Human Genetic Factors and Respiratory Syncytial Virus Disease Severity

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INTRODUCTION

Host genetic factors play a major role in the presentation and outcome of infectious diseases (119). Investigation of these genetic determinants has implications beyond defining individual risks for severe infections but also offers significant insights about the pathogenesis of the offending microbe and may even identify potential targets for therapeutic intervention. The insights afforded by a study of host genetics and its effect on infectious diseases are particularly important for respiratory syncytial virus (RSV), because animal models of RSV are likely poorly reflective of disease pathogenesis in humans (25). The study of RSV host genetics is more complex than those of other well-studied infectious diseases, which affect primarily adults, because it involves the added complexity of age-related immune-developmental genetic processes that have been poorly elucidated to date.

RSV infects over two-thirds of the birth cohort within their first year of life (45) and is the most common cause of lower respiratory tract infection-related hospitalization of children <1 year of age (76, 115). Approximately 20 to 30% of RSV-infected infants will experience lower respiratory tract involvement, and up to 3% of all children in the first year of life suffer an RSV-related hospitalization, typically bronchiolitis or pneumonia (13). Case fatality rates of infants hospitalized with RSV may be as high as 2 to 3%, with mortality rates in infants being approximately 10 times higher than those for influenza virus-infected infants (126). Each year, as many as 1 million children may die from RSV infection worldwide (60, 118). Although infants with prematurity, chronic lung disease, congenital heart disease, or immunodeficiencies are at an increased risk for mortality and other manifestations and sequelae of severe RSV disease, most infants with RSV infection requiring hospitalization were previously healthy (13, 135). Therefore, the spectrum of RSV disease severity within this previously healthy population of infants varies tremendously and indicates that individual host factors or viral factors are likely influencing disease severity. Some minor differences in disease severity may be able to be accounted for by different clinical strains of RSV manifesting different replication kinetics and/or inducing different quantities of proinflammatory cytokines (27, 125). Similarly, correlations between certain strains and/or genotypes of RSV and slight differences in disease severity have been described (14, 55, 87, 88, 94, 134). However, these differences are relatively minor and do not provide sufficient explanation for the extremely wide range of observed disease severities. Here, we will focus on the host genetic factors that may influence primary episodes of RSV infection in previously healthy infants.

The study of genetic associations in RSV pathogenesis have to date been limited to the study of specific individual genes that are currently thought to be involved in the regulation of the immune response and that reflect our current concept of RSV pathogenesis. This is termed the candidate gene approach. These concepts of pathogenesis generally represent two hypotheses. One hypothesis focuses on genes involved in direct pathogen control that alter early viral attachment, fusion, replication, and viral clearance. The other hypothesis focuses on genes that are postulated to modify later immuno-
pathology. There is much dispute in the field regarding the emphasis given to each of these hypotheses based on past observations favoring immunopathogenesis: vaccine-enhanced RSV disease (38, 68, 69), animal models (46), and cytokine measurements in infected children (77) versus insights that favor innate viral growth restrictions (25, 26, 30, 137). These two competing hypotheses are useful for organization, but they clearly are not mutually exclusive. Indeed, the relative merits of each hypothesis (immunopathogenesis versus viral pathogenesis) likely differ, depending upon age, previous RSV experience, and individual host genetics. In an individual patient, degrees of both are likely to combine to produce that individual’s RSV disease. Although the candidate gene approach is limited to the evaluation of individual genes already thought to play a role in RSV pathogenesis and has limited ability to uncover new important genetic targets, it may clarify our understanding of RSV pathogenesis.

A recent study by Thomsen et al. (127) demonstrates an increased concordance of severe RSV infection in identical twins over fraternal twins. That study examined 12,346 twin pairs born in Denmark over a 10-year period and linked information on hospitalizations attributable to RSV. The RSV disease severity concordance rate of identical twins was 0.66 (95% confidence interval [CI], 0.59 to 0.73), which was greater than that of fraternal twins, 0.53 (95% CI, 0.48 to 0.58) (P = 0.02). The genetic contribution for the propensity to develop severe RSV infection was estimated to account for approximately 20% of the variance in RSV disease severity. While environmental influences are obviously important, it is notable that the genetic influence may be profound, even at the individual level.

In order to understand the genetic factors that contribute to the acute infection of infants and children, we identified literature in English listed in the National Library of Medicine database using the keyword RSV and any of the following: susceptibility, polymorphisms, and host genetics. All titles were screened and reviewed. We excluded literature that focused on chronic outcomes such as recurrent wheezing or asthma. Studies that focused on or included high percentages of patients with known major risk factors such as congenital heart disease, immunodeficiency, or prematurity were also excluded.

**GENETIC POLYMORPHISMS AND ASSOCIATION WITH DISEASE SEVERITY**

Well over 20 studies on the topic of genetic polymorphisms and their association with RSV disease severity in infants and children meeting the selection criteria mentioned above have been conducted and published. Table 1 categorizes those studies based on the candidate gene target of interest and tabulates the relevant findings and the specific features of each study. Importantly, the biological relevance of the locus and the phenotype’s direction of effect are also listed to the extent that such data were published. In order to understand those studies, certain common terms need to be reviewed. An allele is a specific variation at a single site on one chromosome. A single-nucleotide polymorphism (SNP) is an example of an allele. A haplotype is a combination of alleles on each chromosome that tend to be inherited together. Thus, a haplotype, in the context of polymorphism analysis, is a combination of SNPs within the candidate gene of interest. Haplotype analysis compares the frequencies of the occurrence of the different haplotypes in populations having different disease severities. Haplotype analysis has the advantage of assessing the influence of a block of SNPs (or genes), which fall in proximity to the SNP being analyzed. Genotype refers to an individual’s combination of their two haplotypes, with each haplotype being on one of their two chromosome pairs. The immunogenetic data in those studies can be analyzed with respect to allele, haplotype, genotype, or any combination of these. The odds ratio (OR) is the frequency of an allele (or of a haplotype or genotype) in the affected population (the population exhibiting severe RSV disease) divided by its frequency in the control population (the population that presumably did not have severe RSV disease). An OR of >1 would mean that the allele is associated with a worse outcome, and an OR of <1 would mean that the allele protects against severe RSV disease.

The majority of studies to date focus on one or a few candidate genes and are designed to maximize the chance of identifying any type of association. This likely gene selection and publication bias makes it difficult to interpret results in a way that allows the construction of a proper model of RSV pathogenesis. Avoiding some of these issues, Janssen et al. reported what is arguably the most informative candidate gene study to date (66). In that study, 384 SNPs in 220 candidate genes across different categories of polymorphic immune response genes in 470 infants, most of whom were hospitalized for RSV infection and were less than 12 months of age, were examined. Results showed that susceptibility to RSV is a complex trait, with the strongest association (demonstrating an association at both the allele and the genotype levels to the phenotype) being found in just a few innate immune genes. These include the vitamin D receptor gene, which has been associated with downregulating interleukin 12 (IL-12) and gamma interferon (IFN-γ) production; nitric oxide synthase (NOS2A), which is well known for its antimicrobial and anti-inflammatory roles; the Jun oncogene (JUN), which is an important transcriptional regulator in innate immune pathways, and IFN-α (IFN5), an important cytokine with antiviral effects. The functionality of each of these SNPs is largely unknown, and the specific interaction of these genes with RSV pathogenesis mechanisms therefore remains to be investigated. However, taken together, this evidence implicates early immune responses that are thought to limit the extent of viral infection as a key factor in host susceptibility to more severe forms of RSV disease.

