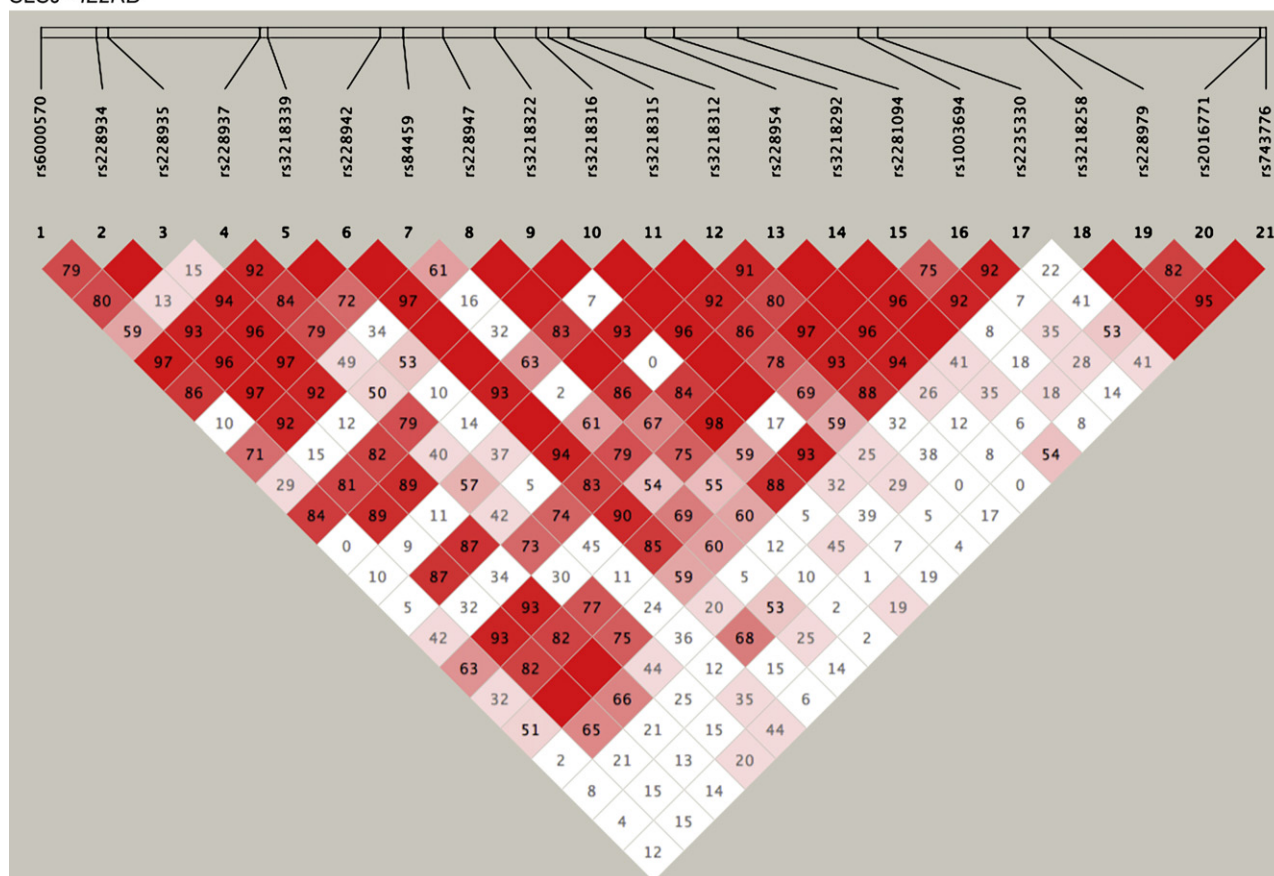


FIG E5. LD pattern for *IL2RB*. **A**, *IL2RB* LD pattern in the SLSJ familial sample. **B**, *IL2RB* LD pattern in the CAPPS cohort. **C**, *IL2RB* LD pattern in the SAGE cohort. **D**, *IL2RB* LD pattern in the Busselton cohort. The location of each tested TagSNP along the chromosome is indicated in the upper part of the figure. The number in each diamond indicates the magnitude of LD (D') as a percentage between respective pairs of SNPs. The strength of LD is depicted by progression of color: the color moves from red to white as D' runs from 1 to 0.

