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Respiratory syncytial virus infection and recurrent wheezing in Chilean infants: A genetic background?

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ABSTRACT

Respiratory syncytial virus (RSV) infection has been associated to recurrent wheezing, but pathogenic mechanisms are unclear. Interleukin-4/Interleukin-13 (IL-4/IL-13) pathway is involved in both conditions. A common host genetic susceptibility may exist in patients whom RSV will trigger severe illness and those who develop recurrent wheezing.

Objective: To assess, by a candidate-gene approach, whether genetic polymorphisms in IL-4/IL-13 pathway are associated with RSV infection severity and its outcome in Chilean children.

A cohort of 118 RSV-infected infants was analyzed and followed for one year. Severity of acute infection and later recurrent wheezing were characterized. Alleles and genotypes frequencies were determined for two SNP in each of the genes IL-4, IL-13 and IL-4Rα. Association tests and interaction analyses were performed.

Enrollment included 60 moderate and 58 severe cases. Two SNP were found associated to severity during acute infection in IL-4Rα gene (Gln551Arg, Ile50Val). The follow up was completed in 71% of patients (84/118). Later recurrent wheezing was 54% in severe group, versus 31% in moderate cases ($p = 0.035$). In relation to outcome, allele Ile50 in IL-4Rα was more frequent in patients with moderate disease and no wheezing outcome. A common protector genotype is proposed for Chilean children: IL-4Rα Ile/Ile.

Conclusion: Genetic variations in the host are associated to infection severity and outcome. A common genetic background might be influencing both pathologies.

1. Introduction

Lower respiratory tract infections (LRTI) are the most common disease in infants under two years of age (Smyth and Openshaw, 2006). In Chile, as worldwide, respiratory syncytial virus (RSV) is the principal etiological agent, reaching up to 80% of hospitalized LRTI during the winter months (Avendaño et al., 2003; Hall et al., 2009). The hospitalization rate of RSV is 2–3% of infected children, with 0.1% of mortality (Avendaño et al., 2003; Palomino et al., 2004). Although RSV infection can cause mild upper respiratory symptoms, in some cases the infection results in severe disease, with respiratory failure that can be lethal (Zorc and Hall, 2010). Risk factors have been described, including prematurity, young age, chronic lung disease, immuno deficiency disorders and congenital heart disease (Hall et al., 2009; Sommer et al., 2011). Nevertheless, the main pathogenic factors that determine severity in previously healthy patients have not been defined.

In addition to the impact of the acute RSV infection, it has been described an association between LRTI in infancy and subsequent airway hyperesponsiveness with recurrent wheezing episodes (Pullan and Hey, 1982; Sigurs et al., 1995, 2005, 2010; Young et al., 1995; Noble et al., 1997; Stein et al., 1999; Schauer et al., 2002; Piippo-Savolainen et al., 2004; Henderson et al., 2005). Despite differences in studies designs, bronchiolitis-especially RSV-induced has been proposed to be a risk factor for the development of wheezing and asthma, even in adulthood (Piippo-Savolainen et al., 2004; Sigurs et al., 2010). A longitudinal cohort study of children that required hospitalization from RSV infection, has demonstrated an increased frequency of wheezing episodes up to 43%,

Abbreviations: LRTI, lower respiratory tract infection; RSV, respiratory syncytial virus; RV, rhinovirus; SNP, single nucleotide polymorphism; IL-4, interleukin 4; IL-13, interleukin 13; IL-4Rα, interleukin 4 receptor alpha; NPA, nasopharyngeal aspirate; ER, emergency room; Ile, Isoleucine; Val, valine; Gln, glutamine; Arg, arginine.

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compared with 10–20% in controls (Sigurs et al., 1995, 2005, 2010). The controversy appears when another relevant study (Stein et al., 1999), analyzed a cohort of patients with LRTI by RSV that did not require hospitalization. Compared with a control group, they found an increased risk of recurrent wheezing by the age of 6 and 11, but no difference was detected after 13 years of pursuit. In our study, considering that virtually 100% of children suffer at least one RSV infection before 2 years of age (Smyth and Openshaw, 2006), we propose the comparison of two groups of hospitalized patients from RSV according to their severity, to assess the possible association of complications during infection with recurrent wheezing and asthma.

The advances in sequencing and genotyping techniques, have allowed investigators to study the role of genetic polymorphisms in common diseases. Asthma or wheezing phenotypes in children, are complex diseases strongly influenced by genetic factors, with single nucleotide polymorphisms (SNP) playing an important role (Vercelli, 2008; Genuneit et al., 2009). Similarly, in relation to RSV infection, human genetics and SNP have been associated to the immune response and the severity of the disease (Hull, 2000; Choi et al., 2002; Hoebee et al., 2003; Wilson et al., 2005; Puthothu et al., 2006a, 2006b; Miyairi and DeVincenzo, 2008; Thomsen et al., 2008; Forton et al., 2009). Based on a candidate gene approach, a potential "genetic background" modulating the pathogenesis of LRTI and asthma in the individuals has been proposed, being a source of intense investigation and discussion nowadays (Goetghebuer et al., 2004; Heinzmann et al., 2004; Ermers et al., 2007; Thomsen et al., 2009). One of the possible mechanisms involved in the pathogenesis of both diseases is located in the IL-4/IL13 interleukin pathway (Kelly-Welch et al., 2003; Ermers et al., 2007; Forton et al., 2009). Polymorphisms described in the genes that encodes or regulate IL-4, IL-13, IL-4Rα have been related not only with the severity of RSV-LRTI (Choi et al., 2002; Hoebee et al., 2003; Puthothu et al., 2006a), but also associated with asthma or wheezing in other studies (Mitsuyasu et al., 1999; Graves et al., 2000; Howard et al., 2002; Beghé et al., 2003; Kabesch et al., 2003; Lee et al., 2004; Isidoro-García et al., 2005; Chan et al., 2006; Tachdjian et al., 2009; Genuneit et al., 2009; Berce and Potocnik, 2010; Bottema et al., 2010; Hesselmar et al., 2010). Only a few of these genetic reports have analyzed the SNP in cohorts (Goetghebuer et al., 2004; Ermers et al., 2007; Thomsen et al., 2009), none of those in latin population. We hypothesized that this genetic link between RSV infection severity and recurrent wheezing is present in Chilean children. By a longitudinal cohort study, the aim is to analyze the genetic variability within the IL-4/IL13 pathway and its relation to disease severity and its outcome in young Chilean children.

2. Methods

2.1. Patients and follow-up

A longitudinal cohort study was performed at Roberto Del Río Children's Hospital in Santiago, Chile. Previously healthy infants, under one year of age, with acute primary infection by RSV acquired in the community were enrolled. The patients were recruited from the Emergency Room (ER) and the Pediatrics Section of the hospital during winter seasons of 2005, 2006 and 2007. The following exclusion criteria were used: previous hospitalization for any cause, prematurity, chronic pulmonary disease, congenital heart disease, primary or secondary immuno deficiency and any previous symptomatic respiratory disease, including common cold and acute otitis media.