**Innate Defenses**

The binding, fusion, and entry of RSV into host cells are dependent on interactions between the RSV surface proteins (primarily the RSV G and F proteins) and numerous host cell molecules, both secreted and surface bound. Some of these interactions may favor RSV infection, while others may illicit immune responses that ultimately result in more effective viral control and clearance. Several studies reported significant associations of polymorphisms in genes involved in innate immunity with RSV disease severity. If these polymorphisms are functionally significant, they may favor adverse RSV disease outcomes. However, a definitive functional correlation of these polymorphisms or a pathogenic link between any polymor-
<table>
<thead>
<tr>
<th>Candidate gene category</th>
<th>Gene</th>
<th>Polymorphism</th>
<th>Association of mutant with primary outcome</th>
<th>Biological significance of polymorphism on RSV pathogenesis</th>
<th>Haplotype&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Trial design (cases and controls)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR</td>
<td>Thr1Met</td>
<td>T allele OR&lt;sup&gt;d&lt;/sup&gt; 1.30; ( P = 0.0017 )</td>
<td>Candidate genes associated with severe RSV were predominantly innate immune genes rather than genes with other functions such as airway mucosal responses, chemotaxis, adaptive immunity, allergic asthma; ( P = 0.046 )</td>
<td>ND</td>
<td>480 children (10 were &gt; 12 mo of age) hospitalized for RSV bronchiolitis, parents, and 1,030 randomly chosen people from a different study (The Netherlands)</td>
<td>66</td>
<td></td>
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<tr>
<td>JUN</td>
<td>G750A</td>
<td>A allele OR, 1.51; ( P = 0.0093 )</td>
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<tr>
<td>IFN5A</td>
<td>C453T</td>
<td>T allele OR, 0.77; ( P = 0.0093 )</td>
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<tr>
<td>NOS2A</td>
<td>G2757A</td>
<td>A allele OR, 1.27; ( P = 0.0031 )</td>
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<tr>
<td>FCER1A</td>
<td>T-66C</td>
<td>C allele OR, 1.25; ( P = 0.0113 )</td>
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<tr>
<td>384 SNPs in 220 candidate genes&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>SPA</td>
<td>SP-A1 Val19Ala</td>
<td>19Ala allele underrepresented; OR, 0.16 (CI, 0.04–0.7); ( P = 0.007 )</td>
<td>Unknown; substitution of glutamine by lysine at amino acid 223 is located in the carbohydrate recognition domain of SPA and may have an impact on its binding and neutralizing capacity for RSV</td>
<td>SP-A1 6A allele underrepresented (OR, 0.17; ( P = 0.0011 )); 6A/1A&lt;sup&gt;a&lt;/sup&gt; haplotypes underrepresented (OR, 0.17; ( P = 0.0011 )); 6A/1A&lt;sup&gt;a&lt;/sup&gt; overrepresented (OR, 10.4; ( P = 0.006 ))</td>
<td>86 infants less than 1 yr of age hospitalized with a diagnosis of RSV bronchiolitis confirmed by antigen testing vs 95 matched healthy control subjects (Finland)</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>SPA</td>
<td>SP-A2 Ala91Pro</td>
<td>91Pro allele underrepresented (OR, 0.29 [CI, 0.1–0.6]; ( P = 0.001 )</td>
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<tr>
<td>SPA</td>
<td>SP-A2 Gln223Lys</td>
<td>223Lys allele overrepresented; OR, 1.78 [CI, 1.1–2.9]; ( P = 0.025 )</td>
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<tr>
<td>SPA</td>
<td>Al2G/T, T131I, T186A, L176F, R272H</td>
<td>No association</td>
<td>Unknown</td>
<td>(-2G/T, T131I, T186A, L176F, R272H in tight LD, association of haplotypes (( P = 0.034 )</td>
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<tr>
<td>Abs</td>
<td>Met11Thr</td>
<td>11 Met allele overrepresented (OR, 163; ( P = 0.033 )); 11 Met/Met genotypes overrepresented (OR, 2.29 [CI, 1.09–4.81]; ( P = 0.028 )</td>
<td>Unknown; those authors speculated that differences in hydrophobic properties between methionine versus threonine in the amino-terminal part may have functional consequences such as less opsonization</td>
<td>ND</td>
<td>131 infants who were hospitalized due to RSV infection and needed supplementary oxygen and/or gavage feeding were compared with 322 children with asthma or 270 randomly chosen adults (Germany)</td>
<td>103</td>
<td></td>
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<tr>
<td>Abs</td>
<td>Ala160Thr, Ser127Thr</td>
<td>No association</td>
<td>Unknown</td>
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<tr>
<td>Abs</td>
<td>Asn138Thr, Asn186Ser</td>
<td>No association</td>
<td>Unknown</td>
<td></td>
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<tr>
<td>Abs</td>
<td>Asn186Ser</td>
<td>No association</td>
<td>Unknown</td>
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<tr>
<td>Pattern recognition receptors</td>
<td>TLR4</td>
<td>Asp299Gly</td>
<td>Gly allele overrepresented (OR, 5.1; ( P = 0.0014 )</td>
<td>Cells that are transfected with the polymorphic alleles with Asp299Gly and Thr399Ile fail to translocate TLR4 to cell surface and have reduced NF-( \kappa )B activity and cytokine production; PBMC of children with variant TLR4 have blunted responses to RSV; the same mutations were previously associated with hypersensitivities to LPS; hypersensitivities of patient’s PBMCs to LPS was a risk factor for intensive care unit hospitalization for RSV</td>
<td>Cosegregation of both mutations (OR, 40; ( P = 0.034 )</td>
<td>99 infants under 12 mo of age hospitalized for RSV</td>
<td>122</td>
</tr>
<tr>
<td>Abs</td>
<td>Thr399Ile</td>
<td>Ile allele overrepresented (OR, 40; ( P = 0.01 )</td>
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<sup>a</sup> 384 SNPs in 220 candidate genes.

<sup>b</sup> SPA SP-A1 Val19Ala 19Ala allele underrepresented; OR, 0.16 (CI, 0.04–0.7); \( P = 0.007 \).

<sup>c</sup> Candidate genes associated with severe RSV were predominantly innate immune genes rather than genes with other functions such as airway mucosal responses, chemotaxis, adaptive immunity, allergic asthma; \( P = 0.046 \).

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<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype or allelic frequencies</th>
<th>Association</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp299Gly</td>
<td>Genotype underrepresented</td>
<td>No association</td>
<td>$P = 0.054$ (HWE)</td>
</tr>
<tr>
<td>Thr399Ile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp299Gly</td>
<td>No mutations found</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Thr399Ile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr399Ile</td>
<td></td>
<td>No association</td>
<td></td>
</tr>
<tr>
<td>CD14</td>
<td>$-159C/T$</td>
<td>No association</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unknown; the SNP at $-159C/T$ is located within a major transcription site and may affect transcription of the CD14 gene; TT genotype is known to have a higher level of soluble CD14 than the CC genotype</td>
</tr>
<tr>
<td>CD14</td>
<td>$-159C/T$</td>
<td>No association</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CD14</td>
<td>$-159C/T-550C/T$</td>
<td>No association</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Patients with CC genotype had higher serum level of soluble CD14; higher soluble CD14 levels may enhance inflammation and cause severe disease</td>
</tr>
<tr>
<td>MBL</td>
<td>Combinations of wild-type or variant structural and promoter alleles</td>
<td>No association</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mutation in the promoter region and exon 1 of MBL results in low serum levels of MBL, which is a common immunodeficiency with rates of up to 10%</td>
</tr>
<tr>
<td>CXC3R1</td>
<td>Amino acid 280; genotype Met/Met plus Thr/Met</td>
<td>Overrepresented (OR, 2.03 [CI, 1.1–3.9]; $P = 0.025$)</td>
<td>Unknown; CXC3R1 gene polymorphisms that substitute valine for isoleucine at position 280 and threonine for methionine at position 280 disrupt CXC3R1 affinity with its ligand fractalkine, which has been shown to be involved in RSV infection</td>
</tr>
<tr>
<td>CXC3R1</td>
<td></td>
<td>Overrepresented (OR, 2.2 [CI, 1.3–3.8]; $P = 0.003$)</td>
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</tbody>
</table>

