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IL10 Family Member Genes *IL19* and *IL20* Are Associated With Recurrent Wheeze After Respiratory Syncytial Virus Bronchiolitis

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ABSTRACT: Mechanisms underlying the increased risk of recurrent wheeze after respiratory syncytial virus lower respiratory tract infection (RSV LRTI) are unclear. Specifically, information about genetic determinants of recurrent wheeze after RSV LRTI is limited. We performed a candidate gene association study to identify genetic determinants of recurrent wheeze after RSV LRTI. We investigated 346 single nucleotide polymorphisms (SNPs) in 220 candidate genes in 166 Dutch infants hospitalized for RSV LRTI. Logistic regression analysis was used to study associations between genotypes and haplotypes and recurrent wheeze after RSV LRTI. We found associations with recurrent wheeze for SNPs in *IL19*, *IL20*, *MUC5AC*, *TNFRSF1B*, *C3*, *CTLA4*, *CXCL9*, *IL4R*, and *IL7* genes. Haplotype analysis of the combined *IL19*/*IL20* genotyped polymorphisms demonstrated an inverse association between the TGG haplotype and recurrent wheeze after RSV LRTI. *IL19* and *IL20* genes were notably associated with recurrent wheeze in infants without asthmatic parents. The association of *IL20* SNP rs2981573 with recurrent wheeze was confirmed in a healthy birth cohort. We concluded that genetic variation in adaptive immunity genes and particularly in *IL19*/*IL20* genes associates with the development of recurrent wheeze after RSV LRTI, suggesting a role for these IL10 family members in the etiology of airway disease during infancy. **(*Pediatr Res* 70: 518–523, 2011)**

Respiratory syncytial virus lower respiratory tract infection (RSV LRTI) during infancy is an independent risk factor for subsequent recurrent wheeze, at least within the first years of childhood (1). Mechanisms underlying the increased incidence of wheeze during the first years after RSV LRTI are unclear. Recurrent wheeze after RSV LRTI was related to signs of airflow limitation during RSV LRTI (2), eosinophilia during RSV LRTI (3), and monocyte IL10-production during the convalescent phase of RSV LRTI (4).

To date, only two studies aimed to identify genetic determinants of recurrent wheeze after RSV LRTI. In one association study of 134 RSV hospitalized infants, a variant of the *IL8* gene was related to the development of subsequent wheeze (5). We previously demonstrated an association between a functional *IL13* polymorphism and wheeze at age 6, whereas no association was found between the *IL13* polymorphism and recurrent wheeze during the first year after RSV LRTI (6).

The availability of analytic tools to study larger numbers of genes and a larger cohort of RSV LRTI-hospitalized infants in whom recurrent wheeze was evaluated enabled us to extend our previous studies. Herein, we describe the results of 346 genotyped single nucleotide polymorphisms (SNPs) on 210 genes, including *IL10* family genes.

METHODS

Subjects and design. The infants included in this study participated in previous studies (4,6–8). In brief, they were hospitalized for RSV LRTI during the winter seasons of 1995–1996 or 2004–2006. Infants included in the winter season of 1995–1996 participated in an observational study investigating the development of recurrent wheeze after RSV LRTI (4,6,7) and infants included in the 2004–2006 seasons received placebo medication in a placebo-controlled trial investigating the role of inhaled beclomethasone to prevent the occurrence of recurrent wheeze after RSV LRTI (8). Identical inclusion criteria were used in both original studies. Infants were hospitalized on suspicion of RSV LRTI, and RSV infection was confirmed by a positive RSV immunofluorescence in nasopharyngeal cells. We included previously healthy infants, *i.e.* infants with a history of cardiac or pulmonary disease were excluded. For this study, we selected infants of native Dutch origin who participated in the follow-up programs and whose parents prospectively recorded the presence of wheeze in a daily log (2). Identical logs were used in both original studies. The primary outcome of this study was predefined as the presence of wheeze during the first 15 mo after RSV LRTI hospitalization (8). We chose this duration of follow-up to capture the second winter season, which showed high incidence of wheeze after RSV LRTI in a previous study (9). Infants who wheezed more than the median counted days with wheeze during the follow-up of 15 mo, *i.e.* 14 or more d, were (arbitrarily) classified as infants with recurrent wheeze; whereas infants who wheezed less than the median counted days with wheeze during follow-up, *i.e.* less than 14 d, were classified as infants without recurrent wheeze. All parents provided written, informed consent. The Ethics Review Committee of the University Medical Centre Utrecht and other participating centers approved the study.

