Genes and Immunity (2006) 7, 632–639

www.nature.com/gene

ORIGINAL ARTICLE

Association study between the CX3CR1 gene and asthma

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CX3CR1, a fractalkine receptor, mediates cell-adhesive and migratory functions in inflammation. Based on CX3CR1 expression observed in bronchial tissues of asthmatic subjects, we hypothesized that genetic variation at this locus may affect susceptibility to asthma. We carried out an association study and a haplotypic analysis with selected polymorphisms of the CX3CR1 in a familial asthmatic sample from a founder population. Genetic analyses performed by FBAT software showed five CX3CR1 single nucleotide polymorphisms (rs938203, rs2669849, rs1050592, T280M and V249I) with significant associations between their common alleles and asthma ($P < 0.004$) in a dominant model. A haplotype formed with common alleles of rs1050592, T280M and V249I is also overtransmitted in asthmatic subjects ($P = 0.005$) under a dominant model. The associations of V249I and rs2669849 have been validated in an independent case–control sample. For V249I, odds ratios (OR) are 2.16 (common homozygous) and 2.11 (heterozygous) in dominant model ($P = 0.031$). For rs2669849, OR are 2.75 (common homozygous) and 1.86 (heterozygous) in additive model ($P = 0.007$) and dominant model ($P = 0.059$). These results suggest an asthma protective effect of the minor alleles in healthy control carriers. Further functional studies of CX3CR1 are needed to document its role in the pathophysiology of asthma.
Genes and Immunity (2006) **7,** 632–639. doi:10.1038/sj.gene.6364340; published online 2 November 2006

Keywords: *association study; asthma; CX3CR1; polymorphisms; haplotypic analyses*

Introduction

Asthma, a multifactorial respiratory disease, involves genetic and environmental factors in its development, chronicity and severity.[1,2] The pathophysiology of asthma is characterized by airway inflammation and remodeling processes modulated by resident and inflammatory cells, notably epithelial cells, eosinophils and T lymphocytes, particularly of the Th2 subset.[3,4] Although some of the underlying mechanisms have been elucidated in recent years, more work is needed to gain a clearer understanding of the genetic and environmental factors that contribute to the development of different forms of asthma.

Numerous genetic linkage and association studies were performed within the last decade (see Ober and Hoffjan[5] for a review), and also a considerable advance in laboratory and statistical methodologies was made. However, more work is needed to characterize genetic risk factors for asthma and its subphenotypes. New technological advances, including large single nucleotide polymorphism (SNP) databases, high-resolution haplotype structure analysis and global gene expression profiling from normal and affected tissues, may be useful in the screening of new genes and pathways relevant to asthma. In this context, we applied high-density microarray technology to bronchial biopsies and identified 79 genes that showed a significant differential expression in asthmatics compared with controls, including numerous genes previously implicated in asthma as well as new potential candidates, such as CX3CR1.[6]

The CX3CR1 gene, localized in the 3p21.3 chromosomal region, encodes for a high affinity seven-transmembrane G-protein-coupled receptor specific for the fractalkine (FKN) chemokine.[7] CX3CR1 has been shown to be expressed on inflammatory leukocytes such as natural killer cells, monocytes and T lymphocytes.[8–11] FKN expression can be observed on interleukin-1β, interferon-γ and tumor necrosis factor-α activated primary endothelial cells,[12,13] on bronchial epithelial cells[14] and on the cells of many other tissues such as lung, brain and intestines.[12] This chemokine exists in two forms; as a membrane-anchored mucin-like stalk proadhesive protein that mediates strong adherence of cytotoxic leukocytes expressing CX3CR1 and leads to their migration into the endothelium to promote tissue damage[8,9,12] or as a soluble chemotactic peptide that recruits CX3CR1 inflammatory expressing cells to induce their cellular