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<th>Haplotypes</th>
<th>Trial design (cases and controls)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>T-589C</td>
<td>589T, -33T, 8375G, 8412A alleles overrepresented (OR, 1.63 [CI, 1.07–2.49]; P = 0.02)</td>
<td>Changes in the promoter region of IL-4 at position -589 leads to higher binding affinity to nuclear transcription factors</td>
<td>Common haplotypes with linked SNP variants (OR, 1.53 [CI, 1.07–2.49]; P = 0.02)</td>
<td>IL-4/IL-13 haplotype associated</td>
<td>105 children less than 24 mo old hospitalized with RSV disease vs 315 healthy blood donors (South Korea)</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>T-33C</td>
<td>No associations</td>
<td>No associations</td>
<td>No association</td>
<td>207 children hospitalized for RSV and both parents or 447 random individuals (The Netherlands)</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GS375A</td>
<td>C allele overrepresented in case-control (OR, 1.43; P = 0.04); overrepresented in TDT (OR, 1.37; P = 0.13)</td>
<td>T allele overrepresented (wild-type allele frequency for cases of 0.713 vs 0.784; P = 0.026)</td>
<td>SNP in the promoter sequence at position -1112 of the IL-13 gene has been shown to alter the binding of nuclear transcription factor and expression of IL-13</td>
<td>Haplotype analysis including 3 IL-13 and 1 IL-4 polymorphism associated with RSV disease (P = 0.0008)</td>
<td>131 infants less than 2 yr of age who were hospitalized due to RSV infection and needed supplementary oxygen and/or gavage feeding were compared with 230 randomly chosen adults (Germany)</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>A-345T</td>
<td>No association</td>
<td>No association</td>
<td>No association</td>
<td>207 children hospitalized for RSV and both parents or 447 random individuals (The Netherlands)</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg10Gln</td>
<td>No association</td>
<td>No association</td>
<td>No association</td>
<td>105 children less than 24 mo old hospitalized with RSV disease vs 315 healthy blood donors (South Korea)</td>
<td>18</td>
<td></td>
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<tr>
<td>IL-5</td>
<td>−746</td>
<td>No association</td>
<td>Unknown</td>
<td>Unknown</td>
<td>207 children hospitalized for RSV and both parents or 447 random individuals (The Netherlands)</td>
<td>59</td>
<td></td>
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<tr>
<td>IL-9</td>
<td>A−345G</td>
<td>No association</td>
<td>No association</td>
<td>No association</td>
<td>207 children hospitalized for RSV and both parents or 447 random individuals (The Netherlands)</td>
<td>58</td>
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<tr>
<td>IL-13</td>
<td>C-1112T</td>
<td>T allele overrepresented</td>
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<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>−1082</td>
<td>Genotypes stratified as &quot;high, intermediate, and low phenotype producers&quot; correlated with development of pneumonia (0%, 11%, and 27%, respectively; P = 0.02)</td>
<td>Stratification of genotype to high, intermediate, and low phenotypes has not been validated in context of RSV infection</td>
<td>ND</td>
<td>77 infants hospitalized for RSV vs 107 randomly selected adults (United States)</td>
<td>41</td>
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<tr>
<td>Gene</td>
<td>SNP(s)</td>
<td>Association/Interaction</td>
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<tr>
<td>IL-10</td>
<td>C-592A</td>
<td>No association overall C allele overrepresented in children &lt;6 mo old (OR 1.61; CI, 1.10–2.35; P = 0.014)</td>
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<tr>
<td></td>
<td>5876, 4949, 1547, 919, -627,</td>
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<tr>
<td></td>
<td>-854, -1111, -3585</td>
<td>SNPs in the promoter studied (−1117, −854, and −627) have been associated with altered transcriptional regulation of IL-10</td>
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<tr>
<td></td>
<td></td>
<td>No association</td>
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<tr>
<td>IL-18</td>
<td>A-607C, G-137C, T133G, C127T,</td>
<td>G-133C associated with severe RSV (P = 0.043)</td>
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<tr>
<td></td>
<td>A5304G, G133C</td>
<td>SNP in the promoter at position −133C/G potentially creates a new binding site for STAT</td>
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<td></td>
<td>Haplotype analysis (P &lt; 0.00001)</td>
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<td>134 infants less than 2 yr of age who were hospitalized due to RSV infection and needed supplementary oxygen and/or gavage feeding were compared with 270 randomly chosen adults (Germany)</td>
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<tr>
<td>IL-6</td>
<td>G-174C</td>
<td>“Higher-producer” genotype −174GG; G/C associated with shorter hospital stay (P = 0.049)</td>
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<td></td>
<td></td>
<td>IL-6 genotype was not related to IL-6 levels in experimentally infected adults (42 adults)</td>
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<tr>
<td>TNF-α</td>
<td>G-308A</td>
<td>No association</td>
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<td></td>
<td>77 infants hospitalized for RSV vs 107 randomly selected adults (United States)</td>
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<tr>
<td></td>
<td>G-308A</td>
<td>Unknown</td>
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<td></td>
<td></td>
<td>Genetic interaction of TNF-α −308A allele with IL4Ra Gln551Arg contributing to OR of 0.40 (P = 0.01)</td>
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<td>ND</td>
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<td>207 children hospitalized for RSV and both parents or 447 random individuals (The Netherlands)</td>
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<td>77 infants hospitalized for RSV vs 107 randomly selected adults (United States)</td>
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<td></td>
<td></td>
<td>Genetic interaction of IL-10−592A allele with IL4Ra G551R allele (OR, 2.03; P = 0.01)</td>
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<td></td>
<td></td>
<td>No association</td>
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<td>207 children hospitalized for RSV and both parents or 447 random individuals (The Netherlands)</td>
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<td>131 infants less than 2 yr of age who were hospitalized due to RSV infection and needed supplementary oxygen and/or gavage feeding were compared with 270 randomly chosen adults (Germany)</td>
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<tr>
<td>TGF-β</td>
<td>Haplotype for codons 10 and</td>
<td>Stratification of genotype to high, intermediate, and low phenotypes has not been validated in context of RSV infection</td>
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<td></td>
<td>25</td>
<td>Associated with oxygen score at presentation (P = 0.008)</td>
<td></td>
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<tr>
<td>IFN-γ</td>
<td>874</td>
<td>Stratification of genotype to high, intermediate, and low phenotype has not been validated in context of RSV infection</td>
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<td>77 infants hospitalized for RSV vs 107 randomly selected adults (United States)</td>
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<tr>
<td>IL-8</td>
<td>T-251A</td>
<td>A allele associated with higher-than-expected rate of transmission of 78% (CI, 62–93%; P = 0.004)</td>
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<td>Unknown</td>
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<td></td>
<td>T-251A</td>
<td>No association</td>
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<tr>
<td></td>
<td>C-781T</td>
<td>No association</td>
<td></td>
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<td></td>
<td></td>
<td>83 infants admitted for RSV bronchiolitis vs their parents (United Kingdom)</td>
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<td>131 infants less than 2 yr of age who were hospitalized due to RSV infection and needed supplementary oxygen and/or gavage feeding were compared with 270 randomly chosen adults (Germany)</td>
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<tr>
<td>Candidate gene category</td>
<td>Gene</td>
<td>Polymorphism</td>
<td>Association of mutant with primary outcome</td>
<td>Biological significance of polymorphism on RSV pathogenesis</td>
<td>Haplotype</td>
<td>Trial design (cases and controls)</td>
<td>Reference</td>
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<tr>
<td>IL8RA</td>
<td>Met32Arg, Ser270Thr, Arg335Cys</td>
<td>No association</td>
<td>Unknown</td>
<td>No association</td>
<td>131 infants less than 2 yr of age, who were hospitalized due to RSV infection and needed supplementary oxygen and/or gavage feeding, were compared with 270 randomly chosen adults (Germany)</td>
<td>108</td>
<td></td>
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<tr>
<td>CCR5</td>
<td>−2554T, −2459G</td>
<td>T allele OR, 1.25 (P = 0.01); G allele OR, 1.21 (P = 0.02); homozygosity enhanced association slightly</td>
<td>SNP in the promoter at position −2459 is associated with both enhanced and decreased expression of CCR5</td>
<td>−2554T and −2459G in complete linkage disequilibrium</td>
<td>580 infants &lt;12 mo of age who had RSV bronchiolitis and who required gavage feeds, intravenous, or oxygen vs 580 infants born consecutively (United Kingdom)</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>RANTES</td>
<td>C-28G, G-403A, TIn1.1C</td>
<td>No association</td>
<td>SNP variants −28G and −403A in the promoter region of RANTES; upregulate RANTES transcription</td>
<td>−28C/C−403G/AIn1.1T/T combined genotype (OR, 2.3; P = 0.035)</td>
<td>106 children aged 1–24 mo hospitalized with RSV-positive lower respiratory disease vs 126 sex-matched healthy adults without a history of lower respiratory tract disease (Crete)</td>
<td>1</td>
<td></td>
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<tr>
<td>ICAM-1</td>
<td>Lys409Glu, A20788G</td>
<td>No association</td>
<td>Unknown</td>
<td>No association</td>
<td>134 infants less than 2 yr of age who were hospitalized due to RSV infection and needed supplementary oxygen and/or gavage feeding, were compared with 270 randomly chosen adults (Germany)</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>VCAM-1</td>
<td>−C833T</td>
<td>No association</td>
<td>Unknown</td>
<td>No association</td>
<td>134 infants less than 2 yr of age who were hospitalized due to RSV infection and needed supplementary oxygen and/or gavage feeding, were compared with 270 randomly chosen adults (Germany)</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>E-selectin</td>
<td>Ser128Arg, His468Tyr</td>
<td>No association</td>
<td>Unknown</td>
<td>ND</td>
<td>134 infants less than 2 yr of age who were hospitalized due to RSV infection and needed supplementary oxygen and/or gavage feeding, were compared with 270 randomly chosen adults (Germany)</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>HLA</td>
<td>HLA A, HLA B</td>
<td>No association</td>
<td>ND</td>
<td>65</td>
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</table>
IGHG1 HGHG2 genotype
Overrepresented (OR, 2.3; n) allele and IGHG2 (/H11002 /H11002 n) genotype were previously shown to be correlated with low antibody response to bacterial agents, immunodeficiencies, and nonatopic phenotype of childhood asthma.

