

## Panel 3: Genetics and Precision Medicine of Otitis Media

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### Abstract

**Objective.** The objective is to perform a comprehensive review of the literature up to 2015 on the genetics and precision medicine relevant to otitis media.

**Data Sources.** PubMed database of the National Library of Medicine.

**Review Methods.** Two subpanels were formed comprising experts in the genetics and precision medicine of otitis media. Each of the panels reviewed the literature in their respective fields and wrote draft reviews. The reviews were shared with all panel members, and a merged draft was created. The entire panel met at the 18th International Symposium on Recent Advances in Otitis Media in June 2015 and discussed the review and refined the content. A final draft was made, circulated, and approved by the panel members.

**Conclusion.** Many genes relevant to otitis media have been identified in the last 4 years in advancing our knowledge regarding the predisposition of the middle ear mucosa to commensals and pathogens. Advances include mutant animal models and clinical studies. Many signaling pathways are involved in the predisposition of otitis media.

**Implications for Practice.** New knowledge on the genetic background relevant to otitis media forms a basis of novel potential interventions, including potential new ways to treat otitis media.

### Keywords

otitis media, genetics, precision medicine

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**G**enetics is critical in otitis media (OM) pathogenesis, an infectious disorder involving inflammation of the middle ear. Family and twin studies have proven that

the heritability of OM is high. Multiple factors and many genes are involved in the predisposition of OM. The genetic risk factors include the transforming growth factor beta (TGF $\beta$ ) signaling pathway, mucin genes, vascular endothelial growth factor, Toll-like receptors (TLRs), and ERK signaling pathways. These pathways overlap and affect innate/adaptive immunity and inflammatory responses of the middle ear mucosa or normal cellular functions (angiogenesis, hypoxia, lymphangiogenesis, mucociliary transport system, mucin production, and mucous cell metaplasia). Increased understanding of these genes and pathways has led to novel therapeutic strategies for OM. Precision medicine (PM)—the use of specific patient genotypic and phenotypic characteristics to inform individualized therapeutic decisions—has also emerged as a consideration for OM management. Tools in PM include molecular diagnostics by panomic analysis and systems biology to understand the specific causes underpinning a patient's OM pathogenesis.

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## Method and Search Strategy

Published literature in PubMed was searched since the last symposium. Search terms included OM, recurrent acute OM, chronic OM, chronic OM effusion, acute OM, heritability, genetic, SNP, genes, genetic polymorphism, GWAS, linkage study, genetics, signaling pathways, mucins, transforming growth factor beta, Toll-like receptor, mucosal metaplasia, chinchilla genome, and animal model. The search included all published literature with available English abstracts. No further exclusion or selection criteria were employed.

## Discussion

### Recent Advances in the Genetics of OM

Chronic infectious diseases, including OM, have been linked to specific genetic profiles. For example, TLR2-deficient mice develop purulent OM because of abundant infiltration of neutrophils related to the absence of goblet cell metaplasia/hyperplasia in the middle ear mucosa.<sup>1</sup> The genetic background interacts with other host and environmental factors to affect the phenotype of OM. Several animal model studies, as well as human studies, have solidified the link between OM phenotypes and genetics.<sup>2</sup>

The branch of PM that addresses OM is referred to as “precision OM,” based on OM properties and genetic backgrounds, such as chronic OM, secretory OM, serous OM, mucous OM, and silent OM. A genetic predisposition or susceptibility to OM is a genetic characteristic feature that influences the possible phenotypic development. Genetic testing and analysis can identify individuals who are genetically predisposed or susceptible to certain OM phenotypes.

### Precision Medicine in OM

Understanding of disease susceptibility factors (intrinsic and extrinsic), the characteristics of the pathogens, and how these potentially affect a patient provides a basis for precision medical care for OM.<sup>3</sup> Recent literature has highlighted the importance of distinguishing patterns of illness in acute OM to enable appropriate precision and individualized care.<sup>4</sup> Currently, these concepts apply primarily to clinical presentations, such as bilateral acute disease, to inform the use of antibiotics or surgical intervention.<sup>5</sup> However, the ability to use patient-specific genetic information to similarly influence treatment decisions has been much studied and published since the last symposium.

In OM, PM offers the potential to tailor a medical treatment to a subgroup of individuals who have similar genetic backgrounds, susceptibilities to pathogens, and responses to external stimuli. Preventive or therapeutic interventions can then be concentrated on those who will benefit, sparing expense and potential complications to those who will not.

### Genetic Aspect of Inflammation

Inflammation is a hallmark of OM. Appropriate inflammation is essential for eradicating pathogens. However, excessive inflammation is clearly detrimental to the host.

Uncontrolled inflammation in the middle ear significantly contributes to the development and progression of OM. Thus, inflammation must be tightly regulated. However, little is known about how inflammation is regulated in the pathogenesis of OM.

A number of studies were carried out to investigate the role of proinflammatory mediators in OM, with in vitro and in vivo approaches. Zhang et al<sup>6</sup> found that blockade of macrophage migration inhibitory factor suppresses inflammation and improves the hearing level in an acute OM model. From a whole genome gene array analysis, Suzukawa et al<sup>7</sup> identified that heparin binding–epidermal growth factor is a key mediator of inflammation-induced mucosal proliferation in a mouse model of OM. Kurabi et al<sup>8</sup> found that mice lacking ASC, the inflammasome adaptor protein, exhibited impairment of IL-1 $\beta$  maturation, reduction in neutrophil recruitment, and bacteria clearance of nontypable *Haemophilus influenzae* (NTHi) in middle ear. Preciado et al<sup>9</sup> found that NTHi challenge in the mouse middle ear led to chronic epithelial mucosal metaplasia and that Cxcl2 was a key proinflammatory mediator.

Investigating the negative regulators of inflammation over the past years also brought novel insights into the tight regulation of inflammatory responses. Woo et al<sup>10</sup> found that IL-10/HMOX1 signaling played a critical role in protecting the cochlea from inflammation-mediated tissue damage through inhibition of MCP-1/CCL2 regulation. Wang et al<sup>11</sup> found that deubiquitinase cylindromatosis (CYLD) suppressed NTHi-induced expression of key proinflammatory chemokine IL-8 via MAP kinase phosphatase 1 (MKP-1)–dependent inhibition of ERK. In addition, Komatsu et al<sup>12</sup> identified phosphodiesterase 4B (PDE4B) as a key negative regulator for CYLD, and inhibition of PDE4B markedly suppressed bacteria-induced inflammation via upregulating CYLD expression by siRNA technology and Cyld-deficient mice. Taken together, all of these studies may lead to the development of new anti-inflammatory therapeutics by targeting key regulators of inflammation in OM.

