Associations and interactions of genetic polymorphisms in innate immunity genes with early viral infections and susceptibility to asthma and asthma-related phenotypes

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Background: The innate immune system is essential for host survival because of its ability to recognize invading pathogens and mount defensive responses.

Objectives: We sought to identify genetic associations of innate immunity genes with atopy and asthma and interactions with early viral infections (first 12 months of life) in a high-risk birth cohort.
Methods: Three Canadian family-based studies and 1 Australian population-based case-control study (n = 5565) were used to investigate associations of 321 single nucleotide polymorphisms (SNPs) in 26 innate immunity genes with atopy, asthma, atopic asthma, and airway hyperresponsiveness. Interactions between innate immunity genes and early viral exposure to 3 common viruses (parainfluenza, respiratory syncytial virus, and picornavirus) were examined in the Canadian Asthma Primary Prevention Study by using both an affected-only family-based transmission disequilibrium test and case-control methods.
Results: In a joint analysis of all 4 cohorts, IL-1 receptor 2 *(IL1R2)* and Toll-like receptor 1 *(TLR1)* SNPs were associated with atopy after correction for multiple comparisons. In addition, an *NFKBIA* SNP was associated with atopic asthma. Six SNPs (rs1519309 *[TLR3]*, rs740044 *[ILIR2]*, rs4543123 *[TLR1]*, rs5741812 *[LBP]*, rs917998 *[IL18RAP]*, and rs3136641 *[NFKBIB]*) were significant ($P < .05$, confirmed with 30,000 permutations) in both the combined analysis of main genetic effects and SNP-virus interaction analyses in both case-control and family-based methods. The *TLR1* variant (rs4543123) was associated with both multiple viruses (respiratory syncytial virus and parainfluenza virus) and multiple phenotypes.
Conclusion: We have identified novel susceptibility genes for asthma and related traits and interactions between these genes and early-life viral infections. (J Allergy Clin Immunol 2012;130:1284-93.)

Key words: *Asthma, atopy, atopic asthma, airway hyperresponsiveness, genetics, association, gene-environment interactions, viral exposures*

The worldwide prevalence of asthma and other allergic diseases has increased during the past 2 decades, particularly in developed countries.[1-3] Currently, asthma affects 8% to 10% of the population in the United States and is the most common chronic disease in children. The innate immune system plays an important role in the development of allergic disorders by sensing various microbial compounds and mounting defensive responses. The hygiene hypothesis states that early childhood exposure to infection or endotoxins alters the T_H1/T_H2 balance, attenuating the T_H2 immune response and thus inhibiting the development of

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Abbreviations used
AHR: Airway hyperresponsiveness
CAPPS: Canadian Asthma Primary Prevention Study
GWIS: Genome-wide interaction study
IL-1R: IL-1 receptor
LD: Linkage disequilibrium
OR: Odds ratio
PIV: Parainfluenza virus
QQ: Quantile-quantile
RSV: Respiratory syncytial virus
SAGE: Study of Asthma Genes and the Environment Study
SLSJ: Saguenay–Lac-Saint-Jean and Quebec City Familial Asthma Collection
SNP: Single nucleotide polymorphism
TLR: Toll-like receptor

asthma and other allergic diseases.[4] In contrast, a microbe-free environment can polarize T_H cells to a T_H2 immune response and therefore predispose to the development of atopy.

A vital part of the innate immune response is the IL-1 receptor (IL-1R)/Toll-like receptor (TLR) superfamily.[5] The genes of the IL-1R/TLR pathways are widely expressed in different cell types in the human lung.[6] The IL-1R subgroup has been implicated in the development of atopy and asthma,[7-9] and genetic associations with atopic diseases have been reported.[8] Single nucleotide polymorphisms (SNPs) of *TLR2*,[10,11] *TLR4*,[11] *TLR6*,[12] *TLR9*,[11,13] and *TLR10*[14] have also been reported to be associated with asthma or allergic diseases. There has been no report yet on genetic variants of Toll/IL-1 receptor adaptors in allergic diseases.

Viral infections are well recognized as an environmental risk factor for both the development of asthma and asthma exacerbations,[15-21] although it is not clear whether viruses are causal, affecting and modifying the growth and development of the immune system, or whether the relationship is indicative of a susceptible subject with impaired lung function and innate immune responses or a combination of the two.[20,22,23] The "2-hit" hypothesis has been postulated, whereby viral infections promote asthma mainly in predisposed children.[20] It is entirely plausible that viral infections initiate asthma in children genetically predisposed to the disease. If we are going to understand the cause of asthma, we need to examine the larger complex picture of genetic susceptibility, environmental exposures, and their interactions.

The aim of this study was to identify novel genetic associations and potential gene-environment interactions between innate immunity genes and early viral infections (first 12 months of life) with 3 common respiratory viruses (parainfluenza virus [PIV], respiratory syncytial virus [RSV], and picornavirus) that have been implicated in the onset or exacerbation of asthma in children. Main genetic effects and interactions were tested for 4 allergic phenotypes: atopy, asthma, atopic asthma, and airway hyperresponsiveness (AHR). Innate immunity genes that were associated with asthma and atopic diseases in other populations were also investigated in our study cohorts, and these data have been previously reported.[24] We considered a variety of analytic approaches, including a genome-wide interaction study (GWIS), but given that we expected interaction effects to be modest (odds ratio [OR] < 2) and our small sample size (approximately 280 children), we believed that the multiple testing burden of a GWIS would overwhelm these modest effects. A more hypothesis-based pathway approach could have been undertaken, and this approach would have increased our power. However, our prior knowledge of biological pathways and gene-virus interactions is limited, and we believed our analyses could benefit from a more general approach. As such, we chose to look at a class of genes (innate immunity) with strong biological plausibility of involvement in gene interactions with the 3 common respiratory tract viruses. This nested hypothesis-based approach represents a compromise, it reduces the number of statistical tests performed compared with a GWIS, but it is not as focused as a genetic pathway approach.

METHODS

Study populations

Four well-characterized groups were used for this study of asthma and asthma-related phenotypes (Table I). Informed consent was obtained from each patient or his or her guardians, and the study was approved by the research ethics board of each recruiting center.