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Received 26 April 2006; revised 1 August 2006; accepted 10 August 2006; published online 2 November 2006

adhesion to the inflammation sites.[9] A role for CX3CR1-FKN-mediated inflammation has been suggested in various inflammatory diseases including vascular injury,[15] atopic dermatitis[16] and allergic airway diseases.[17,18] To our knowledge, no genetic association study has been reported between CX3CR1 and asthma. However, two coding SNPs (T280M and V249I) have been shown to affect risk to other diseases involving immune and inflammatory diseases.[19–23]

Based on our observations that the CX3CR1 gene is less expressed in bronchial biopsies of asthmatic subjects,[6] its biological importance in the recruitment of leukocytes in the inflammatory sites[8,9,12] and its potential role in allergic asthma,[17,18] we hypothesize that CX3CR1 is a candidate gene in asthma pathogenesis. The aims of this study were to carry out an association study and a haplotypic analysis with selected SNPs in CX3CR1 gene in a founder effect familial asthmatic sample from northeastern Quebec.

Results

Family-based association analysis of CX3CR1 SNPs with asthma

One thousand one hundred and thirty nine individuals, aged 3–88 years (Table 1), were included in the study (570 asthmatics, of which 223 were probands and 347 were family members). The median age of asthma onset for cases and their affected siblings was 5 years (2–46 years). The sex ratio (male:female) was 1:1.2 for probands, 1:1.4 for affected family members and 1:1.2 for unaffected family members. The 11 SNPs were tested individually for association with asthma, airway hyperresponsiveness (AHR), atopy and elevated immunoglobulin (Ig)E level under an additive and a dominant genetic model. Given that the analyses showed non-significant results for the AHR, atopy and elevated IgE level phenotypes, the results presented in this section and their interpretation are restricted to the asthma phenotype. Five Mendelian errors have been detected in the entire sample for all the SNPs tested: the genotypes of these families were considered as missing data in the analysis. None of the SNPs gave a significant deviation from equilibrium when considering the unrelated founders of the sample. Calculation of the effective number of independent tests was made using SNPSdP[24] (see Supplementary Information for details on simulation parameters) and allowed to find that the 11 correlated SNPs correspond to eight effective independent ones. The simulation indicates that the four phenotypes studied correspond to between 2.85 and 3.34 effective independent ones, depending on the minor allele frequency of the SNP simulated, with a median of 3.09. Hence, an estimate of the effective number of tests that we performed at this stage is roughly 42 (seven SNPs, three phenotypes and two genetic models). The lowest *P*-value observed (0.00058, rounded up to 0.001 in Table 2) is thus still significant even after allowing for the total number of tests that we performed with a Bonferroni's correction with the effective number of independent tests (corrected *P*-value: $0.00058 \times 42 = 0.0243$).

Under an additive model, no significant associations were found (data not shown). The results obtained under a dominant model are shown in Table 2. Five common alleles (rs1050592, T280M, V249I, rs938203 and

Table 1 Clinical characteristics of the Saguenay–Lac–St-Jean sample subjects

	Families		
	Probands (n = *223*)	*Affected relatives* (n = *347*)	*Unaffected relatives* (n = *569*)
Male:female ratio	1:1.2	1:1.4	1:1.2
Mean age in years (range)	18 (3–46)	40 (2–83)	48 (3–96)
Median age in years	16	41	48
Smoking status			
Never	186 (84%)	176 (51%)	243 (43%)
Ex-smoker	12 (5%)	105 (30%)	199 (35%)
Smoker	25 (11%)	66 (19%)	127 (22%)
FEV_1 as % predicted (s.d.)[a]	92.2 (16.3)	88.9 (22.7)	98.9 (17.0)
PC_{20} in mg/ml (s.d.)[b]	2.66 (3.33)	3.36 (4.53)	26.91 (3.04)
Serum IgE in μg/l (s.d.)[b]	229.1 (4.6)	157.4 (4.6)	80.9 (3.7)
Number of persons with subphenotypes			
Asthma	223 (100%)	347 (100%)[c]	0 (0%)
Atopy[d]	182 (82%)	237 (68%)	218 (38%)
AHR[e]	169 (90%)	217 (82%)	64 (11%)
IgE > 100 mg/l	141 (63%)	185 (53%)	155 (27%)
IgE > 280 μg/l	95 (43%)	106 (31%)	70 (12%)

Abbreviation: IgE, immunoglobulin E.

