

Association of the cysteinyl leukotriene receptor 1 gene with atopy in the British 1958 birth cohort

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Background: Cysteinyl leukotrienes (CysLTs) play an important role in the pathophysiology of many allergic inflammatory disorders. However, data on the contribution of genetic variability of the cysteinyl leukotriene receptor 1 gene (*CYSLTR1*) in asthma and atopy remain conflicting.

Objective: We investigated the association of polymorphisms of interest located at this locus and allergic disease prevalence in a national population with an established DNA archive, the British 1958 birth cohort.

Methods: The British 1958 birth cohort comprises all persons born in Britain during 1 week in 1958. Asthma, wheezy bronchitis, and wheezing were ascertained by interview at ages 7, 11, 16, 23, 33, and 42 years. At age 44 to 45 years, serum total circulating IgE levels were measured and atopy was defined as a serum total IgE level of greater than 30 kU/L and specific IgE levels to 1 or more of dust mite, cat fur, and mixed grass of greater than 0.3 kU/L. DNA samples from 8018 participants were genotyped for 2 variants of the *CYSLTR1* promoter (Xq13-Xq21).

Results: The rare polymorphism C > T (rs7066737) was not associated with any of the phenotypes studied. The common promoter polymorphism A > G (rs2806489) was not associated with total IgE levels or the prevalence or age of onset of asthma, wheezy bronchitis, or wheeze. However, the wild-type allele A was significantly associated with atopy in female subjects ($\chi^2 = 8.30$, $P = .004$), although not in male subjects ($P = .841$).

Conclusions: These data suggest that a *CYSLTR1* polymorphism previously shown to affect the gene transcription *in vitro* might influence the risk of atopy in the female white population with suggestive evidence of heterozygote vigor. (*J Allergy Clin Immunol* 2009;124:566-72.)

Key words: Atopy, asthma, British 1958 birth cohort, cysteinyl leukotriene receptor 1, genetics, leukotriene receptor

Leukotrienes (LTs) are biologically active mediators derived from arachidonic acid.¹ They play a key role in atopic diseases,

Abbreviations used

CysLT: Cysteinyl leukotriene
CysLTR: Cysteinyl leukotriene receptor
CYSLTR1: Cysteinyl leukotriene receptor 1 gene
LD: Linkage disequilibrium
LT: Leukotriene
SNP: Single nucleotide polymorphism

such as asthma² and allergic rhinitis.³ Cysteinyl leukotrienes (CysLTs), including LTC₄, LTD₄, and LTE₄, are the most potent bronchoconstrictors identified to date.⁴ Two human cysteinyl leukotriene receptors (CysLTRs) have been well defined pharmacologically to transduce CysLT signal: CysLTR1 and CysLTR2.⁵ The organization of the genes encoding these 2 G protein-coupled receptors has recently been established.⁶⁻¹⁰ The receptors map to different chromosomes, bear little nucleotide sequence homology, and have only 37% amino acid identity. Classification still relies on their pharmacologic properties, in particular their sensitivity to classical CysLTR antagonists, such as montelukast and BAYU9773.⁵ The human CysLTR1 is highly expressed in the spleen and in cells of relevance to allergic inflammatory processes, such as peripheral blood leukocytes (including eosinophils and mast cells).^{7,9,11} Therefore CysLTR1 is a primary target to better understand the role of CysLTs in atopy.

The human *CYSLTR1* gene is located on chromosome Xq13-Xq21. The coding sequence of the gene is 1014 bp long, intronless and encodes a 337-amino-acid protein (38.5 kd), which shares 24% to 32% identity to members of the purinergic (P2Y) receptor family.^{7,9} One main mRNA of 1537 bp and composed of 3 exons is expressed. However, various minor transcripts have also been described.¹²⁻¹⁴ We previously reported 2 of those transcripts. One was a splice variant of the main transcript. Another rare transcript (<5%) contained 3 additional exons at its 5'-end, suggesting the presence of an alternative promoter (Fig 1, A).¹² Although potentially functional in human airway smooth muscle cells *in vitro*,¹² the alternate promoter did not contain any predicted major transcription factor binding sites or common polymorphisms from the National Center for Biotechnology Information Single Nucleotide Polymorphism database. However, we investigated 4 single nucleotide polymorphisms (SNPs) in a 1133-bp region from the exon 4 3'-end (Fig 1, A) containing the main regulatory elements of the main promoter and observed that the SNP A > G (rs2806489) induced a decrease in *CYSLTR1* mRNA expression *in vitro* in THP1 cells.¹²

Despite the complexity of atopic phenotypes, it is now recognized that atopy is caused by several, perhaps numerous genes, each with a small overall contribution and relative risk and that these genes are necessary but by themselves not sufficient for the development of the disease.¹⁵⁻¹⁷ It is believed that among the