B
CAPPS - *IL2RB*

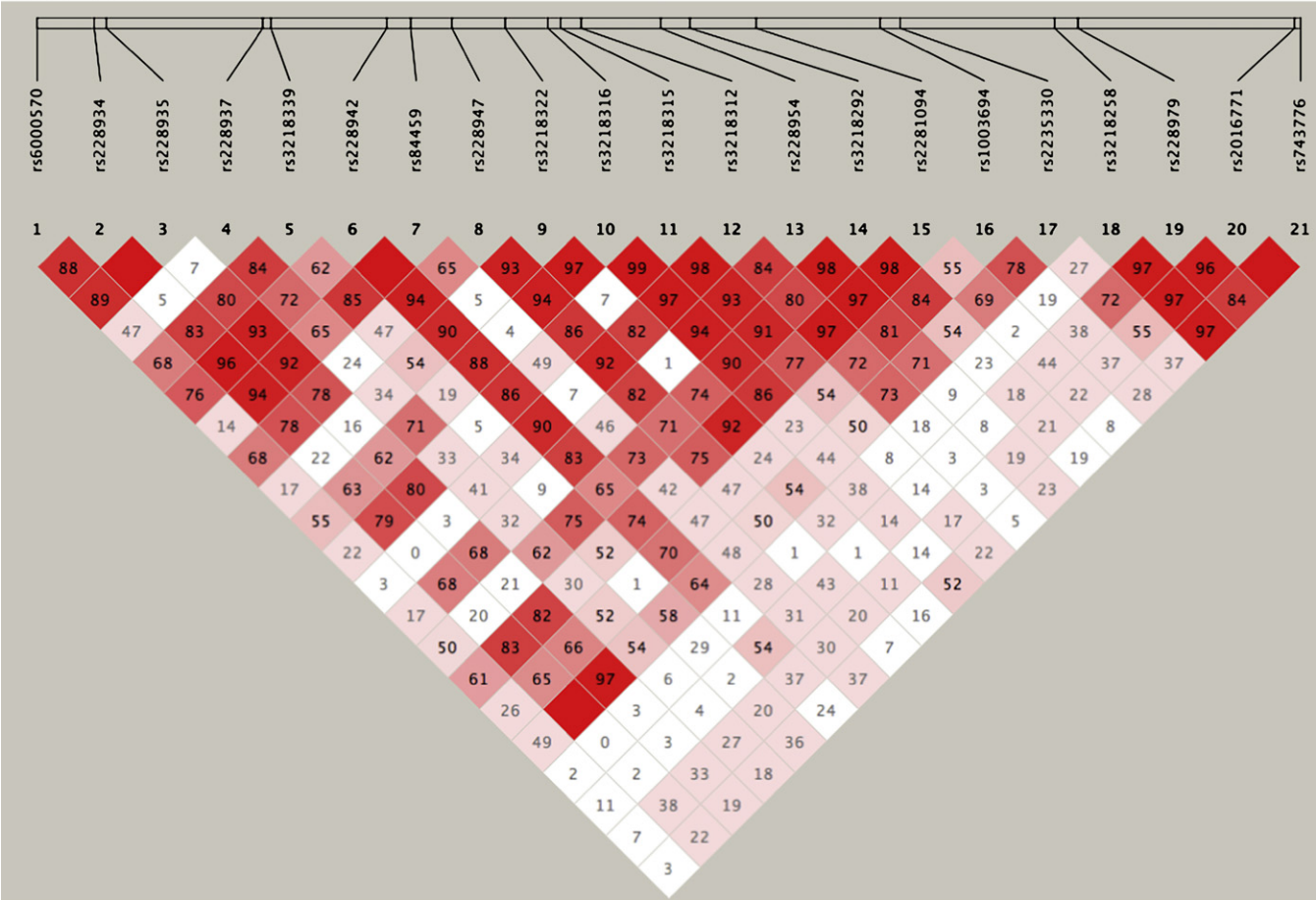


FIG E5. (Continued).

C

SAGE - *IL2RB*

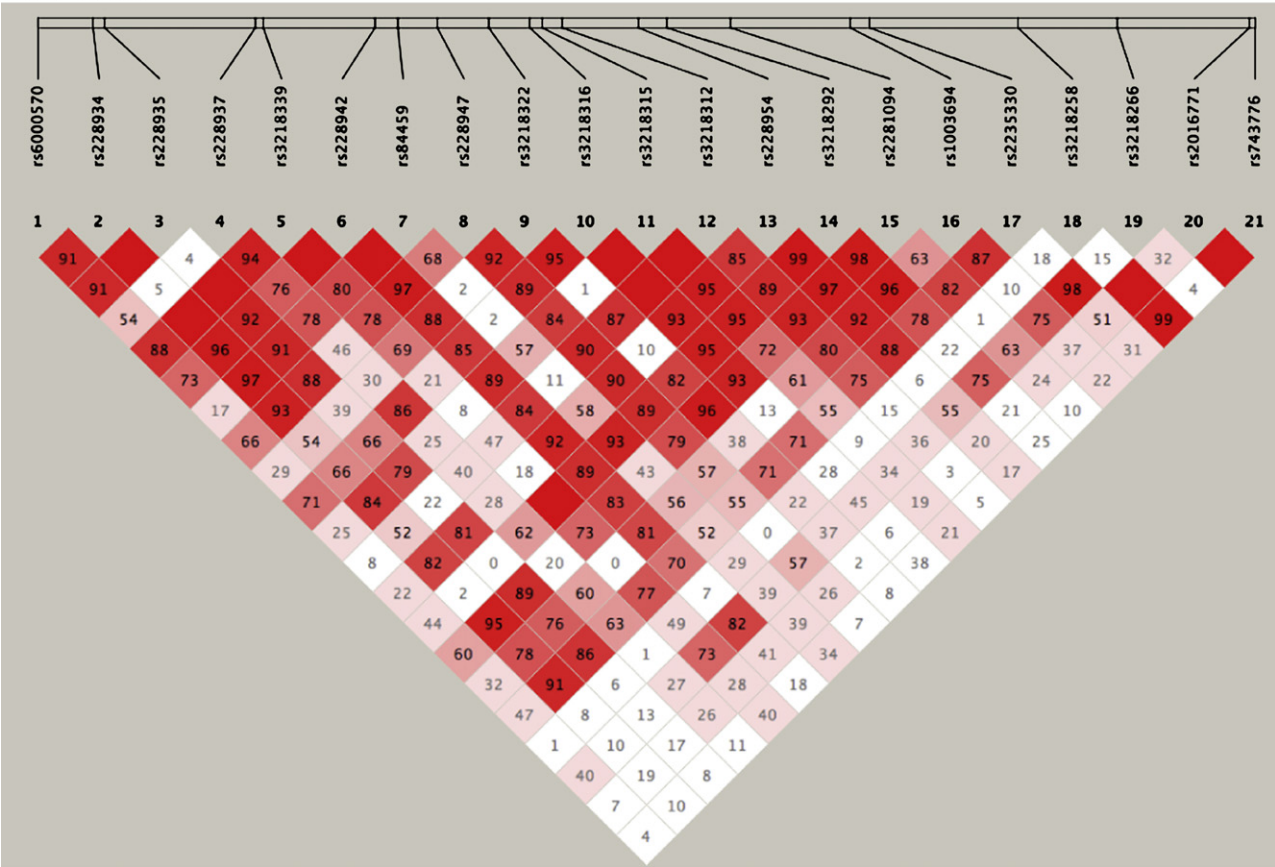


FIG E5. (Continued).