To analyze the clinical outcome after primary infection, patients were invited to participate in a one year follow-up period. A complete follow-up consisted in at least two visits to the Pulmonary Diseases Polyclinic, one month and 1 year after discharge. Complete examination and survey were performed by authors (*LT, MAP, RM, CL*). Patients who developed acute respiratory symptoms during the year of study could get appointments according their requirements. After one year, and based on literature (Schauer et al., 2002), "Recurrent Wheezing" was defined as three or more episodes of physician-verified wheezing and "No Recurrent Wheezing" as two or less events in that period. The study was approved by the Local Ethics Committee of the North Metropolitan Health Service and the Faculty of Medicine, University of Chile. Informed consent was signed by the parents of all study participants.

2.2. Viral diagnosis

A first RSV test from the ER was obtained by immuno fluorescence assay (IFA) from a nasopharyngeal aspirate (NPA) sample. Confirmation assays were completed in our Virology Laboratory on a second fresh sample of NPA taken from each patient during the first 72 h after admission. IFA and virus isolation for RSV, influenza, parainfluenza and adenovirus were conducted as previously described (Avendaño et al., 1991). Reverse transcriptase and real time polymerase chain reaction (PCR) for detection of RSV was also performed. In brief, total RNA of NPA was extracted by the guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 2006). Reverse transcription in a Perkin Elmer Gene Amp® PCR System 2400 was performed using a F gene primer (5′ TGTCTAACTATTTGAACA 3′, nucleotides 844–861 of F gene of Long RSV strain) as published (López et al., 1998). A fragment of the N gene with specific primers (Cane and Pringle, 1992) was amplified by real time PCR in a Light Cycler 1.5 instrument (Roche®).

2.3. Clinical characterization of severity of RSV disease

A clinical score system, previously published by authors (Larrañaga et al., 2009), was used in order to characterize the severity of the RSV infection. The score, calculated after discharge, was developed to create a tool that could evaluate objectively the severity during the entire course of the disease. It includes the most common none invasive parameters used in literature: requirement and length of hospitalization, necessity and days of supplemental oxygen, maximum oxygen requirement, critical care and mechanical ventilation requirement. Considering that our patients were at least admitted to the Observation Room in the ER, score values lower than seven were defined as "Moderate" disease, and scores of seven and more were considered "Severe" cases.

2.4. Genotyping

A total of 5 ml of whole blood was obtained from each patient within 72 h after admission. Sample collection was performed in EDTA tubes and transported to the Virology Laboratory. Specimens were centrifuged at 1.500 rpm for 10 min at 4 °C. Genomic DNA was extracted from pellet using a protocol that included leukocytes isolation by centrifugation in Dextran 5% and treatment with Chomczynski solution (Winkler®). DNA precipitation was performed in absolute ethanol.

Genotyping of six biallelic single nucleotide polymorphisms (SNP) in the genes IL-4, IL-13 e IL-4Rα was performed. Determination of allelic and genotypic frequencies was performed by PCR and restriction fragment length polymorphism (RFLP) analysis on agarose or acrylamide gel electrophoresis. The list of SNP studied, chromosome position, and experimental conditions are shown in Table 1. For genes IL-4 and IL-13, the SNP are localized on promoter region. In the case of gene IL-4Rα, the Ile50 Val (A/G) polymorphism is located in region that codifies the extra cellular domain of the receptor and implicates an amino acid change (isoleucine

Table 1
Polymorphisms studied, primers and restriction enzymes used for genotyping.

Gene (Chromosome position)	**Polymorphism** (SNP identification n°, NCBI)[a]	**PCR Primer**[b]	**Restriction enzyme**	**Fragments length (bp)**	**Refs.**
IL-4 (5q31.1)	**−589 C/T** (rs 2243250)	F: CTAAACTTGGGAGAACAT**G**G R: GTGGCATCTTGGAAACTGTC	**Ava II**	**T**: 124 **C**: 105 + 19	(a)
	−1098 T/G (rs 2243248)	F: GCTGATTTGTAAGTCCGTAAG R: ATCCTCCTACCTCAGTCTCC	**Bsl I**	**T**: 148 **G**: 127 + 21	(a)
IL-13 (5q31)	**−1512 A/C** (rs 1881457)	F: CAACCGCCGCGCCAGCGCCTTCTC R: CCGCTCCTTGGCCGTGTGACC**G**C	**BstU I**	**A**: 133 **C**: 111 + 22	(b)
	−1112 C/T (rs 1800925)	F: GGAATCCAGCATGCCTTGTGAGG R: GTCGCCTTTTCCTGCTCTTCCC**G**C	**BstU I**	**C**: 223 + 23 **T**: 246	(b)
IL-4Rα (16p12.1-p11.2)	**Ile50 Val (A/G)** (rs 1805010)	F: GGCAGGTGTGAGGAGCATCC R: GCCTCCGTTGTTCTCAGG**T**A	**Rsa I**	**A**: 273 **G**: 254 + 19	(c)
	Gln551Arg (A/G) (rs 1801275)	F: GCCCCACCAGTGGCT**C**TC R: CTGGCAAGCAGGCTTGAGAAG	**Dde I**	**A**: 110 + 18 **G**: 128	(c)

SNP: single nucleotide polymorphism. A: adenosine; T: thymidine; C: cytosine; G: guanosine; Ile: isoleucine; Val: valine; Gln: glutamine; Arg: arginine.
(a): Choi et al. 2002 modified (Choi et al., 2002); **(b):** Graves et al. 2000 modified (Graves et al., 2000); **(c):** Hoebbe et al. 2003 (Hoebee et al., 2003)
[a] http://www.ncbi.nlm.nih.gov/SNP/.
[b] Bold letter represent "mismatch" position.

or valine, respectively). The SNP Gln551Arg (A/G) involves also a change of amino acids on the intra cellular domain of IL-4 receptor (Glutamine or Arginine).

2.5. Statistical analyses

2.5.1. Clinical analysis

The number of "Recurrent Wheezing" and "No Recurrent Wheezing" patients, after a year of follow-up, was determined for Moderate and Severe RSV infection groups, considering that patients with moderate disease represent a well-defined control group of milder infection for comparisons. $\chi 2$ and Mann-Whitney Rank Sum tests were performed for group comparisons using SigmaStat® Program (Version 3.5), considering $p < 0.05$ as significant. Relative Risk (RR), with 95% confidential interval, was calculated using Stat Calc® from EpiInfo® Program.

2.5.2. Genetic analyses

Using the QUANTO software (http://hydra.usc.edu/gxe), a sample size of at least 112 RSV-infants was estimated to achieve a statistical power of 80% with a confidence of 95%, considering a genotype risk of 1.8 or higher and an average of minor allele frequency of 0.25, in accordance with the frequencies previously described for IL's SNPs. Hardy–Weinberg equilibrium (HWE) for genotypic frequencies was analyzed (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). The allelic and genotypic association analysis was performed considering each polymorphism as a categorical variable, with two alleles and three genotypes as possible, and the software UNPHASED 3.0.12® was used to determine, by χ^2 statistic, their association with the clinical conditions studied. This program performs a global likelihood-ratio significance test and does not require statistical corrections for multiple comparisons and estimation of linkage parameters. Odds Ratios (OR) are obtained, with 95% confidential interval. Also allows multilocus analysis and is useful for unrelated subjects (Dudbridge, 2008). Thus, haplotype analysis was performed. Finally, a gene–gene interaction analysis was performed to investigate possible relations between different polymorphisms of genes IL-13, IL-4 and IL4Rα that could be associated with clinical phenotypes (Howard et al., 2002; Battle et al., 2007). A regression analysis is especially relevant in this case, because their protein products are functionally related within the intracellular IL-4/IL-13 pathway (Kelly-Welch et al., 2003; Ermers et al., 2007; Forton et al., 2009). The analysis was performed using statistical program STATA 8.0® checking for homogeneity among stratums (Test of homogeneity). In the case of significant difference between stratums ($p < 0.05$), the OR and 95% CI stratified by alleles ($\chi 2$ test) were reported.

