

What Have We Learned from Murine Models of Otitis Media?

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Abstract Otitis media (OM) is a common cause of childhood hearing loss. The large medical costs involved in treating this condition have meant that research to understand the pathology of this disease and identify new therapeutic interventions is important. There is evidence that susceptibility to OM has a significant genetic component, although little is known about the key genetic pathways involved. Mouse models for disease have become an important resource to understand a variety of human pathologies, including OM, due to the ability to easily manipulate their genetic components. This has enabled researchers to create models of acute OM, and has aided in the identification of a number of new genes associated with chronic disease, through the use of mutagenesis programs. The use of mouse models has identified a number of key molecular signalling pathways involved in the development of this condition, with genes identified from models shown to be associated with human OM.

Keywords Mouse · Murine models · Acute otitis media · Chronic otitis media with effusion · *NTHi* · Middle ear · Hearing loss · Deafness · ENU mutagenesis · *Jeff* · *Junbo*

Introduction

Otitis media (OM), inflammation of the middle ear, is a common disease of childhood in both the developed and developing world. It is an often neglected condition, perceived by many to be a transient affliction, which in reality places a substantial social, medical and economic burden on healthcare systems globally [1]. Acute otitis media (AOM) is the most common bacterial infection of childhood. In the UK, and other developed countries, otitis media with effusion (OME) is cited as the most common cause of hearing loss in children under 12 [2] and in the USA, the recorded 2.2 million annual episodes of OME have a cost estimate of \$4 billion [3, 4]. The high prevalence and recurrence of OME, coupled with the progression to chronic disease in a large number of children, results in 30,000 procedures for the insertion of tympanostomy tubes per annum in the UK [5].

OM is a multi-factorial condition with its onset and resolution dependent upon a variety of risk factors that include host immunity, pathogen exposure and genetics [6]. Due to their utility in exploring the genetic basis of human disease, mice have become the preferred model organism in OM research [7–10]. The ease of genetic manipulation [11], relatively short generation times and comparable physiology with humans make mice the ideal model to explore our understanding of disease mechanisms. Furthermore, 99 % of mouse genes have human homologues [12]. The functional and anatomical similarities between human and mouse ears in particular [13] have meant that the mouse has become an excellent tool in OM research (Fig. 1), although not without some limitations [14].

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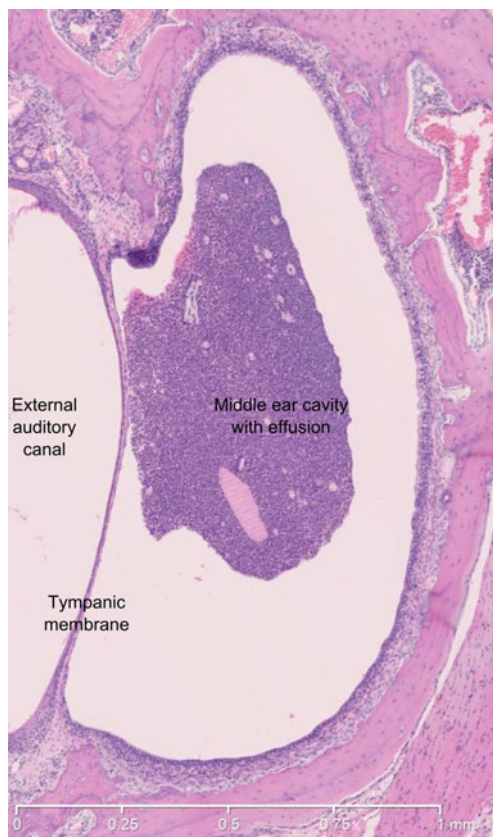


Fig. 1 Haematoxylin and Eosin (H&E) dorsal plane section of *Junbo* (*Jbo/+*) mouse showing the middle ear. *Jbo/+* mice develop otitis media characterised by significant thickening of the middle ear mucosa and an effusion containing neutrophils and macrophages. The functional and anatomical similarities between human and mouse middle ears mean that mice are a good model for hearing research. The pathology of disease in this mouse model is also similar to that of human chronic otitis media, making it an excellent model for otitis media research

Mice as a Model for Otitis Media

Mice have been used to model AOM by inoculation of the middle ear with human otopathogens via trans-bullar injection [9] or pressure-induced translocation from the nasopharynx [15]. Interest is also developing in the use of mice as a model for mucosal vaccination against OM [16]. Models of chronic OM have been identified from mouse mutagenesis programs, including gene-driven knockout approaches [17] and phenotype-driven ENU mutagenesis screens [18, 19]. In this review, we discuss some of the key molecular pathways that have been implicated in OM development through the use of mouse models. A summary of the published murine models is found in Table 1.

