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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