3. Results

3.1. Clinical evaluation

A total of 140 patients were enrolled during the study and confirmation of RSV infection was achieved in 120 cases (85.7%). Of those, 118 accepted to participate in the follow-up. According to the severity score, 60 infants suffered a moderate RSV infection (50.8%) and 58 manifested severe disease (49.2%). Both groups were comparable according to demographic characteristics and relevant epidemiological background as atopic and asthma family history, breastfeeding, maternal smoking and number of siblings (Table 2). Although a total of 55 patients of the moderate group (91.6%) were hospitalized for more than 24 h in the Pediatric Section and the other five were admitted for at least 6 h in an Observation Room in the ER, all clinical parameters included in the scoring system were significantly different between moderate and severe groups as shown in Table 2.

After the follow-up period, complete information was achieved in 84 patients (71.2%), corresponding 45 to moderate RSV infection and 39 to severe disease. No differences of age or gender were detected comparing them with patients who abandoned (data not shown). The percentages of moderate and severe cases that did not complete the follow-up were 25% (15/60) and 32.7% (19/58), respectively ($p = 0.35$). According to collected clinical data, after one year, a total of 14 patients of the moderate RSV infection group (31.1%) had Recurrent Wheezing diagnosis. In the severe group, 21 children had recurrent wheezing episodes, accounting to 53.8%. Thus, according to our score, and compared to a moderate RSV infection group, the RR to present recurrent wheezing after a severe disease was 1.73 (95% CI 1.03–2.92; $p = 0.035$).

3.2. Genetic polymorphisms

3.2.1. Acute infection's severity association analysis

Polymorphisms studied on genes IL-4, IL-13 and IL-4Rα were on Hardy–Weinberg equilibrium for all cases (data not shown). Allelic frequencies for moderate and severe RSV infection groups are shown in Table 3. No direct allelic association with severity was found on SNP of genes IL-4 or IL-13. After comparison of both groups, the allele G (Arginine) on the SNP Gln551Arg of gene IL4-Rα, was found to be more frequent in RSV cases with severe disease

Table 2
Demographic and clinical characteristics of infants with moderate and severe RSV infection.

	Moderate RSV infection ($N = 60$)	Severe RSV infection ($N = 58$)	p-value
Patients profiles			
sex (% male)	55	55.1	0.9[b]
Age (months)[a]	2.3 ± 0.2	2.4 ± 0.3	0.9[c]
Siblings (n°)[a]	1.21 ± 0.2	1.28 ± 0.3	0.98[c]
Breastfeeding (%)[d]	79.5	84.8	0.51[b]
Familiar atopic history (%)	65.9	61.7	0.67[b]
Familiar asthma history (%)	28.5	23.1	0.59[b]
Maternal smoking (%)	34.1	21.3	0.17[b]
Clinical features			
Length of hospitalization (days)[a]	3.43 ± 0.3	9.62 ± 0.5	<0.001[c]
Supplemental oxygen requirement:			
Cases (n°)	33	58	<0.001[b]
Length (days)[a]	1.32 ± 0.2	8.05 ± 0.5	<0.001[c]
Maximal % FiO_2 administrated[a]	25.3 ± 0.5	41.29 ± 1.9	<0.001[c]
Mechanical ventilation (n° of cases)	0	13	<0.001[b]

RSV, respiratory syncytial virus; FiO_2, fraction of inspired oxygen.
[a] Mean ± Standard Error;
[b] Chi square test;
[c] Mann–Whitney Rank Sum Test;
[d] Breast feeding at the moment of hospitalization.

Table 3
Allelic frequencies of studied polymorphisms in infants with moderate and severe RSV infection.

Genetic polymorphism	RSV infection	
	Moderate cases ($N = 120$)[b] N (%)	Severe cases ($N = 116$)[b] N (%)
IL-4		
−589 C/T		
C	82 (68)	80 (69)
T	38 (32)	36 (31)
−1098 T/G		
T	102 (85)	100 (86)
G	18 (15)	16 (14)
IL-13		
−1512 A/C		
A	93 (77)	87 (75)
C	27 (23)	29 (25)
−1112 C/T		
C	90 (75)	87 (75)
T	30 (25)	29 (25)
IL-4 Rα		
Ile50 Val		
Ile	65 (54)	57 (49)
Val	55 (46)	59 (51)
Gln551Arg[a]		
Gln	98 (81)	82 (71)
Arg	22 (19)	34 (29)

[a] Arg: OR 1.85 (1.0–3.4), $p = 0.047$.
[b] Each patient carries 2 alleles, thus N = (cases × 2).

($p = 0.047$; OR 1.85, 95% CI: 1.0–3.4). The analysis of genotypes (Table 4) showed no associations with the infection severity on polymorphisms of IL-4 and IL-13 genes. Nevertheless, in addition to the finding on the allelic analysis, the IL-4Rα genotype 551 Arg/Arg was more frequent in severe cases ($p = 0.045$; OR 3.3 95% CI: 0.96–11.3). Moreover, the analysis of haplotypes showed that in

Table 4
Genotype frequencies of studied polymorphisms in infants with moderate and severe RSV infection

Genetic polymorphism	RSV infection	
	mild cases ($N = 60$) N (%)	Severe cases ($N = 58$) N (%)
IL-4		
−589 C/T		
C/C	28 (46.7)	27 (46.5)
C/T	23 (43.3)	26 (44.8)
T/T	6 (10)	5 (8.6)
−1098 T/G		
T/T	44 (73.3)	44 (75.9)
T/G	14 (23.3)	12 (20.7)
G/G	2 (3.3)	2 (3.4)
IL-13		
−1512 A/C		
A/A	35 (58.3)	33 (56.9)
A/C	23 (38.3)	21 (36.2)
C/C	2 (3.3)	4 (6.9)
−1112 C/T		
C/C	33 (55)	33 (56.9)
C/T	24 (40)	21 (36.2)
T/T	3 (5)	4 (6.9)
IL-4 Rα		
Ile50 Val		
Ile/Ile	17 (28.3)	14 (24.1)
Ile/Val	31 (51.7)	29 (50)
Val/Val	12 (20)	15 (25.9)
Gln551Arg		
Gln/Gln	42 (70)	35 (60.3)
Gln/Arg	14 (23.3)	12 (20.7)
Arg/Arg[a]	4 (6.7)	11 (19)

[a] 551Arg/Arg: OR 3.3 (0.96–11.3), $p = 0.045$.

the gen IL4Rα, the haplotype G–G (Val-Arg) on SNP Ile50Val and Gln551Arg, respectively, was significantly higher in patients with RSV infection ($p = 0.003$; OR 4.26, 95% CI: 1.4–12.8), data not shown.