D
Busselton - *IL2RB*

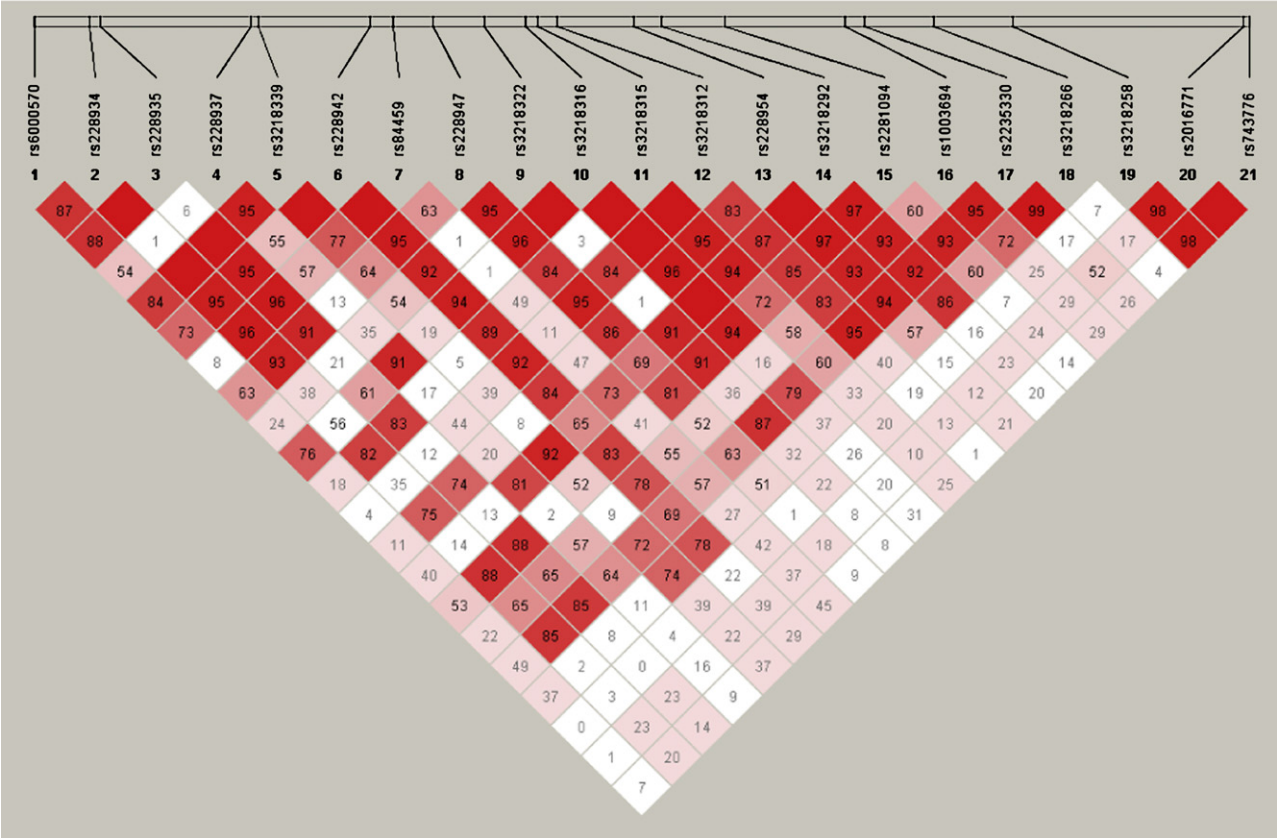


FIG E5. (Continued).