### Genes Relevant to Human OM

Genetic studies are one way to research the pathophysiology behind OM and eventually find new strategies for the therapy and prevention of OM. There are several strategies to conduct human genetic studies.

### Heritability Studies

Heritability is the variance of phenotypic variation due to genotype and has been estimated on twin studies for OM. Hafrén et al estimated heritability from an OM-prone cohort of 590 families, consisting of 2436 subjects. The heritability estimates were 38.5% for recurrent acute OM, 22.1% for OM with effusion, and 47.8% for all OM.<sup>13,14</sup> These results were somewhat lower than heritability estimates from twin studies.

### Linkage Studies

The purpose of linkage studies is to find genomic loci associated to a phenotype. One or several families are genotyped, and with information about the affected status of the

subject and genotype, the loci are calculated. Chen et al did a follow-up linkage study, fine-mapping chromosome 19 on their cohort of 139 families consisting of 607 study subjects. They replicated their previous finding of linkage to loci 19q (LOD<sub>BEST</sub> = 3.75).<sup>15</sup> Rye et al did a linkage study on a cohort of 468 subjects from 101 families. They found significant evidence of linkage to 10q26.3 ( $Z_{lr} = 2.69$ ,  $P = .0036$ ) and borderline linkage to 10q22.3 ( $Z_{lr} = 1.67$ ,  $P = .05$ ). They further fine mapped the region in a cohort of 256 affected cases and 575 controls with imputation data from a previous genome-wide association study (GWAS) that revealed 4 candidate genes: *TCERG1L*, *PP2R2D*, *DOCK1*, and *ADAM12*. Expression analysis and in silico analysis concluded that *PPP2R2D*, part of the TGF $\beta$  pathway, is the most likely candidate gene underlying the linkage result.<sup>16</sup>

**Candidate Gene Association Studies.** In candidate gene studies, the frequencies of a genetic marker is compared between affected study subjects and control subjects. The control subjects can be unrelated healthy controls (case-control study) or healthy siblings or other family members (family study). Sale et al conducted a candidate gene study on 15 genes with separate polymorphisms in 142 families with 618 subjects. Nominal genetic association was found for *MUC5AC* (rs2735733,  $P = .002$ , odds ratio [OR] = 0.646; rs7396030,  $P = .049$ , OR = 1.565; rs2075859,  $P = .041$ , OR = 0.744). Three other single-nucleotide polymorphisms (SNPs) at 3 separate genes showed a trend toward association: *SCN1B* (rs810008,  $P = .013$ ), *SFTPD* (rs1051246,  $P = .039$ ), and *TLR4* (rs2770146,  $P = .038$ ).<sup>17</sup> Rye et al studied the candidate gene *SLC11A1* in 660 affected children from 531 families in a case/pseudo-control study. The best SNP association was in the genetic model at the rs2776631 ( $P = .025$ ). Haplotype analysis revealed the 3\_C\_C\_G haplotype (rs34448891\_rs2276631\_rs3731865\_rs2695343) to be more common in the affected study subjects ( $P = .0008$ ). Functional studies supported *SLC11A1* as a novel candidate gene for OM.<sup>18</sup> A candidate gene study of 192 SNPs from 8 genes was conducted by MacArthur et al on 100 cases and 79 controls. Eight SNPs on 4 genes (*TLR4*, *MUC5B*, *SMAD2*, *SMAD4*) showed a trend toward association ( $P_{BEST\_ADJUSTED} = .625$ , rs10116253 on *TLR4*).<sup>19</sup>

Nokso-Koivisto et al studied 21 SNPs as risk factors for upper respiratory tract infections, OM, and OM proneness in 653 children. An increased risk for OM proneness was associated with the *CX3CR1* gene (Thr280Met, OR = 6.23,  $P = .038$ ). Also white race combined with *IL-1 $\beta$*  (-511) wild type and *IL-10* (-1082) non-wild type were associated with OM proneness.<sup>20</sup> A Greek cohort of 96 children was studied for 8 SNPs of 5 cytokine genes. *IL-10* (-592, -819, and -1082) and TGF $\beta$  (codon 10C>T) were associated with an increased number of acute OM episodes ( $P < .001$ ,  $P < .001$ ,  $P < .001$ , and  $P = .002$ , respectively). *IL-10* (-1082) and TGF $\beta$  (codon 10C>T) were also associated to a later onset of AOM ( $P = .007$  and  $P = .0039$ , respectively).<sup>21</sup> Gessner et al showed in 1032 study subjects from Alaska that those who were homozygous for the arctic variant of *CPT1A* (c.1436C>T) demonstrated a trend to more likely

have OM (OR = 3.6, 95% confidence interval: 1.4-8.9).<sup>22</sup> Primary ciliary dyskinesia is a genetic disease inherited in an autosomal recessive manner. The prevalence of primary ciliary dyskinesia is estimated to be 1 in 20,000 live births. Congenital abnormality of the primary cilia results in situs inversus in 50% of patients. Decreased function of motile cilia causes chronic rhinosinusitis, OM with effusion, bronchiectasis, and infertility.<sup>23</sup> Hafrén et al did a candidate gene study with 53 SNPs on 624 study subjects with recurrent acute OM and/or chronic OM with effusion and 778 control subjects. The positive result for *TLR4* (rs5030717, OR = 1.33,  $P = .003$ ) was further investigated by a tagging SNP analysis, and 2 additional SNPs were identified (rs1329060 and rs1329057). There was an increased association among patients with a more severe phenotype: those with OM starting before the age of 6 months (OR = 2.42,  $P = .0005$ , for rs1329060) and those with repeated insertions of tympanostomy tubes (OR = 1.65,  $P = .00004$  for rs1329060). The result was replicated in a Finnish OM cohort of 205 children (rs1329060, OR = 1.32,  $P = .002$ ; rs1329057, OR = 1.30,  $P = .003$ ; rs5030717, OR = 1.34,  $P = .002$ ). In 3 other cohorts (2 in the United States and 1 in United Kingdom), the 3 SNPs failed to show association with the risk for OM.<sup>25</sup>

A *TLR4* variant (Asp299Gly, rs4986790) is related to the colonization of *Moraxella catarrhalis* in the upper respiratory tract of children (43%) in comparison with the *TLR4* wild type (9%) (RR = 4.91,  $P = .0001$ ).<sup>24,25</sup> The IL-10 rs1800896TC SNP and the IL-1 $\alpha$  rs6746923A and AG SNPs were significantly more and less common, respectively, among children without a history of tympanic membrane perforation than among those who suffered from this complication.<sup>26</sup>

**Genome-wide Association Studies.** Genome-wide association studies (GWASs) require no previous assumption on underlying genetic loci. They are used in the research of common, complex diseases. Hundreds of thousands to millions of SNPs are analyzed in cases and controls to find SNPs associated to the trait. Two GWASs have been published on OM.