[a]FEV_1 = forced expiratory volume in 1 s.

[b]PC_{20} = concentration of methacholine inducing a 20% fall in FEV_1; geometric mean.

[c]Present asthma or past documented clinical history of asthma.

[d]Defined as at least one positive response (wheal diameter $\geqslant$3 mm at 10 min) on skin prick tests.

[e]AHR = Airway hyper-responsiveness as defined in Materials and methods (evaluated for 188 probands, 264 affected family members and 486 unaffected family members).

Table 2 FBAT results for association of CX3CR1 and asthma

SNP	*Allele*	*Allele frequency*	S[a]	E(S)[b]	*Var*(S)	Z[c]	P-*value*
rs1050592	C	0.314	119.000	114.417	39.326	0.731	0.465
	T	0.686	93.000	83.250	11.688	2.852	0.004
T280M	A	0.162	65.000	68.917	30.674	−0.707	0.479
	G	0.838	18.000	14.250	1.188	3.441	0.001
V249I	C	0.704	73.000	63.472	9.751	3.051	0.002
	T	0.296	103.000	100.056	38.147	0.477	0.634
rs2853712	G	0.481	105.000	108.583	27.312	−0.686	NS[d]
	A	0.519	100.000	100.500	25.250	−0.100	NS
rs2669841	C	0.754	54.000	51.500	6.250	1.000	NS
	T	0.246	77.000	76.750	26.562	0.049	NS
rs2853711	C	0.245	97.000	94.833	35.236	0.365	NS
	A	0.755	63.000	61.750	7.812	0.447	NS
rs871610	C	0.770	69.000	69.664	10.238	−0.207	NS
	T	0.230	104.000	101.414	33.628	0.446	NS
rs871144	G	0.261	101.000	97.574	30.674	0.619	NS
	A	0.739	62.000	61.491	8.500	0.175	NS
rs938203	G	0.156	74.000	78.488	26.887	−0.866	0.387
	A	0.844	52.000	45.155	3.994	3.425	0.001
rs3020453	G	0.198	77.000	68.139	27.779	1.681	NS
	A	0.802	42.000	38.222	4.605	1.760	NS
rs2669849	G	0.257	94.000	93.917	36.882	0.014	0.989
	A	0.743	72.000	63.750	7.188	3.077	0.002

Abbreviation: SNP, single nucleotide polymorphism.
[a]S = FBAT statistic.
[b]$E(S)$ = expected FBAT statistic.
[c]Z = Z-score (negative score shows a lower transmission frequency in probands).
[d]NS = not significant (P-value >0.05).

rs2669849) were significantly overtransmitted to asthmatic offspring (P-values <0.004). The associations observed with V249I and rs2669849 have been independently replicated in a case–control sample from Vancouver/Winnipeg. The Armitage test for trend (Table 3) supports the susceptibility to the same alleles. In Table 3, the odds ratios (OR) are expressed under the arbitrary choice to describe the common allele as being a susceptibility allele. Equivalently, the rare allele could be considered to be a protective allele: this would not have an effect on the association results. For V249I, the OR are approximately the same for both common homozygous and heterozygous (2.16 and 2.11, respectively), which supports a dominant model and the association with the common allele (P-value = 0.031). For rs2669849, the dominant model no longer best describes the data (the risks are 2.75 for the common homozygous and 1.86 for the heterozygous); the association is nevertheless also with the common allele ($P = 0.007$ under an additive model; $P = 0.059$ under a dominant model).

Table 3 Statistical analysis of the two SNPs tested in the case–control Vancouver/Winnipeg sample

SNP	*Genotype*	*Case*	*Control*	*OR*[a]	*Additive*[b]		*Dominant*[b]	
					Z[c]	P-*value*	Z[c]	P-*value*
rs2669849	AA	119	151	2.75	2.44	0.0073	1.56	0.059
	AG	55	103	1.86				
	GG	4	14					
V249I	CC	93	134	2.16	1.31	0.095	1.86	0.031
	CT	73	108	2.11				
	TT	8	25					

Abbreviation: SNP, single nucleotide polymorphism.
[a]OR, estimated odds ratio.
[b]Armitage test for trend of association with the common allele[52] under an additive or dominant model.
[c]Z = Z-score.
Shading denotes that OR is common for the two rows.