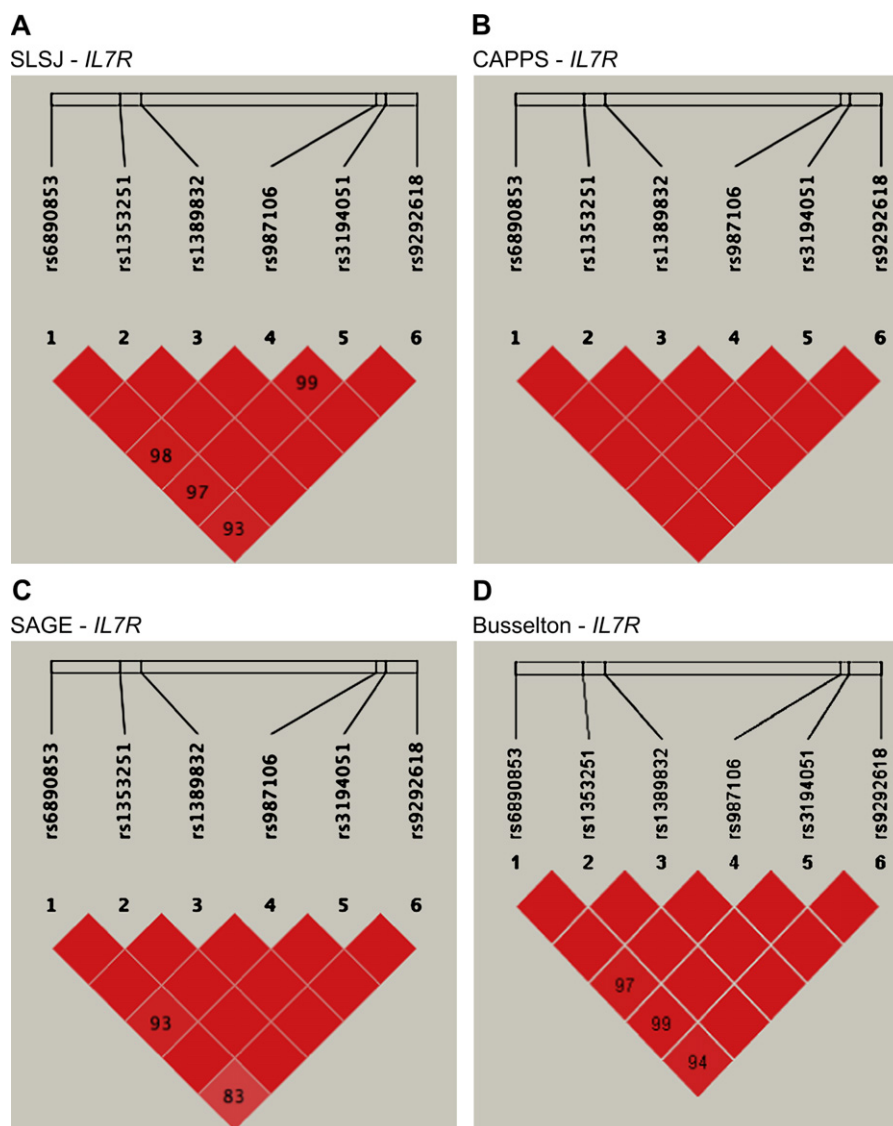


FIG E6. LD pattern for *IL7R*. **A**, *IL7R* LD pattern in the SLSJ familial sample. **B**, *IL7R* LD pattern in the CAPPS cohort. **C**, *IL7R* LD pattern in the SAGE cohort. **D**, *IL7R* LD pattern in the Busselton cohort. The location of each tested TagSNP along the chromosome is indicated in the upper part of the figure. The number in each diamond indicates the magnitude of LD (D') as a percentage between respective pairs of SNPs. The strength of LD is depicted by progression of color: the color moves from red to white as D' runs from 1 to 0.

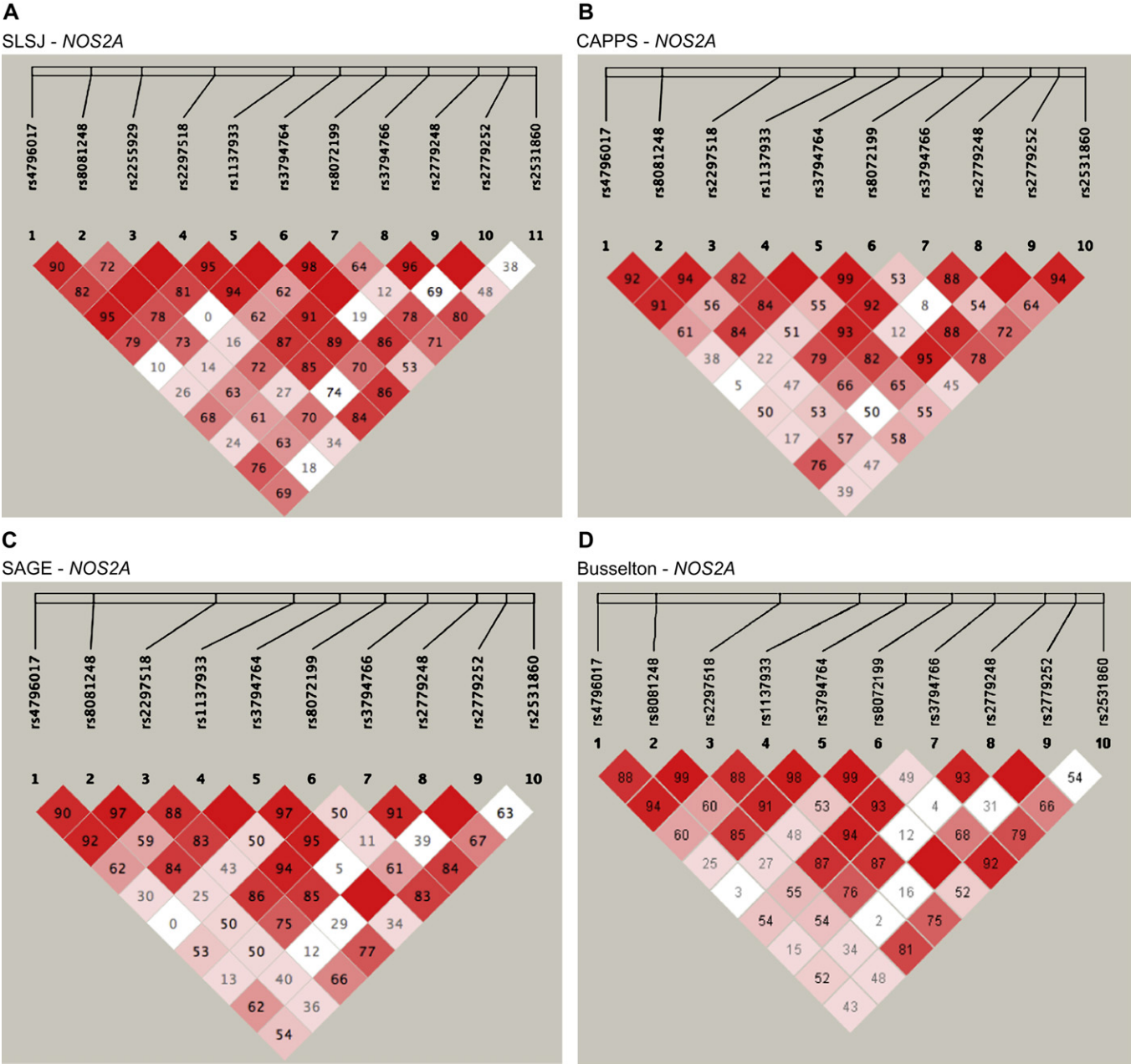


FIG E7. LD pattern for *NOS2A*. **A**, *NOS2A* LD pattern in the SLSJ familial sample. **B**, *NOS2A* LD pattern in the CAPPS cohort. **C**, *NOS2A* LD pattern in the SAGE cohort. **D**, *NOS2A* LD pattern in the Busselton cohort. The location of each tested TagSNP along the chromosome is indicated in the upper part of the figure. The number in each diamond indicates the magnitude of LD (D') as a percentage between respective pairs of SNPs. The strength of LD is depicted by progression of color: the color moves from red to white as D' runs from 1 to 0.