The Canadian Asthma Primary Prevention Study (CAPPS). This prospective birth cohort assessed the effectiveness of a multifaceted intervention program designed to prevent the development of asthma and other atopic disorders in 549 high-risk children who had a family history of allergies.[25] The children were recruited from Vancouver and Winnipeg, Canada, and have been assessed by a pediatric allergist at 3 time points for the presence of allergic phenotypes. A total of 545 families were initially recruited into the study, and 380 families were available for analysis by the end of 7 years of follow-up.[26] The presence of PIV, RSV, or picornavirus was tested at 2 weeks and 3, 8, and 12 months of age, as previously described.[19,27,28] Details of the laboratory protocols have been previously published.[19,27,28] Nasal swabs were tested for each virus, and the sensitivity for detection by means of RT-PCR was 10^2 to 10^3 copies of virus-specific nucleic acid within a specimen.[19]

The Study of Asthma Genes and the Environment (SAGE). This study included 723 children and their parents who were recruited from a population-based sample of 16,320 children born in Manitoba, Canada, in 1995.[29-31] A total of 3,586 children participated in a survey in 2002, of whom 392 had asthma, 192 had hay fever or food allergy, and 3,002 had none of these conditions.

All children with asthma or allergy were invited to participate in a nested case-control study in addition to a sample of control subjects representative of both urban and rural environments.

The Saguenay−Lac-Saint-Jean and Québec City Familial Asthma Collection (SLSJ). This collection is comprised of 306 families from the Saguenay−Lac-Saint-Jean (n = 227) and Quebec City (n = 79) regions of Quebec, Canada.[32-34] A family was deemed acceptable for the study on identification of an asthmatic proband whose 4 grandparents were of French-Canadian ancestry. Each subject completed a modified American Thoracic Society respiratory questionnaire.

The Busselton Health Study Population. Residents of the town of Busselton in the southwest of Western Australia have been involved in a series of health surveys since 1966. Subjects attended one of 6 cross-sectional surveys from 1966 to 1981 and the follow-up survey in 1994. A nested case-control study was designed from this population that included subjects who participated in 1 or more surveys and had a methacholine challenge. Cases and control subjects were defined by the presence (n = 644) or absence (n = 749) of asthma.[35,36]

Phenotype definitions

Asthma was defined as doctor-diagnosed asthma at 7 years of age in the CAPPS and SAGE cohorts based on history and physical examination without knowledge of the results of methacholine and allergy skin tests. In the SLSJ sample asthma was diagnosed by asthma specialists, and in the Busselton Study asthma was defined as self-reported doctor-diagnosed asthma. Atopy was defined as at least 1 positive response (ie, wheal diameter ≥3 mm or more than that elicited by the negative control at 10 minutes). AHR was defined as a PC_{20} FEV_1 of less than 3.2 mg/mL for CAPPS and SAGE subjects or less than

TABLE I. Sample size and characteristics of the 4 study populations

Study design	Family-based trios				Case-control	
Sample	CAPPS	SAGE	SLSJ	Total	Busselton	Total
Families	545	723	306	1574		
Subjects genotyped	1316	1466	1234	4016	1549	1549
				No. of cases	No. of control subjects	
Complete trios						
Asthma*†	57	139	379	575	644	751	1395
Atopy*†	135	190	362	687	620	575	1195
AHR*†	170	120	278	568	213	814	1027
Atopic asthma*†	43	92	305	440	382	400	782
Characteristics						
Sex, male/female	1.05/1	1.26/1	1/1.32		1/1.46	1/1.26	
Ethnicity, white	301 (79%)	525 (73%)	100%		100%	100%	

*Indicates the number of complete trios (genotypes on both parents and the affected child) with this phenotype classification who were eligible for the analysis (ie, passed all QC controls) in each study.
†In the Busselton Study this indicates the number of cases with the phenotype and the number of control subjects for that phenotype.

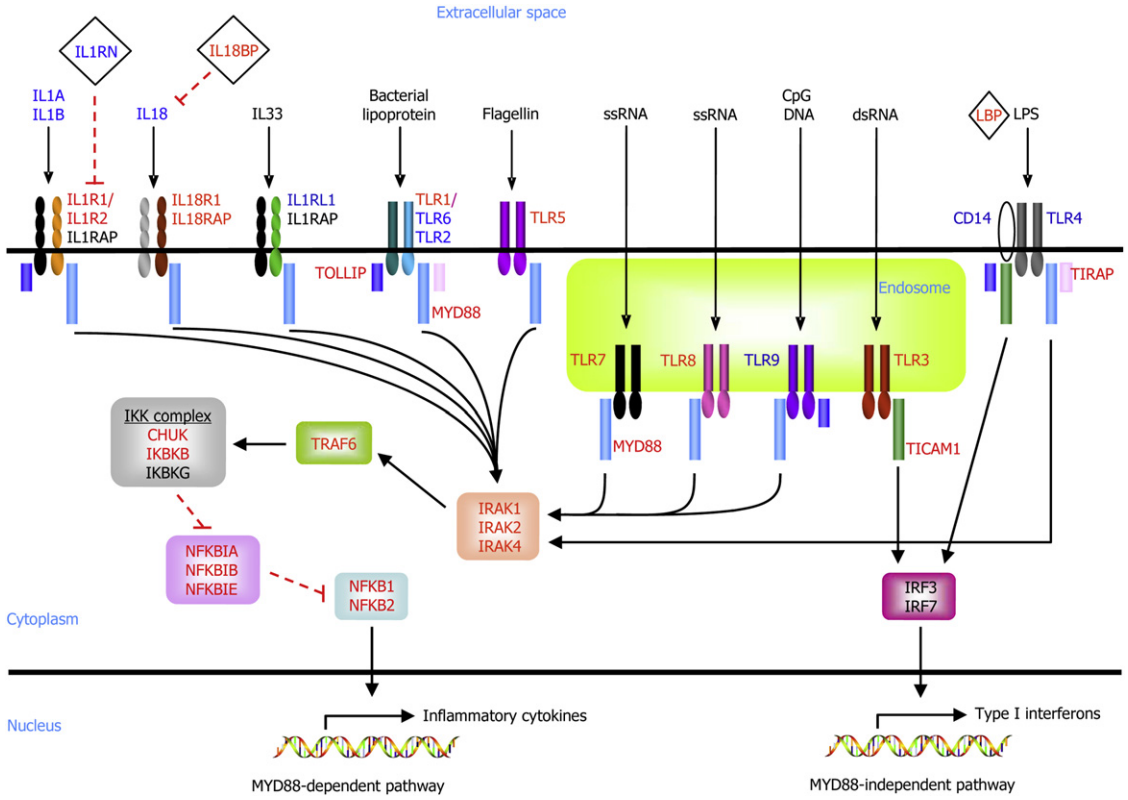


FIG 1. IL-1RI/TLR signaling pathways. Genes reported in the current study are highlighted in *red,* and genes reported in our previous study[24] are highlighted in *blue.*

8 mg/mL for SLSJ subjects or a PD_{20} of 3.9 μmol or less for Busselton subjects. Because AHR is more prevalent in children,[37,38] we used a PC_{20} of less than 3.2 mg/mL because it yields the greatest sum of sensitivity and specificity in children.[37] Atopic asthma was defined as patients given a diagnosis of both asthma and atopy.

