

Genetic susceptibility to otitis media

Margaretha L. Casselbrant^{a,b} and Ellen M. Mandel^{a,b,c}

Purpose of review

Otitis media is a disease that is prevalent in the pediatric population, and recent twin and triplet studies have confirmed that there is a strong genetic component to susceptibility. Here, we summarize the status of current efforts to identify the specific genes underlying otitis media susceptibility and presentation.

Recent findings

Recent studies have focused on identifying candidate genes. For example, IFN- γ polymorphisms, allotypes of the leukocyte IgG (Fc γ R) receptors and certain haplotypes of surfactant genes were linked in pilot studies to otitis media susceptibility. The pattern of gene expression during an episode of otitis media is also being elucidated with the overall goal of providing clues as to which of these modulated genes are polymorphic and thus potentially capable of affecting otitis media susceptibility. Mucin and cathepsin protease genes were shown to exhibit these characteristics.

Summary

In addition to the simple searches for linkages between known genes and otitis media, work is progressing within the context of genome-wide linkage studies. These efforts promise to answer some of the many questions remaining in otitis media susceptibility and pathogenesis.

Keywords

cytokines, genes, otitis media, twin

Introduction

There is incontrovertible evidence from epidemiological, anatomical, physiological and immunological studies that susceptibility to recurrent acute otitis media (AOM) and persistent otitis media with effusion (OME) is genetically determined. The strongest evidence for a genetic predisposition to otitis media has been derived from twin and triplet studies [1,2], a powerful tool for dissecting the genetic contribution to a disease by controlling for the effects of environmental factors. When compared with dizygotic twins, a higher concordance rate in monozygotic twins, those with identical genotypes, evidences a significant genetic component. The estimated otitis media heritability in the retrospective study by Kvaerner *et al.* [1] was 0.74 in females and 0.45 in males and in the prospective study of children followed from birth to 2 years by Casselbrant *et al.* [2] was 0.79 in females and 0.64 in males. Of the original 140 sets for which zygosity was obtained, 114 were followed to the age of 3 years and 83 to the age of 5 years. The inter-twin correlation for the proportion of time with middle-ear effusion (MEE) was significantly higher in monozygotic sets (0.65–0.77) than in dizygotic sets (0.31–0.39) for each year to the age of 3 years and then decreased, a result explicable by the lesser incidence of otitis media in the older children (see Fig. 1). The discordance estimates for the occurrence of three episodes of MEE in monozygotic and dizygotic sets followed to the age of 5 years were 0.02 and 0.40, respectively ($P = 0.07$). The heritability estimate for the proportion of time with MEE in the first 5 years of life was 0.72 ($P < 0.001$). The corresponding estimates for males and females separately were 0.66 and 0.75, respectively. The 5-year results from the study continue to support a strong genetic component to otitis media [3^{*}].

There are single gene disorders/syndromic conditions such as Crouzon's disease [4] in which patients are predisposed to otitis media, and recently, Hardisty *et al.* [5^{*}] reported a single otitis media gene in a deaf mouse mutant, *Jeff*, with chronic proliferative otitis media. The mutation mapped to the distal region of mouse chromosome 17, and in contrast to other inbred mouse strains with otitis media is fully penetrant (all mice are affected). However, for humans, the majority of Mendelian conditions expressing an increased risk of otitis media is characterized by generalized immunodeficiency or is associated with major craniofacial anomalies. Because otitis media is a multifactorial disease with contributions from immunocompetence, inflammatory

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^aENT Research Center, Children's Hospital of Pittsburgh, Pennsylvania, USA; and the Departments of ^bOtolaryngology and ^cPediatrics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Correspondence to Margaretha L. Casselbrant, MD, PhD, Department of Pediatric Otolaryngology, Children's Hospital of Pittsburgh, 3705 Fifth Avenue at DeSoto Street, Pittsburgh, PA 15213, USA
Tel: +1 412 692 6213; fax: +1 412 692 6074;
e-mail: margaretha.casselbrant@chp.edu

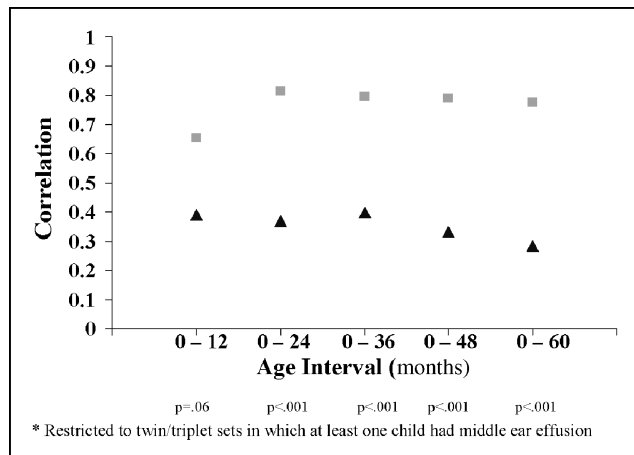
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Abbreviations

AOM	acute otitis media
MEE	middle-ear effusion
OME	otitis media with effusion
RSV	respiratory syncytial virus
RT-PCR	reverse transcriptase-polymerase chain reaction