DNA isolation, genotyping, and selection of SNPs. A candidate gene approach was followed as described in our previous study focusing on genetic determinants of severe acute RSV LRTI (7). Briefly, 384 SNPs in 220 genes were selected based on literature studies in the context of RSV infection and classified into five processes, *i.e.* the airway mucosal response, innate immunity, chemotaxis, adaptive immunity, and allergic asthma. DNA isolation and genotyping of patients and their parents was performed in our previous study (7). SNPs were genotyped using Illumina's Beadarray technology on a 384 Sentrix array matrix. All SNPs were assessed to determine whether the observed genotype frequencies reflected the measured allele frequencies with Hardy-Weinberg equilibrium using χ^2 tests ($p < 0.01$) in control subjects not hospitalized because of RSV LRTI (7). All SNPs were examined for their minor allele frequency (MAF >10%) and call rate (call rate ≥90%). Thirty-

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Abbreviations: FDR, false discovery rate; **LD,** linkage disequilibrium; **LRTI,** lower respiratory tract infection; **RSV,** respiratory syncytial virus; **SNP,** single nucleotide polymorphism

eight SNPs were excluded because of low signal, overlapping of multiple clusters, or scattering of the clusters.

Replication cohort. To confirm our main finding, we genotyped *IL20* SNP rs2981573 (c.379–152 A→G), *IL19* SNP rs2243188 (c.552 + 49 C→A), and *IL19* SNP rs2243191 (Ser213Pro) in 90 infants recruited from an ongoing prospective unselected birth cohort of healthy newborns (10,11). The replication cohort was unselected with regard to RSV LRTI. Infants born to women delivering vaginally at term after uncomplicated pregnancy and delivery were recruited. Recurrent wheeze was measured during the first year of life using identical prospective daily recordings as used in the RSV cohort. Other genetic polymorphisms were not tested in this cohort.

Statistics. We used logistic regression analysis to estimate the OR for genotypes associated with recurrent wheeze after RSV LRTI (SPSS for Windows, Release 15.0: SPSS Inc., Chicago, IL). Significance was set at $p < 0.05$. If less than five infants in either the "recurrent wheeze" or "no recurrent wheeze" groups were homozygous for the minor allele, these infants were analyzed together with heterozygous infants. X-linked SNPs were analyzed separately in boys and girls. We performed sensitivity analyses for observed significant associations in which infants with and without recurrent wheeze were distinguished according to alternative cutoff values [*e.g.* no wheeze at all ($n = 29$) *versus* any wheeze during follow-up ($n = 137$); the quartile of infants with most frequent recurrent wheeze, *i.e.* more than 49 d during follow-up ($n = 42$) *versus* the rest ($n = 124$)]. Because baseline differences existed between infants with and without recurrent wheeze, we performed post hoc stratified analyses for groups of infants with and without asthmatic parents (*i.e.* parental reported physician diagnosed asthma) and for groups of infants with and without signs of airflow limitation during acute RSV LRTI (*i.e.* physician diagnosed wheezing by auscultation).

The global test for groups of genes was used to determine whether the groups of genes involved in different immunological processes, as preclassified in our previous study, were associated with recurrent wheeze after RSV LRTI (7,12). Haplotype analysis was performed in regions with moderate to high-linkage disequilibrium (LD) (0.3–0.8) where multiple SNPs were associated with recurrent wheeze. Pairwise LD was estimated using Haploview (version 4.0, released 21 August 2007, http://www.broad.mit.edu/mpg/haploview), and extent of LD was expressed in terms of standardized R^2 characteristics. Parental and infant SNP information was used to estimate haplotypes of the infants (Unphased software, version 3.0.7) (13). Haplotypes occurring with a frequency of ≥5% were included in haplotype analyses. Logistic regression analysis was used to estimate the OR for haplotypes associated with recurrent wheeze after RSV LRTI. The false discovery rate (FDR) method by Benjamini and Hochberg (14), accepting 5% false discoveries, was used to correct for testing multiple hypotheses.