Acute Otitis Media (AOM)

AOM is usually a consequence of bacterial infection of the middle ear space. It is thought that ascension of commensal

bacterial species normally resident in the nasopharynx occurs with an alteration in mucosal conditions. Certainly, an upper respiratory tract infection precedes most AOM, and is thought to be the trigger for indeterminate mucosal changes that enable bacterial colonisation and proliferation within the middle ear. AOM is commonly associated with one of three human otopathogens; non-typeable *Haemophilus influenzae* (NTHi), *Streptococcus pneumoniae* and *Moraxella catarrhalis*. The use of pneumococcal vaccines in recent years has meant that NTHi has become the most prevalent isolated pathogen in AOM [20].

The relative contributions of the innate or adaptive immune systems to AOM susceptibility have not been ascertained. The innate immune system is easier to experimentally manipulate and to study, and so has been the focus for developing murine models of AOM. In broad terms, the innate immune system is a non-specific response, enabling animals to recognise and subsequently destroy pathogens, including bacteria. Its principal components include granulocytes, macrophages, mast cells, dendritic cells, natural killer cells and complement. Recognition of micro-organisms is made possible through binding of highly conserved epitopes, usually lipids, proteins and nucleic acids, found on the bacterial cell wall. These pathogen-associated molecular patterns (PAMPs) are identified by the pattern recognition family of receptors.

Toll-Like Receptors (TLRs)

One of the most important families of the pathogen recognition receptors is the toll-like receptors (TLRs) [21]. TLRs are present on the surface of cells prominent at mucosal surfaces and include epithelial, mast and dendritic cells. There are 10 and 12 different TLRs in humans and mice, respectively [22], with each TLR possessing distinct functional properties enabling the detection of a broad range of pathogens [23]. Activation of TLRs results in the production of both pro- and anti-inflammatory cytokines including tumour necrosis factor (TNF- α) and interleukin-10 (IL-10) respectively. For most TLRs, this happens via myeloid differentiation primary response gene 88 (MyD88) activation of NF- κ B and/or mitogen-activated protein (MAP) kinase-dependent pro-inflammatory gene expression. However, TLR3 and TLR4 signal via a MyD88-independent pathway, acting via TRIF, leading to the production of type I IFNs. The activation of TLRs and their downstream signalling cascades results in the production of a broad range of cytokines and chemokines to induce the adaptive immune response in addition to innate inflammatory responses.

The deletion of various TLRs in mice, including TLR9 [24], TLR4 [25] and TLR2 [26], has provided important insights into the role these receptors play in mediating inflammation in response to NTHi. Compared to wild-type, *TLR4^{-/-}* and *TLR2^{-/-}* mice have a prolonged and persistent OM, characterised by reduced bacterial clearance, altered mucosal inflammation and