CX3CR1 linkage disequilibrium and haplotype-specific association analyses

Figure 1a shows the linkage disequilibrium (LD) between the SNPs measured by D'. The 11 SNPs separated the region into four blocks of tightly associated SNPs. We used Gabriel's criteria implemented in Haploview 3.2 for block definition (see Materials and methods). Block 1 is located towards the 3′ end and exon 2 and consists of SNPs rs1050592, T280M and V249I. Block 2 is located in the intronic region and consists of SNPs rs2853712 and rs2669841. Block 3 is located towards intronic and 5′-end regions and consists of SNPs rs2853711, rs871610 and rs871144. Block 4 is located towards the 5′ end and consists of SNPs rs938203, rs3020453 and rs2669849. Common haplotypes were observed in each block: block 1, haplotypes TGC (0.69), CAT (0.15) and CGT (0.14); block 2, GC (0.49), AT (0.23) and AC (0.28); block 3, ACA (0.53), CCG (0.23), ATA (0.23) and ACG (0.02); and block 4, AAG (0.25), GAA (0.16), AAA (0.39) and AGA (0.20) (Figure 1b). To characterize common CX3CR1 haplotype transmissions, previously inferred using Haploview, we assessed for nonrandom transmission using FBAT (v1.4) software[25,26] under a dominant genetic model. For asthma, only one