RESULTS

One hundred sixty-six infants were included in this study. The median of counted days with wheeze during follow-up was 14 d (range, 0–279 d). The pattern of wheeze after RSV LRTI in the 1995–1996 and the 2004–2006 cohorts was remarkably similar. Baseline characteristics of infants with and without recurrent wheeze are presented in Table 1. Infants with recurrent wheeze more frequently exhibited signs of airflow limitation during RSV LRTI (63.2 *versus* 44.2%, $p = 0.02$), and more parents of infants with recurrent wheeze tended to suffer from asthma (16.9 *versus* 7.2%, $p = 0.06$).

Table 1. *Baseline characteristics of participating infants*

	No recurrent wheeze ($N = 83$)	Recurrent wheeze ($N = 83$)	p
Sex (% male)	50.6	60.2	0.21
Age at admission in wk (median, range)	10 (1–51)	9 (1–56)	0.33
Duration of pregnancy in wk (median, range)	39.2 (25–42.6)	39.0 (27–42)	0.23
Signs of airway limitation during RSV LRTI (%)	44.2	63.2	0.02
Admission to ICU (%)	13.6	14.6	0.87
Parental asthma (%)	7.2	16.9	0.06
Counted days with wheeze (median, range)	2.5 (0–14)	49 (15–279)	NA

Genotype determination was successful for 346 SNPs. Ten SNPs in nine genes were associated with recurrent wheeze at the genotype level ($p < 0.05$). Results of the genotype-phenotype association study are presented in Table 2. The global test for groups of genes was used to evaluate the importance of the selected processes in susceptibility to recurrent wheeze after RSV LRTI. The group of SNPs in genes involved in adaptive immunity was associated with recurrent wheeze ($p = 0.03$) whereas the other processes were not. Six SNPs within the group of SNPs in genes involved in the adaptive immune system were significantly associated with recurrent wheeze after RSV LRTI. The three associated SNPs in the *IL19* and *IL20* genes were in moderate to high LD with each other (Fig. 1). To test whether individual protective effects of *IL19* and *IL20* polymorphisms could be attributed to a specific haplotypic background, haplotype analysis of the *IL19* and *IL20* genes was executed. A combined haplotype analysis was performed with two of the three genotyped SNPs in the *IL19* and *IL20* genes that were associated with recurrent wheeze [*IL19* SNP rs2243191 (Ser213Pro) and *IL20* SNP rs2981573 (c.379–152 A→G)] and with one IL20 SNP that was not associated with recurrent wheeze after RSV LRTI [*IL20* SNP rs2981572 (c.-1053 T→G)]. The *IL19* SNP rs2243188 (c.552 + 49 C→A) was excluded because of high LD ($R^2 = 0.89$) with *IL19* SNP rs2243191 (Ser213Pro). Three common haplotypes with a frequency ≥5% were identified in the total group of infants (Table 3). These haplotypes comprised 99% of all *IL19/IL20* haplotypes. The combined *IL19/IL20* haplotype TGG had a lower frequency in infants with recurrent wheeze compared with infants without recurrent wheeze [13 *versus* 29%; OR, 0.4 (95% CI, 0.2–0.8); $p = 0.003$].

Baseline differences in the presence of signs of airflow limitation during RSV LRTI and the presence of parental asthma existed between infants with and without recurrent wheeze (Table 1). To test whether associations between the *IL19* and *IL20* SNPs and recurrent wheeze differed for infants with and without an atopic predisposition and for infants with and without signs of airflow limitation, post hoc stratified analyses were performed. Post hoc stratification for the presence of signs of airflow limitation did not alter the associations (data not shown). Post hoc stratification for the presence of parental asthma showed that associations between *IL19* and *IL20* SNPs and recurrent wheeze were limited to the major subgroup of infants without asthmatic parents (Table 4). No association was observed between the *IL19* and *IL20* SNPs and recurrent wheeze in the minor subgroup of infants with asthmatic parents. Similar effect sizes were obtained when the analyses were stratified for other atopic features in parents, *i.e.* infants with and without parents suffering from hay fever and eczema (data not shown).