Table 1 Current mouse models of acute and chronic OM

Pathway	Locus	Chr	Model type	Background strain	General phenotype	OM phenotype	
Acute (induced) otitis media							
Complement	<i>Bf</i>	17	KO	C57BL/6	Mild immune defects	Delayed clearance of acute infection	[30]
	<i>Bf/C2</i>	17	Double KO	C57BL/6	Immune defects	Delayed clearance of acute infection	
	<i>C1qa</i>	4	KO	C57BL/6	Autoimmune disease, increased mortality, glomerulonephritis	Delayed clearance of acute infection	
Toll-like receptors	<i>MyD88</i>	9	KO	C57BL/6	Immune defects	Delayed clearance of acute infection	[27]
	<i>Tlr2</i>	3	KO	C57BL/6	Immune defects	Delayed clearance of acute infection	[26]
	<i>Tlr4</i>	4	SNP or KO	C57BL/6	Immune defects	Delayed clearance of acute infection	[25, 92]
	<i>Tlr9</i>	9	KO	C57BL/6	Immune defects	Delayed clearance of acute infection	[24]
	<i>Ticam1 (Trif)</i>	17	KO	C57BL/6	Immune defects	Delayed clearance of acute infection	[93]
TNF	<i>lpr</i>	19	SNP	MRL/MpJ	Autoimmune disease	Delayed clearance of acute infection	[94]
	<i>TNFA</i>	17	KO	C57BL/6; 129S6	Immune defects	Spontaneous mild mucosal hyperplasia. Delayed clearance of acute infection	[95, 96]
Miscellaneous	<i>Cyld</i>	8	KO	C57BL/6	Lymphoid hyperplasia, susceptibility to colitis and colonic adenocarcinoma	Enhanced inflammation	[97]
	<i>Il-10</i>	1	KO	C57BL/6.129P2	Reduced size, anaemia, chronic enterocolitis	Reduced mucins in acute infection	[98]
Chronic otitis media							
TGF- β	<i>Fbxo11</i>	17	SNP	C3H/HeN	Mild sensorineural hearing loss	Spontaneous chronic in heterozygote	[69, 74]
	<i>Tgfr1</i>	17	KO	C57BL/6J	Smaller size, craniofacial defect	Spontaneous chronic	[70]
NF- κ B	<i>Evi1 (Mecom)</i>	3	SNP	C3H/HeN	Extra digits	Spontaneous chronic in heterozygote	[63]
	<i>IκB$\alpha$$\Delta$N</i>	9	Knock-in	C57BL/6	Defective epithelial appendages, lymph node formation, and macrophage function	Spontaneous chronic	[64]
EYA	<i>Eya1</i>	1	KO	129/SvJ, C57BL/6 or Balb/C	Ossicular and inner ear defects, renal anomalies	Spontaneous chronic in heterozygote	[55]
	<i>Eya4</i>	10	KO	CBA/J	Sensorineural hearing loss	Spontaneous chronic in heterozygote	[56]
Ciliary defects	<i>Cby1</i>	15	KO	C57BL/6	Absence of motile cilia	Spontaneous chronic	[46]
	<i>Dnahc5</i>	15	Transgenic	C57BL/6; CBA/J	Immotile cilia, respiratory infection, left-right defect, hydrocephalus,	Spontaneous acute/chronic	[47]
	<i>Dnahc11</i>	12	SNP	C3H/HeH or mixed	Immotile cilia, sperm dysmotility, rhinitis, sinusitis	Spontaneous chronic	[48]
	<i>Dpcd</i>	19	KO	C57BL/6-Tyrc-Brd	Smaller size, hydrocephalus, situs invertus, sperm dysmotility, rhinosinusitis	Not well characterised	[99]
	<i>Kif27</i>	13	KO	C57BL/6-Tyrc-Brd	Smaller size, hydrocephalus, rhinosinusitis, reduced viability	Not well characterised	[99]
	<i>Odf2</i>	2	KO	C57BL/6	Dysfunctional cilia, sperm dysmotility, hydrocephalus, sinusitis	Not well characterised	[100]
	<i>Stk36</i>	1	O	C57BL/6-Tyrc-Brd	Smaller size, hydrocephalus, craniofacial defects, reduced viability	Not well characterised	[99]
	<i>Till1</i>	15	KO	C57BL/6-Tyrc-Brd	Immotile cilia, sperm dysmotility, rhinitis, sinusitis	Not well characterised	[101]
	<i>Ulk4</i>	9	KO	C57BL/6-Tyrc-Brd	Shortened cilia, smaller size, hydrocephalus, rhinosinusitis, reduced viability	Not well characterised	[99]
	<i>Gus</i>	5		C3H/HeOuJ			[44]

Table 1 (continued)

