1 **Panel 3: Genomics, Precision Medicine and Targeted Therapies**

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Abstract

Objective: To review the most recent advances in human and bacterial genomics as applied to pathogenesis and clinical management of otitis media.

Data Sources: PubMed articles published since the last meeting in June 2015 up to June 2019.

Review Methods: A panel of experts in human and bacterial genomics of otitis media was formed. Each panel member 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 panel met at the 20th International Symposium on Recent Advances in Otitis Media in June 2019, discussed the review and refined the content. A final draft was made, circulated, and approved by the panel members.

Conclusion: Trans-disciplinary approaches applying pan-omic technologies to identify human susceptibility to otitis media and to understand microbial population dynamics, patho-adaptation and virulence mechanisms are crucial to the development of novel, personalized therapeutics and prevention strategies for otitis media.

Implications for Practice: In the future otitis media prevention strategies may be augmented by mucosal immunization, combination vaccines targeting multiple pathogens, and modulation of the middle ear microbiome. Both treatment and vaccination may be tailored to an individual's otitis media phenotype as defined by molecular profiles obtained by using rapidly developing techniques in microbial and host genomics.

Keywords: otitis media, otitis-prone, microbiome, metagenomics, genome-wide association study, precision medicine

1. Introduction

Despite significant decreases in incidence and prevalence worldwide, otitis media (OM) remains a very common diagnosis particularly among children and the elderly. OM continues to impose a major public health burden across human populations and disproportionately affects socially and disadvantaged persons, e.g. indigenous communities. Part of the reason why OM burden cannot be easily eliminated is the incomplete understanding of the OM phenotype and its underlying disease mechanisms.

Human studies of genetic predisposition to experience OM rely on the supposition that the history of diagnosis of OM is accurate. Contamination of the defined susceptible population by individuals who have not experienced OM complicates analysis. The extent of contamination of the susceptible population by "non-susceptibles" currently cannot be known and may vary widely. Precision in diagnosis varies over time for several reasons. The definitions of OM, acute (A)OM and OM with effusion (OME) have changed as recently as 2004 following guidelines from the American Academy of Pediatrics (AAP), then revised again by the AAP in 2013 [1-2]. Moreover, the clinical diagnosis is challenging especially in young children and as such estimates of misdiagnosis can range as high as 50% [3-9]. Clinicians that are specifically trained and tested on their accuracy of diagnosis (proven by tympanocentesis after clinical diagnosis) are termed "validated otoscopists" but they represent <1% of those making OM diagnoses. Tympanocentesis-proven OM is the gold standard of diagnosis but very few clinicians practice this procedure, mostly due to the required skill set for performing tympanocentesis. From a prospective, longitudinal study of children from Rochester, NY, 27% of the children studied met the criteria of otitis-prone (3 AOM infections within 6 months or 4 AOM infections within a year) by age 30 months old if children were diagnosed with AOM by primary care clinicians

[10]. In contrast, if the diagnosis was made by validated otoscopists, 14% met the otitis-prone definition, while if diagnosis was made by tympanocentesis, 6% met the otitis-prone definition [10]. That study gives a perspective on the magnitude of the problem of misclassification of OM. Note that such misclassification may occur not only for AOM but also for chronic (C)OM with effusion especially if with an intact eardrum.

At this time of rapid development in molecular -omic technologies, OM clinicians and scientists must take advantage of these advances to improve understanding and classification of the OM phenotype by integrating knowledge of host, pathogen, and microbiome interactions with overall health status and to enable improvements in OM management and reduction of OM incidence, with the ultimate goal of personalizing treatment and prevention. Here we review the most recent discoveries in human and microbial genomics as applied to OM pathogenesis and precision medicine.

2. Methods

Published literature in PubMed was searched since the last symposium. Search terms included "otitis media", "acute otitis media", "recurrent acute otitis media", "chronic otitis media", "chronic otitis media with effusion", "gene", "genome-wide association studies", "GWAS", "loci", "mouse model", "animal model", "microbiome", "metagenomics", "epigenetic", "personalized medicine", "precision medicine". The search included all published literature with available English abstracts. No further exclusion or selection criteria were employed. Additional inputs were also taken from each panel member on their specific areas of OM expertise.

3. Discussion

3.1 Discovery of OM Susceptibility and Protective Loci in Humans

Twenty-one significant loci have been identified in five genome-wide association studies (GWAS; Table 1). Of these variants, 15 were identified in a 23andMe study that included >120,000 European-descent individuals [12,13]. Additionally four of the loci were either coding variants or in linkage disequilibrium (LD) with coding variants (Table 1): (A) The *PLG* c.112A>G (p. Lys38Glu; rs73015965) variant is pathogenic for autosomal recessive type I plasminogen deficiency that includes COM as a clinical manifestation [16]. *Plg*-knockout mice are also known to spontaneously develop COM [17]. In the GWAS, the heterozygous genotype for the rs73015965 is a risk factor for childhood ear infections (OR=1.43; 95%CI: 1.26,1.63; $p=3.8\text{x}10^{-8}$) [12]. (B) A synonymous variant within *FUT2,* c.249C>T (p.Tyr83=; rs681343), was initially identified as a protective variant in homozygous individuals with OM but was later reported as a risk allele [12,13,18]. The rs681343 variant is in almost complete LD with *FUT2* c.461G>A (p.Trp154*; rs601338) [12,13,18] which has in the genome Aggregation Database (gnomAD.broadinstitute.org) a minor allele frequency (MAF) 0.26-0.50 in various populations except East Asians (MAF=0.002). This stop variant has been well-studied as a protective factor against multiple non-OM infections [18,19]. It also results in higher plasma vitamin B12 levels [20]. Conversely rs601338 increases risk for autoimmune disorders such as Behcet's, celiac and inflammatory bowel diseases [18,21]. In multi-ethnic families and probands with OM, several coding variants within *FUT2* were identified to confer OM susceptibility [18]. This is further supported by the transient expression of *Fut2* in non-typeable *Haemophilus influenzae* (NTHi)-inoculated mouse middle ears and by decreased epithelial A antigen levels due to FUT2 variants [18]. (C) The intronic *TBX1* rs1978060 variant is in almost complete LD with *TBX1* c.1189A>C