Sensitivity analyses in which infants with and without recurrent wheeze were distinguished according to alternative cutoff values revealed comparable results (data not shown). To determine whether the associations between the three *IL19/IL20* SNPs and recurrent wheeze were limited to children with a history of RSV LRTI, we studied the three SNPs in a small

Table 2. *Significant associations with recurrent wheeze after RSV LRTI for genotypic analyses*

SNP	Gene	Process	MAF	AB *vs* AA BB + AB *vs* AA OR (95% CI)	BB *vs* AA OR (95% CI)	*p**
rs11558499	*MUC5AC*	Airway mucosal response	0.19	2.7 (1.4–5.2)†	†	0.002
rs2243191	*IL19*	Adaptive immunity	0.19	0.4 (0.2–0.7)†	†	0.002
rs1061622	*TNFRSF1B*	Innate immunity	0.24	0.4 (0.2–0.8)†	†	0.004
rs2981573	*IL20*	Adaptive immunity	0.20	0.4 (0.2–0.8)†	†	0.004
rs4807893	*C3*	Innate immunity	0.43	0.3 (0.1–0.6)	0.5 (0.2–1.2)	0.004
rs3087243	*CTLA4*	Adaptive immunity and allergic asthma	0.47	1.7 (0.8–3.7)	4.6 (1.7–12.5)	0.008
rs2276886	*CXCL9*	Chemotaxis	0.25	2.1 (1.1–4.0)†	†	0.016
rs2243188	*IL19*	Adaptive immunity	0.20	0.5 (0.3–0.9)†	†	0.026
rs1805015	*IL4R*	Adaptive immunity and allergic asthma	0.14	0.5 (0.2–1.0)†	†	0.037
rs2583762	*IL7*	Adaptive immunity	0.16	1.9 (1.0–3.8)†	†	0.047

* According to χ^2 distribution on genotype frequencies.

† Homo- and heterozygous infants are grouped together because <5 infants in one of the cells.

A, major allele; B, minor allele; AA, homozygous for major allele; AB, heterozygous; BB, homozygous for minor allele.

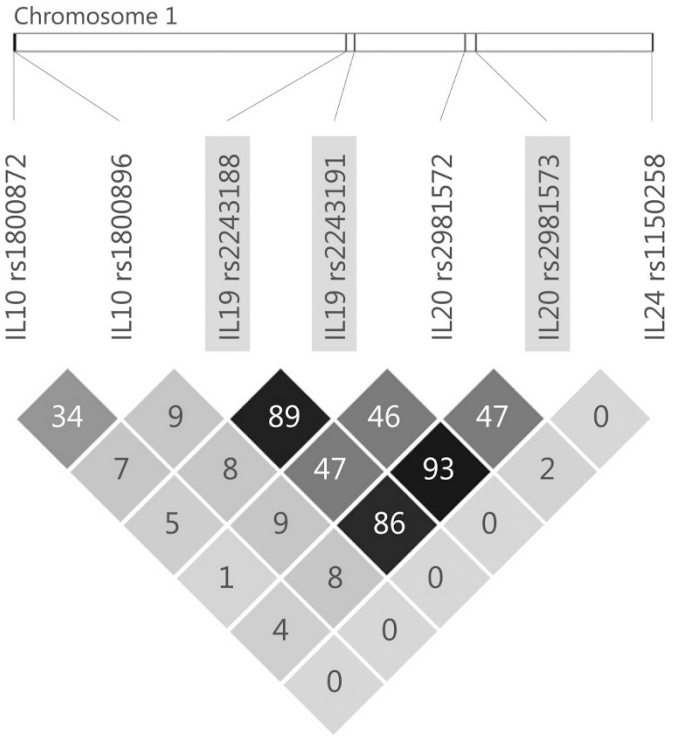

Figure 1. The genes of the *IL10* family on chromosome 1. *IL10*, *IL19*, *IL20*, and *IL24* genes on chromosome 1 and the genotyped SNPs. SNPs rs2243188, rs2243191, and rs2981573 showed significant association with recurrent wheeze after RSV LRTI. Pairwise LD between SNPs is characterized in terms of standardized R^2 characteristics. *Black blocks* indicate high LD between SNPs; *dark* and *light gray blocks* indicate moderate to low LD; and *white blocks* indicate that there is little significant LD.