A haplotype is a combination of alleles on each chromosome that tend to be inherited together. Thus, a haplotype, in the context of polymorphism analysis, is a combination of SNPs within the candidate gene of interest. Haplotype analysis compares the frequency of the occurrence of the different haplotypes in populations having different disease outcomes. Haplotype analysis has the advantage of assessing the influence of a block of SNPs (or genes), which may be in linkage disequilibrium.

Or, the frequency of an allele in the diseased population divided by the frequency of this allele in the control (nondiseased) population. An OR of 1 would mean that the allele is associated with a worse outcome, and an OR of 0.5 would mean that the allele protects against severe RSV disease.

Genes that had associations at both the allele level and genotype level are shown here. The SP-A gene consists of two functional genes, SP-A1 and SP-A2, with five and four polymorphic sites, respectively. Nineteen alleles were identified for SP-A1 (denoted 6A), and 15 alleles were identified for SP-A2 (denoted 1A).

Some of the proteins found within surfactant do not have surface tension lowering properties but rather function as primitive pattern recognition molecules comprising part of the innate immune system. These surfactant proteins are among the collectin subgroups of mammalian C-type lectins thought to be involved in the innate recognition, opsonization, and phagocytosis of various bacterial and viral pathogens. Four studies that identified statistically significant associations of a few surfactant protein gene polymorphisms with infant RSV disease severity have been performed to date.

Surfactant protein A (SP-A) is produced by many cells of the body, especially those cells lining the respiratory and gastrointestinal tracts. This includes respiratory epithelial cells and type I alveolar cells. SP-A has been shown to specifically interact with RSV G and F proteins and to act as an RSV opsonin for macrophages in vitro (10). Furthermore, SP-A binds to the fusion glycoprotein of RSV, effectively neutralizing infection by preventing the virus from entering the target cell and inducing syncytium formation (43). This is thought to occur through the pattern recognition of specific carbohydrates of the highly glycosylated RSV surface proteins. This surfactant’s functionality in RSV infection has also been demonstrated in vivo, where SP-A-deficient mice experimentally infected with RSV show both more severe disease pathology and greater viral loads than do their wild-type littermates (79).

Therefore, SP-A appears to play an important role in pulmonary host defense against RSV (47). The association of SP-A polymorphism with susceptibility to severe RSV infection in infants was investigated (80). Two subunit proteins (SP-A1 and SP-A2) combine into a symmetrical flower-like structure to form the functional SP-A pattern recognition receptor molecule. Polymorphisms of both of these functional SP-A genes were investigated by restriction fragment length polymorphism techniques. The SP-A2 allele 1A, which has a substitution of glutamine by lysine in its carbohydrate recognition domain, was overrepresented in a relatively genetically homogeneous population of RSV-infected infants compared with appropriate control subjects (5% versus 0.5%), with an OR of 10.44 (95% CI of the OR, 1.3 to 83.2; P = 0.006). It is plausible that decreased viral affinity due to this amino acid substitution leads to an increased RSV load, causing more severe disease, but the RSV-binding functionality of the amino acid substitution has not been demonstrated. Unfortunately, these specific polymorphism associations fail to be reproduced in analyses of other infant RSV-infected populations (M. El Saleeby and J. P. DeVincenzo, unpublished data). Other specific haplotypes within SP-A1 and SP-A2 also appear to be under- or overrepresented in the infants who were hospitalized in that study (Table 1).

While the specific interaction of SP-D with the RSV viral G and F proteins is controversial, delayed clearance of RSV and increased inflammation were demonstrated in SP-D knockout mice (78). Furthermore, the intranasal administration of recombinant SP-D to RSV-infected mice significantly reduces the lung viral load by 80% (57). The association of three biallelic SP-D gene polymorphisms with severe RSV infections (74) has been investigated. The frequency of the allele coding for methionine at position 11 was increased in the RSV group.
compared to controls, an association that was independent of other confounding factors that have been shown in multiple other studies to be predictive of more severe RSV disease, such as sex, smoking in the family, length of gestational age at birth, postnatal age during epidemics, and socioeconomic status. Those authors speculated that the difference in hydrophobic properties between methionine and threonine in the amino-terminal region of the molecule may have functional consequences, indicating that such a substitution might decrease RSV opsonization and increase viral load, leading to severe disease. However, no studies of functionality of these polymorphisms have apparently been performed.

The SP-C gene shows a high degree of sequence conservation, but two common polymorphisms have been linked to neonatal respiratory distress syndrome in the Finnish population (75). The 138Thr-186Asn haplotype was overrepresented (frequency of 0.1651 versus 0.0844 for case versus control, respectively; $P = 0.03$) in German children with severe RSV bronchiolitis (107). However, little is currently known about the specific functions of SP-C, and even less is known about the functional impact of the SP-C polymorphism.

SP-B is a hydrophobic protein and an essential constituent of lung surfactant. Studies of SP-B knockout mice and human congenital protein malformations indicate that functional SP-B deficiency is lethal. SP-B polymorphism has been associated with adult diseases of respiratory distress syndrome, chronic obstructive pulmonary disease, and acute respiratory failure. Haplotype analysis of SP-B gene polymorphism demonstrates a significant association with RSV severity (103); however, as the associations with multiple adult respiratory diseases suggest, and as is likely true for other gene polymorphisms as well, this SP-B effect on RSV disease severity may simply be a reflection of the overall fitness of respiratory function in these individuals rather than any direct interaction of RSV and SP-B.