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estimated 11 million human genetic polymorphisms with a minor allele frequency of greater than 1%,¹⁸ the majority of those that influence disease risk lie outside of the coding regions of genes and affect the regulation of gene expression.^{19,20} It was also estimated that a large proportion (58.9%) of those regulatory variants lie within the first 500 bp upstream of the transcription start sites of genes.^{19,21} There have been some small recent studies of *CYSLTR1* genetic variation in allergic disease (see the Discussion section for further details). These studies have provided discrepant results, probably because of lack of power. The aim of the current study was therefore to address this issue in a definitive population study. Specifically, we hypothesized that the regulatory polymorphism that affects *CYSLTR1* transcription *in vitro* might contribute to the development of asthma, atopy, or both. Therefore after conducting a linkage disequilibrium (LD) analysis of the SNPs at this locus, we genotyped the selected tag promoter SNPs in the British 1958 birth cohort and estimated their contribution to variability in asthma/wheeze prevalence, serum total IgE levels, and atopy.

METHODS

Study population and procedures

The British 1958 birth cohort (also known as the National Child Development Study) is a longitudinal study of more than 17,000 persons born in England, Scotland, and Wales during the week of March 3-9, 1958, who were originally recruited for a perinatal mortality survey. From its original focus on the circumstances and outcomes of birth, the 1958 study has broadened in scope to chart many aspects of the health, educational, and social development of cohort members. The cohort was followed up at ages 7, 11, and 16 years by parental interviews and examinations by school medical officers and at ages 23, 33, and 42 years in interviews. Immigrants with the same dates of birth were identified at ages 7, 11, and 16 years and followed up into adulthood, but adult immigrants (after age 16 years) have not been included.

Asthma and wheezy bronchitis were ascertained by means of parental interview in childhood, as described in more detail elsewhere.²² In adulthood questions were related to asthma ever, wheezing ever, and wheezing episodes in the past year. At age 34 to 35 years (1992-1993), a subsample of the cohort, enriched for history of childhood wheezing illness, were examined in their homes by a team of trained research nurses. Spirometry was done in the standing position without nose clips by using a Vitalograph bellows spirometer (Vitalograph Ltd, Buckingham, United Kingdom). At least 3 blows were recorded, and up to 5 were done if the best-test variation (assessed by the sum of FEV₁ and forced vital capacity) was greater than 5%. Spirometry was done before and 20 minutes after a dose of 400 µg of salbutamol administered by means of dry powder inhaler. Subjects were asked to refrain from bronchodilator medication for 6 hours before the test.

Of 18,558 subjects eligible for inclusion in the British 1958 birth cohort study (17,638 born in Britain and 920 immigrants with the same dates of birth), 12,069 were still in contact with the study team in 2002 and were invited to participate in the biomedical study at age 44 to 45 years.²² Nine thousand three hundred seventy-seven were visited, and adequate blood samples were obtained from 8018 participants, with consent for DNA extraction. These participants were resident in England, Scotland, or Wales at the time of examination and were predominantly of white ethnicity (97% [7752/8018]). The lifetime histories of asthma and wheezing illness for those who contributed DNA and thus are included in the analyses in this study are similar to those who are not included: at age 42 years, the lifetime prevalence of asthma or wheeze in the full cohort was 49.9%, and in the cohort with DNA, it was 49% (for both male and female subjects). Serum total circulating IgE levels were measured with the HYTEC enzyme immunoassay (Hycor Biomedica, Garden Grove, Calif). Concentrations of specific IgE to house dust mite, mixed grass pollen, and cat fur were also measured if serum total IgE levels were greater than 30 kU/L. Seven thousand seven hundred four (3771 male and 3723 female patients) of the 8018 had valid total IgE measurements,