3.2.2. Recurrent wheezing association analysis

The comparison of allelic and genotypic frequencies between Recurrent Wheezing and No Recurrent Wheezing groups is shown in Table 5. No direct associations were found on genotypes or alleles for genes IL-4 or IL-13. The analysis of IL-4Rα gene, detected that the genotype 50 Ile/Ile was more frequent in No Recurrent Wheezers, tending to act as a protection factor ($p = 0.036$; OR 0.31, 95% CI: 0.09–1.06). In Table 6 is shown the analysis of wheezing outcome related to the severity of the acute RSV infection. Briefly, the groups of severe and moderate RSV infection were analyzed separately according to the development of recurrent wheezing symptoms, looking for a common genetic background associated to those clinical conditions. In addition, the genotype Ile/Ile on SNP IL-4Rα Ile50Val had a higher frequency in moderate RSV cases who did not developed recurrent wheezing ($p = 0.046$; OR: 0.14, 95% CI: 0.01–1.3), compared with those who had a recurrent wheezing outcome.

No haplotypes were found significantly associated to recurrent wheezing or no recurrent wheezing outcome after RSV bronchiolitis.

3.2.3. Gene–gene interaction analysis

The regression analysis to investigate a possible interaction between polymorphisms of genes IL-13, IL-4 and IL4Rα within intracellular IL-4/IL-13 pathway, showed that comparing moderate vs. severe RSV infection, the SNP IL-13 −1512 A/C interacts with IL-4Rα Ile50Val (Test of homogeneity, $p = 0.0077$). When in gene IL-4Rα the allele 50 Ile is present, the allele −1512C in gene IL-13 acts as a risk factor for severity of RSV infection ($p = 0.049$; OR: 3.24,

Table 5
Allelic and genotypic frequencies of polymorphisms in infants according to Recurrent Wheezing Diagnosis.

Genetic polymorphism	Recurrent wheezing diagnosis	
	No recurrent wheezing (cases = 49; alleles = 98) N (%)	Recurrent wheezing (cases = 35; alleles = 70) N (%)
IL-4		
−589 C/T		
Allele C	63 (64)	49 (70)
C/C	21 (42.9)	15 (42.9)
C/T	21 (42.9)	19 (54.3)
T/T	7 (14.3)	1 (2.8)
−1098 T/G		
Allele T	84 (86)	60 (86)
T/T	37 (75.5)	26 (74.3)
T/G	10 (20.4)	8 (22.9)
G/G	2 (4.1)	1 (2.8)
IL-13		
−1512 A/C		
Allele A	72 (73)	54 (77)
A/A	26 (53.1)	21 (60)
A/C	20 (40.8)	12 (34.3)
C/C	3 (6.1)	2 (5.7)
−1112 C/T		
Allele C	71 (72)	54 (77)
C/C	25 (51)	21 (60)
C/T	21 (42.9)	12 (34.3)
T/T	3 (6.1)	2 (5.7)
IL-4 Rα		
Ile50Val		
Allele A = Ile	55 (56)	30 (43)
Ile/Ile	17 (34.7)	5 (14.3)[a]
Ile/Val	21 (42.9)	20 (57.1)
Val/Val	11 (22.4)	10 (28.6)
Gln551Arg		
Allele G = Gln	74 (76)	54 (77)
Gln/Gln	31 (63.3)	24 (68.6)
Gln/Arg	12 (24.5)	6 (17.1)
Arg/Arg	6 (12.2)	5 (14.3)

[a] IL-4Rα 50Ile/Ile: OR: 0.31, $p = 0.036$.

95% CI: 0.93–11.27). In contrast, according to subsequent wheezing, the analysis showed a trend towards an interaction between the SNP IL-4 −589 C/T and IL-4Rα Ile50Val (Test of homogeneity, $p = 0.056$). In the presence of allele 50 Ile in the gene IL-4Rα, the allele −589T results associated to No Recurrent Wheezing group as a protection factor ($p = 0.031$; OR 0.24, 95% CI: 0.06–0.97).

4. Discussion

4.1. Clinical outcome

Prospective cohort studies have analyzed the association between severe RSV infection during infancy and recurrent wheezing (Stein et al., 1999; Piippo-Savolainen et al., 2004; Sigurs et al., 2010), but there is still no real consensus in the acceptance of this issue. One of the main differences between publications is related to the characterization of severity during the acute episode and the definition of control groups. In our study, we performed a comparison between cases classified in two objectively different severities. Although we did not include mild outpatients or parents as control, we compared in a longitudinal prospective approach a real severe RSV infection group with a milder disease. This comparison, different from those published, allowed us to conform groups that are closer to what is occurring in hospitalized children and will be useful to analyze RSV infection of that almost 100% of previously healthy infants, where the question about which factors determine the severity of the disease and its outcome is still unclear.

Table 6
Allelic and genotypic frequencies of polymorphisms in infants with Moderate and Severe RSV infection according to Recurrent Wheezing Diagnosis.

Genetic polymorphism	RSV infection outcome			
	Moderate RSV infection		Severe RSV infection	
	No recurrent wheezing (cases = 31; alleles = 62) N (%)	Recurrent wheezers (cases = 14; alleles = 28) N (%)	No recurrent wheezing (cases = 18; alleles = 36) N (%)	Recurrent wheezers (cases = 21; alleles = 42) N (%)
IL-4				
−589 C/T				
Allele C	41 (66)	18 (64)	22 (61)	31 (74)
C/C	14 (45.2)	5 (35.7)	7 (38.9)	10 (47.6)
C/T	13 (41.9)	8 (57.1)	8 (44.4)	11 (53.4)
T/T	4 (12.9)	1 (7.1)	3 (16.7)	0 (0)
−1098 T/G				
Allele T	52 (84)	25 (89)	32 (89)	35 (83)
T/T	23 (74.2)	11 (78.6)	14 (77.8)	15 (71.4)
T/G	6 (19.4)	3 (21.4)	4 (22.2)	5 (23.8)
G/G	2 (6.4)	0 (0)	0 (0)	1 (4.7)
IL-13				
−1512 A/C				
Allele A	44 (71)	24 (86)	28 (78)	30 (71)
A/A	15 (48.4)	10 (71.4)	11 (61.1)	11 (52.4)
A/C	14 (45.2)	4 (28.6)	6 (33.3)	8 (38.1)
C/C	2 (6.4)	0 (0)	1 (5.5)	2 (9.5)
−1112 C/T				
Allele C	44 (71)	24 (86)	27 (75)	30 (71)
C/C	15 (48.4)	10 (71.4)	10 (55.5)	11 (52.4)
C/T	14 (45.2)	4 (28.6)	7 (38.9)	8 (38.1)
T/T	2 (6.4)	0 (0)	1 (5.5)	2 (9.5)
IL-4 Rα				
Ile50Val				
Allele A = Ile	35 (56)	12 (43)	20 (56)	18 (43)
Ile/Ile	11 (35.5)	1 (7.1)[a]	6 (33.3)	4 (19.1)
Ile/Val	13 (41.9)	10 (71.4)	8 (44.4)	10 (47.6)
Val/Val	7 (22.6)	3 (21.4)	4 (22.2)	7 (33.3)
Gln551Arg				
Allele G = Gln	48 (77)	24 (86)	26 (72)	30 (71)
Gln/Gln	19 (61.3)	11 (78.6)	12 (66.7)	13 (61.9)
Gln/Arg	10 (32.2)	2 (14.3)	2 (11.1)	4 (19.1)
Arg/Arg	2 (6.5)	1 (7.1)	4 (22.2)	4 (19)

[a] IL-4Rα 50Ile/Ile: OR: 0.14, $p = 0.046$.