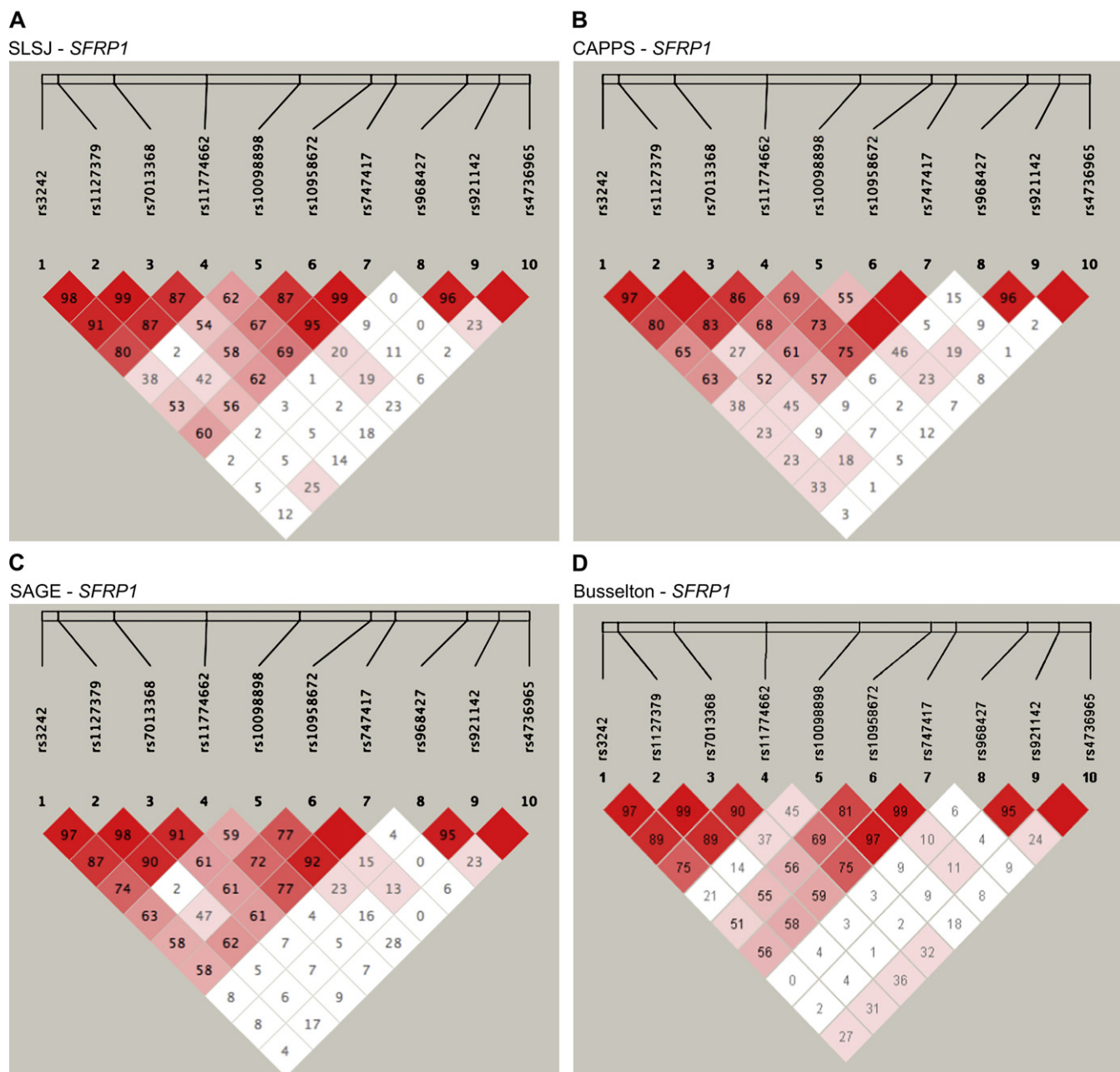


FIG E8. LD pattern for *SFRP1*. **A**, *SFRP1* LD pattern in the SLSJ familial sample. **B**, *SFRP1* LD pattern in the CAPPS cohort. **C**, *SFRP1* LD pattern in the SAGE cohort. **D**, *SFRP1* LD pattern in the Busselton cohort. The location of each tested TagSNP along the chromosome is indicated in the upper part of the figure. The number in each diamond indicates the magnitude of LD (D') as a percentage between respective pairs of SNPs. The strength of LD is depicted by progression of color: the color moves from red to white as D' runs from 1 to 0.

TABLE E1. FBAT values for all TagSNPs of the SLSJ sample that generated significant associations in any 1 population for 1 or more of the studied phenotypes