Pathway	Locus	Chr	Model type	Background strain	General phenotype	OM phenotype
Lysosomal Disorders			Spontaneous transposition		(MPS VII) Widespread lysosomal storage, dysostosis	Spontaneous chronic, lysosomal granules
	<i>Ids</i>	X	KO	C57BL/6.129	(MPS II) Widespread lysosomal storage.	Spontaneous chronic, lysosomal granules [42, 45•]
	<i>Idua</i>	5	KO	C57BL/6	(MPS I) Widespread lysosomal storage, dysostosis	Spontaneous chronic, lysosomal granules [41]
	<i>Naglu</i>	11	KO	C57BL/6	(MPS IIIB) Widespread lysosomal storage, neurological defects.	Spontaneous chronic, lysosomal granules [43]
Miscellaneous	<i>Chd7</i>	4	Spontaneous deletion	BALB/cByJ	Keratoconjunctivitis, circling behavior, growth retardation	Spontaneous chronic [102]
	<i>Dbh</i>	2	KO	C57BL/6; 129/SvCPJ	altered metabolism, thermoregulation, motor function, learning	Spontaneous chronic [103]
	<i>E2f4</i>	10	KO	C57BL/6	Erythropoietic defects, chronic rhinitis	Spontaneous chronic [104]
	<i>Fgfr1</i>	8	SNP	C3HeB/FeJ	Pinna and ossicle malformation, cochlear defects, shortened skull	Spontaneous chronic in heterozygote [105, 106]
	<i>Isl1</i>	13	SNP	C3HeB/FeJ	Ossicular fusion	Spontaneous chronic in heterozygote [107]
	<i>Lmna</i>	3	KO	C57BL/6J	enlarged nuclear morphology of macrophages, hyperphosphatemia	Spontaneous chronic [108]
	<i>Mcph1</i>	8	KO	C57BL/6	Eye defects, erythrocyte defects, infertile	Spontaneous chronic [109]
	<i>Nf2</i>	11	Conditional KO	FVB/N C57BL/6	Schwannomas, osteosarcomas, other tumours, craniofacial defects	Spontaneous chronic [110]
	<i>Oxgr1</i>	14	KO	C57BL/6J-TyrC-Brd; 129 Sv/EvBrd	Not well characterised	Spontaneous chronic in homozygote [111]
	<i>p73</i>	4	KO	C57BL/6 or BALB/c	Lethal gastrointestinal and intracranial haemorrhage, neurological malformation	Spontaneous chronic [112]
	<i>Phex</i>	X	Spontaneous deletion	BALB/cAnBomUrd	Craniofacial, cochlear, and bone defects, endolymphatic hydrops, deformity of epithelial cilia	Spontaneous chronic [113, 114]
	<i>Plg</i>	17	KO	Mixed C57BL/6 & DBA/1J	Widespread fibrin deposition in organs	Spontaneous chronic. Fibrin deposition. [115]
	<i>Rpl38</i>	11	Spontaneous deletion	TsJ/LeJ.BlackSwiss	Shortened, kinked tail, reduced body weight, anaemia, skeletal anomalies	Spontaneous chronic in heterozygote, with crystal deposition [116]
	<i>Sall4</i>	2	KO	C57BL/6J-TyrC-Brd; 129/SvEvBrd	Exencephaly, renal hypoplasia, anogenital defects	Not well characterised [117]
	<i>Sh3pxd2b</i>	11	Spontaneous deletion	B10.A-H2h4/(4R)SgDvEg	Reduced growth, craniofacial and ocular defects, infertility	Spontaneous chronic [118, 119]
	<i>Tbx1</i>	16	Transgenic	C57BL/6.129	Cardiovascular defects, craniofacial and pharyngeal arch anomalies	Spontaneous chronic [32]
	MMU16 (Ts65Dn)	16	Partial trisomy	B6EiC3Sn	Neurological deficits, craniofacial malformation	Spontaneous chronic [33]

KO Knockout, *SNP* single nucleotide polymorphism

leukocyte infiltration kinetics [26]. *TLR9*^{-/-} mice also demonstrate a delay in inflammatory cell recruitment and bacterial clearance in the middle ear after *NTHi* challenge [24]. Likewise, *MyD88*^{-/-} mice demonstrate prolonged middle ear mucosal thickening compared to wild-type mice after challenge with *NTHi*. They also demonstrate a delay in the recruitment of macrophages and neutrophils, with an attenuation of these cells' ability to internalise and phagocytose bacteria [27]. While the absence of MyD88 does not result in a complete failure of *NTHi* eradication through potential MyD88-independent mechanisms, its presence is required to prevent

AOM becoming persistent [27]. The finding that *MyD88*^{-/-} mice have a more severe phenotype than *TLR9*, *TLR2* and *TLR4* knockouts alone has suggested functional redundancy between mammalian TLRs to compensate for their loss [28].