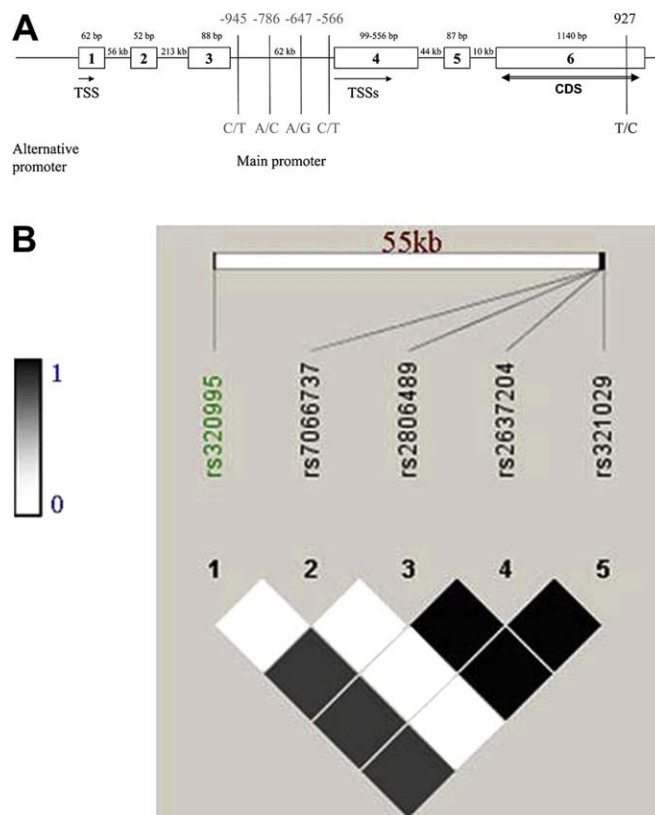


FIG 1. Genetic variations in *CYSLTR1*. **A**, Genomic organization of *CYSLTR1*. *CYSLTR1* genomic organization is shown as described previously (figure not to scale).¹² The intronless coding region is in exon 6 (CDS). The polymorphisms C/T (rs321029), A/C (rs2637204), A/G (rs2806489), C/T (rs7066737), and T/C (rs320995) observed by means of direct sequencing are indicated. Positions are given from the exon 4 3'-end for the promoter SNPs and from the coding ATG for the coding exon. TSS, Transcription start site. **B**, LD plot on markers in *CYSLTR1*. Shown is the pairwise LD between *CYSLTR1* SNPs genotyped in the Nottingham population ($n = 48$). LD was estimated by using the program Haploview. All pair D' value were equal to 1 (see text for comments). Markers are plotted equidistantly. R^2 measures of LD are shown. Scales for measures of LD are shown on the left side of the plot.

and 7483 (3761 male and 3722 female patients) had specific IgE data. Subjects with at least 1 specific IgE level of greater than 0.3 kU/L were classified as atopic.²³ Forty-eight percent of the population (55% and 42% among male and female subjects, respectively) showed increased serum total IgE levels (>30 kU/L), and 29% of the cohort was atopic (34% and 24% among male and female subjects, respectively).

Protocols for the 2002-2004 biomedical examination and the 1992-1993 special study of asthma and lung function were approved by the South East MultiCentre Research Ethics Committee. All participants provided informed written consent to participate in genetic association studies, and the present study was approved by the South East MultiCentre Research Ethics Committee and the Oversight Committee for the biomedical examination of the British 1958 birth cohort.

Genotyping

The coding variant T > C (rs320995) was genotyped in a population of 48 white subjects from the Nottingham area.¹² Ethical approval for the use of relevant samples was obtained from the regional ethics committee. Genotyping was initially performed by using RFLP with the primers 5'-GCCAGG TTTGTGTGTGTAGGT-3' (forward) and 5'-TGGTTTGGACTGGAAATG GTT-3' (reverse) and the restriction enzyme *Hpy*188I (New England Biolabs,

TABLE I. Allele frequencies of *CYSLTR1* polymorphisms in a white population from Nottingham

Position in the promoter	-945		-786		-647		-566		+927	
refSNP ID	rs321029		rs2637204		rs2806489		rs7066737		rs320995	
Allele	C	T	A	C	A	G	C	T	T	C
No. of alleles and frequency (n = 48 subjects)	51 0.797	13 0.203	51 0.797	13 0.203	51 0.797	13 0.203	61 0.953	3 0.047	54 0.844	10 0.156

Forty-eight white British subjects (32 male and 16 female subjects) selected at random from an in-house DNA archive (Nottingham, United Kingdom) were genotyped for the promoter polymorphisms rs321029, rs2637204, rs2806489, and rs7066737 and the coding SNP rs320995. Because *CYSLTR1* is located on the X chromosome, twice as many male as female subjects were screened to ensure an equal number of alleles between the 2 sexes. All the polymorphisms had similar frequencies in male and female subjects (χ^2 test of allele counts, $df = 1$, $P > .4$) and were in Hardy-Weinberg equilibrium ($P > .1$).

Ipswich, Mass). Twelve percent of the genotypes (6/48 subjects) were also assessed by using direct sequencing as a control.

DNA samples from the 8018 participants in the 45-year follow-up were genotyped for the 2 *CYSLTR1* promoter variants A > G (rs2806489) and C > T (rs7066737) by Geneservice Ltd (Cambridge, United Kingdom) with Taqman methodology for allelic discrimination (Applied Biosystems, Foster City, Calif). All genotyping was performed blind to clinical status. The genotyping call rate was 98% for both polymorphisms.

LD analysis

In order to assess linkage disequilibrium (LD) between the polymorphisms studied, haplotype frequencies in the Nottingham population were estimated using the program GENECOUNTING version 2.2.²⁴ For each pair of loci, 3 measures of LD (Lewontin's D, D' with its confidence bounds, and r^2) were measured by using the program Haploview version 3.32.

Statistical analysis

For the continuously distributed outcome, log-transformed total IgE levels (adjusted for both sex and height), regression modeling analysis was performed by using STATA version 8.0 software (StataCorp, College Station, Tex). Regression models were fitted with each genotype at a given locus assigned a separate parameter estimate (2 df ANOVA models). The proportion of residual variance statistically explained by each polymorphism was derived from the ANOVA table for each model. Data related to serum total IgE levels were also analyzed as a categorical outcome. The analysis was limited to white nontwin subjects.