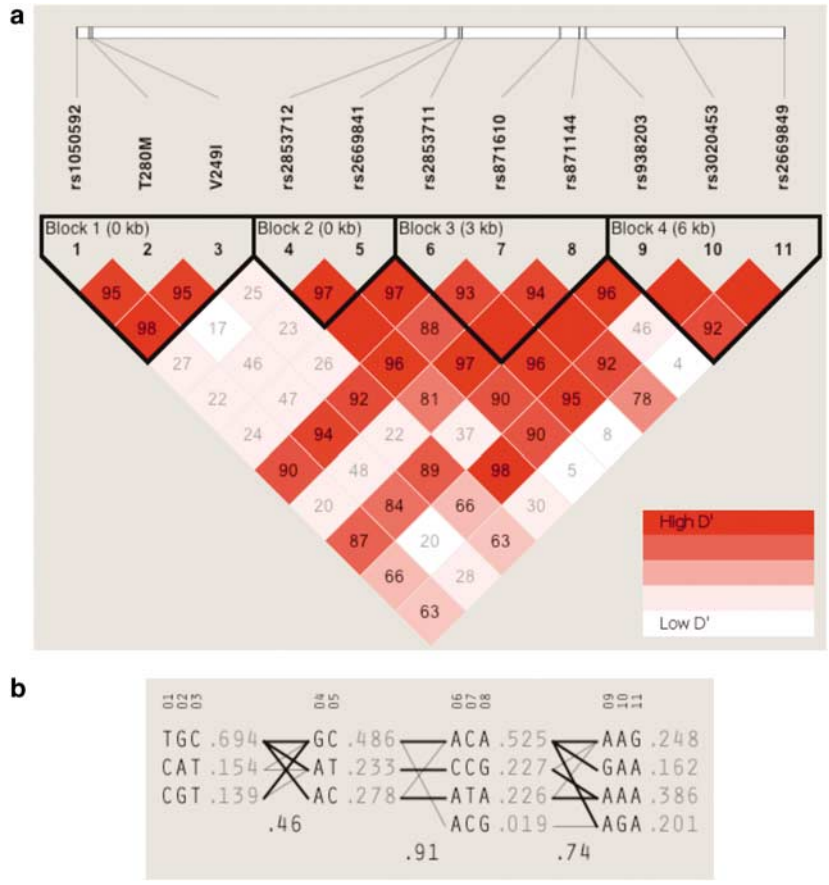

Figure 1 Pairwise LD pattern of CX3CR1 measured by D' and the common haplotypes of the pairwise LD regions. (**a**) The location of each tested SNP along the chromosome is indicated on top. The number in each diamond indicates the magnitude of LD in percent between respective pairs of SNPs. For example, the pairwise LD for SNPs rs2853712 and rs2669841 is 0.97. Squares without values represent perfect LD ($D' = 1.0$). Strength of LD is depicted by progression of color; for all D' with LOD > 2, the color moves from red to white as D' runs from 1 to 0; D' with LOD < 2 are represented in white. (**b**) Common haplotypes of the four blocks are listed with their frequencies in parentheses. Thick lines joining haplotypes from each block represent combined haplotypes with frequency >0.1 and thin lines for frequency <0.1.

Table 4 FBAT results for block 1 haplotype transmission patterns related to asthma

Block 1			S[a]	E(S)[b]	Z[c]	P-*value*
rs1050592	*T280M*	*V249I*				
T	G	C	45.968	37.191	2.823	0.005
C	A	T	45.969	42.939	0.616	NS[d]
C	G	T	40.021	42.532	−0.479	NS

[a]S = FBAT statistic.
[b]$E(S)$ = expected FBAT statistic.
[c]Z = Z-score.
[d]NS = not significant (P-value >0.05).

haplotype (TGC) of the first block showed nonrandom transmission (overtransmitted, $P = 0.005$) (Table 4). The other haplotypes tested (including all block combinations) were nonsignificant (data not presented).

Discussion

We report an association between five genetic variants and one haplotype of the CX3CR1 gene and asthma in a French-Canadian sample. The selection of CX3CR1 was based on our previous report of its differential expression in asthmatic bronchial biopsies compared to control subjects using microarrays,[6] its biological functions in inflammatory processes such as in allergic asthma[17] and its genetic association with other inflammatory complex traits.[19–21] This study demonstrates how microarray studies can be used to motivate original areas of investigation and to provide new research hypotheses to highlight candidate genes that have not been identified by classical genome screens.[27]

This genetic association study is the first to investigate the association of CX3CR1 with asthma. However, the CX3CR1 gene has been tested with other complex traits where the less common alleles of two SNPs in strong LD, I249 and M280,[22] were associated with both increased risk of human immunodeficiency virus (HIV) infection, and accelerated HIV disease progression,[22,23] with reduced prevalence of coronary events and atherosclerosis[19–21] but not with peripheral arterial diseases[28] or cerebrovascular diseases.[29] The associations with HIV infection[22,23] have not been confirmed by an independent study of McDermott *et al*.[30] We tested these SNPs in the present sample and found positive associations with asthma. According to our results, the common alleles of these two SNPs (V249 and T280) are overtransmitted in

asthmatic probands. These single-marker association results were further supported by the pairwise disequilibrium pattern analysis that showed strong LD (0.98) between these two SNPs and with the rs1050592 in the block 1 haplotype. Haplotype TGC spanning this block was also overtransmitted in asthmatic probands, suggesting a significant nonrandom distribution of this haplotype in asthma. As the minor alleles were undertransmitted to asthmatic probands, this positive association suggests that the less common I249/M280 haplotype could represent a protective factor in asthma. Concerning the association with the three other SNPs involving variants in the promoter region (rs938203, rs3020453 and rs2669849), these genetic markers could be in LD with the CX3CR1 V249I/T280M variants studied (LD = 0.88, 0.68 and 0.63, respectively) or could be associated with regulatory variants affecting expression. The latter is also suggested by the reported imbalance between expressed alleles of CX3CR1 in heterozygous lymphoblastoid cell lines.[31,32]

Family-based tests of association are not robust in the presence of Mendelian-consistent genotyping errors: these undetected errors give the false impression of overtransmission of the common alleles from parents to offspring.[33] Overtransmission of common alleles is precisely what we observed. It is thus reassuring to see a validation in a case–control study: even though undetected genotyping errors can change the outcome of association results, they do not inflate the type I error of the test.[34] It could be suggested that these common alleles are susceptibility alleles in asthma, which moreover seem to act in a dominant fashion, indicating a role of this CX3CR1–FKN pathway in the disease. The association of V249I and rs2669849 has been validated in the Vancouver/Winnipeg case–control sample in our preliminary works, and additional association studies are in process using three Canadian asthma samples with 3600 individuals (AllerGen Network of Excellence).