Gene/TagSNP selection and genotyping

Twenty-six candidate innate immunity genes that had comprehensive SNP discovery data available from the Innate Immunity and SeattleSNPs Programs for Genomic Applications were selected (see Table E1 in this article's Online Repository at www.jacionline.org). Fig 1 shows genes in the innate immune system, and those included in this study are indicated in red. We have previously studied[24] 11 other innate immunity genes in the same study populations (see Table E1); for these genes, main genetic effects are not presented, but we do present the SNP-virus interaction effects.

TagSNPs were selected by using an "LD-tag SNP" approach and a linkage disequilibrium (LD) selection algorithm developed by Carlson et al.[39] The details of the genotyping and data cleaning have been previously published.[24] A total of 321 SNPs were studied, and SNPs were retained for analysis if they had a minimum call rate of 90%, a maximum of 4 Mendelian errors, and a maximum of 1 reproducibility error and showed consistency with Hardy-Weinberg equilibrium at a P value of greater than .001.

TABLE II. Clinical characteristics of subjects in the viral analyses

		Asthma		Atopy		Atopic asthma		AHR	
	All	Control subjects	Cases	Control subjects	Cases	Control subjects	Cases	Control subjects	Cases
No. included	280	225	55	158	115	144	40	100	155
Sex (% male)	53.9	50.7	69.1	47.5	60.9	45.8	70.0	53.0	55.5
Mother allergic (%)	60.0	56.9	72.7	53.8	70.4	52.1	75.0	62.0	62.6
Father allergic (%)	50.4	53.8	36.4	50.6	51.3	53.5	40.0	50.0	52.3
Maternal education (% high)	62.9	62.2	65.5	62.7	60.9	63.2	65.0	59.0	62.6
Mother smoking during pregnancy (%)	5.7	6.2	3.6	6.3	6.1	6.9	2.5	9.0	3.9
Duration of breast-feeding (%)									
Never	7.5	7.6	7.3	8.2	7.8	7.6	7.5	9.0	5.8
<4 mo	12.5	11.6	16.4	10.8	13.9	10.4	17.5	17.0	10.3
≥4 mo	80.0	80.8	76.3	81.0	78.3	82.0	75.0	74.0	83.9
Smoke exposure in the house (%)	20.0	19.6	21.8	21.5	20.0	21.5	20.0	22.0	20.6
Dog at home (%)	27.5	26.7	30.9	31.6	21.7	29.9	22.5	36.0	22.6
Cat at home (%)	20.4	19.6	23.6	21.7	20.0	21.5	27.5	26.0	16.1
Other children <12 y at home (%)									
0	7.5	6.2	12.7	7.0	7.8	6.9	15.0	9.0	6.5
1	56.1	56.4	54.5	53.2	59.1	52.1	52.5	54.0	56.1
≥2	26.8	27.1	25.5	29.7	23.5	31.3	27.5	28.0	28.4
Day care age 1 (%)	7.9	7.6	9.1	7.6	7.0	6.3	5.0	6.0	8.4
RSV (%)									
All	39.3	38.7	41.8	39.9	39.1	38.2	37.5	43.0	38.0
Center, Vancouver	13.9	15.1	9.1	17.1	10.4	17.4	7.5	16.0	12.9
Intervention	7.9	8.4	5.5	10.1	5.2	10.4	5.0	9.0	7.7
Control	6.0	6.7	3.6	7.0	5.2	7.0	2.5	7.0	5.2
Center, Winnipeg	25.4	23.6	32.7	22.8	28.7	20.8	30.0	27.0	25.1
Intervention	13.6	14.2	10.9	11.4	17.4	12.5	15.0	14.0	14.2
Control	11.8	9.4	21.8	11.4	11.3	8.3	15.0	13.0	10.9
PIV (%)									
All	46.8	45.8	50.9	47.5	45.2	47.2	50.0	48.0	47.7
Center, Vancouver	28.2	29.3	23.6	33.5	20.9	33.3	17.5	32.0	29.0
Intervention	15.7	16.9	10.9	17.7	13.0	18.1	10.0	20.0	15.5
Control	12.5	12.4	12.7	15.8	7.9	15.2	7.5	12.0	13.5
Center, Winnipeg	18.6	16.5	27.3	14.0	24.3	13.9	32.5	16.0	18.7
Intervention	8.9	8.9	9.1	5.7	13.9	6.3	12.5	8.0	9.0
Control	9.7	7.6	18.2	8.3	10.4	7.7	20.0	8.0	9.7
Picornavirus (%)									
All	66.8	68.9	58.2	67.1	65.2	68.1	55.0	65.0	67.1
Center, Vancouver	35.0	37.8	23.6	40.5	27.0	42.4	22.5	37.0	35.5
Intervention	19.3	21.3	10.9	22.2	14.8	23.6	12.5	23.0	19.4
Control	15.7	16.5	12.7	18.3	12.2	18.8	10.0	14.0	16.1
Center, Winnipeg	31.8	31.1	34.6	26.6	38.2	25.7	32.5	28.0	31.6
Intervention	16.8	17.3	14.5	10.2	26.1	11.1	20.0	15.0	16.1
Control	15.0	13.8	20.1	16.4	12.1	14.6	12.5	13.0	15.5
Center, Vancouver (%)	44.6	48.0	30.9	51.3	34.8	52.1	25.0	47.0	45.2
Center, Winnipeg (%)	55.4	52.0	69.1	48.7	65.2	47.9	75.0	53.0	54.8
Intervention (%)	52.9	55.6	41.8	47.5	60.0	50.7	52.5	51.0	56.1

Note: This table includes information for 280 CAPPS white children having asthma status and viral information.
Day care age 1 (%), Percentage attending day care before age 1 y; *Smoke exposure in the house (%)*, percentage with smokers living in the home.

Statistical analysis and correction for multiple testing

Associations between SNPs and the 4 phenotypes were tested by using a general additive allelic likelihood ratio test, as implemented in UNPHASED,[40,41] which uses a retrospective conditional likelihood similar to the transmission disequilibrium test[42] for the family-based studies and a standard retrospective case-control likelihood for case-control designs.