Gene	TagSNP	Base change	MAF	Asthma			Atopy			AHR			Allergic asthma		
				N	Z	P value	N	Z	P value	N	Z	P value	N	Z	P value
<i>ALOX15</i>	rs4790210	T > C	0.18	102	0.96	.34	86	0.53	.60	88	0.33	.74	90	0.53	.60
	rs916055	T > C	0.35	136	-0.55	.58	115	-0.40	.69	116	-0.68	.50	115	-0.70	.49
	rs743646	T > C	0.10	59	-0.12	.90	45	0.00	1.00	48	0.26	.80	48	0.20	.84
	rs7217186	A > G	0.47	130	0.68	.49	115	1.13	.26	113	0.44	.66	114	0.64	.52
<i>CD14</i>	rs2664593	G > C	0.19	95	0.31	.75	77	0.33	.74	79	1.12	.26	82	0.45	.65
	rs778583	C > T	0.26	113	0.76	.45	91	0.39	.70	93	0.07	.94	95	0.85	.39
	rs4914	G > C	0.11	64	-0.88	.38	54	-0.60	.55	53	-1.15	.25	54	-0.41	.68
<i>CD27</i>	rs7970260	A > C	0.45	117	0.40	.69	93	0.38	.71	97	0.97	.33	94	0.34	.74
	rs3136551	A > G	0.04	29	0.43	.67	24	0.57	.57	26	0.65	.52	24	0.00	1.00
<i>CXCL12</i>	rs2861442	A > G	0.25	113	-0.47	.64	96	-0.78	.44	92	-0.85	.40	98	-0.74	.46
<i>IL2RB</i>	rs6000570	G > A	0.42	117	0.85	.39	102	1.34	.18	99	0.94	.35	103	1.38	.17
	rs3218312	A > G	0.19	83	-1.54	.12	68	-1.17	.24	65	-1.49	.14	70	-0.97	.33
	rs228954	A > G	0.42	118	-0.10	.92	105	0.66	.51	106	-0.25	.80	105	0.78	.43
	rs2281094	C > T	0.25	109	-0.40	.69	97	0.46	.65	95	-0.44	.66	101	0.14	.89
	rs228979*	C > A	0.27	109	-1.59	.11	92	-2.10	.0358 (>1.0)	92	-1.20	.23	94	-2.29	.0220 (.9240)
	rs2016771	G > T	0.34	128	2.26	.0236 (.9912)†	109	1.46	.14	106	1.36	.17	113	1.61	.11
<i>NOS2A</i>	rs4796017	T > C	0.41	123	-1.60	.11	111	-1.29	.20	108	-1.20	.23	112	-1.44	.15
	rs2297518	G > A	0.22	107	-0.70	.48	96	-0.90	.37	94	-0.07	.94	94	-1.13	.26
	rs3794766	G > A	0.26	127	-1.56	.12	111	-1.38	.17	105	-0.87	.39	109	-2.13	.0334 (.8016)
	rs2779248	A > G	0.45	132	-1.26	.21	112	-1.92	.06	102	-0.51	.61	111	-2.34	.0194 (.4656)
	rs2779252	G > T	0.05	36	0.44	.66	33	0.47	.64	30	0.72	.47	34	0.33	.74
	rs2531860	C > T	0.24	107	-0.40	.69	93	-0.07	.95	92	-0.15	.88	92	-0.10	.92
<i>SFRP1</i>	rs1127379	T > C	0.47	132	-0.43	.67	111	0.04	.97	110	-0.59	.55	116	-0.49	.62
	rs7013368	C > T	0.33	124	0.64	.52	101	0.98	.33	105	0.22	.82	108	0.57	.57
	rs11774662	T > C	0.34	125	1.19	.23	106	1.45	.15	105	1.13	.26	112	1.39	.16
	rs968427	T > C	0.48	130	-0.07	.95	114	-0.59	.55	118	0.25	.80	119	-0.43	.67
	rs921142	T > C	0.38	125	-0.18	.86	110	-0.19	.85	106	0.20	.84	115	-0.43	.66

P values of .05 or less are marked in bold. Gray shading indicates that the TagSNP has at least 1 P value of less than .05. Corrected P values are indicated in parentheses. MAF, Minor allele frequency; N, number of families contributing the statistic; Z, Z score.

*This TagSNP has been tested in SLSJ and CAPPS only.

†The number of independent SNPs is estimated to be 14 for *IL2RB* and 8 for *NOS2A*. The number of independent phenotypes is estimated to be 3.

TABLE E2. FBAT values for all TagSNPs of the CAPPS sample that generated significant associations in any 1 population for 1 or more of the studied phenotypes

Gene	TagSNP	Base change	MAF	Asthma			Atopy			AHR			Allergic asthma		
				N	Z	P value	N	Z	P value	N	Z	P value	N	Z	P value
<i>ALOX15</i>	rs4790210	T > C	0.21	29	-2.61	.0090 (.1620) [†]	61	-2.85	.0044 (.0792)	101	-2.06	.0397 (.7146)	22	-2.75	.0060 (.1080)
	rs916055	T > C	0.34	29	-1.33	.18	78	-1.69	.09	129	-2.59	.0095 (.1710)	21	-1.57	.12
	rs743646	T > C	0.10	26	0.58	.56	44	0.44	.66	66	2.15	.0314 (.5652)	21	0.43	.67
	rs7217186	A > G	0.47	43	-2.21	.0269 (.4842)	99	-1.31	.19	154	-3.24	.0012 (.0216)	32	-1.94	.05
	rs2664593	G > C	0.21	34	1.27	.21	69	0.66	.51	107	1.98	.0482 (.8676)	26	1.06	.29
<i>CD14</i>	rs778583	C > T	0.26	34	1.12	.26	72	-0.21	.83	109	0.88	.38	25	0.56	.58
	rs4914	G > C	0.11	17	0.47	.64	47	-0.71	.48	65	2.02	.0436 (.5232)	12	-0.28	.78
<i>CD27</i>	rs7970260	A > C	0.40	40	0.67	.50	89	0.29	.78	142	-0.73	.47	32	1.07	.29
	rs3136551	A > G	0.05	6	NA	NA	22	0.00	1.00	32	-0.69	.49	4	NA	NA
<i>CXCL12</i>	rs2861442	A > G	0.27	38	0.00	1.00	79	-0.40	.69	123	0.98	.33	29	-0.33	.74
<i>IL2RB</i>	rs6000570	A > G	0.48	40	-0.14	.89	95	-0.64	.52	143	0.58	.56	31	-0.16	.87
	rs3218312	A > G	0.22	32	0.48	.63	67	1.13	.26	112	0.00	1.00	23	1.35	.18
	rs228954	A > G	0.43	42	0.53	.60	84	-1.17	.24	142	1.03	.30	30	-0.16	.87
	rs2281094	C > T	0.25	40	0.83	.41	79	-1.11	.27	119	1.07	.28	29	0.65	.52
	rs228979*	C > A	0.29	41	-1.24	.22	79	-1.58	.11	133	0.08	.94	28	-2.27	.0231 (>1.0)
	rs2016771	G > T	0.40	43	0.79	.43	95	0.36	.72	140	0.74	.46	32	1.07	.29
	rs4796017	T > C	0.43	44	0.94	.35	98	0.62	.54	150	-0.63	.53	32	0.95	.34
<i>NOS2A</i>	rs2297518	G > A	0.19	30	2.14	.0326 (.7824)	68	1.33	.19	110	0.00	1.00	21	2.20	.0278 (.6672)
	rs3794766	G > A	0.23	35	0.95	.34	76	0.52	.60	124	-0.83	.40	25	1.46	.14
	rs2779248	A > G	0.35	45	-0.39	.70	87	0.20	.84	138	-0.83	.41	34	0.15	.88
	rs2779252	G > T	0.08	12	1.16	.25	38	-0.32	.75	51	0.69	.49	9	NA	NA
	rs2531860	C > T	0.15	27	-1.92	.06	43	-1.54	.12	80	-1.56	.12	18	-2.29	.0218 (.5232)
	rs1127379	T > C	0.47	41	0.40	.69	100	-1.69	.09	154	-1.34	.18	30	0.62	.54
<i>SFRP1</i>	rs7013368	C > T	0.36	40	-0.41	.69	89	-1.64	.10	149	-0.91	.36	30	-0.31	.76
	rs11774662	T > C	0.36	40	-1.07	.29	90	-1.81	.07	150	-1.48	.14	28	-1.23	.22
	rs968427	T > C	0.43	44	-0.52	.61	102	0.69	.49	156	-1.10	.27	34	-0.30	.77
	rs921142	T > C	0.38	42	-0.53	.60	103	0.85	.39	150	-1.33	.18	31	-0.46	.65