**Host cell receptor genes.** The heavily glycosylated RSV G protein has been identified as being the binding protein crucial for viral attachment to host cells. Alterations in host attachment site sequences have been shown to confer resistance against other important pathogens such as the Duffy coat antigen promoter polymorphisms against *Plasmodium vivax* and CD4 coreceptor CCR5 mutations in human immunodeficiency virus (HIV)-infected long-term nonprogressors. Thus, there is precedence for such receptor polymorphisms to have a biological significance in RSV disease. For RSV, the cell surface binding sites on epithelial cells remain unclear; however, there is evidence that the binding of the RSV G protein to leukocytes involves the host CX3C receptor (CX3CR) (129). CX3CR is a leukocyte receptor for fractalkine, a member of the CX3C chemokine family. Fractalkine binding of the CX3CR on leukocytes produces downstream effects, which include proinflammatory and other effects mediated through substance P (128). Fractalkine expression is thought to contribute to inflammatory diseases such as rheumatoid arthritis, transplant rejection, glomerulonephritis, and others. Additionally, fractalkine is important in T-helper cell 1 (Th1)-type cellular and natural killer cell responses. The RSV G protein contains a CX3C chemokine attachment motif at amino acid positions 182 to 186, and this motif has been shown to interact with the CX3C chemokine receptor. The RSV G glycoprotein may compete with fractalkine for binding to the CX3CR, producing possible deleterious immunomodulatory effects promoting deleterious Th2-type responses (see “Immunopathology” below). Thus, the host CX3CR appears to be central to several putative RSV pathogenesis pathways. Two nonsynonymous, single-nucleotide genetic polymorphisms, Val249Ile and Thr280Met, of the CX3CR1 gene that disrupt fractalkine’s affinity to its CX3C receptor have been identified. Genotyping revealed an overrepresentation of the 280Met-containing genotypes (Met/Met or Thr/Met) in children hospitalized with RSV lower respiratory tract disease compared to sex-matched healthy adults without a history of lower respiratory tract disease (37.8% versus 20.8%, respectively; OR, 2.03; 95% CI, 1.1 to 3.9; $P = 0.025$) (2). This finding demonstrates an association between the common CX3CR1-Thr280Met variations and an increased risk of severe RSV bronchiolitis. Competing hypotheses exist regarding the possible mechanism of this effect. The mutation either may result in higher viral affinity facilitating viral entry or may alternatively modulate host immune responses, decreasing the effectiveness of viral clearance or of the stimulation of inappropriate and immunopathogenic immune responses.

Efficient recognition of viruses, including RSV, by innate immune receptors, most notably the Toll-like receptors, ultimately activates NF-κB, which in turn stimulates the transcription of genes directly involved in the antiviral response (56). The pattern recognition receptor’s role in the innate immune response to respiratory viruses has been recently reviewed (136). These antiviral responses include the release of nitric oxide and cytokines that recruit T cells, leading to the development of adaptive antiviral immunity, which ultimately clears the pathogen. The binding of RSV to Toll-like receptor 4 (TLR4) is clearly involved in RSV-induced NF-κB activation (51, 92), although the signals between cell surface/receptor binding and NF-κB translocation are not yet well defined. In addition to the TLR4 pathway, RSV-ligated receptors may also induce the activation of other signaling cascades such as phosphatidylinositol 3-kinase and the p38 mitogen-activated protein kinase pathways independent of the IRAK-containing signaling module. The biological importance of TLR4 and another pattern recognition receptor, CD14, in the innate control of RSV infection and disease has been confirmed in knockout mouse studies (73). However, a subsequent in vivo study challenges the role of TLR4 in RSV pathogenesis. The authors of that study performed RSV infection using several mouse strains with TLR4 deletions on different genetic backgrounds and found no effect on disease outcome (32). Furthermore, TLR4 does not appear to be important for the pathogenesis of closely related viruses such as Sendai virus and murine pneumovirus (35, 132). Thus, confirmation of the role of TLR4 in RSV disease deserves further study.

The findings for TLR4 knockout mice have also been evaluated for humans by investigating naturally occurring polymorphism frequencies. Perhaps the most striking known association between any genetic polymorphism and RSV severity, and one of the few in which any functional relevance is shown, is with regard to TLR4 mutations (122). TLR4 mutations Asp299Gly and Thr399Ile, either alone or in combination, are overrepresented in infants under 12 months of age hospitalized for RSV bronchiolitis compared to infants who have mild RSV
bronchiolitis being cared for in ambulatory settings (OR of 4.1). This genetic association is also noteworthy since it is one of only a few to predict disease severity once an individual is infected. This is shown by the control (comparator) group being composed of similarly aged infants with documented mild RSV infection. Most other studies used control groups composed of adult blood donors, making it impossible to determine whether any genetic association found is related to increased disease once an individual is infected or is related only to increasing the chances of becoming RSV infected. Importantly, the functional significance of these TLR4 SNPs has been investigated. Tulic et al. recently reported that human bronchial epithelial cells transfected with 299Gly and 399Ile alleles fail to efficiently translocate TLR4 receptor to the cell surface. This is associated with reduced NF-κB signaling. Also, peripheral blood mononuclear cells (PBMC) from children expressing TLR4 variants demonstrated blunted responses to RSV (130). These same mutations were previously associated with hyporesponsiveness to lipopolysaccharide (LPS) and an increased incidence of gram-negative sepsis (5, 83). Consistent with this observation, a separate study by those authors demonstrated that the hyporesponsiveness of a patient’s PBMC to LPS was a risk factor for intensive care unit hospitalization by RSV, which also tended to correlate with the presence of these TLR4 mutations (85). Numerous translational studies associating these TLR4 polymorphisms to various other disease entities have been described, thus strengthening the likelihood that this polymorphism is relevant in RSV pathogenesis as well. This includes a study that shows that these polymorphisms are found in close to 90% of patients who are at high risk (mostly prematurity) for severe RSV infection, versus 10% of controls (9). However, to its detriment, in that study, it was impossible to distinguish whether the polymorphism is related to prematurity itself, the survival of being born prematurely, or the susceptibility to RSV in this highly selected study population.

Not all studies of RSV and TLR4 polymorphism resulted in consistent findings. The TLR4 mutation Asp299Gly was found to have either no effect or even a possible protective effect against severe RSV infection in studies from Germany and Canada (100, 104). Differences with respect to the background population, age of patients, selection of controls, and allele frequency may have influenced such outcomes. An obvious example of a population bias was seen where TLR4 mutations were not identified in either cases or controls in a Japanese study (64). Despite such considerations, Paulus et al. also demonstrated that levels of proinflammatory cytokine production in PBMC from patients who were heterozygous for Asp299Gly were not significantly elevated (100). Thus, the TLR4 mutation’s effects on RSV severity continues to deserve scrutiny, especially in varied and heterogeneous populations.

The pattern recognition receptor CD14, found in vivo to play a role in innate immunity to RSV, has genetic polymorphisms in humans. However, no association between the CD14 promoter polymorphism −159C/T and RSV bronchiolitis was found in any of the three studies performed to date (64, 104, 122). However, the CC alleles of the CD14 promoter polymorphism −550C/T was found to be associated with the hospitalization of children for RSV bronchiolitis (64). Children with risk factors such as cardiac disease, pulmonary disease, and prematurity were appropriately excluded. Some functional significance of this promoter polymorphism was also suggested by showing that serum levels of soluble CD14 were higher in children with the CC genotype. One pathogenesis hypothesis is that CD14, which has been reported to bind to LPS and enhance the transfer of LPS to membranous CD14, ultimately enhances the production of cytokines such as tumor necrosis factor alpha (TNF-α), thus exacerbating disease (52, 63, 70, 102). However, even the hypothesis that increased TNF-α production worsens human RSV disease is by no means clear and may not even be correct.

Mannan-binding lectin (MBL) is also a member of the collectin family and is believed to play an important role in pattern recognition and innate immune defense. Mutation in the promoter region and in exon 1 of MBL results in low serum levels of MBL. These sets of mutations are the genetic basis for a recognized common immunodeficiency syndrome occurring in up to 10% of the U.S. population (123). However, no association between low levels of MBL or MBL variant alleles and increased occurrences of RSV lower respiratory tract infections or more severe RSV disease has been found (71).