The details of the statistical analysis and correction for multiple testing have been previously published.[24,43]

Viral main effects

Main effects of viral infection were tested in CAPPS. A child was considered exposed to a specific viral subtype if they had a positive PCR result for that virus during the first 12 months of life. ORs were calculated by using logistic regression, with viral exposure as the predictor and asthma, atopy, atopic asthma, and AHR as dependent variables. Risks for asthma, atopy, and atopic asthma were evaluated at 3 times points (12 and 24 months and 7 years of life). Clinical characteristics for the CAPPS cohort are provided in Table II. Because the incidence for asthma, atopy, and atopic asthma (all 7 year phenotypes) differed significantly by recruitment center (Table II) and intervention status, a center and intervention covariates were included in the regression equation to adjust for these effects.

Viral interactions

Interactions between viral exposures and SNPs were tested with the inclusion of an interaction term (SNP-virus exposure) in the logistic regression

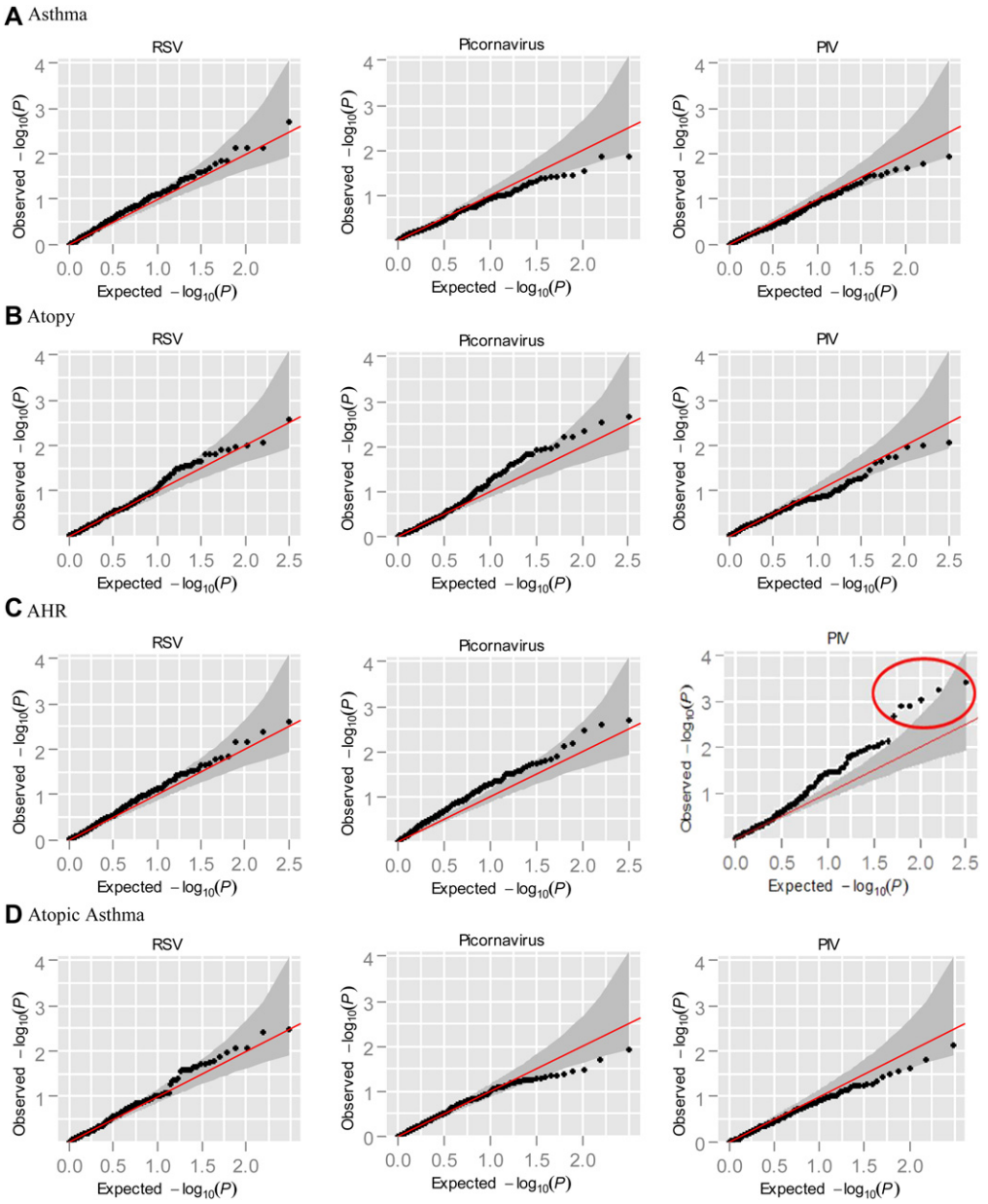


FIG 2. QQ plots showing the *P* value distributions for the interaction term (SNP-virus) plotted against the expected distributions (indicated by the *red diagonal line*). The 95% CI is indicated by the *shaded area*. QQ plots have been provided for each virus and phenotype.

by using only 7-year phenotypes because these are the most reliable. Interactions were tested by using both case-control and family-based methods. All results were adjusted for recruitment center and intervention group. For SNPs and interaction terms with *P* values of .05 or less, a permutation procedure was performed to determine the *P* value from an empiric distribution because the nominal *P* values obtained from statistical software are based on the asymptotic large sample theory. The empiric distribution was obtained by randomly assigning affectation (case/control) and infection status to subjects (exposed vs unexposed). An association test is then performed, and the *P* value is recorded; this procedure was repeated 30,000 times. The number of times a *P* value is less than or equal to the observed *P* value was determined and is represented as the permutation *P* value. For our family-based analysis, the affectation status and exposure values were randomly assigned to 1 of 4 possible offspring genotypes based on parental mating type, association tests were performed and repeated 30,000 times, and the number of times a *P* value was less than or equal to the observed *P* value was determined. Quantile-quantile (QQ) plots, which show the *P* value distribution for the interaction term (SNP-virus) plotted against the expected distribution, are given for each virus and phenotype (Fig 2); the expected distribution is denoted by the line, and the 95% CI has been shaded. When a large number of tests are performed, a review of the QQ plots, which evaluate the distribution of the observed *P* values, might be more informative than evaluating statistically significant observations.