unselected prospective birth cohort using identical log-based methodologies as used in the RSV cohort to quantify infant wheeze during the first year of life (10,11). The *IL20* SNP rs2981573, *IL19* SNP rs2243188, and *IL19* SNP rs2243191 were genotyped in 90 infants. *IL20* SNP rs2981573 was significantly associated with recurrent wheeze during the first year of life (OR, 0.39; 95% CI, 0.16–0.96, $p = 0.04$). For others SNPs, we could not confirm an association with recurrent wheeze, although similar trends were observed for both *IL19* SNPs rs2243191 (OR, 0.64; 95% CI, 0.26–1.53, $p = 0.31$) and rs2243188 (OR, 0.74; 95% CI, 0.31–1.74, $p = 0.49$) and for the *IL19/IL20* TGG haplotype (OR, 0.69; 95% CI, 0.37–1.27, $p = 0.15$). Post hoc stratification for the presence of parental asthma showed similar results (data not shown).

DISCUSSION

This study demonstrates that genetic variation in adaptive immunity genes and particularly in *IL19* and *IL20* genes seems to be associated with the occurrence of recurrent wheeze after RSV LRTI. The prevalence of recurrent wheeze was lower in infants with the combined *IL19/IL20* TGG haplotype compared with infants with the CTA haplotype. The relationship between the *IL20* SNP rs2981573 and recurrent wheeze was confirmed in a small healthy birth cohort.

We previously demonstrated the importance of SNPs in innate immune genes to determine susceptibility to RSV LRTI (7). These genes are not associated with the development of recurrent wheeze after RSV LRTI, which we now show to be determined by variation in *IL10*-related genes. Relationship between the *IL10* family member genes *IL19* and *IL20* and recurrent wheeze after RSV LRTI or any other chronic airway disease have not yet been described in literature. Of the two other studies that reported on genetic susceptibility of recurrent wheeze after RSV LRTI, one study showed no association (6). The other study of Goetghebuer *et al.* (5) reported an association between the *IL8* -251 C→T polymorphism and recurrent wheeze, which we could not confirm. However, Goetghebuer *et al.* analyzed the occurrence of wheeze after RSV LRTI in infants with a mean age of 6.5 y, whereas our study focused on recurrent wheeze during the first year after RSV LRTI only. These differences in wheeze phenotypes might have influenced the results because our previous study suggested that recurrent wheeze during the first year after RSV LRTI and recurrent wheeze at the age of 6 y are distinct entities with distinct immunological and genetic characteristics (6).

The major strength of our study is that polymorphisms in genes involved in different biological pathways were studied in a cohort of RSV LRTI hospitalized infants that was prospectively followed to evaluate the occurrence of recurrent wheeze. Some of our findings deserve further discussion.

Table 3. *Results of combined IL19-IL20 haplotype analysis in patients with recurrent wheeze after RSV LTRI*

Haplotype	rs2243191	rs2981572	rs2981573	No wheeze ($N = 83$)	Wheeze ($N = 83$)	Haplotypic, OR (95% CI)	p
HT 1	C	T	A	60%	66%	*	
HT 2	C	G	A	10%	19%	1.72 (0.89–3.22)	0.106
HT 3	T	G	G	29%	13%	0.43 (0.24–0.75)	0.003
Other HT†				1%	2%		

Haplotype frequencies (%) and haplotypic ORs with their 95% CIs and p compared with the reference haplotype are indicated in patients with recurrent wheeze ($N = 83$) and in patients without recurrent wheeze ($N = 83$).