As CX3CR1 is preferentially expressed in Th1 cells compared to Th2 cells in response to FKN, cell infiltration via FKN–CX3CR1 interaction promotes Th1 response and may contribute to the pathogenesis of numerous vascular and tissue injuries.[13,15,35,36] FKN expressed on inflamed endothelium may play the role of a vascular gateway for cytotoxic effector cells expressing CX3CR1 by rapidly capturing them from the blood and promoting their migration into the tissue, where inflammation and Th1 polarization may occur through interferon-γ production.[15] CX3CR1 I249/M280 haplotype has been associated with a reduced risk of coronary events,[19–21] which could be explained by a decreased ability of circulating monocytes harboring CX3CR1 I249/M280 haplotype to adhere to vascular endothelium as a result of a decreased binding affinity of CX3CR1 for its ligand.[15] This decrease in monocyte infiltration results in a decreased inflammation and Th1 response responsible for this protective effect in cardiovascular diseases.[21] On the other hand, HIV-infected individuals carrying this I249/M280 haplotype also showed a decreased binding affinity between CX3CR1 and FKN, resulting in a decreased Th1 response and host cellular defense that would lead to the more rapid development of AIDS.[22] Based on these genetic and functional studies and because FKN is also expressed on human bronchial epithelial cells,[14] we hypothesize that common CX3CR1 V249/T280 haplotype may be a genetic risk factor in asthma, possibly by supporting inflammatory leukocytes entry in the bronchial airway walls and by enhancing the inflammatory response. Then, the individuals carrying the I249/M280 haplotype are protected against the development of asthma by this decrease in their inflammatory response, as observed in cardiovascular diseases. Delineation of the real contribution of the genetic risks associated with CX3CR1 in asthma pathogenesis awaits further characterization of the molecular structure of the gene and of the functional consequences of the identified variants in lung physiology.

In this regard, we plan to compare CX3CR1 level of protein expression in bronchial biopsies of asthmatic and control subjects and to characterize the cellular source of their mRNA expression. As FKN is constitutively expressed in pulmonary endothelial and epithelial cells contributing to the recruitment and the persistence of inflammatory cells in the airways of allergic individuals,[17] we believe that further functional works on CX3CR1 and FKN will help delineate the impact of this immune-inflammatory pathway in the pathophysiology of asthma.

In conclusion, microarrays allowed us to identify CX3CR1 as a new candidate gene for asthma and our association study showed a potential susceptibility risk if carrying CX3CR1 common alleles of five SNPs, suggesting an asthma-protective effect of the minor alleles in healthy control carriers. Based on previous genetic and functional studies, we hypothesize that CX3CR1 could be implicated in the asthma pathophysiology, but we actually pursue the functional studies of either associated SNPs in asthma pathogenesis to elucidate the real biological relevance of CX3CR1 in asthma.

Materials and methods

Samples

The study sample, described in a recent report,[37] comprises asthmatic probands residing in Saguenay–Lac-St-Jean (SLSJ), a region of northeastern Quebec (Canada). In this region resides a founder population[38–41] of approximately 287 000 individuals who descend predominantly from approximately 2500 founders originating from France who settled in Quebec in the 17th century.[42] All the information concerning the recruitment and clinical evaluation of the subjects, as well as the information concerning the Vancouver/Winnipeg case–control sample[43] used to validate associations, is available as Supplementary Information.