The first was published by Australian colleagues Rye et al in 2012. They used the Illumina 660W-Quad to study 416 affected cases and 1075 controls. No marker showed genome-wide significance ( $P < 10^{-8}$ ), but there was a trend toward association in the genes *CAPN14* and *GALNT14* on chromosome 2p23.1 and *BPIFA3* and *BPIFA1* on chromosome 20q11.21. The top results did not replicate in an independent cohort of 793 affected study subjects from 645 families, nor did they replicate in a North American cohort of 596 subjects and 370 affected.<sup>27</sup>

The second GWAS study on OM was conducted on a North American cohort of 602 study subjects from 143 families, with 373 affected subjects. Allen et al used the Illumina Human CNV370-Duo. No SNP reached genome-wide significance, but they conducted a replication study on 53 SNPs in an independent cohort of 1584 study subjects

from 441 families. In an intergenic region on chromosome 2, the SNP rs10497394 replicated, thus giving a significant association to OM ( $P_{\text{META-ANALYSIS}} = 1.52 \times 10^{-8}$ ). Three other SNPs on chromosomes 15 and 5 showed a trend toward association.<sup>15</sup> Allen et al<sup>28</sup> noted that diagnostic approaches such as human linkage studies and GWASs may provide new insights to OM research for the development of novel therapeutic strategies.

**Sequencing.** Sequencing is becoming more affordable these days and is mainly used to find rare variants in rare diseases. In recent years, there has also been an increasing number of studies on complex diseases. In 2015 Santos-Cortez et al published the first sequencing study on OM.<sup>29</sup> They did exome sequencing on 2 second cousins in a Filipino isolate population where the prevalence of OM is almost 50%. They found a variant of *A2ML1* ( $\alpha$ -macroglobulin-like 1) as a new risk variant for OM. The result was replicated in 51 other subjects in the same isolate (LOD, 7.5). The variant is due to a duplication in the gene (p.Ser829Trpfs\*9). *A2ML1* is expressed in murine middle ear and is a rare, high genetic-risk variant for OM.

### Genes Involved in Hearing Loss and OM in Animal Models

The mouse model is a useful tool to study and identify the genes relevant to OM. The advance in genetic studies through mutant mice is summarized in **Table 1**.<sup>30-61</sup>

### Genes Whose Deficiency Results in OM

There are genes that are essential for the maintenance of the middle ear epithelial cell integrity and health. The deficiency of the following genes results in OM. (1) *Tgfr1*—it functions through several routes as a negative regulator of the TGF $\beta$  signaling pathway. *Tgfr1* mutant mice have significantly raised auditory thresholds due to a conductive deafness arising from OM.<sup>37</sup> (2) *Phex*—a mutation in the *Phex* gene is linked to predisposition to OM in mice. The mutation in the *Phex* gene primarily upregulates the expression level of the *FGF23* gene in the middle ears.<sup>39</sup>

There is a report where *FGF23* mutant mice showed mixed hearing loss and middle ear malformation.<sup>62</sup> (3) *Oxgr1*—the *Oxgr1* gene encodes oxoglutarate receptor 1. The ligand for *Oxgr1* was reported to be involved in regulation of vascular endothelial growth factor, an important inducer of angiogenesis and vascular permeability. *Oxgr1* deficiency demonstrated the presence of inflammatory cells, changes in the mucosal epithelium, and middle ear fluid in mice.<sup>38</sup> (4) *Mcp1*—the *Mcp1*-deficient mice had mild to moderate hearing impairment (around 70% penetrance). Other defects of *Mcp1*-deficient mice included small skull sizes, increased micronuclei in red blood cells, increased B cells, and ocular abnormalities.<sup>36</sup> (5) *Dfl*—the *Dfl* gene is cytogenetic risk factor for schizophrenia and can cause hearing difficulties as well as a variety of other medical problems.<sup>63</sup> (6) *Lmna*—mutant *Lmna* mice exhibited profound early-onset hearing deficits and abnormal positioning of the eustachian tube

accompanied by OM.<sup>40</sup> (7) *Spag6*—mammalian sperm-associated antigen 6 (*Spag6*) is required for normal flagella and cilia motility. The deficiency of this gene in humans causes OM due to accumulation of fluid and mucociliary abnormality.<sup>32</sup>

### Genes Associated with Virulence in OM

(1) *Hfq*—in the chinchilla model of OM, the *hfq* mutant of 86-028NP exhibited impaired competitive fitness when compared with its wild-type progenitor but exhibited no apparent defect in virulence to elicit OM.<sup>64</sup> (2) *HemR*—this gene is involved in the uptake of iron and iron-containing moieties, essential for *Haemophilus*. Variability of the *HemR* polymorphism may result in a reduced ability to acquire iron, rendering NTHi unable to survive in the middle ear.<sup>65</sup> (3) *Fur*—this gene is also essential for *Haemophilus* to grow in the middle ear.<sup>66</sup> (4) *VapBC-1 VapXD ToxAVapA*—deletions in *vapBC-1*, *vapXD*, and *ToxAVapA* significantly decreased the survival of NTHi in the chinchilla model of OM.<sup>67,68</sup>

### Signaling Pathways and Inflammatory Factors Involved in OM

(1) *Asc*—mice lacking the *Asc* gene showed a reduction in leukocyte recruitment and infiltration to the cavity, and their macrophages exhibited reduced phagocytosis of NTHi.<sup>8</sup> (2) *Pai-1*—plasminogen activator inhibitor (PAI-1) regulates inflammatory cell migration. *PAI-1* knockout mice showed significant pathologic changes of tympanosclerosis.<sup>30</sup> (3) *C5a*—mice deficient in the *C5a* gene showed the reduced levels of IL-6, mKC, and MCP-1, in association with reduced inflammatory cell recruitment, mucosal inflammation, and bacterial clearance.<sup>69</sup> (4) *Il-17a*—the function of interleukin 17A (IL-17A), a neutrophil-inducing factor. Mice lacking the *IL-17A* gene were associated with abnormal recruitment and apoptosis of neutrophils.<sup>70</sup>