Complement

Activation of complement in response to pathogens culminates in the cleavage of the central C3 protein. This produces the C3a and C5a components and formation of the

membrane attack complex, all mediated via the classical, alternative and mannose binding lectin pathways. Analysis of paediatric middle ears with recurrent acute otitis media (rAOM) have demonstrated the presence of complement components from all three complement cascades [29]. Mice with knockout of complement factors C1qa, Factor B and Factor B/C2 demonstrate increased susceptibility to OM caused by *S. pneumoniae* [30]. Defects in complement also prolong the time mice spend with OM [30, 31].

Chronic Otitis Media with Effusion (COME)

In a significant number of children, OME can follow an episode of AOM. OME is a non-purulent middle ear effusion without the symptoms of acute infection, and it can become chronic (COME). Models of chronic OM have been recovered from both gene- and phenotype-driven mouse mutagenesis programs. Most of these models are syndromic and display a multitude of anatomical and functional deficits as well as middle ear inflammation, and mirror the phenotypes of some human syndromes. A smaller number of mouse models are non-syndromic, without the wide-ranging phenotypic effects of syndromic models. These non-syndromic models represent better models for non-syndromic human OM.

Syndromic Otitis Media

Children with certain congenital diseases have a higher prevalence of chronic OM than the general population, with OM developing as one of a number of phenotypic abnormalities. The genetic polymorphisms that underlie such syndromes are becoming increasingly known.

In several syndromes, the susceptibility to COME is associated with craniofacial abnormalities, and this is reflected in mouse models of the same syndromes. For example, the *Tbx1* mouse [32] is a model of 22q11.2 deletion syndrome, and the *Ts65Dn* mouse [33] is a model of Down syndrome. Much of the existing literature suggests that the association of chronic OM in craniofacial malformation is due to dysmorphism and consequent dysfunctional opening of the Eustachian tube (ET). However, whereas ET dysmorphology is probably important in aetiology, it may not be the only or the main factor. Susceptibility to OM is more likely complex, heterogeneous, and to some extent specific to each syndrome. A factor not often recognised is the co-existence of additional overt or subtle immunodeficiency in craniofacial malformation, which has for example been demonstrated in chromosome 22q.11.2 deletion [34, 35], Kabuki syndrome [36], CHARGE syndrome [37] and Down syndrome [38, 39].

Mucopolysaccharidoses are a group of lysosomal storage diseases caused by the absence of or defects in 11 lysosomal enzymes required for the breakdown of glycosaminoglycans (GAGs). Accumulation of GAGs in the middle ear triggers chronic inflammation [40]. Mucopolysaccharidosis (MPS) I [41], MPS II [42], MPS IIIB [43] and MPS VII [44] mouse models have been characterised, with similar pathologies as human disease. The MPS II mouse model is caused by knockout of iduronate sulfatase (IDS) with IDS-KO mice having increased hearing threshold with thickening of the tympanic membrane and middle ear exudates at 17 weeks compared to wild-type littermates [45]. Enzyme replacement therapy with recombinant human IDS resulted in the prevention of the hearing deficits in the MPS II mouse model [45], highlighting the importance of OM mouse models when evaluating therapies.

The *Cby1*- [46], *Dnahc5*- [47] and *Dnahc11*- [48] deficient mice are well-characterised models of primary ciliary dyskinesias (PCD) that have ciliary defects and develop a spontaneous chronic OM. In PCD, there is a chronic middle ear effusion, but it is not clear if this is due to a simple failure of clearance of mucus or to another mechanism. The evidence of disrupted ciliary function in non-syndromic COME is contradictory [49–54], and, where disruption has been observed, it is not clear whether this is cause or effect.

The relevance of some of these mouse models in understanding non-syndromic OM is debatable, as craniofacial syndromes, mucopolysaccharidoses or primary ciliary dyskinesia probably invoke mechanisms of disease that are unique to these syndromes. However, OM in humans can occur as part of a syndrome, so these models are relevant for elucidating the underlying molecular pathways and mechanisms of syndromic OM.