The definition of the severity in acute RSV infection, which is mainly determined by the degree of respiratory failure, has not been sufficiently addressed in the literature and hospitalization requirement has been frequently used as a severity factor. Although 96% of the children enrolled in our study were hospitalized during their clinical course, a 50.8% of cases corresponded to moderate disease according to the severity score. Moreover, of the 55 patients hospitalized with moderate disease, 40% (22 cases) did not require supplemental oxygen during their stay, being a group of cases that eventually could have been treated as outpatients. Consequently, the use of hospitalization as a single criterion of severity is not appropriate in our population. In this context, we used a scoring system previously published by authors (Larrañaga et al., 2009), which considers different clinical and none invasive parameters to characterize the severity of the infection in view of the entire course of the disease. Thus, the clinical presentation was typified by a sum of factors that reflected the disease, giving an objective and consistent final diagnosis of severity. The score was able to distinguish significantly between the two groups. Therefore, it is a useful tool, which may be used in future analysis, not only related to RSV infection, but as a system for assessing severity of bronchopulmonary diseases that determine respiratory failure in children.

Once characterized the acute RSV infection, the establishment of the follow-up allowed us to confirm, in Chilean infants, the association of severe RSV primary infection with recurrent wheezing during the first year after the event. Severe cases showed 1.73-fold higher risk of having three or more episodes of wheezing during the year after the infection. Thus, in Chilean population, the severity of the infection by the virus behaves as a marker of patients who have almost twice the chance of developing recurrent wheezing at one-year term.

Different publications have defined and classified the wheezing episodes or phenotypes during the first years of life (Martinez et al., 1995; Kurukulaaratchy et al., 2003; Brand et al., 2008; Spycher et al., 2010). Although there is still no complete agreement about those definitions, it is accepted that at young age the reasons behind wheezing symptoms can be LRTI caused by viral infections and atopic wheeze frequently exacerbated by viral infections, especially RSV and rhinovirus (RV). In our study, since we used a strict definition of primary respiratory tract infection, the patients enrolled corresponded to young infants, 96% of them younger than 6 months old (113/118) with an average of 2.4 months. With this, according to one of the most accepted definitions in literature (Martinez et al., 1995), the severity of the acute RSV infection in our patients was not affected by the expression of atopic or asthmatic wheezing phenotype.

A limitation of our study is that no RV detection was performed. Although a recent prospective multicenter report has shown that RSV and/or RV are the major etiological causes (84.5%) of bronchiolitis in children under 2 years of age (Mansbach et al., 2012), their cohort differs from ours in including older patients, with bronchiolitis (no matter if it was first episode), and including children with comorbidities. Based in other reports, we can infer that percentages of RSV-RV infection in the moderate and severe groups are similar. It has been demonstrated that infection by RV is present in similar rates on asymptomatic infants and hospitalized cases of LRTI (Iwane et al., 2011), and no significant clinical differences were detected comparing both viruses (Calvo et al., 2010). A percentage of 8.6% of co-infection with RV was detected in young infants (median age: 3.3 months) hospitalized for RSV bronchiolitis (Salvador García et al., 2012) and higher rates have been detected in children older than one year (Korppi et al., 2004; Iwane et al., 2011), preterm infants (van Piggelen et al., 2010), very low birth weight infants (Miller et al., 2012) and in cases of severe LRTI from intensive care units especially those with underlying diseases (Louie et al., 2009). Although it is not possible to re-analyze the samples included in this report, our group is still working in the field, now including respiratory virus molecular diagnosis, and we will be repo in more recent trials and we will be reporting this findings

4.2. Genetic polymorphisms

Genetic association studies, specially the analysis of SNP in relation to host-susceptibility, require local validation in different human populations to assess their real impact. No previous publications have addressed the question about how genetic variability in the IL-4/IL-13 pathway can influence the acute disease and later outcome of RSV infection in Chile, therefore this study is relevant to replicate previous findings by others (Choi et al., 2002; Howard et al., 2002; Hoebee et al., 2003; Park et al., 2004; Puthothu et al., 2006a; Ermers et al., 2007; Berce and Potocnik, 2010; Hesselmar et al., 2010). About the ethnic background of the group analyzed in this study, the Chilean population is constituted, in more than 90%, by the admixture of Caucasian and South Amerindian people. This percentage is even higher in public hospitals, especially in Santiago of Chile, where this study was performed (Valenzuela, 1988; Instituto Nacional de Estadísticas, 2002). With this, we can postulate that there is no effect of ethnicity variability influencing the distribution of SNPs in our children.

After the analysis of association of polymorphisms in our patients, the allele G in Gln551Arg SNP (rs 1801275) was shown as a risk factor of severe respiratory disease during primary RSV infection, and this association was detected in the analysis of alleles, genotypes and haplotypes. At our knowledge, only one previous report had shown the relation of 551Arg with severe infection for RSV (Hoebee et al., 2003), in a group of hospitalized infants older than 6 months of age. The substitution of adenosine for guanine causes an amino-acid change in the intracellular domain of the receptor (from glutamine to arginine) and it has been documented the association of 551Arg with atopic asthma (Beghé et al., 2003; Lee et al., 2004), non-atopic asthma (Berce and Potocnik, 2010) and rhinitis (Bottema et al., 2010). Potential functional implications have been described in a mouse model, where the presence of arginine at that position synergize with signal transducer and activator of transcription (STAT) 6, and enhance the expression of IL-4 and IL-13 regulated genes, promoting allergic inflammation and asthma (Tachdjian et al., 2009). Moreover, a recent pharmacogenetic analysis has shown that this variant might influence in the response to an IL-4/IL-13 antagonist candidate for asthma therapy, adding evidence that this polymorphism plays an important role in the pathogenesis of the disease (Slager et al., 2010). In our analysis, after the follow-up, the patients with recurrent wheezing did not show increased frequency of allele 551Arg. A possible reason is that, after one year of pursuit, our recurrent wheezing patients may belong to any of the phenotype groups described (Martinez et al., 1995). The only group that is clearly identified is the No Recurrent Wheezers. It is a period in life of wheezing infants when is not possible to know whether that child will continue towards the development of asthma or have transient episodes. A longer follow up in a larger cohort where phenotype subgroups can be differentiated is required.