### Mucin OM

*Cxcl2* is identified as the most significant increased inflammatory mediator during the early acute phase of infection.<sup>9</sup> *Math1*, a critical transcription factor, with proinflammatory cytokine tumor necrosis factor alpha (TNF $\alpha$ ) and retinoic acid, promoted differentiation of normal mouse middle ear epithelium into mucus-producing cells via upregulation of mucins and trefoil factors. This results in pathologic mucous cell metaplasia and increased susceptibility of chronic OM.<sup>34</sup> GalNAc residue essential for binding of bacteria to respiratory epithelium is localized in mucin-producing epithelium and submucosal glands of normal rat eustachian tube.<sup>71</sup> Combination of certain NTHi and *Streptococcus pneumoniae* strains, but not all, worked synergistically to upregulate the gel-forming mucin transcripts. Mucins play a protective role in the epithelium but with their dysregulation can lead to mucus stasis.<sup>72</sup> Acrolein, a hazardous air pollutant in tobacco smoke, promoted inflammatory responses, mucin gene expression, and cell death in human middle ear epithelium culture.<sup>73</sup> Other air-suspended urban particles, both artificial and naturally occurring, induced COX-2 and MUC5AC expression in

**Table 1.** Important Genes Involved in OM of Animals.

Gene	Variant and Strain Background	Finding
<i>PAI-1</i>	Type I plasminogen activator inhibitor (PAI-1) knockout PAI-1 KO mice (B6.129S2-Serpine1 <sup>tm1Mlg</sup> )	Low levels of inflammation against NTHi at the early stage of OM and fail to terminate inflammation. <sup>30</sup>
<i>JNK1</i>	JNK1 knockout, C57BL/6	Exhibit enhanced mucosal thickening, with delayed recovery, enhanced neutrophil recruitment early in OM, and delayed bacterial clearance. <sup>31</sup>
<i>JNK2</i>	JNK2 knockout C57BL/6	Delayed mucosal hyperplasia, delayed recruitment of neutrophils, and failure of bacterial clearance. <sup>31</sup>
<i>SPAG6</i>	Sperm-associated antigen 6 (Spag6)-deficient mice	Associated with disordered cilia orientation, leading to uncoordinated cilia beating. <sup>32</sup>
<i>HB-EGF</i>	C57BL/6:CB F1 hybrid mice	Inducing mucosal epithelial hyperplasia in vitro. <sup>7</sup>
<i>TBX21</i>	Tbx21-deficient mice (C.129S-Tbx21tm1Glm/J)	TBX21 regulating innate immune responses, via TLR2 during pneumococcal infections. <sup>33</sup>
<i>Math1</i>	Mouse middle ear epithelial cells	Plays a critical role in the pathogenesis of OM by induction of mucous cell differentiation, in the presence of TNF $\alpha$ and retinoid acid. <sup>34</sup>
<i>Pax9</i>	Pax9 mutant homozygosity for Slc25a21 <sup>tm1a(KOMP)Wtsi</sup>	OM and hearing impairment in mice may be due to disrupted Pax9 expression. <sup>35</sup>
<i>McpH1</i>	A knockout-first allele in which a promoter-less cassette including LacZ and neo genes were inserted in intron 3-4 of the McpH1 gene, McpH1-deficient (McpH1 <sup>tm1a(EUCOMM)Wtsi</sup> )	Underlying genetic predisposition to OM. <sup>36</sup>
<i>TGF<math>\beta</math></i>	TGF $\beta$ knockout, Tgfi1 homozygous mutant mice on a C57BL/6J background	A role of TGF $\beta$ signaling and its impact on responses to hypoxia in the inflamed middle ear. <sup>37</sup>
<i>Oxgr1</i>	Mutant (Oxgr1 <sup>-/-</sup> )	A trend of increase in Muc5B and Muc19 expression in the middle ear. <sup>38</sup>
<i>Phex</i>	A spontaneous intragenic deletion involving at least 30 kb containing Phex exons 13 and 14, BALB/c-Phex <sup>Hyp-Duk/Y</sup>	Affecting the FGF23 mediated pathways in the middle ears, predisposition to OM. <sup>39</sup>
<i>Lmna</i>	Mutant Lmna mice heterozygous (Lmna <sup>Dhe/+</sup> )	A model of human OM and laminopathy. <sup>40</sup>
<i>Chd7</i>	Spontaneous deletion of Chd7 exons 2-312	OM with effusion. <sup>41</sup>
<i>Tc1</i>	Tc1 mouse model of Down syndrome, C57BL/6J6129S8 background	A limited region of human chromosome 21 involved in OM. <sup>42</sup>
<i>HIF</i>	Junbo and Jeff mouse mutant models	The role of hypoxia and HIF-mediated pathways. <sup>43</sup>
<i>Isl1</i>	A nonconservative tyrosine to cysteine (Y71C) missense mutation in the Isllet1 gene, Isl1 <sup>Drsh</sup> . C3HeB/FeJ background	Predisposition carriers to OM. <sup>44</sup>
<i>Sh3pxd2b</i>	Sh3pxd2b <sup>nee</sup> heterozygous mice	All mice that had the Sh3pxd2bnee mutation went on to develop craniofacial dysmorphologies and subsequently OM, by as early as 11 days of age. <sup>45</sup>
Factor B	C57BL/6 mice	Factor B deficiency decreases C3 activation during acute pneumococcal OM. Complement C3 activation and opsonophagocytosis of <i>S pneumoniae</i> were greatly attenuated in factor B- and factor B/C2-deficient mice. <sup>46</sup>
<i>TNFA</i>	TNFA deletion	The TNF and TNF receptor superfamilies mediate both inflammation and apoptosis during OM. TNFA is also required for appropriate regulation of caspase genes. <sup>47</sup>
<i>TLR9</i>	Toll-like receptor 9 <sup>-/-</sup> mice on a C57BL/6 background	DNA sensing via TLR9 plays a role in OM pathogenesis and recovery. <sup>48</sup>

(continued)