Non-Syndromic Otitis Media

Mouse models of chronic spontaneous OM that do not have an association to a known human syndrome could be considered of greater importance in implicating potential roles for pathways involved in driving chronic inflammation.

EYA

EYA pathways have been implicated as playing a role in chronic OM through mouse models deficient in *Eya1* [55] and *Eya4* [56], which develop a spontaneous chronic OM. Murine *Eya4* has been shown to play an important role in the innate immune response [57], and *Eya1* may have similar immune functions, although this has not been demonstrated [58]. In man, mutations in EYA are known to lead to middle or inner malformation or degeneration, but not inflammation [59–61].

NF- κ B

The NF- κ B family of transcription factors are involved in stress-induced, immune and inflammatory responses [62]. The *Junbo* mutant [63] and *I κ B α Δ N* knockin [64] mouse models suggest a possible role for NF- κ B in chronic OM. The *Junbo* mouse carries a mutation in *Evi1* and, in specific pathogen-free conditions, develops spontaneous chronic OM usually by 54 days after birth (DAB). *Evi1* is a nuclear transcription factor that has interaction with several signalling pathways, such as TGF- β [65, 66] and JNK [67]. However, more recent work has suggested that *Evi1* also acts as an inducible negative-feedback regulator of NF- κ B by inhibiting its binding to DNA through inhibition of p65 acetylation [68]. It is predicted that the mutation in *Junbo* results in over-expression of NF- κ B, which most likely leads to the chronicity of inflammation in the middle ear.

Conversely, the *I κ B α Δ N* knockin mouse suppresses NF- κ B and still spontaneously develops chronic OM [64]. The suppression of NF- κ B in these mice leads to diffuse embryonic disruption, with malformation of epithelial appendages and defective lymphatic development, including only rudimentary development of secondary lymphoid organs. It is likely that OM in this model is the regional manifestation of a more generalised defect in lymphocytes. Together, these models suggest that NF- κ B signalling cascades may play a pivotal role in the transition from acute to chronic OM.

TGF- β

The *Jeff* mutant [69] and *Tgif1* knockout [70] mice provide strong evidence that TGF- β signalling also plays an important role in regulating chronic inflammation in the middle ear. TGF- β is a key regulator of inflammatory response in a number of organs [71, 72]. TGF- β acts through specific receptors activating multiple intracellular pathways, which in turn results in phosphorylation of receptor-regulated Smad2/3 proteins that associate with Smad4 [73]. These complexes translocate to the nucleus and bind DNA to regulate transcription.

Chronic OM spontaneously develops in the *Jeff* heterozygote mouse [69], which carries a mutation in *Fbxo11* [74]. *Fbxo11* is a member of the large F-box family of proteins, which are specificity factors for the SCF E3 ubiquitin ligases and are responsible for the targeted degradation of proteins [75]. In *Jeff* homozygotes (*Jf/Jf*), nuclear accumulation of phospho-Smad2 (pSmad2) is observed and is significantly upregulated in epithelia. Even though mice heterozygous for *Jeff* and *Smad2* mutations recapitulate the phenotype of *Jf/Jf* mice, an interaction between *Fbxo11* and *Smad2* was not observed. *Fbxo11* is also known to neddylate p53, and therefore inhibit its activity [76]. p53 is a transcriptional cofactor of Smad2 [77], and it is thought that the *Jeff*

mutation impacts upon TGF- β signalling, possibly via p53, leading to its abundant activity. However, reduced rather than elevated levels of p53 are found in the *Jeff* mouse [78], suggesting *Fbxo11* has a role in stabilising p53.

TGF- β promotes transcription of *Tgif1*, which in turn has been found to function through several paths as a negative regulator of TGF- β signalling. TGIF was initially identified as a Smad2-binding protein and a co-repressor of TGF- β -induced transcription [79], but it can also inhibit Smad2 phosphorylation by an alternative mechanism to its association with Smad2 in partnership with c-Jun [80]. Spontaneous chronic OM is observed in the *Tgif1* knockout mouse, with onset at around 21 DAB [70]. Both pSmad2 and p21 levels were lowered in the homozygous mutant, demonstrating a suppression of the TGF- β pathway.