RESULTS

Joint analysis for SNP main effects

In the joint analyses of all 4 cohorts, SNPs in *IL1R2* (rs740044) and *TLR1* (rs4543123) were associated with atopy after

TABLE III. Significant associations in CAPPS, SAGE, SLSJ, and the Busselton Health Study (Busselton) for the phenotypes of asthma, atopy, atopic asthma, and AHR

				CAPPS			SAGE			SLSJ			Busselton			Combined		
Type*	Gene	SNP†	Phenotype	OR	*P* value	c*P* value‡	OR	*P* value	c*P* value‡	OR	*P* value	c*P* value‡	OR	*P* value	c*P* value‡	OR	*P* value	c*P* value‡
A	*IL1R2*	rs740044	Atopy	0.73	.1528	1.0000	0.81	.2407	1.0000	0.77	.0349	.8417	0.84	.0438	.7932	0.80	.0007	**.0153**
A	*TLR1*	rs4543123	Atopy	0.87	.5223	1.0000	0.75	.1014	.9126	0.87	.2866	1.0000	0.81	.0351	.3161	0.82	.0043	**.0387**
A	*NFKBIA*	rs3138052	AA	0.71	.3162	1.0000	0.44	.0017	**.0303**							0.53	.0019	**.0337**
A	*LBP*	rs2232580	Atopy	4.00	.0007	**.0141**	1.05	.8728	1.0000	0.92	.6798	1.0000				1.20	.2394	1.0000
		rs5741812	Atopy	2.26	.0020	**.0423**	1.00	1.0000	1.0000	0.69	.3345	1.0000	0.94	.6096	1.0000	1.05	.5944	1.0000
A	*NFKBIB*	rs3136641	Atopy	1.87	.0014	**.0260**	1.05	.7868	1.0000	1.16	.2193	1.0000	1.03	.7748	1.0000	1.14	.0423	.7609
A	*CHUK*	rs3808916	AA	§	.0018	**.0221**	1.30	.5310	1.0000	1.35	.1116	1.0000	1.05	.8200	1.0000	1.14	.3203	1.0000
A	*NFKBIA*	rs2233419	AA	1.08	.8415	1.0000	0.41	.0016	**.0294**	0.97	.8597	1.0000	1.04	.7815	1.0000	0.92	.3539	1.0000
A	*TLR5*	rs2241096	AHR	0.86	.5860	1.0000	2.40	.0028	**.0255**	1.20	.3932	1.0000	0.91	.6116	1.0000	1.14	.2630	1.0000
A	*IL18RAP*	rs917998	AHR	1.17	.6308	1.0000	1.83	.0840	1.0000	0.48	.0006	**.0178**	0.58	.0059	.1599	0.70	.0033	.0907
A	*NFKBIE*	rs520639	Atopy	0.92	.7728	1.0000	0.90	.6910	1.0000	0.97	.8206	1.0000	1.45	.0036	**.0408**	1.15	.0988	1.0000
A	*TLR3*	rs1519309	AA	0.67	.2044	1.0000	1.06	.8137	1.0000	0.98	.8613	1.0000	1.41	.0012	**.0220**	1.15	.0602	1.0000
A	*IKBKB*	rs3747811	AHR	0.94	.6860	1.0000	0.92	.6920	1.0000	1.06	.6051	1.0000	0.71	.0017	**.0248**	0.90	.1118	1.0000
		rs10958713	AHR	1.00	1.0000	1.0000	1.21	.3880	1.0000	0.90	.3757	1.0000	0.68	.0011	**.0169**	0.86	.0270	.4054
A	*LBP*	rs12624843	AHR	0.91	.5763	1.0000	0.89	.5599	1.0000	0.89	.3921	1.0000	1.40	.0025	**.0443**	1.07	.3134	1.0000
C	*IL1R2*	rs740044	Atopy	0.80	.3453	1.0000	0.88	.4944	1.0000	0.77	.0349	.8417	0.84	.0438	.7932	0.82	.0021	**.0447**
C	*TLR5*	rs2241096	Atopy	1.00	1.0000	1.0000	1.67	.0816	.7347	1.28	.2072	1.0000	1.33	.0377	.3398	1.33	.0055	**.0494**
C	*CHUK*	rs3808916	AA	§	.0039	**.0471**	1.00	1.0000	1.0000	1.35	.1116	1.0000	1.05	.8200	1.0000	1.12	.3823	1.0000
C	*IL18R1*	rs7558013	AHR	1.06	.7630	1.0000	2.50	.0011	**.0341**	0.64	.0039	.1168	0.85	.2049	1.0000	0.90	.1986	1.0000
C	*IL18RAP*	rs6543135	AHR	1.06	.7606	1.0000	2.50	.0011	**.0307**	0.66	.0065	.1988	0.85	.1953	1.0000	0.90	.2227	1.0000

P values that are statistically significant ($P < .05$) after correction for multiple testing are shown in boldface.
AA, Atopic asthma.
*Analysis type A indicates all subjects, and analysis type C indicates white subjects only.
†SNPs listed using the unique rs (reference SNP) number assigned by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).
‡*P* value after correction for the number of SNPs being tested and the number of phenotypes.
§Stable OR estimate not available.

correction for multiple comparisons (Table III). In addition, rs3138052 in *NFKBIA* was associated with atopic asthma in the CAPPS and SAGE cohorts (genotypic information was unavailable for this SNP in the other cohorts). In all 3 associations the minor allele was protective (ORs, 0.53-0.82). The complete analysis of all SNPs in the cohorts is shown in Table E2 in this article's Online Repository at www.jacionline.org.

Analysis by individual study sample

In separate analyses of each of the 4 cohorts after correction for multiple comparisons, 2 SNPs in *LBP* (rs2232580 and rs5741812) were associated with atopy in the CAPPS cohort (Table III). Other associations in the CAPPS cohort were rs3136641 in *NFKBIB* with atopy and rs3808916 in *CHUK* with atopic asthma.

Two associations were observed in the SAGE cohort: 1 SNP in *NFKBIA* (rs2233419) with atopic asthma and rs2241096 in *TLR5* with AHR (Table III). In the SLSJ cohort 1 polymorphism (rs917998) in *IL18RAP* was associated with AHR.

There were 5 significant associations observed in analyses of the Busselton cohort (Table III). The rs520639 polymorphism in the *NFKBIE* gene was associated with atopy, and the rs1519309 SNP in *TLR3* was associated with atopic asthma. Two SNPs in the *IKBKB* gene (rs3747811 and rs10958713) were associated with AHR. The LD between these polymorphisms in this cohort was an r^2 value of 0.59. One polymorphism in the *LBP* gene (rs12624843) was also associated with AHR.

Analysis of samples of white subjects

As in the joint analysis of all 4 cohorts described above, *IL1R2* rs740044 was associated with atopy in white subjects only (Table III). The associations with the *TLR1* (rs4543123) and *NFKBIA* (rs3138052) SNPs were not seen in the white subjects only. An additional association (rs2241096 SNP in *TLR5*) was seen in the white subjects only in all 4 cohorts. The complete analysis of the white subjects for all SNPs is shown in Table E3 in this article's Online Repository at www.jacionline.org.