**Table 1.** (continued)

Gene	Variant and Strain Background	Finding
<i>RPL38</i>	An 18-kb deletion/insertion of the <i>Rpl38</i> gene, encoding a ribosomal protein of the large subunit. Tail-short mouse	Skeletal defects, conductive hearing impairment, ectopic mineralization in the middle ear and chronic OM with effusion. <sup>49</sup>
<i>MCP-1/CCL2</i>	C57BL/6, <i>MCP-1</i> <sup>-/-</sup> , <i>CCR2</i> <sup>-/-</sup> , and <i>TNF</i> <sup>-/-</sup> mice	Deficiency of <i>MCP-1/CCL2</i> or <i>CCR2</i> alone was limited to inhibit OM-induced inner ear inflammation due to compensation of alternative genes. <sup>50</sup>
<i>CCL3</i>	<i>TNF2/2</i> mice (B6/129S6- <i>Tnf</i> <sup>tm1Gkl/J</sup> )	<i>CCL3</i> is a potent downstream effector of TNF-mediated inflammation in vitro and in vivo. <sup>51</sup>
<i>TLR</i>	<i>TLR2</i> <sup>-/-</sup> and <i>TLR4</i> <sup>-/-</sup> mice on a C57BL/6 background	<i>TLR2</i> and <i>TLR4</i> signaling critical to the regulation of NTHi-induced OM and critical for bacterial clearance. <sup>52</sup>
<i>Fbxo11</i>		A role for <i>Fbxo11</i> in TGF- $\beta$ signaling is involved in chronic inflammation of the middle ear.
TGF- $\beta$		Defects in TGF- $\beta$ signaling or associated pathways may underlie the development of chronic OM. <sup>53</sup>
<i>MyD88</i>	<i>MyD88</i> <sup>-/-</sup> mice	Prolonged ME mucosal thickening and delayed recruitment of neutrophils and macrophages. <sup>54</sup>
Lysozyme M	Lysozyme M <sup>-/-</sup> mice	Lysozyme M plays an important role in protecting the middle ear from invading pathogens, particularly in the early phase. <sup>55</sup>
<i>Eya4</i>	<i>Eya4</i> -deficient ( <i>Eya4</i> <sup>-/-</sup> ) mice	Severe hearing deficits, developed OM with effusion, abnormal middle ear cavity and eustachian tube dysmorphology. <sup>56</sup>
<i>Fbxo11</i>	Jeff mouse	The heterozygotes develop chronic suppurative OM. The homozygotes show cleft palate, facial clefting and perinatal lethality. <sup>57</sup>
<i>Evil</i> locus	A missense change in the C-terminal zinc finger region of the transcription factor <i>Evil</i> Junbo mutant	Hearing loss due to chronic suppurative OM and otorrhea. <sup>58</sup>
<i>Enpp1</i>	<i>Enpp1</i> asj/asj	Otitis media with effusion (OME) in all of the hearing-impaired <i>Enpp1</i> asj/asj mice by anatomic and histological examinations. <sup>59</sup>
<i>C3H/HeJ</i>	A single amino acid substitution in its Toll-like receptor 4, making it insensitive to endotoxin	Reduced lipopolysaccharide responsiveness, less capable of reacting immunologically to bacterial infection and clearance. <sup>60</sup>
<i>Dp(16)1 yey</i>	The <i>Dp(16)1 Yey</i> mouse is associated with Down syndrome	Middle ear suppressed cellular hypoxia and inflammation in the middle ear. <sup>61</sup>
<i>Slc25a21</i>	The homozygous <i>Slc25a21</i> (tm1a(KOMP)Wtsi) mice	Associated with OM and hearing impairment. <sup>35</sup>

Abbreviations: CCL3, Chemokine (C-C motif) ligand 3; CCR2, C-C chemokine receptor type 2; HB-EGF, heparin-binding-epidermal growth factor; KO, knockout; MCP-1, monocyte chemoattractant protein-1; OM, otitis media; Y71C, a nonconservative tyrosine to cysteine.

human middle ear epithelium culture.<sup>74</sup> Injection of urban particle solution into rat middle ear induced mucosal changes and dysregulation of genes associated with inflammatory response and mucin production.<sup>75</sup> The impact of these environmental exposures on mucin regulation and mucosal changes conferred the development of OM.

### Immune Tolerance of Middle Ear Mucosa

Clinically, the middle ear is considered an immune-privileged area.<sup>76</sup> Homografts of the tympanic member

usually survive for a long time without administration of any immunosuppressive drugs after surgery.<sup>77</sup> For this reason, the middle ear is tolerant to the invading upper respiratory commensals that reside and thrive, frequently causing OM. Consistent with this, immune- and inflammatory-related cytokines, such as TNF $\alpha$  and interferon gamma,<sup>78,79</sup> are induced, which regulate the immunity of the middle ear mucosa against infectious agents. Programmed death ligand 1 (PD-L1), an inducible protein in the middle ear epithelial cells, is induced, which inhibits

middle ear immunity when PD-L1 binds to its receptor, programmed death 1 (PD-1), on the surface of mucosal lymphocytes.<sup>80-82</sup> This way, the PD pathway is formed in the middle ear mucosa to suppress the immunity of the middle ear, making a regional immunotolerance at this specific tissue while the entire immune system is normal.

PD-L1 negatively regulates the activity of effector T lymphocytes, including intraepithelial lymphocytes, in the mucosa,<sup>80,83</sup> reducing the production of IL-2, which is a major cytokine to expand T cells. T cells participate in the innate and adaptive immunity. Due to the negative effects of PD-L1 on the T-cell activity, the innate and adaptive immune systems are both suppressed, and invading microorganisms survive and thrive in the middle ear. This is a unique phenotype of OM characterized by the activation of the PD pathway. In this particular phenotype of OM, the PD pathway may serve as a novel target for the treatment of OM.

### New Intervention for OM

Nonconventional approaches to treatment were examined in an OM animal model. The noninvasive transcutaneous immunization to induce protective immune responses against NTHi-induced OM was conducted in the chinchilla model. The vaccine formula, utilizing a new chimeric immunogen, targeted 2 important bacterial outer membrane proteins and effectively removed mucosal surface bacteria and mucosal biofilm in the middle ear. The increase of activated T cells and the release of host defense peptide contributed to a rapid disease resolution.<sup>84</sup> Antimicrobial activity of photodynamic therapy with porfimer sodium (Photofrin) was assessed against clinical isolates of Mcat. The treatment demonstrated significant reduction in planktonic bacteria and viable bacteria in the biofilm structure.<sup>85</sup>

Various drug delivery methods were attempted to improved efficacy of OM treatment. OTO-201, a sustained-release formula of ciprofloxacin administered via intratympanic injection, was proved to be more effective and convenient in OM treatment.<sup>86</sup> Levofloxacin thermosensitive gel allowed on-site delivery and prolonged the release of antibiotics in the middle ear space. This characteristic provides effective treatment of suppurative OM without recurrence.<sup>87</sup>

In summary, there have been more animal models available for genetic studies regarding OM in recent years and more genes in animal models and human genetic linkage studies relevant to the pathogenesis of OM. In general, these studies are evolving, and the relationship between these genes and human OM remains to be clarified in the years to come.