P values of .05 or less are marked in bold and underlined if they remain significant after correction for multiple testing. Gray shading indicates that the TagSNP has at least 1 *P* value of less than .05. Corrected *P* values are indicated in parentheses.

MAF, Minor allele frequency; N, Number of families contributing the statistic; Z, Z score; NA, not available because the number of families was too low (<10) to contribute to the test.

*This TagSNP has been tested in SLSJ and CAPPS only.

[†]The number of independent SNPs is estimated to be 6 for *ALOX15*, 4 for *CD14*, 21 for *IL2RB*, and 8 for *NOS2A*. The number of independent phenotypes is estimated to be 3.

TABLE E3. FBAT values for all TagSNPs of the SAGE sample that generated significant associations in any 1 population for 1 or more of the studied phenotypes

Gene	TagSNP	Base change	MAF	Asthma			Atopy			AHR			Allergic asthma		
				N	Z	P value	N	Z	P value	N	Z	P value	N	Z	P value
<i>ALOX15</i>	rs4790210	T > C	0.20	63	2.19	.0282 (.5076) [†]	93	1.73	.08	74	2.16	.0310 (.5580)	43	2.38	.0173 (.3114)
	rs916055	T > C	0.35	92	0.83	.41	132	0.31	.76	103	0.69	.49	61	0.78	.44
	rs743646	T > C	0.10	44	-1.13	.26	54	-0.38	.71	45	-0.94	.35	24	-1.35	.18
	rs7217186	A > G	0.48	101	0.61	.54	145	0.58	.56	114	0.16	.87	67	1.39	.16
	rs2664593	G > C	0.23	71	-2.09	.0370 (.6660)	109	-2.23	.0258 (.4644)	84	-2.05	.0404 (.7272)	47	-2.99	.002755 (.04959)
<i>CD14</i>	rs778583	C > T	0.27	75	0.31	.76	106	-0.61	.54	84	-0.19	.85	53	0.87	.39
	rs4914	G > C	0.11	36	-2.92	.0035 (.0420)	51	-1.60	.11	41	-1.68	.09	25	-1.57	.12
<i>CD27</i>	rs7970260	A > C	0.41	90	0.09	.93	127	1.49	.14	100	0.71	.48	58	0.69	.49
	rs3136551	A > G	0.04	14	0.00	1.00	23	0.21	.83	20	0.45	.65	8	NA	NA
<i>CXCL12</i>	rs2861442	A > G	0.26	74	1.92	.06	104	2.03	.0429 (>1.0)	83	1.85	.06	49	1.34	.18
<i>IL2RB</i>	rs6000570	G > A	0.47	105	0.43	.66	139	1.96	.0500 (>1.0)	116	1.24	.22	71	0.42	.67
	rs3218312	A > G	0.20	63	-0.47	.64	99	-0.37	.72	74	0.11	.92	45	-0.54	.59
	rs228954	A > G	0.45	93	-0.71	.48	125	-1.55	.12	106	-1.03	.30	62	-0.66	.51
	rs2281094	C > T	0.31	83	0.78	.44	107	-0.78	.44	87	-0.29	.78	50	1.12	.26
	rs3218266*	C > G	0.38	90	2.44	.0149 (.9387)	129	-0.40	.69	103	0.09	.93	62	1.73	.08
	rs2016771	G > T	0.44	90	1.46	.14	126	-0.39	.70	104	0.42	.67	56	0.93	.35
<i>NOS2A</i>	rs4796017	T > C	0.46	82	2.35	.0187 (.4488)	124	1.64	.10	100	1.29	.20	53	2.33	.0201 (.4824)
	rs2297518	G > A	0.18	68	1.25	.21	88	1.12	.26	69	1.59	.11	42	1.16	.25
	rs3794766	G > A	0.24	75	0.43	.67	105	0.27	.79	90	1.17	.24	47	0.14	.89
	rs2779248	A > G	0.37	92	-1.11	.27	128	-0.62	.54	109	-0.59	.55	58	-0.58	.56
	rs2779252	G > T	0.06	25	-1.13	.26	37	-0.32	.75	27	-1.98	.0482 (>1.0)	17	-1.61	.11
	rs2531860	C > T	0.15	52	-1.03	.30	69	-1.44	.15	58	-1.18	.24	30	-0.51	.61
<i>SFRP1</i>	rs1127379	T > C	0.44	95	-0.79	.43	127	-2.11	.0348 (.6264)	111	-1.10	.27	62	-0.22	.83
	rs7013368	C > T	0.34	93	-1.38	.17	119	-2.21	.0270 (.4860)	100	-1.38	.17	63	-0.56	.57
	rs11774662	T > C	0.32	90	-2.03	.0428 (.7704)	111	-1.58	.11	92	-1.30	.19	58	-0.92	.36
	rs968427	T > C	0.48	99	-0.43	.66	139	1.08	.28	108	-2.06	.0392 (.7056)	71	0.31	.76
	rs921142	T > C	0.42	96	-0.72	.47	135	0.68	.50	106	-2.23	.0258 (.4644)	68	0.32	.75