There were 3 associations observed in the analysis of white subjects only in the individual cohorts: rs3808916 in *CHUK* with atopic asthma, rs7558013 in *IL18R1* with AHR, and rs6543135 in *IL18RAP* with AHR. The SLSJ and Busselton cohorts only contained white subjects, and therefore the associations in these cohorts when analyzed individually are as shown in the previous section.

Viral main effects

There were no significant main effects for the association of any viral infection with asthma, atopy, atopic asthma, and AHR (see Table E4 in this article's Online Repository at www.jacionline.org). However, we note that the main effect of the virus often differs depending on the recruitment center. For example, for atopic asthma, the OR for picornavirus infection was 1.98 (95% CI, 0.23-17.12) in Vancouver, whereas there was a protective effect in Winnipeg (OR, 0.68; 95% CI, 0.29-1.62). This is likely due to the striking differences in the incidence rates for atopic asthma and the prevalence of picornavirus infection between the 2 recruitment centers (Table II).

Viral interactions

Table IV lists all significant ($P \leq .05$ confirmed by 30,000 permutations) SNP-virus interactions for asthma, atopy, atopic asthma, and AHR at 7 years of age, with both nominal *P* values

TABLE IV. Significant viral interactions (must be significant in both case-control and family-based analysis and verified as significant by means of permutation) for asthma, atopy, atopic asthma, and AHR at 7 years adjusted for recruitment center and intervention group

Phenotype	Virus	SNP	Gene	Main effect of SNP, OR (95% CI)	*P* value	Main effect of virus, OR (95% CI)	*P* value	Interaction virus-SNP, OR (95% CI)	*P* value	pp value*
Asthma	Picornavirus	rs4608848	*TLR3*	3.69 (1.62-8.41)	.002	2.05 (0.67-6.24)	.206	0.27 (0.10-0.76)	.013	.0151
		rs11721827	*TLR3*	4.49 (1.60-12.63)	.004	1.03 (0.47-2.29)	.935	0.27 (0.07-0.99)	.048	.0951
	RSV	rs543810	*IL18*	0.19 (0.04-0.83)	.027	0.57 (0.27-1.20)	.138	17.70 (2.86-109.42)	.002	.0006
		rs3771156	*IL18RAP*	1.62 (0.84-3.16)	.152	2.00 (0.87-4.57)	.101	0.23 (0.07-0.76)	.016	.0135
		rs3771172	*IL18R1*	1.63 (0.84-3.16)	.151	1.97 (0.86-4.50)	.107	0.24 (0.07-0.80)	.020	.0170
		rs1041973	*IL1RL1*	0.67 (0.33-1.38)	.276	0.53 (0.22-1.26)	.151	3.44 (1.16-10.16)	.026	.0337
		rs949963	*IL1R1*	0.77 (0.35-1.69)	.512	0.56 (0.24-1.33)	.192	3.57 (1.10-11.57)	.034	.0439
		rs913930	*TLR4*	0.49 (0.25-0.97)	.040	0.53 (0.22-1.28)	.158	2.82 (1.07-7.43)	.035	.0381
Atopy	Picornavirus	rs5743551	*TLR1*	2.01 (1.01-4.00)	.047	1.84 (0.91-3.74)	.091	0.26 (0.10-0.63)	.003	.002
	PIV	rs4648004	*NFKB1*	2.27 (1.32-3.90)	.003	2.09 (0.95-4.60)	.068	0.45 (0.21-0.95)	.035	.033
	RSV	rs10004195	*TLR10*	0.52 (0.29-0.95)	.034	0.50 (0.26-0.99)	.046	3.04 (1.24-7.47)	.015	.013
		rs10776483	*TLR10*	0.45 (0.22-0.92)	.028	0.54 (0.29-1.03)	.061	3.29 (1.26-8.58)	.015	.013
		rs440286	*IL1RN*	2.11 (1.25-3.56)	.005	1.48 (0.74-2.95)	.267	0.34 (0.13-0.86)	.023	.020
		rs671492	*TIRAP*	1.72 (1.04-2.86)	.036	1.39 (0.71-2.71)	.341	0.37 (0.15-0.87)	.023	.023
		rs4543123	*TLR1*	0.51 (0.28-0.93)	.028	0.53 (0.27-1.04)	.065	2.84 (1.14-7.07)	.025	.022
		rs3213448	*IL1RN*	2.33 (1.07-5.05)	.032	1.12 (0.62-2.03)	.696	0.26 (0.08-0.86)	.028	.025
		rs646005	*TIRAP*	0.80 (0.50-1.27)	.343	0.39 (0.17-0.92)	.030	2.53 (1.10-5.81)	.029	.028
		rs5743794	*TLR6*	0.69 (0.37-1.27)	.232	0.55 (0.29-1.05)	.068	2.78 (1.10-6.98)	.030	.028
		rs315931	*IL1RN*	2.15 (1.28-3.60)	.004	1.50 (0.72-3.14)	.283	0.42 (0.19-0.96)	.040	.039
Atopic asthma	Picornavirus	**rs1519309**	***TLR3***	**0.69 (0.41-1.17)**	**.171**	**0.47 (0.22-1.01)**	**.054**	**2.31 (1.03-5.21)**	**.043**	**.041**
		rs740044	***IL1R2***	**1.71 (0.75-3.92)**	**.203**	**1.55 (0.53-4.48)**	**.420**	**0.24 (0.07-0.89)**	**.033**	**.031**
	PIV	rs4251431	*IRAK4*	11.45 (0.94-139.23)	.056	0.97 (0.42-2.25)	.944	0.04 (0.00-0.82)	.037	.383
		rs1056890	*NFKB2*	1.30 (0.60-2.80)	.502	5.57 (1.74-17.80)	.004	0.20 (0.05-0.73)	.015	.014
	RSV	rs10776483	*TLR10*	0.24 (0.06-0.88)	.032	0.35 (0.13-0.97)	.044	12.99 (2.33-72.40)	.003	.004
		rs5743562	*TLR1*	0.31 (0.08-1.16)	.082	0.33 (0.11-0.93)	.036	12.25 (2.24-66.95)	.004	.004
		rs5743794	*TLR6*	0.32 (0.09-1.16)	.082	0.37 (0.13-1.03)	.056	8.50 (1.71-42.19)	.009	.008
		rs5743551	*TLR1*	0.53 (0.21-1.34)	.179	0.30 (0.09-0.98)	.046	6.15 (1.47-25.79)	.013	.015
		rs11096957	*TLR10*	0.44 (0.19-1.01)	.052	0.29 (0.09-0.97)	.044	5.04 (1.36-18.72)	.016	.020
		rs4543123	***TLR1***	**0.45 (0.16-1.21)**	**.114**	**0.39 (0.13-1.15)**	**.088**	**5.34 (1.21-23.52)**	**.027**	**.027**
AHR	Picornavirus	**rs5741812**	***LBP***	**0.38 (0.14-1.03)**	**.056**	**0.84 (0.45-1.58)**	**.588**	**3.48 (1.01-11.97)**	**.048**	**.060**
		rs352163	*TLR9*	0.60 (0.33-1.09)	.095	0.53 (0.20-1.36)	.184	2.10 (1.01-4.39)	.048	.050
	PIV	rs1039559	*TLR6*	0.48 (0.27-0.83)	.008	0.28 (0.11-0.70)	.007	4.11 (1.88-8.99)	.0004	.0003
		rs11096957	*TLR10*	1.95 (1.08-3.53)	.028	2.85 (1.26-6.44)	.012	0.24 (0.10-0.56)	.0009	.0008
		rs10008492	*TLR10*	1.78 (1.00-3.16)	.049	3.08 (1.30-7.29)	.010	0.25 (0.11-0.59)	.0013	.0015
		rs10776483	*TLR10*	2.14 (1.01-4.53)	.046	1.91 (0.98-3.74)	.059	0.21 (0.08-0.57)	.0021	.0022
		rs917998	***IL18RAP***	**1.09 (0.47-2.54)**	**.845**	**1.50 (0.81-2.76)**	**.198**	**0.16 (0.04-0.62)**	**.0078**	**.0087**
		rs1558642	*IL1R1*	2.60 (1.28-5.26)	.008	1.73 (0.88-3.37)	.110	0.27 (0.10-0.71)	.0080	.0077
		rs2192752	*IL1R1*	2.60 (1.28-5.27)	.008	1.73 (0.88-3.38)	.110	0.28 (0.10-0.73)	.0093	.0091
		rs4543123	***TLR1***	**1.99 (0.99-4.00)**	**.052**	**1.73 (0.87-3.45)**	**.117**	**0.32 (0.13-0.80)**	**.0147**	**.0155**
		rs3213733	*IL18R1*	0.57 (0.30-1.09)	.089	0.71 (0.37-1.36)	.300	2.69 (1.07-6.77)	.0349	.0388
		rs10176820	*IL18RAP*	0.57 (0.30-1.09)	.089	0.71 (0.37-1.36)	.300	2.69 (1.07-6.77)	.0349	.0409
		rs2075186	*IL18RAP*	0.57 (0.30-1.09)	.089	0.71 (0.37-1.36)	.300	2.69 (1.07-6.77)	.0349	.0388
		rs6531670	*TLR6*	1.58 (0.92-2.72)	.100	1.79 (0.85-3.79)	.127	0.44 (0.19-0.98)	.0349	.0389
	RSV	**rs3136641**	***NFKBIB***	**0.74 (0.44-1.25)**	**.266**	**0.46 (0.22-0.98)**	**.044**	**2.78 (1.12-6.91)**	**.028**	**.031**