**Immunopathology**

Mounting evidence from infants and experimentally infected adults suggests that the increased RSV load drives both an increased disease severity and higher proinflammatory cytokine concentrations (29, 30). However, this concept is not accepted by all, and a controversy as to the relative contributions of viral load versus immunopathology to the production of RSV disease continues to swirl. The controversy is illustrated by clinical observations of the relative effectiveness (or lack thereof) of antivirals compared to that of corticosteroids in the attempted treatment of RSV in infants. It is clear that corticosteroids do not substantially improve the outcome of RSV disease, as was shown by several well-designed randomized placebo-controlled clinical trials. Their use is also associated with a worsening viral load (15). Aerosolized ribavirin, the only approved antiviral for RSV, has been studied in several small, randomized trials as well and does not produce a substantial clinically relevant benefit either. However, a measurable improvement in viral load, accompanied by measurable improvements in disease severity outcomes, occurs with aerosolized ribavirin. Considering that ribavirin produces only a modest antiviral effect and observing that no antiviral study has evaluated treatments starting early in the course of the disease, the available evidence suggests that ribavirin provides a proof of concept that controlling early viral replication reduces RSV disease (28). Even with this proof of concept, the question of immunopathology remains relevant, however, because for any given viral load, variations in the degree of inflammation due to genetic differences are likely able to alter disease outcome.

Much evidence from studies of mice and multiple lines of evidence from studies of humans illustrate the importance of immune differentiation toward Th1 responses in the control of viral infections including RSV. Specific cytokine profiles are the hallmarks of these responses, with Th1 responses producing IFN-γ, IL-12, and others, whereas Th2 responses produce IL-4 and IL-10. A carefully conducted prospective birth cohort
study investigated whether RSV bronchiolitis is associated with excess Th2 and/or deficient Th1 cytokine responses (77). In that study, the IL-4/IFN-γ and IL-10/IL-12 ratios in nasal lavage fluids from infants with acute bronchiolitis were elevated compared to those for infants with upper respiratory tract infection alone despite the lack of a difference in upper respiratory tract RSV load in the few infants for whom it was quantified. The prevailing concept of RSV disease being immune mediated has stimulated a number of genetic association studies targeting Th1 or Th2 cytokine genes and neutrophil responses, which have in general shown low-magnitude and equivocal associations with RSV severity. Overall, the accumulating evidence of associations with a number of cytokine gene polymorphisms and haplotypes suggests that a well-known disease-modifying locus on chromosome 5q31 may be involved in RSV disease severity. This locus contains a cluster of Th2 cytokine genes (IL-4, IL-13, and IL-5) as well as other relevant immune-related genes such as IFN regulatory factor 1 (IRF1) and granulocyte-macrophage colony-stimulating factor (CSF2). Therefore, this locus and the genetic polymorphisms in this area have been the subject of numerous candidate gene studies. Polymorphisms in this locus have been associated with susceptibility to a variety of infectious diseases including malaria (113), schistosomiasis (24), and leishmaniasis (67) as well as asthma (82) and Crohn’s disease (117). However, the crucial link between the polymorphisms in this area, biological markers (cytokine measurements), and RSV disease outcome has yet to be made.

Th1 versus Th2 cytokine response genes. The concept that Th2-biased immune responses play a major deleterious role in RSV disease has driven the investigation of cytokines such as IL-4 and IL-13 that mediate the differentiation of Th2 cells. In an investigation of common SNPs of IL-4, IL-13, and IL-5 genes, a common IL-4 haplotype was overrepresented in children hospitalized with RSV disease versus healthy adult blood donors (OR, 1.63) (18). This haplotype includes the −589T promoter variant, previously shown to be associated with increased immunoglobulin E levels in American and Japanese populations (98, 111). An association of variants of genes encoding IL-4 and the IL-4 receptor α chain in infants hospitalized with RSV bronchiolitis has also been found when the parents of these children were used as controls (59). The IL-4 −590T allele was found at a higher frequency among 200 children hospitalized with RSV than in random controls (OR, 1.43; \( P = 0.04 \)). While the association was most significant in infants older than 6 months of age who demonstrated an OR of 2.09 (\( P = 0.02 \)) (59), there was no association with infants less than 6 months of age, suggesting, as was previously proposed, that perhaps the pathological process in these two age groups may be different. Older infants with a more mature effective antiviral immune response and/or an immune response that is not RSV naïve may be more prone to Th2-mediated immunopathology. IL-4 receptor polymorphisms have been further evaluated. The IL-4 receptor α chain Gln551Arg allele is a gain-of-function polymorphism that is located in the intracellular domain of the receptor in a region known to be involved with the IL-4-induced activation of STAT6 DNA-binding activity (112). STAT6 stimulates the transcription of a number of genes related to Th2 responses and is thought to play a key role in the Th2 polarization of the immune system. The Arg551 (also known as Arg576) allele was associated with functionally enhanced signaling and changed the binding specificity of this receptor to specific downstream signal-transducing molecules. This polymorphism was again associated with severe RSV infection in infants >6 months of age but not in infants <6 months of age even though the study had greater statistical power in this <6-month-old age group (59). Those results indicated that possible gain-of-function variants of Th2 cytokine genes may play a role in increasing the severity of RSV disease, which appears more pronounced after the first half-year of life. These findings highlight one of the limitations of the numerous RSV association studies where either a genetic effect is age related and thus not studied properly or where a genetic effect is present only in RSV-experienced populations. Not all clinical evaluations of IL-4 polymorphisms are consistent in their findings. Contrary to the data described above, a study of German children failed to show an independent association of the IL-4 589C/T polymorphism with disease severity (106).

The Th2 cytokine IL-13 gene has also been examined for its RSV disease association. An independent association between the IL-13 polymorphism −1112C/T and more severe RSV disease has also been observed (\( P = 0.026 \)) (106). This polymorphism has been shown to functionally alter the expression of IL-13 and the binding of nuclear factors to its promoter (131). Furthermore, haplotype analysis found that the combination of IL-13 and IL-4 polymorphisms strengthened the RSV severity association (\( P = 0.0008 \)). A region on chromosome 5q31-33 that encodes IL-3, -4, -5, -9, and -13 was found to be a disease-modifying locus for Schistosoma mansoni in an early genomewide study (86) and has been associated with various infectious and inflammatory disorders (109). It is possible that the associations observed for the polymorphisms in the respective cytokine genes are merely a marker for this disease-modifying locus with haplotypes in linkage disequilibrium with each marker. Further evaluation is ongoing.

Polymorphism in the IL-18 promoter −137G/C has been associated with severe RSV disease in German children (105). While it was suggested that there may be a potential increase in IL-18 production in carriers of the mutant allele, thus contributing to the severity of RSV bronchiolitis, this is not an association that is supported well by data reported in the existing literature (12, 39).

IL-10 is a central regulatory cytokine that is known to suppress Th1 and promote Th2 immune responses. It downregulates cell-mediated immune function by the inhibition of cytokines, chemokines, and antigen presentation. IL-10 levels in nasal secretions are markedly increased during RSV infection, although studies correlating IL-10 levels with RSV severity are limited (29, 77). The role that IL-10 plays in RSV disease was evaluated by examining variations in the IL-10 gene (138). Although the promoter polymorphisms studied (−1117, −854, and −627) have been associated with the altered transcriptional regulation of IL-10 (22, 31, 44, 93), none of the SNPs or haplotypes evaluated were statistically over- or underrepresented in infants hospitalized for RSV bronchiolitis compared to controls. However, in a subgroup analysis, two SNPs were associated (OR, 1.7; \( P = 0.004 \)) with the need for RSV-induced mechanical ventilation. An expanded study was performed to investigate polymorphisms in genes encoding IL-9,
IL-10, and TNF-α using both a transmission/disequilibrium test and a case-control approach (58). In that study, children homozygous for the promoter polymorphism IL-10 −592C or −592A allele had a higher risk of hospitalization for RSV bronchiolitis than did heterozygous carriers (OR, 1.73 and 2.55, respectively). However, the effects that these specific IL-10 polymorphisms have on the functionality of IL-10 are not understood, and it is therefore unclear whether these polymorphisms have direct relevance to disease pathogenesis. In children hospitalized at <6 months of age, a significant association between RSV bronchiolitis and the IL-10 −592C allele was found (OR, 1.61). No significant associations of TNF-α and IL-9 polymorphisms with RSV bronchiolitis were observed.