Regarding to the SNP Ile50Val (rs 1805010), after the analysis of allele frequencies, we detected that allele G (Valine) tends to be associated with Recurrent Wheezing group of patients ($p = 0.089$; OR: 1.7 CI: 0.92–3.2, not shown). In addition with the above, we find that the genotype A/A (Ile/Ile) was more frequent in the group of no recurrent wheezers after one year of follow-up; and moreover, it was more frequent in those who had moderate disease and did not developed a recurrent wheezing phenotype during the first year after RSV infection. A previous study had shown that the presence of the valine at the position 50 in the IL-4 receptor located in the extracellular domain, results to an increased activation of STAT6 and increased production of IgE in B cells (Mitsuyasu et al., 1999). This genetic variant has also been associated with eczema, atopy and asthma (Beghé et al., 2003; Chan et al., 2006; Genuneit et al., 2009). Furthermore, Ermers et al. (Ermers et al., 2007) found that the G allele (Val) was more frequent in patients who have recurrent wheezing after 6 years from a RSV infection, when compared with a group of non-wheezers after infection (OR = 2.54, CI 1.00–6.44). Our results support those findings, and allow us to propose that a common genetic background is present in those children who manifest a milder RSV disease and do not develop a recurrent wheezing phenotype in Chilean population.

By the analysis of gene–gene interactions within the IL-4/IL-13 pathway we obtained additional information, confirming the importance of this approach especially in genes associated in a functional way. Briefly, by a logistic regression analysis we can obtain information of possible related effects of these single genetic variants in a common pathway. Although a borderline p-value and a CI including the value 1 were detected, probably due to the size of the sample, we postulate that polymorphisms in IL-13 and IL-4 genes interact with IL-4Rα SNP, affecting susceptibility to severe RSV infection and to later recurrent wheezing in Chilean

population. With respect of severity, the presence of IL4Rα Ile50 determined the allele IL13 −1512C as a risk factor, confirming the importance of this immunological pathway in our patients (Puthothu et al., 2006a, 2006b). Previous studies have demonstrated interactions between IL4Rα, IL-4 and IL-13 genes in asthma susceptibility (Howard et al., 2002; Lee et al., 2004; Chan et al., 2006; Battle et al., 2007). The analysis allowed us to detect that SNP IL4 −589T acts as a protection factor for recurrent wheezing in the presence of Ile50 (IL-4Rα), differing to what was previously describe for this variant (Beghé et al., 2003). Although opposite variations can occur according the ethnicity of the population studied, future functional studies analyzing this pathway and its genetic variants are needed to suggest a possible dominance of one SNP over the others.

RSV is still a main public health concern. The use of a score to define RSV infection severity, a clear definition of recurrent wheezing outcome, and the analysis by a candidate gene approach allowed us to find genetic variations in the host that are affecting the course of the acute disease and its outcome in Chilean children. A characterization of a larger cohort for a longer period is needed to define the possible phenotypic groups and to detect new genetic markers associated with susceptibility or protection to the infection.

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References

Avendaño, L.F., Larrañaga, C., Palomino, M.A., Gaggero, A., Montaldo, G., Suarez, M., Diaz, A., 1991. Community-and hospital-acquired respiratory syncytial virus infections in chile. Pediatr. Infect. Dis. J. 10, 564–568.

Avendaño, L.F., Palomino, M.A., Larranaga, C., 2003. Surveillance for respiratory syncytial virus in infants hospitalized for acute lower respiratory infection in chile (1989–2000). J. Clin. Microbiol. 41, 4879–4882.

Battle, N.C., Choudhry, S., Tsai, H.-J., Eng, C., Kumar, G., Beckman, K.B., Naqvi, M., Meade, K., Watson, H.G., Lenoir, M., Burchard, E.G., 2007. Ethnicity-specific gene–gene interaction between IL-13 and IL-4R alpha among african americans with asthma. Am. J. Respir. Crit. Care Med. 175, 881–887.

Beghé, B., Barton, S., Rorke, S., Peng, Q., Sayers, I., Gaunt, T., Keith, T.P., Clough, J.B., Holgate, S.T., Holloway, J.W., 2003. Polymorphisms in the interleukin-4 and interleukin-4 receptor alpha chain genes confer susceptibility to asthma and atopy in a caucasian population. Clin. Exp. Allergy 33, 1111–1117.

Berce, V., Potocnik, U., 2010. Association of Q551R polymorphism in the interleukin 4 receptor gene with nonatopic asthma in slovenian children. Wien. Klin. Wochenschr. 122 (Suppl), 11–18.

Bottema, R.W.B., Nolte, I.M., Howard, T.D., Koppelman, G.H., Dubois, A.E.J., de Meer, G., Kerkhof, M., Bleecker, E.R., Meyers, D.A., Postma, D.S., 2010. Interleukin 13 and interleukin 4 receptor-α polymorphisms in rhinitis and asthma. Int. Arch. Allergy Immunol. 153, 259–267.

Brand, P.L.P., Baraldi, E., Bisgaard, H., Boner, A.L., Castro-Rodriguez, J., Custovic, A., de Blic, J., de Jongste, J.C., Eber, E., Everard, M.L., Frey, U., Gappa, M., Garcia-Marcos, L., Grigg, J., Lenney, W., Le Souëf, P., McKenzie, S., Merkus, P.J.F.M., Midulla, F., Paton, J.Y., Piacentini, G., Pohunek, P., Rossi, G.A., Seddon, P., Silverman, M., Sly, P.D., Stick, S., Valiulis, A., van Aalderen, W.M.C., Wildhaber, J.H., Wennergren, G., Wilson, N., Zivkovic, Z., Bush, A., 2008. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur. Respir. J. 32, 1096–1110.

Calvo, C., Pozo, F., García-García, M.L., Sanchez, M., Lopez-Valero, M., Pérez-Breña, P., Casas, I., 2010. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. Acta Paediatr. 99, 883–887.

Cane, P.A., Pringle, C.R., 1992. Molecular epidemiology of respiratory syncytial virus: rapid identification of subgroup a lineages. J. Virol. Methods 40, 297–306.

Chan, I.H.S., Leung, T.F., Tang, N.L.S., Li, C.Y., Sung, Y.M., Wong, G.W.K., Wong, C.K., Lam, C.W.K., 2006. Gene–gene interactions for asthma and plasma total IgE concentration in Chinese children. J. Allergy Clin. Immunol. 117, 127–133.

Choi, E.-H., Lee, H.J., Yoo, T., Chanock, S.J., 2002. A common haplotype of interleukin-4 gene IL4 is associated with severe respiratory syncytial virus disease in korean children. J. Infect. Dis. 186, 1207–1211.

Chomczynski, P., Sacchi, N., 2006. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nat. Protocols 1, 581–585.

Dudbridge, F., 2008. Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. Hum. Hered. 66, 87–98.

Ermers, M.J., Hoebee, B., Hodemaekers, H.M., Kimman, T.G., Kimpen, J.L.L., Bont, L., 2007. IL-13 genetic polymorphism identifies children with late wheezing after respiratory syncytial virus infection. J. Allergy Clin. Immunol. 119, 1086–1091.

Forton, J.T., Rowlands, K., Rockett, K., Hanchard, N., Herbert, M., Kwiatkowski, D.P., Hull, J., 2009. Genetic association study for RSV bronchiolitis in infancy at the 5q31 cytokine cluster. Thorax 64, 345–352.