SNPs that are also significant in the analysis of main genetic effects (see Table III) are shown in boldface.
*Permutation *P* value for interaction tests, *P* value was determined using 30,000 permutations.

and those determined by using permutation. SNPs must be significant in both case-control and family-based analyses to be included in this table. Six SNPs (rs1519309 *[TLR3]*, rs740044 *[ILIR2]*, rs4543123 *[TLR1]*, rs5741812 *[LBP]*, rs917998 *[IL18-RAP]*, and rs3136641 *[NFKBIB]*) are significant in both the combined analysis of main genetic effects (Table III) and SNP-virus interaction analyses in both case-control and family-based methods, and the significance of the *P* values has been confirmed by using permutation testing (Table IV). Additionally, the *TLR1* variant (rs4543123) is associated with both multiple viruses (RSV and PIV) and multiple phenotypes (AHR and atopic asthma). QQ (Fig 2) plots indicate that there are fewer significant SNP-virus (picornavirus and PIV) interactions than would have been expected given the number of tests performed for asthma and an excess number of SNP-viral infections (picornavirus and RSV) for atopy than would have been expected based on the number of tests performed, as evidenced by the early deviation of the observed line at approximately 1.0 on the x-axis. This early deviation borders the 95% CI. For atopic asthma, the QQ plots demonstrate fewer interactions than would be expected for picornavirus and PIV, whereas there was an increased number of interactions between 1.1 and 2.0 $-\log_{10}(p)$ than would have been expected for RSV. For AHR, the QQ plots show a deviation from the expected distribution for all 3 viruses, with the most

striking deviation being for PIV and AHR. SNPs with minor allele frequencies of 0.05 or less were removed and the QQ plots were redrawn to determine the dependency and sensitivity of our findings to rare variants. The observed distributions and our conclusions did not change (data not shown). A complete table of significant interaction results is provided in Table E5 in this article's Online Repository at www.jacionline.org, and results for all SNPs are provided in Tables E6 to E17 in this article's Online Repository at www.jacionline.org (case-control approach).

Tables E17 to E21 in this article's Online Repository at www.jacionline.org list all nominally significant ($P \leq .05$) SNP-virus interactions by using a family-based approach with both nominal *P* values and those determined by using permutation.

Supplemental Web resource

Given the volume of results, a supplemental Web resource is available at http://genapha.icapture.ubc.ca/viraltables/viraltables.html.

DISCUSSION

This is the first comprehensive assessment of innate immunity genes and asthma-related phenotypes that includes SNP-virus interactions in a prospective study of children at high risk for asthma and related phenotypes. Strengths of our approach include the prospective longitudinal design, comprehensive assessment of innate immunity genes, use of permutation to determine interaction *P* values, and use of both case-control and family-based models demonstrating that interaction effects are not due to population stratification.[44]

We found 2 novel associations (rs740044 in *IL1R2* and rs4543123 in *TLR1*) with atopy in joint analysis. *IL1R2* encodes the type II receptor for IL-1, and the soluble form inhibits the actions of IL-1 by acting as a decoy receptor.[45] The rs740044 polymorphism is located in the region upstream of *IL1R2* approximately 8 kb from the start of transcription. This polymorphism is in strong LD with several other SNPs in the 5′ region (see Table E3). Further investigation will be required to determine whether any of these polymorphisms affect the regulation of the gene and are therefore likely to be causal for the association.