For the categorical outcomes (k categories), associations were assessed both with the genotype and number of minor alleles at each locus. Statistical significance for heterogeneity of outcome by genotype (χ^2 test), and for heterogeneity of minor allele frequency across outcome categories (χ^2 test of allele counts) were calculated to assess association. The proportion of disease burden statistically explained by each polymorphism (population-attributable risk) was derived by subtracting the disease prevalence among the lowest-risk genotype from the overall disease prevalence and expressing the difference as a proportion of the overall prevalence. The power of the association study was estimated by using the program QUANTO version 1.1.1.²⁵

Genetic association studies were performed in sex-stratified groups because *CYSLTR1* is on the X chromosome. A logistic regression analysis was performed by using 2 possible scoring conventions for hemizygous male subjects. While coding the female subjects as 0, 1, or 2 minor alleles, male subjects were coded first as 0 or 2 (assumption of X chromosome inactivation) and then as 0 or 1 (no X inactivation). Heterosis was tested for statistical significance by including in the model for female subjects a binary parameter (0 for homozygotes and 1 for heterozygotes). The improvement in model fit, compared with the simple (per-allele) model, was used to derive a likelihood ratio χ^2 test for significant departure from additivity. If the per-allele effect in the combined model is close to null, then the comparison of heterozygotes/homozygotes provides a measure of the direction and magnitude of heterosis.

TABLE II. Allele frequencies of the *CYSLTR1* promoter tag SNPs in the British 1958 birth cohort

Position in the promoter	-647		-566	
refSNP ID	rs2806489		rs7066737	
Alleles	A	G	C	T
No. of alleles and frequency	3078	861	3877	68
in male subjects	0.781	0.219	0.983	0.017
No. of alleles and frequency	6082	1730	7664	152
in female subjects	0.779	0.221	0.981	0.019
No. of alleles and frequency	9160	2591	11541	220
in population sample	0.780	0.220	0.981	0.019

Because *CYSLTR1* is located on the X chromosome, data are given separately for male and female subjects in this and the following tables. Eight thousand eighteen white British subjects (4040 male and 3978 female subjects) were genotyped for the polymorphisms rs2806489 and rs7066737. Frequencies were calculated from successfully genotyped subjects. None of the SNPs showed allele frequencies significantly different between female and male subjects (χ^2 test of allele counts, $df = 1$, $P > .40$).

RESULTS

LD analysis

Analysis of *CYSLTR1* by using Haploview showed, in agreement with previous data, that the gene is located in a recombination cold spot.^{12,26} Because the main focus of this study was to investigate potential regulatory region SNPs, an initial analysis of *CYSLTR1* polymorphisms (4 in the promoter region and 1 in the coding region; Fig 1, A) was performed to select the best tag SNPs for the gene. In the Nottingham population the minor allele frequency of the well-characterized synonymous coding SNP T > C (rs320995, 927 bp from the coding ATG) was 15.6% (Table I). When predicted from the genotyping data, 4 haplotypes were identified: CAACT, CAATT, TCGCT, and TCGCC, numbered I to IV (see Table E1 in this article's Online Repository at www.jacionline.org). The frequency of the most common mutant haplotype, TCGCC, was estimated at 15.6%, whereas haplotypes II and III were rare.

LD was also estimated for each pair of SNPs, and an LD plot was constructed (Fig 1, B). The 3 other promoter SNPs (rs321029, rs2637204, and rs2806489) were in perfect LD with each other ($D' = 1$; $r^2 = 1$; $P < .001$, χ^2 test). The coding SNP rs320995 was in high but not perfect LD ($r^2 < 1$) with those 3 loci. The D' 95% CI was tight (0.74-1.00), r^2 was 0.73, and the LD goodness-of-fit test results were significant ($P < .001$). Although only SNPs rs321029 and rs320995 were genotyped as part of the HapMap project, those 2 SNPs showed an LD similar to ours in the CEPH population ($r^2 = 0.82$).

These data indicate that knowing the genotype at only the 2 most downstream loci of the *CYSLTR1* main promoter enables

TABLE III. Genotype and prevalence of minor alleles for *CYSLTR1* promoter SNPs in relation to prevalence or age of onset of asthma, wheezy bronchitis, or wheeze: male subjects

Wheeze only or asthma prevalence by 0-42 y	rs2806489			rs7066737		
	A	G	%G	C	T	%T
All cohort members	3078	861	21.9	3877	68	1.7
No asthma or wheeze by 42 y of age, no. (%)	1557 (50.6)	446 (51.8)	22.3	1969 (50.8)	30 (44.1)	1.5
Wheeze but not asthma by 42 y of age, no. (%)	1232 (40.0)	327 (38.0)	21.0	1537 (39.6)	30 (44.1)	1.9
Asthma by 42 y of age, no. (%)	289 (9.4)	88 (10.2)	23.3	371 (9.6)	8 (11.8)	2.1
Tests for heterogeneity (<i>P</i> value)	.498			.533		

Data on asthma/wheeze were available for 83% and 100% of the male subjects successfully genotyped for the markers A > G (rs2806489) and C > T (rs7066737), respectively.