To date, few studies have examined the effect of both Th1 and Th2 cytokine gene polymorphisms in combination (41). Infants who had a genotype associated with a higher level of production of IFN-γ had more severe lower respiratory illness and a longer duration of intensive care unit stay but less otitis media. In that same study, an IL-6 high-producer genotype was associated with a shorter length of oxygen supplementation and a shorter hospital stay. That study also shows that genotypes producing lower transforming growth factor β1 and IL-10 levels were associated with clinical parameters of increased RSV disease severity. Although such associations appear to agree with our current understanding of RSV pathogenesis, a separate study from the same group of investigators failed to show a correlation of genotype to their respective cytokine levels in adults experimentally challenged with RSV, questioning the validity of this stratification scheme (42). Although this was a negative study with limitations, this attempt to provide a biological link and to replicate the results in a different population is crucial to a meaningful candidate gene association study and raises appropriate questions regarding the biological relevance of other studies.

**Neutrophil response genes.** A well-recognized pathological feature of RSV bronchiolitis is prominent neutrophilic infiltration within the airways (15, 33, 137). RSV-infected airway epithelial cells secrete high levels of neutrophil-recruiting chemokine, IL-8, as well as other proinflammatory cytokines (36, 96). Infants with RSV bronchiolitis also have high levels of IL-8 in their nasal secretions and plasma samples (11, 97). Therefore, neutrophil-recruiting chemokines such as IL-8 and their respective chemokine receptors are a reasonable target for RSV immunogenetic investigation. Here again, results are intriguing but not consistent.

A common SNP exists 251 bp upstream of the IL-8 transcription start site (62) (designated the −251A site) and is associated with functionally increased levels of IL-8 production by LPS-stimulated whole blood cells. The association of this allele with RSV disease was analyzed by the transmission disequilibrium test, a test measuring the overtransmission of an allele from heterozygous parents to their offspring. The frequency of this allele was significantly increased (deviated from the expected transmission rate of 50%) in infants hospitalized with RSV bronchiolitis (transmission, 62%; 95% CI, 53 to 71; P = 0.014) and particularly in those without known risk factors (transmission, 78%; 95% CI, 62 to 93; P = 0.004). In a subsequent report, those authors showed that a significant increase in IL-8 transcript levels was seen in human respiratory epithelial A549 cells in an IL-8 haplotype containing the same promoter polymorphism, −251A (50), although the −251A polymorphism itself did not appear to affect transcription directly. The hypothesis is that individuals with this promoter site polymorphism secrete greater amounts of IL-8 upon RSV infection, which ultimately causes more lung damage. However, this remains speculative and has not been confirmed in studies of different populations. In a separate study from Germany, the IL-8 polymorphisms −251A and −781T did not show significant associations with severe RSV disease (108). Likewise, in that same study, no associations with RSV disease severity were seen when three SNPs within the IL-8 receptor (IL8RA) were evaluated.

CCR5 is the receptor for the chemokines CCL5 (RANTES) and CCL3 (MIP-1α) that recruit T cells, monocytes, basophils, eosinophils, and, to a lesser degree, neutrophils (84, 91). CCL5 and CCL3 are present in peripheral blood and in respiratory secretions at higher concentrations in infants with severe RSV disease (90, 95). Additionally, higher concentrations of CCL3 correlate with an increased severity of RSV disease in infants with bronchiolitis (40) and increased symptom scores in adults experimentally infected with RSV (29). Therefore, variations in CCR5, CCL5, or CCL3 may play a role in the immunopathology of RSV infections. Several promoter region variants of the CCR5 gene that have been described are associated with several diseases such as chagasic cardiomyopathy (16) and reduced progression to AIDS after infection with HIV, although this may not be functionally relevant to RSV since CCR5 is a coreceptor for HIV but is not thought to be a cellular receptor for RSV (20). Case-control and family-based association studies show that a −2459G variant and a 2554T polymorphism of CCR5 are associated with severe bronchiolitis (61). Data describing the functional impact of the −2459G promoter polymorphism on CCR5 are conflicting at this point and have been associated with both enhanced and decreased expression of CCR5 (89, 116). The functional significance of the position 2554 variation is unknown.

The genetic polymorphism of RANTES itself has also been studied with respect to the severity of several infectious and allergic diseases including HIV, atopic dermatitis, life-threatening asthma, and hepatitis. Two alleles within the promoter region of the gene (−28G and −403A) have been shown to upregulate RANTES transcription, while an additional variant within the first intron (In1.1C) downregulates this transcriptional activity. The frequencies of these alleles in a homogenous population of children hospitalized with RSV bronchiolitis were compared with those of a population of adults without histories of respiratory disease. The association of the −28G allele could not be ascertained due to the infrequency of its occurrence. No association with RSV disease severity for the other functional alleles was found. However, a particular combined genotype of RANTES was statistically associated with severe RSV disease (1). It is unclear to what degree this and other similar findings are influenced by the problem of multiple-hypothesis testing (Table 2).

The activation of adhesion molecules such as ICAM-1, VCAM-1, and selectin occurs in epithelial cells infected with RSV and direct leukocytes to the site of infection. Additionally, the RSV-induced expression of ICAM-1 and other cellular adhesion molecules on respiratory epithelial cells augments the adhesion of pathogenic bacteria to these cells (8). Poly-
TABLE 2. Problems and pitfalls commonly seen in studies of RSV immunogenetics

<table>
<thead>
<tr>
<th>Problem</th>
<th>RSV study problem</th>
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<tbody>
<tr>
<td>Problems associated with multiple-hypothesis testing</td>
<td>Little validation for associations in more than one population data set</td>
</tr>
<tr>
<td>Proposed physiological mechanism often not validated with human (especially infant) in vitro systems</td>
<td>Use of mouse models, which are relatively nonparallel to human RSV disease, to identify targets for human genetic studies</td>
</tr>
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<td>Use of mouse models, which are relatively nonparallel to human RSV disease, to identify targets for human genetic studies</td>
<td>RSV quantity or cytokine levels not used as outcome variables even though the pathogenic mechanisms proposed to account for these gene associations may involve alterations in viral replication or cytokines</td>
</tr>
<tr>
<td>Little use of standardized or objective measurements of RSV disease severity</td>
<td>Differing levels of disease severity may be influenced by polymorphisms in different genes; for example, within the mechanically ventilated population, genes controlling surfactant production itself may well be important, but this effect may not be seen if the population studied involves only those hospitalized (but not mechanically ventilated) compared to adult healthy controls</td>
</tr>
<tr>
<td>Failure to account for linkage disequilibrium; the studied polymorphism may simply be a marker for the presence of a variant of a different gene that is close in proximity or is in linkage disequilibrium</td>
<td>Little attempt to differentiate between primary vs secondary episodes of RSV infection</td>
</tr>
<tr>
<td>Frequent use of comparator groups (controls) that may not have been exposed to RSV in the neonatal period; most studies select controls as never having had an episode of respiratory hospitalization, but more rigorous attempts to control for differences in RSV exposure should be incorporated into study design; alternatively, study designs could be employed to compare two groups of known RSV-infected children, each having different documented degrees of RSV severity</td>
<td>No attempt to control for seasonality of birth (a method for age adjusting the RSV exposure); infants who are &lt;3–6 mo of age during an RSV epidemic are more likely to have severe disease than are those infants who are born at times placing them outside this age susceptibility window</td>
</tr>
<tr>
<td>Little attempt to control for other clinical factors well known to be associated with RSV disease severity (presence of other children in home, tobacco exposure, gestational age)</td>
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...morphism in ICAM-1 has been associated with rheumatoid arthritis, inflammatory bowel disease, and asthma. No association of severe RSV disease with any of the polymorphisms investigated was found (72). However, these polymorphisms were rarely identified in the population studied (both cases and controls), thus raising the possibility that a true genetic association was not detected simply because of a lack of statistical power.