Phenotyping

Following the American Thoracic Society's criteria,[44] we defined participants as asthmatics if (1) they had a reported history of asthma (validated by a physician), or (2) they showed a positive methacholine provocation test (PC_{20}) and asthma-related symptoms. AHR was defined as a PC_{20} methacholine $\leqslant 8$ mg/ml. If PC_{20} was unavailable, AHR was defined as either a rise in the post-bronchodilator forced expiratory volume in 1 s (FEV_1) of $\geqslant 15\%$ from baseline or a variation of the morning–evening peak expiratory flow rate of $\geqslant 12\%$ over a 2-week period.[45] Atopy was defined as at least one positive response (wheal diameter $\geqslant 3$ mm at 10 min) on skin

prick tests, and elevated serum IgE level was fixed at a concentration of 100 μg/l following the sample distribution.

Genotyping

Blood samples were drawn from all participants and DNA was extracted from whole blood leukocytes using the Genomic-tip 100/G kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. A panel of 12 SNPs was selected from public databases (NCBI and The SNP Consortium) according to the presence of allelic imbalance in an SLSJ sample previously noted by our group (data not shown) and tested for association with each asthma subphenotype. One SNP, rs2669850, was excluded due to poor performance of the genotyping assay. SNPs described in this report are cited using their reference SNP identifier (rs#) from the NCBI database. See Table 5 for CX3CR1 SNP characteristics and Figure 2 for CX3CR1 genomic organization. Among the 11 working SNPs, there were two nonsynonymous coding variants in the transmembrane domain of the receptor, T280M (a G-to-A substitution changed Thr^{280} to Met) and V249I (a C-to-T substitution changed Val^{249} to Ile), five 5′ untranslated region (UTR) variants, one 3′UTR variant and three non-coding variant. SNPs genotyping was performed using SNPstream UHT (Orchid Biosciences, Princeton, NJ, USA)[46] and HEFP (Molecular Devices, Sunnyvale, CA, USA).[47] All protocols, primers and conditions used are available in Supplementary Information.

Statistical analysis

For the SLSJ familial sample, allele distribution patterns and Mendelian errors were assessed by FBAT software (v 1.4)[25,26,48] under an additive and a dominant genetic model for each CX3CR1 SNP. To account for the inclusion of multiple affected family members, an empirical estimate of the variance was used.[48] Hardy–Weinberg equilibrium was tested for each SNP with a χ^2 test using one degree of freedom. The calculation of the effective number of independent tests is described in Supplementary Information. Strength of LD between pairs of

Table 5 CX3CR1 polymorphism characteristics

SNP	*Base change*	*Gene map locus*	*MAF*[a]	*Chromosome location*[b]	*Genomic features*
rs1050592	T/C	3p21	0.314	39281787	3′UTR
rs3732378	G/A	3p21	0.162	39282165	Nonsynonymous T280M
rs3732379	C/T	3p21	0.296	39282259	Nonsynonymous V249I
rs2853712	A/G	3p21	0.481	39293291	Intron
rs2669841	C/T	3p21	0.246	39293707	Intron
rs2853711	A/C	3p21	0.245	39293800	Intron
rs871610	C/T	3p21	0.230	39296870	5′UTR
rs871144	A/G	3p21	0.261	39297469	5′UTR
rs938203	A/G	3p21	0.156	39297668	5′UTR
rs3020453	A/G	3p21	0.198	39300526	5′UTR
rs2669849	A/G	3p21	0.257	39303877	5′UTR

Abbreviations: SNP, single nucleotide polymorphism; UTR, untranslated region.
[a]MAF = minor allele frequency.
[b]Chromosome location for each SNP was obtained from the November 2005 freeze of the University of California Santa Cruz Genome Browser database using (NM_001337) (http://genome.ucsc.edu/).