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Figure 1. Correlations of the cumulative proportion of time with middle-ear effusion during selected month intervals for children within a twin/triplet set in which at least one child had middle-ear effusion, by zygosity



■ Monozygotic; ▲ dizygotic. Adapted from Casselbrant *et al.* [3*].

regulation and Eustachian tube functioning, the underlying genetic determinants are likely to be complex, involving at least several loci with both known and unknown functions. The identification of otitis media susceptibility genes will allow for the development of molecular assays that can be used by physicians to assign relative otitis media risk for a given child and to modify follow-up and treatments accordingly. Moreover, such knowledge will lay the foundation for a better understanding of otitis media pathogenesis, which can be translated into better treatments to prevent or resolve the disease.

Single gene candidates

It is well known that viral upper respiratory infection caused by respiratory syncytial virus (RSV), influenza, parainfluenza, adenovirus and rhinovirus, among others predisposes to otitis media [6]. Suggested mechanisms include in-situ viral infection of the middle-ear mucosa, hydrops *ex vacuo* secondary to virus-induced Eustachian tube inflammation or in-situ bacterial infection of the middle ear secondary to virus-induced immune suppression. Of the viruses associated with otitis media, RSV has been studied extensively. RSV infection is extremely common in infants and young children, and in most children the disease course is self-limiting. However, otitis media is a common complication of RSV infection [7], and RSV virus, genomes or proteins can be detected in effusions recovered from the middle ears of those patients at variable frequencies [7,8]. RSV can also be found in lesser frequency in the middle ears of children with AOM [9] and OME [10] but without respiratory RSV infection. Gentile *et al.* [11*] recently studied polymorphisms for TNF- α , IFN- γ , IL-6, IL-10 and transforming growth factor beta production in 77 infants hospitalized with RSV infection. They found that the genotype for

low IFN- γ production (vis-à-vis high IFN- γ production) was associated with a predisposition to otitis media, and concluded that the IFN- γ polymorphism may represent one member of a family of genes that contributes to the measured heritability of otitis media.

A gene polymorphism linked to otitis media caused by bacterial infection is that coding for the leukocyte IgG (Fc γ /R) receptor that plays a role in IgG-facilitated phagocytosis of bacteria [12*]. An immunocompetent response to *Streptococcus pneumoniae* requires that antibodies, primarily of the IgG2 subclass, bind to capsular polysaccharides on the bacteria and also to Fc receptors on lymphoid cells. Several polymorphisms in Fc γ R are known. For Fc γ RIIa, the only variant capable of binding IgG2 antibodies, binding depends on the presence of histidine at position 131; the substitution of arginine at that position prevents binding. Also, Fc γ RIIIb, which can bind IgG1 and IgG3, displays the neutrophil antigen polymorphisms NA1 and NA2. The frequencies of the Fc γ RIIa-H131 homozygous allotype and the Fc γ RIIIb-NA1 allotype were less in children with four or more episodes of AOM compared with children with fewer AOM episodes suggesting that these polymorphisms may be risk factors for recurrent, pneumococcal otitis media [13].

The surfactants are mucosally secreted mixtures of four surfactant proteins, SP-A, SP-B, SP-C and SP-D, each with distinct properties and functions [14]. SP-A increases phagocytosis of *S. pneumoniae*, *Haemophilus influenzae*, RSV and other pathogens. The SP-A protein is fully functional only with the participation of two genes, SP-A1 and SP-A2, both located on chromosome 10q22-q23. Using a candidate gene approach, R  met *et al.* [15] reported an over-representation of the 6A⁴-1A⁵ haplotype in children with recurrent otitis media and in children diagnosed with their first episode of AOM before 6 months; there was also an underrepresentation of the 6A²-1A⁰ haplotype in the latter subgroup. Also, two studies reported a possible association between surfactant gene polymorphisms and severe RSV infection in infants [16,17]. In the first, genotype analysis of DNA from 86 children with RSV bronchiolitis requiring hospital admission and 95 healthy controls showed a significant difference in the allele distribution of the SP-A2 gene ($P = 0.006$) and a trend towards a significant difference in allele distribution of the SP-A1 gene ($P = 0.071$) [16]. In the second study [17], DNA from 84 infants requiring hospital admission for the treatment of bronchiolitis caused by RSV and 93 healthy controls was genotyped for three biallelic SP-D polymorphisms. The frequency of the allele coding for Met 11 ($P = 0.033$) was greater in the severe RSV group. Given that RSV infection is a known precipitant of otitis media, the results of the studies suggest that the SP genotype may be a risk factor for a limited number of otitis media episodes.

In a recent clinical report, Manolidis *et al.* [18] described the presence of chronic otitis media in only one case in a population of 2362 surgically confirmed cases of otosclerosis. Albeit a clinical association, the authors hypothesized that genes associated with otosclerosis may protect against bacterial ear infections.