* The haplotype combining the most frequent alleles at each site is chosen as the reference haplotype (CTA).

† Haplotypes occurring with a frequency of ≤5% were excluded from the haplotype analyses.

Table 4. *Results of post hoc stratified analyses of IL19-IL20 SNPs and recurrent wheeze after RSV LRTI in infants with and without asthmatic parents*

SNP	No asthmatic parent ($N = 146$) OR (95% CI)	p*	Asthmatic parent ($N = 20$) OR (95% CI)	p*	Interaction p†
rs2243188	0.3 (0.2–0.7)	0.003	1.3 (0.2–9.7)	0.83	0.25
rs2243191	0.2 (0.1–0.5)	0.00009	1.3 (0.2–9.8)	0.83	0.13
rs2981573	0.3 (1–0.5)	0.00025	1.3 (0.2–9.8)	0.83	0.16

ORs with their 95% CIs, p and interaction terms between IL19 and IL20 SNPs and recurrent wheeze after RSV LRTI in the subgroups of infants with ($N = 20$) and without ($N = 146$) asthmatic parents.

* p value of logistic regression test.

† p value of interaction.

First, the presence of false-positive results cannot be precluded because most of the observed associations lost significance after correction for multiple testing using the FDR method by Benjamini and Hochberg (14). However, based on the number of associated SNPs in a process, genes involved in adaptive immunity were overrepresented. Furthermore, the association of *IL19* SNP rs2243191 and *IL20* SNP rs2981573 with recurrent wheeze in the major subgroup of infants without asthmatic parents remained significant after FDR correction. A haplotype analysis of the SNPs on the *IL19/IL20* region showed association of the TGG haplotype and recurrent wheeze, potentially pointing at a functional variant located on this haplotype. Finally, we confirmed and expanded our conclusion on the association between the *IL20* SNP rs2981573 and recurrent wheeze in a replication cohort that was unselected for RSV LRTI.

Second, the post hoc observation that associations between *IL19* and *IL20* SNPs and recurrent wheeze were particularly detected in infants without an atopic background gave the impression that IL19 and/or IL20 cytokines are involved in nonatopic wheeze during infancy. The baseline observation that parental asthma was more common in infants with recurrent wheeze might refer to the heritability of atopic wheeze. It is known that RSV LRTI is a risk factor for subsequent recurrent wheeze independent of atopic status (1). We hypothesize that IL19 and IL20 cytokines are predominantly involved in nonatopic viral-induced recurrent wheeze. This hypothesis is further supported by a recent trial demonstrating reduced wheeze after antibody-mediated RSV prevention in nonatopic but not in atopic preterm infants (15). However, in our study, interaction terms between *IL19* and *IL20* SNPs and atopic features did not reach significance (Table 4). In addition, this study is relatively small and weak genetic effects may remain undetected. The OR of the observed genetic associations with recurrent wheeze was 0.4 (*IL19* SNP rs2243191 and *IL20* SNP rs2981573) and 0.5 (*IL19* SNP rs2243188), respectively. Using QUANTO 1.1 (16), we calculated that the power to detect associations with these effect sizes was 75% (OR, 0.4) and 54% (OR, 0.5), respectively, in this study. The power to detect smaller genetic effects in children with or without asthmatic parents is low, and therefore lack of significant association does not preclude a smaller, still relevant, association.

Third, this study aimed to explain recurrent wheeze after RSV LRTI but does not address the relationship between LRTI caused by other viruses and development of reactive airway disease. It is of particular interest that the *IL20* SNP rs2981573 association with recurrent wheeze was replicated in a cohort that was unselected for RSV LRTI. This might signify that *IL19* and *IL20* genes have a role in infant wheeze in the general population, potentially regardless of RSV LRTI. For instance, rhinovirus-associated wheezing illness is strongly linked to recurrent wheeze and allergic asthma development (17). New studies are required to study the role of genetic variation in *IL19* and *IL20* genes and recurrent wheeze after LRTI caused by other viruses including rhinovirus.