Genuneit, J., Cantelmo, J.L., Weinmayr, G., Wong, G.W.K., Cooper, P.J., Riikjärv, M., Gotua, M., Kabesch, M., von Mutius, E., Forastiere, F., Crane, J., Nystad, W., El-Sharif, N., Batlles-Garrido, J., García-Marcos, L., García-Hernández, G., Morales-Suarez-Varela, M., Nilsson, L., Bråbäck, L., Saraçlar, Y., Weiland, S.K., Cookson, W.O.C., Strachan, D., Moffatt, M.F., 2009. A multi-centre study of candidate genes for wheeze and allergy: the international study of asthma and allergies in childhood phase 2. Clin. Exp. Allergy 39, 1875–1888.

Goetghebuer, T., Isles, K., Moore, C., Thomson, A., Kwiatkowski, D., Hull, J., 2004. Genetic predisposition to wheeze following respiratory syncytial virus bronchiolitis. Clin. Exp. Allergy 34, 801–803.

Graves, P.E., Kabesch, M., Halonen, M., Holberg, C.J., Baldini, M., Fritzsch, C., Weiland, S.K., Erickson, R.P., von Mutius, E., Martinez, F.D., 2000. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. J. Allergy Clin. Immunol. 105, 506–513.

Hall, C.B., Weinberg, G.A., Iwane, M.K., Blumkin, A.K., Edwards, K.M., Staat, M.A., Auinger, P., Griffin, M.R., Poehling, K.A., Erdman, D., Grijalva, C.G., Zhu, Y., Szilagyi, P., 2009. The burden of respiratory syncytial virus infection in young children. N. Engl. J. Med. 360, 588–598.

Heinzmann, A., Ahlert, I., Kurz, T., Berner, R., Deichmann, K.A., 2004. Association study suggests opposite effects of polymorphisms within IL8 on bronchial asthma and respiratory syncytial virus bronchiolitis. J. Allergy Clin. Immunol. 114, 671–676.

Henderson, J., Hilliard, T.N., Sherriff, A., Stalker, D., Al Shammari, N., Thomas, H.M., 2005. Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. Pediatr. Allergy Immunol. 16, 386–392.

Hesselmar, B., Bergin, A.-M., Park, H., Hahn-Zoric, M., Eriksson, B., Hanson, L., Padyukov, L., 2010. Interleukin-4 receptor polymorphisms in asthma and allergy: relation to different disease phenotypes. Acta Paediatr. 99, 399–403.

Hoebee, B., Rietveld, E., Bont, L., Oosten, M.V., Hodemaekers, H.M., Nagelkerke, N.J.D., Neijens, H.J., Kimpen, J.L.L., Kimman, T.G., 2003. Association of severe respiratory syncytial virus bronchiolitis with interleukin-4 and interleukin-4 receptor alpha polymorphisms. J. Infect. Dis. 187, 2–11.

Howard, T.D., Koppelman, G.H., Xu, J., Zheng, S.L., Postma, D.S., Meyers, D.A., Bleecker, E.R., 2002. Gene–gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. Am. J. Hum. Genet. 70, 230–236.

Hull, J., 2000. Association of respiratory syncytial virus bronchiolitis with the interleukin eight gene region in UK families. Thorax 55, 1023–1027.

Instituto Nacional de Estadísticas., Censo 2002, 2003. Comisión Nacional del XVII Censo de Poblaciones y VI de Vivienda. Empresa Periodística La Nación (Eds.), Santiago de Chile. pp. 1–50. <http://www.ine.cl/cd2002/sintesiscensal.pdf>.

Isidoro-García, M., Dávila, I., Laffond, E., Moreno, E., Lorente, F., González-Sarmiento, R., 2005. Interleukin-4 (IL4) and Interleukin-4 receptor (IL4RA) polymorphisms in asthma: a case control study. Clin. Mol. Allergy 3, 15.

Iwane, M.K., Prill, M.M., Lu, X., Miller, E.K., Edwards, K.M., Hall, C.B., Griffin, M.R., Staat, M.A., Anderson, L.J., Williams, J.V., Weinberg, G.A., Ali, A., Szilagyi, P.G., Zhu, Y., Erdman, D.D., 2011. Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. J. Infect. Dis. 204, 1702–1710.

Kabesch, M., Tzotcheva, I., Carr, D., Höfler, C., Weiland, S.K., Fritzsch, C., Mutius, E.V., Martinez, F.D., 2003. A complete screening of the IL4 gene: novel polymorphisms and their association with asthma and IgE in childhood. J. Allergy Clin. Immunol. 112, 893–898.

Kelly-Welch, A.E., Hanson, E.M., Boothby, M.R., Keegan, A.D., 2003. Interleukin-4 and interleukin-13 signaling connections maps. Science 300, 1527–1528.

Korppi, M., Kotaniemi-Syrjanen, A., Waris, M., Vainionpaa, R., Reijonen, T.M., 2004. Rhinovirus-associated wheezing in infancy: comparison with respiratory syncytial virus bronchiolitis. Pediatr. Infect. Dis. J. 23, 995–999.

Kurukulaaratchy, R.J., Fenn, M.H., Waterhouse, L.M., Matthews, S.M., Holgate, S.T., Arshad, S.H., 2003. Characterization of wheezing phenotypes in the first 10 years of life. Clin. Exp. Allergy 33, 573–578.

Larrañaga, C., Ampuero, S., Luchsinger, V., Carrión, F., Aguilar, N., Morales, P., Palomino, M.A., Tapia, L., Avendaño, L.F., 2009. Impaired immune response in severe human lower tract respiratory infection by respiratory syncytial virus. Pediatr. Infect. Dis. J. 28, 867–873.

Lee, S.-G., Kim, B.-S., Kim, J.-H., Lee, S.-Y., Choi, S.-O., Shim, J.-Y., Hong, T.-J., Hong, S.-J., 2004. Gene–gene interaction between interleukin-4 and