TABLE IV. Genotype and prevalence of minor alleles for *CYSLTR1* promoter SNPs in relation to prevalence or age of onset of asthma, wheezy bronchitis, or wheeze: female subjects

Wheeze only or asthma prevalence by 0-42 y	rs2806489				rs7066737		
	AA	AG	GG	%G	CC	CT + TT	%T
All cohort members	2367	1348	191	22.1	3762	146	2.0
No asthma or wheeze by 42 y of age, no. (%)	1200 (50.7)	707 (52.4)	109 (57.1)	22.9	1937 (51.5)	75 (51.4)	1.9
Wheeze but not asthma by 42 y of age, no. (%)	880 (37.2)	479 (35.5)	61 (31.9)	21.2	1366 (36.3)	55 (37.7)	2.0
Asthma by 42 y of age, no. (%)	287 (12.1)	162 (12.0)	21 (11.0)	21.7	459 (12.2)	16 (11.0)	1.8
Tests for heterogeneity (<i>P</i> value)		.467		.204	.882		.916

Tests of heterogeneity *P* values are given per allele and per genotype. Data on asthma/wheeze were available for 100% of the female subjects successfully genotyped for both markers A > G (rs2806489) and C > T (rs7066737).

TABLE V. Genotype and prevalence of minor alleles for *CYSLTR1* promoter SNPs in relation to levels of serum total IgE: male subjects

Total IgE	rs2806489			rs7066737		
	A	G	%G	C	T	%T
All cohort members	2885	800	21.7	3626	63	1.7
<10 kU/L, no. (%)	461 (16.0)	132 (16.5)	22.3	582 (16.1)	13 (20.6)	2.2
10-30 kU/L, no. (%)	833 (28.9)	247 (30.9)	22.9	1065 (29.4)	15 (23.8)	1.4
31-99 kU/L, no. (%)	889 (30.8)	220 (27.5)	19.8	1084 (29.9)	21 (33.3)	1.9
≥100 kU/L, no. (%)	702 (24.3)	201 (25.1)	22.3	895 (24.7)	14 (22.2)	1.5
Tests for heterogeneity (<i>P</i> value)	.333			.602		

Data on serum total IgE levels were available for 94% of the subjects successfully genotyped for both markers A > G (rs2806489) and C > T (rs7066737).

one to reconstitute the haplotype of this promoter with high probability.

Genetic association studies

Because the haplotype of the *CYSLTR1* promoter can be deduced from the 2 tag SNPs A > G (rs2806489) and C > T (rs7066737), those loci were genotyped in the British 1958 birth cohort (Table II). The allele frequencies observed were in keeping with those seen in the Nottingham population (Table I). Although the genotyping error rate was not specifically estimated for these 2 SNPs, it was validated for other SNPs genotyped in this cohort and, in general, was less than 1%.²⁷ However, as a consequence of allele discrimination errors, a small number of male subjects were miscalled as a heterozygote (1 of 3946 male subjects for SNP rs7066737 and 11 of 3950 male subjects for SNP rs2806489). Because of the uncertainty regarding the genotype of these subjects, they were omitted from all further analyses. The marker A > G (rs2806489) was in Hardy-Weinberg equilibrium ($P = .956$) but not the SNP rs7066737 ($P < .001$). This deviation from Hardy-Weinberg equilibrium is probably due to the rarity of the T allele. Although this study had low power to

detect an odds ratio for atopy of less than 0.5 or greater than 1.5 in the recessive model, it had greater than 89% power to detect a major effect of the marker rs7066737 on atopy in both the dominant and log additive models. Therefore, for this SNP, we only performed an analysis of CC homozygotes against the T allele carriers. Whether analyzed per allele or per genotype, the polymorphism C > T rs7066737 was not associated with atopy, serum total IgE level, or asthma/wheeze prevalence and age of onset (Tables III-VIII and see Tables E2 and E3 in this article's Online Repository at www.jacionline.org).

Among the male population, none of the phenotypes studied was associated with rs2806489 (see Table E2 and Tables III, V, and VII). In the female population, the marker was not associated with prevalence or age of onset of asthma, wheezy bronchitis, or wheeze (see Table E3 and Table IV) but was weakly associated with log serum total IgE level ($P = .024$, 2 *df*). However, when IgE levels were grouped into 4 graded categories, a consistent association could not be detected, irrespective of whether analyzed per allele or per genotype ($P > .28$; Table VI). It is thus likely that this association is a false-positive result caused by multiple testing.