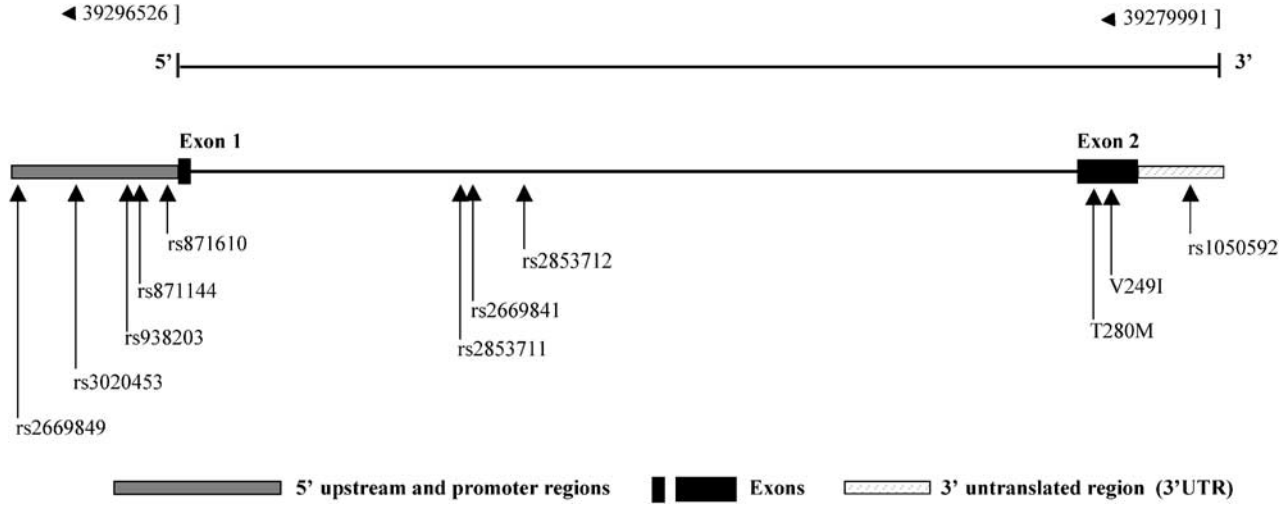

Figure 2 Genomic organization of CX3CR1 (shown on reverse complement strand). Positions and names of the 11 SNPs analyzed are represented by arrows below the gene structure. Source: November 2005 Genome Browser (NM_001337) (http://genome.ucsc.edu/) and NCBI (gene ID: 1524) (http://www.ncbi.nlm.gov/) databases.

SNPs was measured as D',[49] using Haploview software (v 3.2).[50] Regions of strongly associated markers (LD blocks) were inferred using the definition proposed by Gabriel *et al*.[51] and implemented in Haploview. Haplotype-specific associations were investigated under a dominant genetic model using the 'haplo' command implemented in FBAT[25,26] and an empirical variance estimator was used.[48] For the Vancouver/Winnipeg case–control sample, the Armitage test for trend[52] was used to test for association between asthma and the rs2669849 and V249I SNPs. OR and confidence intervals (CI) were estimated from 2×2 contingency tables (baseline is homozygous rare). The R statistical software (http://www.R-project.org) was used to evaluate these tests.

Acknowledgements

We thank all families of the SLSJ population for their enthusiastic participation in this study. We also thank Paul Bégin, Jean-Pierre Leblanc, Christian Allard, Mario Laforte, Hélène Archibald and Diane Gagné for their invaluable participation in the ascertainment of subjects, and Nathalie Pagé from the Laval Hospital for her manuscript revision. K Tremblay and V Provost are supported by the Respiratory Health Network (RHN) of the Fonds de la recherche en santé du Québec (FRSQ). K Tremblay is also supported by a grant from AllerGen (to TJ Hudson, A Sandford and C Laprise). TJ Hudson is the recipient of an Investigator Award from the Canadian Institutes of Health Research (CIHR) and a Clinician-scientist Award in Translational Research from the Burroughs Wellcome Fund. A Sandford is the chairholder of the Canadian Research Chair (www.chairs.gc.ca) on genetic susceptibility to inflammatory airway diseases. C Laprise is the chairholder of the Canada Research Chair on genetic determinants in asthma and the director of the Genetics & Genomics Thematic Unit of the RHN of the FRSQ. This work was supported by the Canadian Institutes of Health Research (CIHR) and the Respiratory Health Network (RHN) of the Fonds de la recherche en santé du Québec (FRSQ).

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