IL19 and IL20 are members of the IL10 family that were initially identified during a sequence database search aimed to find potential *IL10* gene homologs (18,19). IL10 is a pleiotropic anti-inflammatory cytokine known to suppress Th1-like immune responses and promote Th2 responses (20). IL10 levels measured during acute RSV LRTI related to disease severity in one study (4), whereas another study showed no association (21). We previously showed that monocyte IL10 production during the convalescent phase of RSV LRTI is predictive of the subsequent development of recurrent wheeze (4). Monocyte IL10 levels measured during acute RSV LRTI were not associated with the *IL19/IL20* haplotypes in a subgroup of 40 patients (data not shown). Several studies focused on the role of genetic variation in the *IL10* gene locus in the pathophysiology of acute RSV LRTI. Overall, the frequency of *IL10* polymorphisms in infants with RSV LRTI did not differ from controls (22–25). However, in infants hospitalized ≤6 mo of age, the *IL10* -592C allele was related to RSV LRTI hospitalization (23). In addition, genetic variation at the *IL10* gene locus was associated with the need for mechanical ventilation (24) and with the frequency of pneumonia (25) in RSV LRTI-hospitalized infants. The SNPs that were associated with recurrent wheeze after RSV LRTI in this study, *i.e.*

particularly SNPs in adaptive immunity genes, were not associated with acute RSV LRTI (7), suggesting that RSV LRTI and the subsequent occurrence of recurrent wheeze have a different genetic etiology.

IL19 and *IL20* genes are clustered together with *IL10* and *IL24* genes on chromosome 1q31–32 and have similar genomic structures and similar primary and secondary protein structures (26). Both IL19 and IL20 bind to the IL20 receptor complex, consisting of the IL20R1 and IL20R2 subunits. IL20 also binds to a heterodimeric receptor consisting of IL22R1 and IL20R2 (27). The receptors for IL19 and IL20 are widely expressed, but only lung and skin tissue express both receptors (28). Both receptors signal through STAT3 (18,27). IL10 family members cross-regulate expression of other IL10 family members. IL19 induces selective expression of IL10 by monocytes and myeloid dendritic cells (29). IL19 induces IL19 expression by an auto-feedback mechanism, which is not yet fully understood. Control of IL19 expression is provided by IL10, strongly interfering with *IL19* gene transcription. The *IL19* and *IL20* genes contain a highly polymorphic, informative repeat sequence useful for genotyping (30). Genotyped SNPs in this study are located in the intron, exon, and promoter region. Only the *IL19* SNP rs2243191 resulted in an amino acid change, *i.e.* Ser → Pro. Previous studies showed associations of the genotyped *IL19* and *IL20* SNPs with Hepatitic C virus clearance (31), psoriasis (32), palmoplantar pustulosis (33), and juvenile idiopathic arthritis (34), suggesting that IL19 and IL20 play a role in the pathology of inflammatory disorders. It is still to be determined whether the polymorphisms have differential effects on the function of the encoded protein or levels of gene expression and thus contribute to disease etiology. Limited data were available on the role of IL19 and IL20 in the etiology of airway diseases. In asthmatics, IL19 serum levels are increased, but no human data on levels in bronchoalveolar lavages have been published (35). In mice and humans, IL19 overexpression enhanced allergic airway inflammation by the induction of Th2 cytokines (35,36). However, nonallergic mechanisms by which IL19 and IL20 induce airway inflammation have been considered. Adenosine-induced IL19 production by primary bronchial epithelium cells enhanced monocyte TNFα production (37). In line with these literature data, we hypothesize that our findings underscore a central role of bronchial epithelial cells in the pathogenesis of recurrent wheeze after RSV LRTI.

In conclusion, genetic variation in adaptive immunity genes and particularly in *IL10* family member genes *IL19* and *IL20* genes seems to be associated with recurrent wheeze after RSV LRTI, and perhaps infant wheeze in the general population, suggesting a role for IL19 and IL20 cytokines in airway disease. Investigations of how the *IL19* and *IL20* gene polymorphisms affect the function of the encoded protein or gene expression levels are needed to evaluate the pathophysiological mechanism underlying the protective effect of the TGG haplotype on recurrent wheeze after RSV LRTI.

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