Interestingly, the wild-type allele A of SNP rs2806489 was associated with atopy in female subjects (Table VIII). The sex

TABLE VI. Genotype and prevalence of minor alleles for *CYSLTR1* promoter SNPs in relation to levels of serum total IgE: female subjects

Total IgE	rs2806489				rs7066737		
	AA	AG	GG	%G	CC	CT + TT	%T
All cohort members	2218	1265	177	22.1	3526	135	1.9
<10 kU/L, no. (%)	543 (24.5)	325 (25.7)	44 (24.9)	22.6	874 (24.8)	30 (22.2)	1.7
10-30 kU/L, no. (%)	734 (33.1)	434 (34.3)	53 (29.9)	22.1	1181 (33.5)	49 (36.3)	2.1
31-99 kU/L, no. (%)	574 (25.9)	322 (25.5)	57 (32.2)	22.9	920 (26.1)	31 (23.0)	1.7
≥100 kU/L, no. (%)	367 (16.5)	184 (14.5)	23 (13.0)	20.0	551 (15.6)	25 (18.5)	2.3
Tests for heterogeneity (<i>P</i> value)		.306		.282		.608	.495

Tests of heterogeneity *P* values are given per allele and per genotype. Data on serum total IgE levels were available for 94% of the subjects successfully genotyped for both markers A > G (rs2806489) and C > T (rs7066737).

TABLE VII. Genotype and prevalence of minor alleles for *CYSLTR1* promoter SNPs in relation to incidence of atopy: male subjects

Atopy	rs2806489			rs7066737		
	A	G	%G	C	T	%T
All cohort members	2879	797	21.7	3617	63	1.7
No atopy, no. (%)	1911 (66.4)	526 (66.0)	21.6	2397 (66.3)	42 (66.7)	1.7
Atopy, no. (%)	968 (33.6)	271 (34.0)	21.9	1220 (33.7)	21 (33.3)	1.7
Tests for heterogeneity (<i>P</i> value)	.841			.947		

Data on atopy were available for 94% of the subjects successfully genotyped for both markers A > G (rs2806489) and C > T (rs7066737).

TABLE VIII. Genotype and prevalence of minor alleles for *CYSLTR1* promoter SNPs in relation to incidence of atopy: female subjects

Atopy	rs2806489				rs7066737		
	AA	AG	GG	%G	CC	CT + TT	%T
All cohort members	2217	1265	177	22.1	3525	135	1.9
No atopy, no. (%)	1652 (74.5)	1009 (79.8)	136 (76.8)	22.9	2692 (76.4)	102 (75.6)	1.9
Atopy, no. (%)	565 (25.5)	256 (20.2)	41 (23.2)	19.6	833 (23.6)	33 (24.4)	2.1
Tests for heterogeneity (<i>P</i> value)		.002		.004		.827	.598

Tests of heterogeneity *P* values are given per allele and per genotype. Data on atopy were available for 94% of the subjects successfully genotyped for both markers A > G (rs2806489) and C > T (rs7066737).

difference was statistically significant ($P < .05$) in a combined sex*SNP interaction model, irrespective of whether the hemizygous male subjects were coded as having 0/1 or 0/2 minor (G) alleles. In female subjects, the per-allele odds ratio for atopy was 0.82 (95% CI, 0.72-0.94; $P = .004$), and 11.4% of all atopic cases were potentially attributable to the A allele, although this was mainly attributable to a lower prevalence of atopy in heterozygotes. A formal test for departure from linear trend was significant ($P = .042$), providing suggestive evidence of a protective effect of heterosis ("heterozygote vigor").

DISCUSSION

In this study we detected an association between reduced risk of atopy in female subjects and the presence of the G allele at rs2806489. We also confirmed that *CYSLTR1* promoter and coding polymorphisms are in strong LD. Two other studies looking at the 3 most common promoter SNPs have recently been published, which have also examined LD at this locus.^{14,28} Zhang et al¹⁴ screened 32 unrelated female subjects with seasonal allergic rhinitis (allele frequencies not given), whereas Kim et al²⁸ screened 340 Korean subjects. Because allele frequencies were estimated only from a population of nonasthmatic control subjects, frequencies are not directly comparable with those reported in this study.

However, both groups drew the same conclusions regarding LD patterns for these SNPs. Our results are also in keeping with data from the HapMap project, where the minor allele of the SNP rs321029 had a frequency of 25.8% in the CEU population (ie, white subjects from Northern and Western Europe).

Because of this strong linkage, only the 2 tag SNPs A > G (rs2806489) and C > T (rs7066737) were genotyped in the British 1958 birth cohort. The polymorphism C > T (rs7066737) was not associated with atopy, serum total IgE level, or asthma/wheeze prevalence and age of onset, although this could still be a false-negative result because of the lack of power given the low minor allele frequency. No association of the polymorphism A > G (rs2806489) was detected with any of the phenotypes studied in male subjects. In female subjects, the SNP rs2806489 did not affect the prevalence or age of onset of asthma, wheezy bronchitis, or wheeze. In addition, although a weak association was seen between this SNP and log serum total IgE levels, this was not consistent when analyzed as a categorical outcome, suggesting this might be a false-positive result. In contrast, the G allele was associated with a reduced risk of atopy in female subjects ($P = .004$).

A formal test for departure from linear trend also provided support for heterosis with a protective effect at this locus. This suggests that the *CYSLTR1* gene is not inactivated and female

TABLE IX. Genetic association studies of the *CYSLTR1* gene with atopic phenotypes.