This is the first description of an association of *IL1R2* polymorphisms with atopy and the first report of rs740044 in the context of asthma-related phenotypes, although a previous study of a Japanese population demonstrated association of an *IL1R2* polymorphism with aspirin hypersensitivity in asthmatic patients.[46] This same polymorphism was identified as interacting with picornavirus infection (Table IV) to modify the risk for atopic asthma in children from the CAPPS cohort.

TLR1 functions as a heterodimer with TLR2 to act as a receptor for bacterial lipoproteins.[47] The rs4543123 polymorphism is located in the region flanking the 3′ end of the *TLR1* gene approximately 5 kb from the termination of transcription. The rs4543123 SNP is in strong LD with several nearby polymorphisms, including variants in the *TLR10* gene. The function of TLR10 is not well defined, but the results of a recent study suggest that this receptor binds to a similar repertoire of lipoproteins as TLR1.[48] The rs4543123 SNP is in perfect LD with rs4833095, an amino acid substitution ($Asn^{248} \rightarrow Ser$) in the *TLR1* coding region.

Three *TLR1* polymorphisms were tested for association with asthma-related phenotypes in a German population, and 2 of them (rs5743595 and rs4833095) were associated with childhood atopic asthma after correction for multiple comparisons.[49] Both variants were protective against atopic asthma and resulted in increased TLR1 mRNA and protein levels. Therefore these results are consistent with our data, although the causal SNP underlying these associations remains to be determined. We also found evidence to support that *TLR1* (rs4543123) interacts with RSV infection to modify the risk for atopic asthma and evidence to support an interaction with PIV infection that modifies the risk for AHR.

We found a novel association of rs3138052 in *NFKBIA* with atopic asthma in the CAPPS and SAGE cohorts combined. NFKBIA is one of the inhibitory IκB proteins that form dimers with nuclear factor κB and thus maintain the transcription factor in the cytoplasm in an inactive form. Interestingly, there were 3 additional polymorphisms in IκB genes that were associated with asthma-related phenotypes in the individual cohort. Further associations were found with variants in genes that code for the IKK complex (ie, *CHUK* [also known as *IKBKA*] and *IKBKB*), suggesting an important role for polymorphisms in genes involved in nuclear factor κB regulation in the development of allergy and asthma phenotypes.

We also found that 3 polymorphisms in the *LBP* gene were associated with atopy or AHR in the individual cohorts. LBP protein is an acute-phase serum protein that binds to the LPS in the cell wall of Gram-negative bacteria.[50] LBP binds to LPS in a complex with bactericidal permeability-increasing protein, and this ternary complex interacts with membrane-bound CD14.[51,52] Polymorphisms in *LBP* have not previously been associated with asthma or related phenotypes.

We observed associations with variants in 3 receptor genes in the IL-1R/TLR superfamily (*TLR3*, *TLR5*, and *IL18RAP*) in the individual cohorts. There have been no previous reports of polymorphisms in *TLR3* or *TLR5* being associated with asthma or atopy. Previous studies have found that polymorphisms in the *IL1RL1*, *IL18R1*, and *IL18RAP* gene cluster on chromosome 2q were associated with asthma, AHR, and total serum IgE levels.[53,54] Determination of causality for these associations is difficult because of the strong LD that is found in this chromosomal region.

We found that the distribution for SNP-virus interactions deviated from expected, with an excess of associations early in the QQ distribution for all 3 viruses (PIV, RSV, and picornavirus) and AHR in our case-control analyses. The QQ plot for interactions with SNP-PIV for the AHR phenotype indicates extreme deviation at the tail end of the distribution. These SNPs are located in 2 genes (*TLR6* and *TLR10*) and were identified in both case-control and family-based analyses, and permutation testing confirmed the significance of these findings. RSV was associated with an increased number of SNP-virus interactions for atopy and atopic asthma, but again, this was early in the distribution, and we observed no excess in the tails of the distribution. We observed fewer SNP-virus interactions than expected by chance for asthma and atopic asthma for picornavirus infection. Beyond a few highly significant observations for PIV-SNPs for AHR, the QQ plots demonstrate an excessive number of observations early in the distribution, suggesting that our study is underpowered to distinguish causal SNPs. Six SNPs (rs1519309 *[TLR3]*, rs740044 *[ILIR2]*, rs4543123 *[TLR1]*, rs5741812 *[LBP]*, rs917998 *[IL18RAP]*, and rs3136641 *[NFKBIB]*) were significant ($P < .05$, confirmed with 30,000 permutations) in both the combined analysis of main genetic effects and SNP-virus interaction analyses in both

case-control and family-based methods. The *TLR1* variant (rs4543123) was associated with both multiple viruses (RSV and PIV) and multiple phenotypes.

We observed important differences in the incidence of asthma, atopy, atopic asthma, and AHR and large differences in infection rates for RSV and PIV between the 2 recruitment centers in the CAPPS cohort. These observations demonstrate the variability/heterogeneity of environmental exposures and the importance of controlling for these differences when estimating the effects of gene-environment interactions. Heterogeneity is likely to pose a major obstacle in the replication of gene-environment interactions, and careful consideration of the metrics of study design, exposure assessment, and measurement will be crucial in the analyses of gene-environment interaction effects.

Limitations to our study include a small sample size for interaction effects (280 children), likely misclassification of viral exposures because of the cross-sectional nature of the exposure assessment (nasal swabs), and the number of viral subtypes that could be detected at the time versus current technology. Additional limitations include the lack of symptom assessment at the time of nasal swab collection, which prohibits the correlation of respiratory symptoms, such as wheeze with viral infection. Finally, greater than 92% of the children had an older sibling in the home (Table II), which might confound some of our results and interpretations.

In conclusion, we have found several novel associations of variants in innate immunity genes with asthma, AHR, and atopy. This is preliminary evidence to support the concept that innate immunity genes and early viral exposure (first 12 months of life) interact to convey a predisposition to AHR in the absence of asthma or atopy. Ideally, we would have liked to replicate the gene-virus interactions reported in this article, but to our knowledge, there is no available comparable study in which genetics and viral testing have been done. Our future directions include the evaluation of these effects in the CAPPS cohort at the 15-year time point and replicating our findings in the Canadian Healthy Infant Longitudinal Development (CHILD) study, which is another Canadian prospective birth cohort that is currently underway and will be assessing viral exposures in the first year of life. Before firm conclusions can be drawn from these data, replication in other cohorts must be observed.

Key messages

- **Polymorphisms in innate immunity genes increase susceptibility to asthma, atopy, atopic asthma, and AHR.**
- **Polymorphisms in innate immunity genes interact with early viral infections to increase susceptibility to AHR.**
- **Understanding of the mechanisms whereby genetic susceptibility and viral infections predispose to asthma and related traits might inform clinical medicine.**

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