Genome-wide studies to identify gene–environment interactions

We are currently aware of two large-scale studies using global genetic linkage analysis to identify candidate genes/gene markers associated with otitis media susceptibility. Daly *et al.* [19] are recruiting families with 150 affected sibling pairs for their study. Here, in the ENT Research Center at the Children's Hospital of Pittsburgh, we are collecting DNA samples from 500 sibling pairs with a history of tympanostomy tube insertion, their parent(s) and unaffected siblings. Both studies are currently ongoing and no results have been presented or published.

Genes identified by expression analysis

The disease course of an otitis media episode is orchestrated by the up and downregulation of a large number of genes to yield a coordinated, feedback modulated immunoinflammatory response consisting of the recognition of the local presence of a pathogen, followed by the initiation of specific and non-specific proinflammatory pathways to neutralize the pathogen, and finally the initiation of anti-inflammatory pathways that promote healing. Many of the signaling molecules used in controlling this process are known to have polymorphic variants (e.g. cytokines, see above), and it is reasonable to suggest that the disease presentation may be affected by the individual's genotypes or haplotypes for those variants. Recent work has begun to clarify the patterns of gene activation for various stimuli, and a summary of this work is presented here.

Middle-ear mucosal inflammation is associated with the synthesis and/or release of a wide variety of inflammatory mediators, which, in turn, cause the upregulation of mucin genes and the production/secretion of mucin-containing fluid. Mucin is a glucoprotein whose specific rheological properties allow for functional coupling to the mucociliary transport system and consequently the clearance of debris and detritus from the middle ear cavity. Takeuchi *et al.* [20] studied mucin gene expression in MEEs obtained from five patients with OME using reverse transcriptase–polymerase chain reaction (RT–PCR). A wide variety of mucin genes, including *MUC1*, *MUC4*, *MUC5AC*, *MUC6*, *MUC7*, *MUC8*, *MUC9*, *MUC11* and *MUC12*, was expressed to various degrees. Interestingly, *MUC5AC* was only expressed in the two mucopurulent samples but not in the three serous samples. These results suggest that the upregulation of certain mucin genes may determine the rheological characteristics of the effusion and the effectiveness of mucociliary

clearance. In a second study [21], chinchilla middle-ear epithelial cell cultures were incubated with and without exposure to IL-1 β and TNF- α , and were assayed by RT–PCR for *MUC2*. Compared with controls, these two proinflammatory cytokines caused a differential upregulation and expression of *MUC2*.

In a broad survey using complementary DNA microarrays in combination with RT–PCR, middle-ear mucosal gene expression was assayed 6 weeks after infectious challenge with *S. pneumoniae* in rats [22]. The results showed greater than baseline levels in a number of genes, including those coding for mitogenic signaling proteins, helix–loop–helix transcriptional regulators, ion channels and mucin glycoproteins (*MUC2* and *MUC5*). The upregulation of these genes may be responsible for the development of thick effusion and mucous cell hyperplasia/metaplasia after an AOM episode, especially when complicated by other factors such as Eustachian tube dysfunction.

A second survey using similar techniques was performed on mucosal tissue recovered at 24 and 48 h after *S. pneumoniae* infection in Sprague–Dawley rats [23]. Comparing infected with control middle-ear mucosa, a large number of genes were identified whose expression was changed, including those whose products included cytokines, ion channels, and the cathepsin proteases [cathepsin B (*Ctsb*), L (*Ctsl*) and K (*Ctsk*)]. Of interest to inflammatory processes, the cathepsins are involved in the intracellular digestion of macromolecules, proenzyme activation, enzyme inactivation, antigen presentation, hormone maturation, tissue remodeling and bone matrix resorption. In that study, *Ctsk* and *Ctsl* messenger RNA, both present in the normal middle ear mucosa, was downregulated whereas *Ctsb* mRNA was upregulated. The investigators hypothesized that early intervention with *Ctsb* inhibitors may decrease the production of proinflammatory cytokines and abort disease progression to OME.

Of importance to this review is the possibility that functional polymorphisms in these various genes may lead to differing levels of control and regulation over the inflammatory process. The inadequate upregulation of the protective mechanisms in response to an identified pathogen or the downregulation of the inflammation after neutralization of the pathogen may be responsible for the different patterns of otitis media expression. Further work on these pathways may serve to explain the role of candidate genes for otitis media susceptibility identified by the methods described above.

Conclusion

With the publication of the results for the two large-scale twin studies documenting a high heritability for otitis

media, there has been a renewed interest in identifying the contributing gene variants. It is doubtful that polymorphic single genes will account for a large amount of the heritable component, but, as demonstrated by the above reviewed studies, single genes may contribute to disease susceptibility under specified conditions (e.g. RSV infection) or for defined 'high-risk' subgroups (e.g. FcγRIIa). Two large-scale, genome-wide gene linkage studies are currently in progress and hold the promise of identifying a large number of candidate susceptibility genes. A mechanistic interpretation as to how those candidate genes may affect disease susceptibility or presentation can be gained from ongoing parallel studies focused on the role of gene expression in coordinating and orchestrating middle-ear mucosal inflammation. Through this improved understanding of the gene–environmental interaction, medical science can identify new targets for possible intervention that will lead to better treatments and preventive methods.

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