Overall, the findings in the *Jeff* mutant and *Tgif1* knockout mouse models provide considerable support for the view that defects in the TGF- β /SMAD pathway impact on susceptibility to chronic OM.

Mouse to Man

A key advantage of studies using mouse models is that the findings can be used to examine the effects of these same genes in the human population. This is commonly achieved through candidate gene association studies. Genetic polymorphisms in the human population have been found for *IL6*, *IL10*, *TLR4* and *TNFA* [81]. Some of these polymorphisms are associated with a reduction or increase in infection susceptibility. Mutations in *TLR2* and *TLR4* have been found in patients with OM [82]. Two different candidate gene studies have identified an association with polymorphisms in *FBXO11* [83, 84]. Evidence for association at rs2134056 was found in the Minnesota COME/ROM Family Study cohort [83, 85] and two different polymorphisms within *FBXO11* were identified for association in the Western Australian Family Study of Otitis Media [84]. *SMAD2* and *SMAD4* were also identified for association in the latter study. Thus, findings from mouse models for a number of innate immune system and TGF- β pathway components have led to the identification of their importance in human OM. However, results obtained using this approach require caution in human studies due to the frequent non-replication of findings from genetic association studies or because of the failure of the method to undertake comprehensive testing of candidate loci [86].

Mouse models have also been exploited to explore disease mechanisms and therapy. Within the chronically inflamed ear of both the *Junbo* and *Jeff* mouse models, the mucosa and leukocytes have been shown to be hypoxic and targeting of hypoxia inducible factor–vascular endothelial growth factor

(HIF-VEGF) pathways with small molecule inhibitors can lead to resolution of inflammation and improve hearing [87].

Limitations of Mouse Models in OM Research

Despite the wealth of information obtained from murine models of OM, there are several limitations to using murine models for OM research. Firstly, the human otopathogens *S. pneumoniae* and *NTHi* are not known to cause murine OM other than under experimentally induced conditions, whereas *M. catarrhalis* is cleared rapidly from the mouse middle ear [88]. Importantly, many induced models utilise single *NTHi* strains. While this is informative, the population in reality is heterogenous, so findings from one strain may not be representative. Secondly, functional equivalence of genes cannot

be assumed from homology in mouse and man, which may in part be due to species-specific alternate splicing [89]. Thirdly, although mice have been used to identify highly penetrant loci underlying many Mendelian disorders [90], they have been less successful for complex trait analysis [91]. Finally, although the toolkit to manipulate the mouse genome has proven valuable, it is still a poor mimic of the allelic variations underlying common heritable disease. In particular, gene-driven knockout approaches produce amorphic rather than hypo- or hypermorphic alleles. Most knockout models for OM result in pleiotropic effects and many can mimic forms of syndromic OM. Phenotype-driven ENU mutagenesis avoids some of the problems of the knockout approach; however, 30 % of ENU mutants are embryonic lethal, suggesting that unnaturally severe genotypic effects can be produced.