Polymorphisms studied	Population	Association	Reference
-945 C > T (rs321029) -786 A > C (rs2637204) -647A > G (rs2806489)	Korean 105 AIA, 110 ATA, 125 C	Yes with TCG haplotype with AIA vs. ATA and C (males, $P = .02$ and $.03$) Yes with total IgE and atopy (females, $P = .003$ and $.03$) No with BHR and FEV ₁	Kim et al 2006 ²⁸
	Japanese 137 families with A, n = 466, 48 families with AR, n = 188	No with asthma and AR	Zhang et al 2006 ¹⁴
-945 C > T (rs321029)	Korean 159 AIA, 116 AICU	Yes with AIA vs. AICU ($P = .015$)	Kim et al 2007 ³⁴
	Korean 93 AIA, 181 ATA, 123 C	No with AIA and ATA	Choi et al 2004 ³¹
	Spanish white 130 A, 78 C	Yes, alone and in combination with LTC4S SNP -444 C allele with asthma (males, $P = .039$)	Sanz et al 2006 ²⁹
927 T > C (rs320995)	Spanish white 87 A (41 with AD), 79 C	Yes with asthma and asthma + AD vs. A+no AD (males, $P < .022$)	Arriba-Mendez et al 2006 ³⁰
	UK white 341 families	No with asthma, atopy and related phenotypes Yes, atopy severity * (females, $P = .0087$)	Hao et al 2006 ³²
899 G > A (G300S)	Tristan de Cunha 52 atopy vs. 60 C, 54 A vs 58 C	Yes with atopy ($P < .0001$) Yes with asthma (females, $P = .005$)	Thompson et al 2007 ³³
-647 A > G (rs2806489) -566 C > T (rs7066737)	UK white 2101 atopy, 5234 C	No with total IgE rs2806489: Yes with atopy * ($P = .004$)	Duroudier et al this paper

subjects express 2 copies of the gene. Although it is common for X-linked genes to not undergo X inactivation, the mechanisms underlying this phenomenon are not fully understood. However, against that hypothesis, if heterozygotes are also protected, one would also expect protection in male subjects. Another possibility is that *CysLTR1* expression might be under the influence of a sex-specific regulator, such as estrogen. This possibility has not been investigated to date.

There have been a few studies of *CYSLTR1* genetic variation in allergic diseases in the past 2 years (Table IX).^{14,28-34} There are discrepancies among these studies, which is probably due to their small size. In a white Spanish population, Sanz et al²⁹ reported the minor allele of the coding SNP T > C (rs320995) to be associated with asthma, but Arriba-Mendez et al³⁰ observed such an association only in male subjects, whereas 2 other groups could not detect it in a white British and a Korean population.^{31,32} Although not seen in a Japanese study,¹⁴ Kim et al²⁸ detected an association of the 3 most common *CYSLTR1* promoter SNPs with aspirin-intolerant asthma among male Korean subjects and with atopy among female subjects with aspirin-intolerant asthma. Interestingly, we found the promoter polymorphism rs2806489 to protect female subjects from atopy. In addition, in a family-based association study (341 British white subjects), the coding SNP T > C (rs320995), which is in high LD with the promoter SNP rs2806489, was associated with atopy severity among female subjects, although not with atopy risk.³² In a highly asthmatic population with a founder effect, Thompson et al³³ observed the variant of a new coding polymorphism, 899 G > A (G300S), to

be associated with both asthma and atopy in female subjects. Other studies have detected an association of the promoter or coding SNPs with allergic phenotypes, such as high total IgE level,²⁸ and atopic dermatitis,³⁰ which could not be reproduced elsewhere.^{14,32} Overall, those results suggest a possible sex-specific association of *CYSLTR1* genetic variations with allergic phenotypes.

The strength of our study is the size of the population studied and the objective phenotypic information available. By using the British 1958 birth cohort, our study was considerably larger than any previous study and hence a robust way of assessing the possible contribution of polymorphisms at this locus to risk of atopy.

Because the genomic region of *CYSLTR1* is a recombination cold spot,^{12,26} the positive associations seen could potentially originate from other functional polymorphisms located in the *CYSLTR1* gene or in its region, making it difficult to identify the true causal polymorphism or polymorphisms. However, we have previously reported inhibition of *CYSLTR1* transcription *in vitro* in THP1 cells because of the variant G at -647 (rs2806489): this effect could potentially be explained by the allele introducing a binding site for the transcription factor c-myb, which is responsible for negative regulation of transcription.³⁵ The same substitution A/G also introduces a CpG motif. Because this CpG site is not associated with a CpG island, it is likely to undergo methylation,³⁶ leading to repression of *CYSLTR1* transcription. In addition, this polymorphism is only 6 bp upstream of a predicted nuclear factor κ B (NF- κ B) binding site. The substitution G/A might affect binding of nuclear factor κ B to its site,

thus reducing *CYSLTR1* transcription. Those data suggest that in female subjects the presence of the G allele might cause a decrease in *CYSLTR1* transcription and hence a diminished responsiveness to LTs in relevant tissues, leading to a reduced risk of atopy.