interleukin-4 receptor alpha in korean children with asthma. Clin. Exp. Allergy 34, 1202–1208.
Louie, J.K., Roy-Burman, A., Guardia-Labar, L., Boston, E.J., Kiang, D., Padilla, T., Yagi, S., Messenger, S., Petru, A.M., Glaser, C.A., Schnurr, D.P., 2009. Rhinovirus associated with severe lower respiratory tract infections in children. Pediatr. Infect. Dis. J. 28, 337–339.
López, J.A., Bustos, R., Orvell, C., Berois, M., Arbiza, J., García-Barreno, B., Melero, J.A., 1998. Antigenic structure of human respiratory syncytial virus fusion glycoprotein. J. Virol. 72, 6922–6928.
Mansbach, J.M., Piedra, P.A., Teach, S.J., Sullivan, A.F., Forgey, T., Clark, S., Espinola, J.A., Camargo, C.A., 2012. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. Arch. Pediatr. Adolesc. Med. 166, 700–706.
Martinez, F.D., Wright, A.L., Taussig, L.M., Holberg, C.J., Halonen, M., Morgan, W.J., 1995. Asthma and wheezing in the first six years of life. N. Engl. J. Med. 332, 133–138.
Miller, E.K., Bugna, J., Libster, R., Shepherd, B.E., Scalzo, P.M., Acosta, P.L., Hijano, D., Reynoso, N., Batalle, J.P., Coviello, S., Klein, M.I., Bauer, G., Benitez, A., Kleeberger, S.R., Polack, F.P., 2012. Human rhinoviruses in severe respiratory disease in very low birth weight infants. Pediatrics 129, e60–e67.
Mitsuyasu, H., Yanagihara, Y., Mao, X.Q., Gao, P.S., Arinobu, Y., Ihara, K., Takabayashi, A., Hara, T., Enomoto, T., Sasaki, S., Kawai, M., Hamasaki, N., Shirakawa, T., Hopkin, J.M., Izuhara, K., 1999. Cutting edge: dominant effect of Ile50Val variant of the human IL-4 receptor alpha-chain in IgE synthesis. J. Immunol. 162, 1227–1231.
Miyairi, I., DeVincenzo, J.P., 2008. Human genetic factors and respiratory syncytial virus disease severity. Clin. Microbiol. Rev. 21, 686–703.
Noble, V., Murray, M., Webb, M.S.C., Alexander, J., Swarbrick, A.S., Milner, A.D., 1997. Respiratory status and allergy nine to 10 years after acute bronchiolitis. Arch. Dis. Child 76, 315–319.
Palomino, M.A., Larrañaga, C., Villagra, E., Camacho, J., Avendaño, L.F., 2004. Adenovirus and respiratory syncytial virus-adenovirus mixed acute lower respiratory infections in Chilean infants. Pediatr. Infect. Dis. J. 23, 337–341.
Park, B.L., Kim, L.H., Choi, Y.H., Lee, J.-H., Rhim, T., Lee, Y.M., Uh, S.-T., Park, H.-S., Choi, B.W., Hong, S.-J., Park, C.-S., Shin, H.D., 2004. Interleukin 3 (IL3) polymorphisms associated with decreased risk of asthma and atopy. J. Hum. Genet. 49, 517–527.
Piippo-Savolainen, E., Remes, S., Kannisto, S., Korhonen, K., Korppi, M., 2004. Asthma and lung function 20 years After wheezing in Infancy. Arch. Pediatr. Adolesc. Med. 158, 1070–1076.
Pullan, C.R., Hey, E.N., 1982. Wheezing, asthma, and pulmonary dysfunction 10 years after infection with respiratory syncytial virus in infancy. BMJ 284, 1665–1669.
Puthothu, B., Krueger, M., Forster, J., Heinzmann, A., 2006a. Association between severe respiratory syncytial virus infection and IL13/IL4 haplotypes. J. Infect. Dis. 193, 438–441.
Puthothu, B., Krueger, M., Heinze, J., Forster, J., Heinzmann, A., 2006b. Impact of IL8 and IL8-receptor alpha polymorphisms on the genetics of bronchial asthma and severe RSV infections. Clin. Mol. Allergy 4, 2.
Salvador García, C., Moreno Docón, A., Piñero, J.A., Alfayate Miguelez, S., Iborra Bendicho, M.A., 2012. Aetiology of bronchiolitis in hospitalised children in South-East Spain. An. Pediatr. (Barc) 77, 386–390.
Schauer, U., Hoffjan, S., Bittscheidt, J., Kochling, A., Hemmis, S., Bongartz, S., Stephan, V., 2002. RSV bronchiolitis and risk of wheeze and allergic sensitisation in the first year of life. Eur. Respir. J. 20, 1277–1283.
Sigurs, N., Aljassim, F., Kjellman, B., Robinson, P.D., Sigurbergsson, F., Bjarnason, R., Gustafsson, P.M., 2010. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. Thorax 65, 1045–1052.
Sigurs, N., Bjarnason, R., Sigurbergsson, F., Kjellman, B., Bjorksten, B., 1995. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. Pediatrics 95, 500–505.
Sigurs, N., Gustafsson, P.M., Bjarnason, R., Lundberg, F., Schmidt, S., Sigurbergsson, F., Kjellman, B., 2005. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am. J. Respir. Crit. Care Med. 171, 137–141.
Slager, R.E., Hawkins, G.A., Ampleford, E.J., Bowden, A., Stevens, L.E., Morton, M.T., Tomkinson, A., Wenzel, S.E., Longphre, M., Bleecker, E.R., Meyers, D.A., 2010. IL-4 receptor α polymorphisms are predictors of a pharmacogenetic response to a novel IL-4/IL-13 antagonist. J. Allergy Clin. Immunol. 126, 875–878.
Smyth, R.L., Openshaw, P.J.M., 2006. Bronchiolitis. Lancet 368, 312–322.
Sommer, C., Resch, B., Simões, E.A., 2011. Risk factors for severe respiratory syncytial virus lower respiratory tract infection. Open Microbiol. J. 5, 144–154.
Spycher, B.D., Silverman, M., Kuehni, C.E., 2010. Phenotypes of childhood asthma: are they real? Clin. Exp. Allergy 40, 1130–1141.
Stein, R.T., Sherrill, D.L., Morgan, W.J., Holberg, C.J., Halonen, M., Taussig, L.M., Wright, A.L., Martinez, F.D., 1999. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 354, 541–545.
Tachdjian, R., Mathias, C., Al Khatib, S., Bryce, P.J., Kim, H.S., Blaeser, F., O'Connor, B.D., Rzymkiewicz, D., Chen, A., Holtzman, M.J., Hershey, G.K., Garn, H., Harb, H., Renz, H., Oettgen, H.C., Chatila, T.A., 2009. Pathogenicity of a disease-associated human IL-4 receptor allele in experimental asthma. J. Exp. Med. 206, 2191–2204.
Thomsen, S.F., Stensballe, L.G., Skytthe, A., Kyvik, K.O., Backer, V., Bisgaard, H., 2008. Increased concordance of severe respiratory syncytial virus infection in identical twins. Pediatrics 121, 493–496.
Thomsen, S.F., van der Sluis, S., Stensballe, L.G., Posthuma, D., Skytthe, A., Kyvik, K.O., Duffy, D.L., Backer, V., Bisgaard, H., 2009. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. Am. J. Respir. Crit. Care Med. 179, 1091–1097.
Valenzuela, C.Y., 1988. On sociogenetic clines. Ethol. Sociobiol. 9, 259–268.
van Piggelen, R.O., van Loon, A.M., Krediet, T.G., Verboon-Maciolek, M.A., 2010. Human rhinovirus causes severe infection in preterm infants. Pediatr. Infect. Dis. J. 29, 364–365.
Vercelli, D., 2008. Discovering susceptibility genes for asthma and allergy. Nat. Rev. Immunol. 8, 169–182.
Wilson, J., Rowlands, K., Rockett, K., Moore, C., Lockhart, E., Sharland, M., Kwiatkowski, D., Hull, J., 2005. Genetic variation at the IL10 gene locus is associated with severity of respiratory syncytial virus bronchiolitis. J. Infect. Dis. 191, 1705–1709.
Young, S., Keeffe, P.T.O., Arnott, J., Landau, L.I., 1995. Lung function, airway responsiveness, and respiratory symptoms before and after bronchiolitis. Arch. Dis. Child 72, 16–24.
Zorc, J.J., Hall, C.B., 2010. Bronchiolitis: recent evidence on diagnosis and management. Pediatrics 125, 342–349.