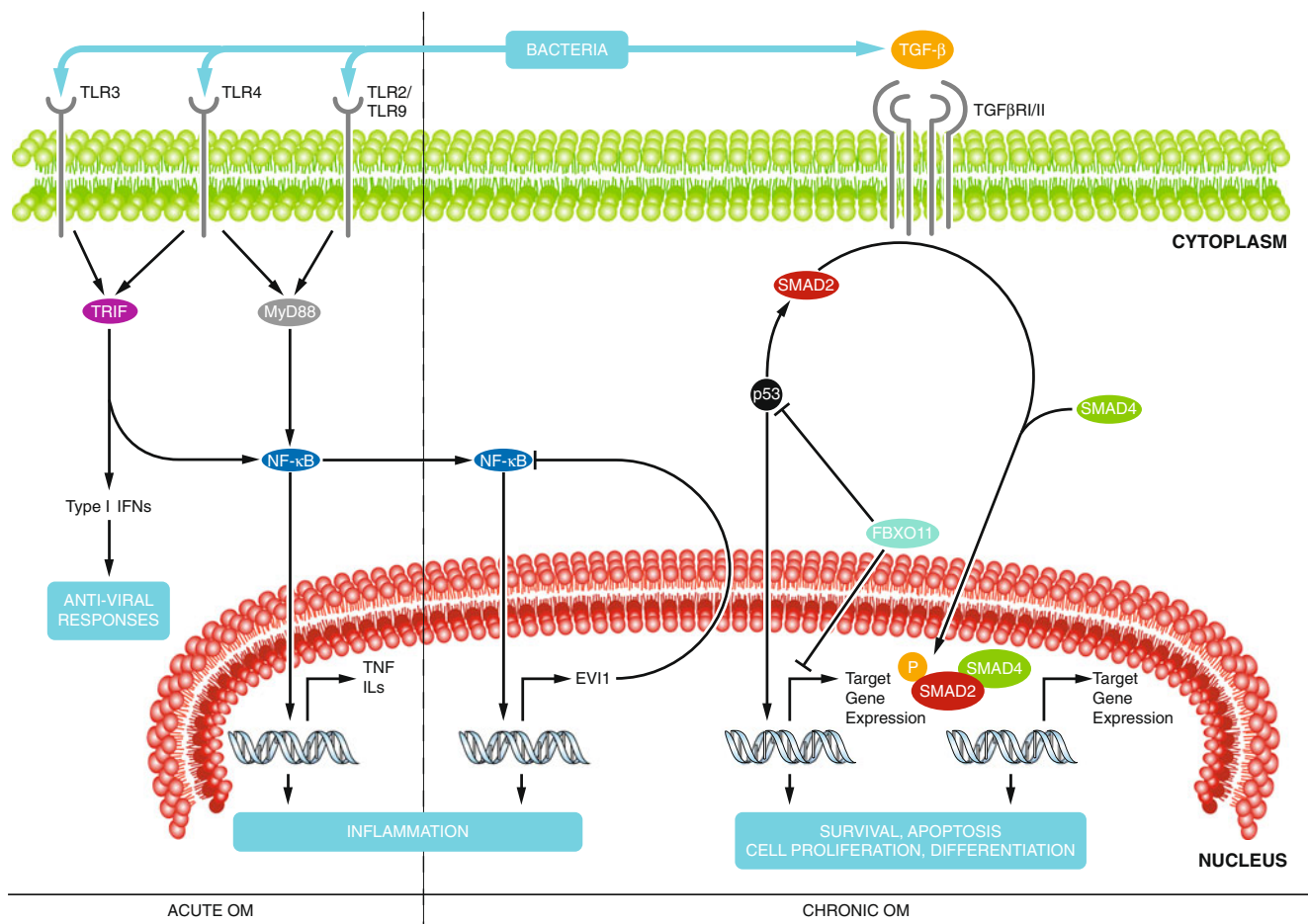


Fig. 2 Schematic indicating the potential roles of interacting pathways related to otitis media (OM) susceptibility identified through mouse models of acute and chronic OM. Bacteria such as non-typeable *Haemophilus influenzae* (*NTHi*) can induce a signalling cascade through a number of TLRs, via TRIF and MyD88 to NF-κB. This results in downstream induction of TNF and interleukins. Furthermore, TRIF mediated signalling leads to production of type I IFNs. NF-κB can be regulated by EVI1, which can act as an inducible negative-feedback

regulator. OM susceptibility can also occur via changes in TGF-β signalling pathways. TGF-β induces SMAD2 phosphorylation, which complexes with SMAD4 and accumulates in the nucleus, where they regulate the expression of target genes. SMAD2 has a transcriptional cofactor, p53. Fbxo11 can suppress p53 function and inhibits its transcriptional activity. See the text for a more detailed description of pathways

Conclusions

At present, the use of mouse models is the most promising method to identify candidate loci underlying susceptibility to OM. Mouse models have highlighted the role of TLRs in AOM, in particular the protection against commensal and pathogenic bacteria, and that persistent NF- κ B or TGF- β signalling could be two mechanisms leading to the overactive pro-inflammatory response seen in chronic OM (Fig. 2).

On-going mutagenesis screens will no doubt highlight new models and new pathways, and also further elaborate the pathways already implicated in the genetic susceptibility of OM. Such knowledge may in the future allow targeted or individualised molecular therapy for these diseases.

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Compliance with Ethics Guidelines

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Michael Crompton declares that he has no conflict of interest.

Mahmood F. Bhutta declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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