### Author Contributions

**Jizhen Lin**, overall writing; **Lena Hafrén**, writing in paragraphs regarding human genetics of otitis media; **Joseph Kerschner**, writing in paragraphs regarding precision medicine of otitis media, mucins, genetics, intervention etc; **Jian-Dong Li**, writing in paragraphs regarding inflammation and genetics; **Steve Brown**, writing in paragraphs and tables regarding the genes involved in otitis media; **Qing Y. Zheng**, writing in paragraphs and tables regarding the genes

involved in otitis media; **Diego Preciado**, contributing to mucins in human otitis media; **Yoshihisa Nakamura**, contributing to paragraphs regarding gene polymorphisms and susceptibility; **Qihong Huang**, contributing to the molecular genetics of otitis media and related discussion and writing; **Yan Zhang**, contributing to the molecular genetics of otitis media and related discussion and writing.

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**Competing interests:** Joseph Kerschner, AventaMed—scientific advisor; Steve Brown, Audition Therapeutics Ltd—co-chief scientific officer, shareholder; Pulmagen Therapeutics Ltd—shareholder; CNR Monterotondo, Rome—consultant.

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### References

1. MacArthur CJ, Hausman F, Kempton JB, Choi D, Trune DR. Otitis media impacts hundreds of mouse middle and inner ear genes. *PLoS One*. 2013;8:e75213.
2. Casselbrant ML, Mandel EM. The genetics of otitis media. *Curr Allergy Asthma Rep*. 2001;1:353-357.
3. Ruben RJ. Otitis media: the application of personalized medicine. *Otolaryngol Head Neck Surg*. 2011;145:707-712.
4. Sibbald AD. Acute otitis media in infants: the disease and the illness. Clinical distinctions for the new treatment paradigm. *Otolaryngol Head Neck Surg*. 2012;147:606-610.
5. Pichichero ME, Casey JR, Almudevar A. Reducing the frequency of acute otitis media by individualized care. *Pediatr Infect Dis J*. 2013;32:473-478.
6. Zhang J, Xu M, Zheng Q, Zhang Y, Ma W, Zhang Z. Blocking macrophage migration inhibitory factor activity alleviates mouse acute otitis media in vivo. *Immunol Lett*. 2014;162:101-108.
7. Suzukawa K, Tomlin J, Pak K, et al. A mouse model of otitis media identifies HB-EGF as a mediator of inflammation-induced mucosal proliferation. *PLoS One*. 2014;9:e102739.
8. Kurabi A, Lee J, Wong C, et al. The inflammasome adaptor ASC contributes to multiple innate immune processes in the resolution of otitis media. *Innate Immun*. 2015;21:203-214.
9. Preciado D, Burgett K, Ghimbovski S, Rose M. NTHi induction of Cxcl2 and middle ear mucosal metaplasia in mice. *Laryngoscope*. 2013;123:E66-E71.
10. Woo JI, Kil SH, Oh S, et al. IL-10/HMOX1 signaling modulates cochlear inflammation via negative regulation of MCP-1/CCL2 expression in cochlear fibrocytes. *J Immunol*. 2015;194:3953-3961.
11. Wang WY, Komatsu K, Huang Y, et al. CYLD negatively regulates nontypeable *Haemophilus influenzae*-induced IL-8 expression via phosphatase MKP-1-dependent inhibition of ERK. *PLoS One*. 2014;9:e112516.
12. Komatsu K, Lee JY, Miyata M, et al. Inhibition of PDE4B suppresses inflammation by increasing expression of the deubiquitinase CYLD. *Nat Commun*. 2013;4:1684.
13. Hafrén L, Kentala E, Einarsdottir E, Kere J, Mattila PS. Current knowledge of the genetics of otitis media. *Curr Allergy Asthma Rep*. 2012;12:582-589.