In summary, this study is a comprehensive assessment of polymorphisms at the *CYSLTR1* locus and suggests that a regulatory polymorphism (A > G, rs2806489) at this locus contributes to the risk of atopy with evidence of heterozygote vigor among female subjects.

We thank all members of the 1958 birth cohort, particularly those who provided consent to use of their DNA for genetic epidemiologic analyses and the research nurses who contributed to the successful completion of both field studies.

Key message

- *CYSLTR1* genetic variability might contribute to the risk of the development of atopy in female subjects.

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TABLE E1. *CYSLTR1* haplotypes estimates in the Caucasian population sample from Nottingham

Haplotype	<u>−945</u> rs321029	<u>−786</u> rs2637204	<u>−647</u> rs2806489	<u>−566</u> rs7066737	<u>+927</u> rs320995	Estimated frequency	Expected frequency (linkage equilibrium)
I	C	A	A	C	T	0.750	0.407
II	C	A	A	T	T	0.047	0.020
III	T	C	G	C	T	0.047	0.007
IV	T	C	G	C	C	0.156	0.001

Haplotype frequencies for the *CYSLTR1* promoter polymorphisms rs321029, rs2637204, rs2806489, and rs7066737 and the coding SNP rs320995 (−945, −786, −647, and −566 from the exon 4 3′ end and +927 from the coding ATG, respectively) were estimated from genotyping data in the Nottingham population (n = 48 subjects).

TABLE E2. Genotype and prevalence of minor alleles for *CYSLTR1* promoter SNPs in relation to prevalence or age of onset of asthma, wheezy bronchitis, or wheeze: male subjects

	rs2806489			rs7066737		
	A	G	%G	C	T	%T
All cohort members	3078	861	21.9	3877	68	1.7
Prevalence, 0-42 y						
No asthma or wheeze by 42 y of age, no. (%)	1557 (50.6)	446 (51.8)	22.3	1969 (50.8)	30 (44.1)	1.5
Any history of asthma or wheeze by 42 y of age, no. (%)	1521 (49.4)	415 (48.2)	21.4	1908 (49.2)	38 (55.9)	2.0
Tests for heterogeneity (P value)		.528			.276	
Age of onset						
No asthma or wheeze by 42 y of age, no. (%)	1557 (50.6)	446 (51.8)	22.3	1969 (50.8)	30 (44.1)	1.5
Onset of asthma or wheeze 0-16 y of age, no. (%)	682 (22.2)	180 (20.9)	20.9	851 (21.9)	15 (22.1)	1.7
Onset of asthma or wheeze 17-42 y of age, no. (%)	467 (15.2)	126 (14.6)	21.2	585 (15.1)	9 (13.2)	1.5
Asthma or wheeze by 42 y, age at onset unknown, no. (%)	372 (12.1)	109 (12.7)	22.7	472 (12.2)	14 (20.6)	2.9
Tests for heterogeneity (P value)		.804			.205	

Data on asthma/wheeze were available for 83% and 100% of the male subjects successfully genotyped for the markers A > G (rs2806489) and C > T (rs7066737), respectively.

TABLE E3. Genotype and prevalence of minor alleles for *CYSLTR1* promoter SNPs in relation to prevalence or age of onset of asthma, wheezy bronchitis, or wheeze: Female subjects

	rs2806489				rs7066737		
	AA	AG	GG	%G	CC	CT + TT	%T
All cohort members	2367	1348	191	22.1	3762	146	2.0
Prevalence, 0-42 y							
No asthma or wheeze by 42 y of age, no. (%)	1200 (50.7)	707 (52.4)	109 (57.1)	22.9	1937 (51.5)	75 (51.4)	1.9
Any history of asthma or wheeze by 42 y of age, no. (%)	1167 (49.3)	641 (47.6)	82 (42.9)	21.3	1825 (48.5)	71 (48.6)	2.0
Tests for heterogeneity (<i>P</i> value)		.178		.08		.978	.970
Age of onset							
No asthma or wheeze by 42 years of age, no. (%)	1200 (50.7)	707 (52.4)	109 (57.1)	22.9	1937 (51.5)	75 (51.4)	1.9
Onset of asthma or wheeze at 0-16 y of age, no. (%)	421 (17.8)	255 (18.9)	29 (15.2)	22.2	682 (18.1)	26 (17.8)	1.8
Onset of asthma or wheeze 17-42 y of age, no. (%)	451 (19.1)	241 (17.9)	35 (18.3)	21.4	704 (18.7)	25 (17.1)	1.8
Asthma or wheeze by 42 y, age at onset unknown, no. (%)	295 (12.5)	145 (10.8)	18 (9.4)	19.8	439 (11.7)	20 (13.7)	2.4
Tests for heterogeneity (<i>P</i> value)		.332		.173		.875	.738

Tests of heterogeneity *P* values are given per allele and per genotype. Data on asthma/wheeze were available for 100% of the female subjects successfully genotyped for both markers A > G (rs2806489) and C > T (rs7066737).