**Genetic effectors of adaptive immunity.** Although it is clear that children with the classic primary immunodeficiencies are at risk of prolonged and/or severe infection with RSV (54), few studies have investigated the impact of more subtle genetic polymorphisms affecting the adaptive immune response to RSV infection. The immunoglobulin heavy G2 chain (IGHG2) gene variation in infants from Finland hospitalized with RSV lower respiratory tract disease has been investigated and compared to a characterized population of healthy adult controls from Sweden. The Gm allotype was investigated by enzyme-linked immunosorbent assay, which revealed a predominance of the IGHG2(−n) allele and IGHG2(−n/n) genotype (7). This genotype was previously shown to correlate with low antibody responses to bacterial agents, immunodeficiencies, and the nonatopic phenotype of childhood asthma. The question of the comparability of the genetic backgrounds of the two populations evaluated in that study opens the results to some skepticism. Infants’ antibody responses to a natural RSV infection are generally poorer than those of adults, and even adults have muted antibody responses to RSV compared to responses to most other pathogens. Typical antibody titer rises of fourfold are not seen after a single RSV infection in infants and even in healthy adults. However, it is very clear that passive RSV antibody, derived from a small percentage of adults who do have high neutralizing RSV titers, significantly protects infants from RSV disease (48). This genetic association is therefore intriguing but needs further evaluation, especially at a functional level.

Cytotoxic T lymphocytes recognize virus-infected cells and eliminate pathogens but may contribute to immunopathology through bystander killing effects (6). The HLA class I antigens that mediate this process were investigated using 26 Australian infants who suffered RSV bronchiolitis and required mechanical ventilation (65). No difference in the incidences of any HLA type I allele was seen among the cases versus two reference populations, both local and international. The small sample size in this study certainly raises the question of a possible medically significant association remaining undetected due to low statistical power. Future studies are warranted in this important area to examine this possibility.

**Potential Problems**

Despite the relatively short list of genetic polymorphisms studied, few consistent results have been reported, illustrating the complexity and difficulty of performing genetic association studies. This lack of reproducibility has many potential causes, including those related to study design, sample size, publication bias, as well as true variability. Table 2 lists some potential problems with RSV immunogenetic studies. Arguably, the most pernicious problem associated with these studies is that of multiple-hypothesis testing. Evaluation of multiple RSV disease endpoints and their possible associations with several polymorphisms or even combinations of polymorphisms quickly leads to the testing of multiple hypotheses. As indicated by our multifaceted concepts of RSV pathogenesis and also well-illustrated by demonstrable differences in risks according to age groups, severe RSV infections likely represent heterogeneous pathogenic consequences influenced by many genes. Immunogenetic studies that define disease by measurable parameters directly associated with disease processes, such as early viral load, viral clearance, or actual cytokine measurements, may better capture and define the pathogenic link between genotype and phenotype. On the other hand, true variability among study results can be caused by the well-recognized differences in the frequencies of specific polymorphisms in the different populations studied, making comparisons of results derived from different genetic pools difficult (23). This is especially true in diseases affected by multiple genes. Several studies have dealt with this problem by using a family-based association study design called the transmission disequilibrium test. The
transmission disequilibrium test measures the overtransmission of an allele from heterozygous parents to their diseased offspring and is not affected by population structure. Unfortunately, other problems including that of multiple-hypothesis testing inherent in the candidate gene approach remain. Other factors causing variability of results of different studies include environmental interactions, interactions among genes, and the choice of statistical models used for analysis.

**FUTURE POSSIBILITIES**

**Meta-Analysis of Genetic Association Studies**

Genetic association studies can be considered to be more similar to randomized trials than other types of observational epidemiological studies because of Mendelian randomization (Mendel’s law of independent assortment), which states that the inheritance pattern of one trait does not affect the inheritance pattern of another. The rationale and methodology for synthesizing randomized trials are highly relevant to the meta-analysis of genetic association studies. This topic has recently been reviewed and will not be discussed in detail here (81, 114). Complete and consistent study reporting and making data readily available are a prerequisite for sound meta-analysis. A common access point for information sharing among the different groups performing these RSV studies should be considered to facilitate such meta-analyses.

**Human Genome-Wide Association Studies**

With the accumulating collection of DNA samples obtained from a population with a well-defined group of RSV diseases, genome-wide association studies may become possible. Genome-wide association studies are a powerful method for identifying susceptibility genes for common diseases and have become quite feasible with the commercially available chips that capture information from two-thirds of the common variations in the human genome (19). The statistical association of thousands of SNP markers with a disease phenotype allows the identification of disease-defining regions or loci from the entire human genome. Such studies take advantage of the numerous meiotic recombinations that have occurred in the human population, which enable high-precision mapping. Genome-wide association studies are completely unbiased with respect to the investigator’s preconceived concept of pathogenesis and therefore can potentially identify totally novel susceptibility genes as well as multiple disease genes with modest effects. Such is the type of risk association that one expects for common disorders such as RSV. A few examples of the successful application of genome-wide disease association studies include type 1 and type 2 diabetes and Crohn’s disease (37, 53, 99, 136a).

**Single Versus Multiple Gene Defects**

RSV disease susceptibility is likely to be determined by a combination of multiple common genetic traits, each with mild to moderate effects predisposing to a more severe form of disease. These putative polymorphisms may have a major impact on the population as a whole but a relatively minor effect at the individual level. The extreme ends of the clinical spectrum of RSV disease severity may be resistance traits or single-gene defects that confer profound susceptibility to RSV. Recently, a number of single-gene defects in innate immunity that confer a propensity for fulminant disease by a limited number of common pathogens have been identified. These include IRAK4 deficiency, conferring susceptibility to pneumococcus, and defects in the IFN-γ–IL-12 axis, causing susceptibility to severe and fatal mycobacterial diseases (101). Due to the fact that nearly all individuals become RSV infected within the first 2 years of life, individuals who develop the most severe forms of RSV but are otherwise healthy may serve as a pool to identify such single-gene defects of immunity. Although there is a need for a general functional screening method to direct the genetic analyses for these individuals, data from recent publications (133) suggest that we may be able to screen individuals with defects in that particular pathway. Genetic screening for RSV susceptibility may be especially important given the availability but high cost of effective passive-antibody prophylaxis regimens.

**Animal Models**

Because the pathogenesis of RSV infections in animals, i.e., mouse models, appears to differ significantly from that in humans in a number of aspects, the identification of a novel class of host genes in animals that confers resistance against RSV in humans is a significant challenge. Additional challenges and questions of translatability would need to be overcome when attempting to establish the biological plausibility of such identified genes. Despite this fact, much of the biological confirmation of the aforementioned effectors was derived from murine models and suggests some similarities to human RSV disease. In this regard, forward genetics of mouse strain variability is a potential approach to identify novel susceptibility genes. A recent example of this approach identified the UNC93B1 gene, mediating TLR7, -8, and -9 signaling, conferring susceptibility to herpesviruses in mice with clinical correlates in humans (17, 121). Investigation of RSV susceptibility in inbred mice has identified resistant (C57BL/6) and susceptible (AKR/J) strains. The distribution of RSV load in backcross progeny suggested that susceptibility was influenced by more than one gene (120). These RSV susceptibility traits are amenable to resolution by genomic analyses such as mouse studies of forward genetics.

However, since infection with human RSV fails to produce severe disease in small-animal models, genetic markers of disease severity in these models may be difficult to delineate. Alternative animal models include natural or experimental infections of bovine RSV in calves, which produces a disease very similar to that seen in infants (124). Alternative small-animal models would include rodent models that use Sendai virus or pneumonitis virus, the latter virus having significant similarity with the RSV virus and yet causing severe bronchiolitis in mice (110). Significant variabilities in susceptibilities to these two viruses among different inbred mouse strains have been reported, which could be subjected to a forward genetic analysis (4, 34).
CONCLUSION
The wide range of disease severity caused by RSV coupled with its relative paucity of recognized viral differences makes it an attractive target for host immunogenetic studies. Furthermore, defining the entire set of putative genes associated with RSV susceptibility has the potential for improving our knowledge of disease pathogenesis, identifying potential therapeutic targets, and even allowing the practical genetic identification of high-risk populations, which could be candidates for existing effective RSV prophylactic regimens (3, 63a).

Future research using more powerful and consistently reproducible techniques including genome-wide approaches will result in separate populations will help solidify the findings. Confirming the multiple-candidate gene approach results in separate populations will help solidify the findings. Future research using more powerful and consistently reproducible techniques including genome-wide approaches will allow us to not to cause suffering but rather to understand and ultimately to relieve it.

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