14. Hafren L, Kentala E, Jarvinen TM, et al. Genetic background and the risk of otitis media. *Int J Pediatr Otorhinolaryngol.* 2012;76:41-44.
15. Allen EK, Chen WM, Weeks DE, et al. A genome-wide association study of chronic otitis media with effusion and recurrent otitis media identifies a novel susceptibility locus on chromosome 2. *J Assoc Res Otolaryngol.* 2013;14:791-800.
16. Rye MS, Scaman ES, Thornton RB, et al. Genetic and functional evidence for a locus controlling otitis media at chromosome 10q26.3. *BMC Med Genet.* 2014;15:18.
17. Sale MM, Chen WM, Weeks DE, et al. Evaluation of 15 functional candidate genes for association with chronic otitis media with effusion and/or recurrent otitis media (COME/ROM). *PLoS One.* 2011;6:e22297.
18. Rye MS, Wiertsema SP, Scaman ES, et al. Genetic and functional evidence for a role for SLC11A1 in susceptibility to otitis media in early childhood in a Western Australian population. *Infect Genet Evol.* 2013;16:411-418.
19. MacArthur CJ, Wilmot B, Wang L, Schuller M, Lighthall J, Trune D. Genetic susceptibility to chronic otitis media with effusion: candidate gene single nucleotide polymorphisms. *Laryngoscope.* 2014;124:1229-1235.
20. Nokso-Koivisto J, Chonmaitree T, Jennings K, Matalon R, Block S, Patel JA. Polymorphisms of immunity genes and susceptibility to otitis media in children. *PLoS One.* 2014;9:e93930.
21. Ilija S, Goulielmos GN, Samonis G, Galanakis E. Polymorphisms in IL-6, IL-10, TNF-alpha, IFN-gamma and TGF-beta1 genes and susceptibility to acute otitis media in early infancy. *Pediatr Infect Dis J.* 2014;33:518-521.
22. Gessner BD, Gillingham MB, Wood T, Koeller DM. Association of a genetic variant of carnitine palmitoyltransferase 1A with infections in Alaska Native children. *J Pediatr.* 2013;163:1716-1721.
23. Takeuchi K, Kitano M, Ishinaga H, et al. Recent advances in primary ciliary dyskinesia [published online October 30, 2015]. *Auris Nasus Larynx.*
24. Vuononvirta J, Peltola V, Mertsola J, He Q. Risk of repeated *Moraxella catarrhalis* colonization is increased in children with Toll-like receptor 4 Asp299Gly polymorphism. *Pediatr Infect Dis J.* 2013;32:1185-1188.
25. Hafren L, Einarsdottir E, Kentala E, et al. Predisposition to childhood otitis media and genetic polymorphisms within the Toll-like receptor 4 (TLR4) locus. *PLoS One.* 2015;10:e0132551.
26. Esposito S, Marchisio P, Orenti A, et al. Genetic polymorphisms of functional candidate genes and recurrent acute otitis media with or without tympanic membrane perforation. *Medicine (Baltimore).* 2015;94:e1860.
27. Rye MS, Warrington NM, Scaman ES, et al. Genome-wide association study to identify the genetic determinants of otitis media susceptibility in childhood. *PLoS One.* 2012;7:e48215.
28. Allen EK, Manichaikul A, Sale MM. Genetic contributors to otitis media: agnostic discovery approaches. *Curr Allergy Asthma Rep.* 2014;14:411.
29. Santos-Cortez RL, Chiong CM, Reyes-Quintos MR, et al. Rare A2ML1 variants confer susceptibility to otitis media. *Nat Genet.* 2015;47:917-920.
30. Shin SG, Koh SH, Woo CH, Lim JH. PAI-1 inhibits development of chronic otitis media and tympanosclerosis in a mouse model of otitis media. *Acta Otolaryngol.* 2014;134:1231-1238.
31. Yao W, Frie M, Pan J, et al. C-Jun N-terminal kinase (JNK) isoforms play differing roles in otitis media. *BMC Immunol.* 2014;15:46.
32. Li X, Xu L, Li J, et al. Otitis media in sperm-associated antigen 6 (Spag6)-deficient mice. *PLoS One.* 2014;9:e112879.
33. Woo CH, Shin SG, Koh SH, Lim JH. TBX21 participates in innate immune response by regulating Toll-like receptor 2 expression in *Streptococcus pneumoniae* infections. *Mol Oral Microbiol.* 2014;29:233-243.
34. Nakamura Y, Komori M, Yamakawa K, et al. Math1, retinoic acid, and TNF-alpha synergistically promote the differentiation of mucous cells in mouse middle ear epithelial cells in vitro. *Pediatr Res.* 2013;74:259-265.
35. Maguire S, Estabel J, Ingham N, et al. Targeting of Slc25a21 is associated with orofacial defects and otitis media due to disrupted expression of a neighbouring gene. *PLoS One.* 2014;9:e91807.
36. Chen J, Ingham N, Clare S, et al. Mcph1-deficient mice reveal a role for MCPH1 in otitis media. *PLoS One.* 2013;8:e58156.
37. Tateossian H, Morse S, Parker A, et al. Otitis media in the Tgif knockout mouse implicates TGFbeta signalling in chronic middle ear inflammatory disease. *Hum Mol Genet.* 2013;22:2553-2565.
38. Kerschner JE, Hong W, Taylor SR, et al. A novel model of spontaneous otitis media with effusion (OME) in the Oxgr1 knock-out mouse. *Int J Pediatr Otorhinolaryngol.* 2013;77:79-84.
39. Han F, Yu H, Li P, et al. Mutation in Phex gene predisposes BALB/c-Phex(Hyp-Duk)/Y mice to otitis media. *PLoS One.* 2012;7:e43010.
40. Zhang Y, Yu H, Xu M, et al. Pathological features in the LmnaDhe/+ mutant mouse provide a novel model of human otitis media and laminopathies. *Am J Pathol.* 2012;181:761-774.
41. Tian C, Yu H, Yang B, et al. Otitis media in a new mouse model for CHARGE syndrome with a deletion in the Chd7 gene. *PLoS One.* 2012;7:e34944.
42. Kuhn S, Ingham N, Pearson S, et al. Auditory function in the Tc1 mouse model of Down syndrome suggests a limited region of human chromosome 21 involved in otitis media. *PLoS One.* 2012;7:e31433.
43. Cheeseman MT, Tyrer HE, Williams D, et al. HIF-VEGF pathways are critical for chronic otitis media in Junbo and Jeff mouse mutants. *PLoS Genet.* 2011;7:e1002336.
44. Hilton JM, Lewis MA, Grati M, et al. Exome sequencing identifies a missense mutation in Isl1 associated with low penetrance otitis media in dearisch mice. *Genome Biol.* 2011;12:R90.
45. Yang B, Tian C, Zhang ZG, et al. Sh3pxd2b mice are a model for craniofacial dysmorphism and otitis media. *PLoS One.* 2011;6:e22622.
46. Li Q, Li YX, Stahl GL, Thurman JM, He Y, Tong HH. Essential role of factor B of the alternative complement pathway in complement activation and opsonophagocytosis during acute pneumococcal otitis media in mice. *Infect Immun.* 2011;79:2578-2585.
47. Ebmeyer J, Leichtle A, Hernandez M, et al. TNFA deletion alters apoptosis as well as caspase 3 and 4 expression during otitis media. *BMC Immunol.* 2011;12:12.