P values of .05 or less are marked in bold and underlined if they remain significant after correction for multiple testing. Gray shading indicates that the TagSNP has at least 1 P value of less than .05. Corrected P values are indicated in parentheses.

MAF, Minor allele frequency; N, number of families contributing the statistic; Z, Z score; NA, not available because the number of families is too low (<10) to contribute to the test.

*This TagSNP has been tested in the SAGE and Busselton cohorts only.

†The number of independent SNPs is estimated to be 6 for *ALOX15*, 4 for *CD14*, 8 for *CXCL12*, 21 for *IL2RB*, 8 for *NOS2A*, and 6 for *SFRP1*. The number of independent phenotypes is estimated to be 3.

TABLE E4. Odds ratios for all TagSNPs of the Busselton cohort that generated significant associations in any 1 population for 1 or more of the studied phenotypes

Gene	TagSNP	Base change	MAF	Asthma		Atopy		AHR		Allergic asthma	
				OR	P value	OR	P value	OR	P value	OR	P value
<i>ALOX15</i>	rs4790210	T > C	0.18	0.99	.89	0.89	.28	0.83	.21	0.91	.47
	rs916055	T > C	0.31	0.99	.92	0.85	.07	0.90	.37	0.86	.15
	rs743646	T > C	0.12	1.00	.98	1.07	.61	1.13	.48	1.04	.81
	rs7217186	A > G	0.46	0.95	.47	0.83	.026 (.39)	0.90	.35	0.82	.0488 (.7320)
	rs2664593	G > C	0.22	1.10	.28	1.25	.028 (.42)	1.18	.19	1.26	.06
<i>CD14</i>	rs778583	C > T	0.28	1.03	.71	0.91	.32	0.72	.0079 (.0711)	0.91	.43
	rs4914	G > C	0.12	1.14	.27	1.03	.80	0.90	.5410	1.09	.57
<i>CD27</i>	rs7970260	A > C	0.38	0.99	.94	1.02	.81	1.30	.0182 (.2730)	1.00	1.00
	rs3136551	A > G	0.04	0.59	.0075 (.1125)†	0.71	.10	1.14	.63	0.45	.0037 (.0555)
<i>CXCL12</i>	rs2861442	A > G	0.31	0.95	.51	0.94	.49	0.98	.86	0.91	.3902
<i>IL2RB</i>	rs6000570	G > A	0.49	1.04	.65	1.08	.38	1.09	.43	0.90	.28
	rs3218312	A > G	0.23	1.07	.47	1.02	.88	0.76	.0393 (>1.0)	1.04	.74
	rs228954	A > G	0.44	1.10	.20	1.06	.47	1.32	.0108 (.4536)	1.14	.19
	rs2281094	C > T	0.27	1.10	.27	1.19	.06	1.31	.0255 (>1.0)	1.25	.0513
	rs3218266*	C > G	0.33	1.03	.70	1.14	.15	1.20	.11	1.17	.14
	rs2016771	G > T	0.41	1.01	.95	1.03	.75	1.00	.98	1.03	.79
	rs4796017	T > C	0.43	1.03	.75	1.02	.84	0.96	.69	1.02	.85
<i>NOS2A</i>	rs2297518	G > A	0.19	1.06	.58	0.98	.84	0.85	.27	1.05	.71
	rs3794766	G > A	0.23	1.15	.12	1.07	.48	0.86	.27	1.19	.15
	rs2779248	A > G	0.37	1.01	.90	1.13	.14	0.97	.80	1.14	.20
	rs2779252	G > T	0.06	1.26	.16	0.94	.72	0.72	.20	1.13	.58
	rs2531860	C > T	0.15	1.15	.18	1.18	.16	1.15	.35	1.33	.0454 (.9534)
	rs1127379	T > C	0.42	0.92	.26	1.01	.95	1.19	.11	0.94	.57
<i>SFRP1</i>	rs7013368	C > T	0.37	0.90	.17	1.02	.82	1.01	.93	0.93	.50
	rs11774662	T > C	0.36	0.89	.16	0.98	.81	0.96	.75	0.89	.28
	rs968427	T > C	0.44	1.06	.45	1.04	.66	1.01	.93	1.05	.62
	rs921142	T > C	0.39	1.04	.63	1.03	.73	0.94	.56	1.04	.71

P values of .05 or less are marked in bold. Gray shading indicates that the TagSNP has at least 1 P value of less than .05. Corrected P values are indicated in parentheses.

MAF, Minor allele frequency; OR, 95% CI odd ratio.

*This TagSNP has been tested in the SAGE and Busselton cohorts only.

†The number of independent SNPs is estimated to be 5 for *ALOX15*, 3 for *CD14*, 5 for *CD27*, 14 for *IL2RB*, and 7 for *NOS2A*. The number of independent phenotypes is estimated to be 3.