48. Leichtle A, Hernandez M, Lee J, et al. The role of DNA sensing and innate immune receptor TLR9 in otitis media. *Innate Immun.* 2012;18:3-13.
49. Noben-Trauth K, Latoche JR. Ectopic mineralization in the middle ear and chronic otitis media with effusion caused by RPL38 deficiency in the Tail-short (Ts) mouse. *J Biol Chem.* 2011;286:3079-3093.
50. Woo JI, Pan H, Oh S, Lim DJ, Moon SK. Spiral ligament fibrocyte-derived MCP-1/CCL2 contributes to inner ear inflammation secondary to nontypeable *H influenzae*-induced otitis media. *BMC Infect Dis.* 2010;10:314.
51. Leichtle A, Hernandez M, Ebmeyer J, et al. CC chemokine ligand 3 overcomes the bacteriocidal and phagocytic defect of macrophages and hastens recovery from experimental otitis media in TNF-/- mice. *J Immunol.* 2010;184:3087-3097.
52. Leichtle A, Hernandez M, Pak K, et al. TLR4-mediated induction of TLR2 signaling is critical in the pathogenesis and resolution of otitis media. *Innate Immun.* 2009;15:205-215.
53. Tateossian H, Hardisty-Hughes RE, Morse S, et al. Regulation of TGF-beta signalling by Fbxo11, the gene mutated in the Jeff otitis media mouse mutant. *Pathogenetics.* 2009;2:5.
54. Hernandez M, Leichtle A, Pak K, et al. Myeloid differentiation primary response gene 88 is required for the resolution of otitis media. *J Infect Dis.* 2008;198:1862-1869.
55. Shimada J, Moon SK, Lee HY, et al. Lysozyme M deficiency leads to an increased susceptibility to *Streptococcus pneumoniae*-induced otitis media. *BMC Infect Dis.* 2008;8:134.
56. Depreux FF, Darrow K, Conner DA, et al. Eya4-deficient mice are a model for heritable otitis media. *J Clin Invest.* 2008;118:651-658.
57. Hardisty-Hughes RE, Tateossian H, Morse SA, et al. A mutation in the F-box gene, Fbxo11, causes otitis media in the Jeff mouse. *Hum Mol Genet.* 2006;15:3273-3279.
58. Parkinson N, Hardisty-Hughes RE, Tateossian H, et al. Mutation at the Evi1 locus in Junbo mice causes susceptibility to otitis media. *PLoS Genet.* 2006;2:e149.
59. Tian C, Harris BS, Johnson KR. Ectopic mineralization and conductive hearing loss in Enpp1asj mutant mice, a new model for otitis media and tympanosclerosis. *PLoS One.* 2016;11:e0168159.
60. Mitchell CR, Kempton JB, Scott-Tyler B, Trune DR. Otitis media incidence and impact on the auditory brain stem response in lipopolysaccharide-nonresponsive C3H/HeJ mice. *Otolaryngol Head Neck Surg.* 1997;117:459-464.
61. Bhutta MF, Cheeseman MT, Brown SD. Myringotomy in the Junbo mouse model of chronic otitis media alleviates inflammation and cellular hypoxia. *Laryngoscope.* 2014;124:E377-E383.
62. Lysaght AC, Yuan Q, Fan Y, et al. FGF23 deficiency leads to mixed hearing loss and middle ear malformation in mice. *PLoS One.* 2014;9:e107681.
63. Fuchs JC, Zinnamon FA, Taylor RR, et al. Hearing loss in a mouse model of 22q11.2 deletion syndrome. *PLoS One.* 2013;8:e80104.
64. Hempel RJ, Morton DJ, Seale TW, Whitby PW, Stull TL. The role of the RNA chaperone Hfq in *Haemophilus influenzae* pathogenesis. *BMC Microbiol.* 2013;13:134.
65. LaCross NC, Marrs CF, Gilsdorf JR. Otitis media associated polymorphisms in the hemin receptor HemR of nontypeable *Haemophilus influenzae*. *Infect Genet Evol.* 2014;26:47-57.
66. Harrison A, Santana EA, Szelestey BR, Newsom DE, White P, Mason KM. Ferric uptake regulator and its role in the pathogenesis of nontypeable *Haemophilus influenzae*. *Infect Immun.* 2013;81:1221-1233.
67. Ren D, Walker AN, Daines DA. Toxin-antitoxin loci vapBC-1 and vapXD contribute to survival and virulence in nontypeable *Haemophilus influenzae*. *BMC Microbiol.* 2012;12:263.
68. Ren D, Kordis AA, Sonenshine DE, Daines DA. The ToxAvapA toxin-antitoxin locus contributes to the survival of nontypeable *Haemophilus influenzae* during infection. *PLoS One.* 2014;9:e91523.
69. Tong HH, Lambert G, Li YX, et al. Deletion of the complement C5a receptor alleviates the severity of acute pneumococcal otitis media following influenza A virus infection in mice. *PLoS One.* 2014;9:e95160.
70. Wang W, Zhou A, Zhang X, et al. Interleukin 17A promotes pneumococcal clearance by recruiting neutrophils and inducing apoptosis through a p38 mitogen-activated protein kinase-dependent mechanism in acute otitis media. *Infect Immun.* 2014;82:2368-2377.
71. Kirkeby S, Friis M, Mikkelsen HB, Caye-Thomasen P. Bacterial adherence in otitis media: determination of N-acetylgalactosamine (GalNAc) residues in the submucosal glands and surface epithelium of the normal and diseased Eustachian tube. *Microb Pathog.* 2011;51:48-57.
72. Kerschner JE, Hong W, Khampang P, Johnston N. Differential response of gel-forming mucins to pathogenic middle ear bacteria. *Int J Pediatr Otorhinolaryngol.* 2014;78:1368-1373.
73. Song JJ, Lee JD, Lee BD, Chae SW, Park MK. Effect of acrolein, a hazardous air pollutant in smoke, on human middle ear epithelial cells. *Int J Pediatr Otorhinolaryngol.* 2013;77:1659-1664.
74. Im GJ, Park MK, Song JJ. Effect of urban particles on human middle ear epithelial cells. *Int J Pediatr Otorhinolaryngol.* 2014;78:777-781.
75. Park MK, Chae SW, Kim HB, Cho JG, Song JJ. Middle ear inflammation of rat induced by urban particles. *Int J Pediatr Otorhinolaryngol.* 2014;78:2193-2197.
76. Hong S, Van Kaer L. Immune privilege: keeping an eye on natural killer T cells. *J Exp Med.* 1999;190:1197-1200.
77. Alford BR, McFarlane JR, Neely JG. Homograft replacement of the tympanic membrane. *Laryngoscope.* 1976;86:199-208.
78. Lasisi AO, Olayemi O, Arinola OG, Omilabu SA. Interferon-gamma in suppurative otitis media: significance of otorrhoea type and disease outcome. *J Laryngol Otol.* 2009;123:1103-1107.
79. Si Y, Zhang ZG, Chen SJ, et al. Attenuated TLRs in middle ear mucosa contributes to susceptibility of chronic suppurative otitis media. *Hum Immunol.* 2014;75:771-776.
80. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med.* 2002;8:793-800.
81. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. *Annu Rev Immunol.* 2011;29:235-271.

82. Scanduzzi L, Ghosh K, Zang X. T cell costimulation and coinhibition: genetics and disease. *Discov Med*. 2011;12:119-128.
83. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med*. 1999;5:1365-1369.
84. Novotny LA, Clements JD, Bakaletz LO. Kinetic analysis and evaluation of the mechanisms involved in the resolution of experimental nontypeable *Haemophilus influenzae*-induced otitis media after transcutaneous immunization. *Vaccine*. 2013;31:3417-3426.
85. Luke-Marshall NR, Mang TS, Hansen LA, Campagnari AA. *Moraxella catarrhalis* is susceptible to antimicrobial photodynamic therapy with Photofrin. *Lasers Surg Med*. 2014;46:712-717.
86. Wang X, Fernandez R, Tsivkovskaia N, et al. OTO-201: nonclinical assessment of a sustained-release ciprofloxacin hydrogel for the treatment of otitis media. *Otol Neurotol*. 2014;35:459-469.
87. Li C, Gu J, Mao X, Ao H, Yang X. Preparation of levofloxacin thermo-sensitive gel and clinical application in the treatment of suppurative otitis media. *Acta Otolaryngol*. 2014;134:468-474.