1 (p.Asn397His; rs72646967) [13]. *Tbx1*-knockout mice have COM that is characteristic of
2 22q11.2 deletion syndrome in humans [22,23]. In the 23andMe GWAS the *TBX1* rs1978060
3 variant was identified as a risk factor for both childhood ear infections and myringotomy [12,13].
4 (D) The intronic variant *CDHR3* rs114947103 is in almost complete LD with *CDHR3*
5 c.1586G>A (p.Cys529Tyr; rs6967330) [13]. Previously *CDHR3* rs6967330 was implicated in a
6 GWAS for asthma, particularly for wheezing after rhinovirus infection [24-26]. On the contrary
7 for childhood ear infections, the *CDHR3* rs114947103 variant was identified as a protective
8 factor (OR=0.94; 95%CI:0.91,0.96; p=5.4x10^{-9}) [12].
9 From these GWAS only five genes or variants were replicated in an independent OM
10 dataset (Table 1): (a) *FUT2* [12,13,18]; (b) *TBX1* variant rs1978060 [12,13]; (c) *ABO* [12,27,28];
11 (d) intergenic variant rs10497394 on chromosome 2q31.1 which lies between genes *CDCA7* and
12 *SP3* [14]; and (e) a 3'UTR variant in *FNDC1* [15]. ABO blood types were previously known to
13 be associated with different otitis media types, such that type O is protective while type A
14 increases risk for OM [27,28]. The initial GWAS for the rs10497394 and *FNDC1*-3'UTR
15 variants reported independent replication, indicating that they are credibly associated with OM
16 [14,15]. Moreover *FNDC1* variants were positively correlated with *FNDC1* expression levels but
17 negatively correlated with methylation status of *FNDC1* [15]. To date, the *FNDC1* variant
18 rs2932989 is the only significant epigenetic signal identified for OM in humans.
19 All of the five replicated GWAS loci were identified as expression quantitative trait loci
20 (eQTL) for various mucosal and epithelial tissues in the Genotype-Tissue Expression (GTEx)
21 database (gtexportal.org, accessed 21 August 2019), suggesting a regulatory function for these
22 variants (Table 2). Unfortunately middle ear mucosal epithelium is not represented in GTEx.
23 While the majority of these eQTL variants affect expression levels of genes where they lie, some

of the variants have more distant effects (e.g. variant rs885932 at 6p22.1 is 0.87Mb proximal to target gene *IER3* at 6p21.33). Of note, four of the ten eQTL-GWAS loci affect expression of multiple genes in various tissues (Table 2). The rs4329147 variant not only primarily affects *HLA-DRB5* expression but also influences the expression of six other HLA genes and four additional non-HLA genes, including complement gene *C4A* (Table 2). While the initial 23andMe study identified the rs4329147 variant as the most significant locus among HLA alleles (Table 1), a follow-up study on the same dataset demonstrated that this association is lost when conditioning for the *HLA-DRB1* Gln96 allele is performed, implying that the association is largely driven by the amino acid variant within *HLA-DRB1* [12,13].

When the genes associated with eQTL variants are analyzed in NetworkAnalyst [29], significant pathways based on protein-protein interactions included the following: protein binding, MHC class II receptor activity, antigen processing and presentation, endocytosis, immune response, regulation of binding, regulation of TGF-β receptor signaling, pattern specification process, endoderm development, collagen and chromatin binding, chromatin remodeling, and regulation of Ras GTPase activity and of catabolic process. These results provide a glimpse of which pathways are important in the development of OM based on the genes that have been identified so far by GWAS.

3.2 Host Genomics in Animal Models for OM

Several animal models of OM have been developed over the years (Table 3). The chinchilla, *Chinchilla lanigera*, has long been used as model of OM because it recapitulates the human condition very faithfully with respect to pathogen detection [30] and the severity of disease induced by different strains of the same bacterial species [31]. Based on the demonstrated

usefulness of this model, the ISOM community convinced the National Human Genome Research Institute to conduct a chinchilla genome project which culminated in the publication of a comprehensive chinchilla genomic resource [32] which is being exploited for host-pathogen interactions. The mouse has also become one of the favored models for genetic studies because of its lifespan, easy breeding and well-established methods for introducing genetic modifications. In addition, the similarities in auditory structure between mouse and human and the close evolutionary relationship of these two genomes, make the mouse a valuable model to study the genetics of hearing [52], although there are also limitations in inter-species comparisons, and comparison of syndromic to non-syndromic disease. In the past four years, mouse studies have identified several genes and genetic pathways involved in the predisposition to OME including the following examples.

FLI1 and *ETS1* are transcription factors from the ETS (E26 transformation-specific) family, known to be hemizygous in Jacobsen syndrome. It has been demonstrated that these genes have a role in the development of the nose, middle ear cavity and ossicles. The *Fli1*$^{+/-}$ and *Ets1*$^{+/-}$/*Fli1*$^{+/-}$ mice exhibit hearing impairment associated with COM, inflamed middle ear epithelium, abnormally small middle ear cavity, fusion of ossicles to the middle ear wall, and deformed stapes [41].

Mouse mutants called *asj* (ages with stiffened joints), which have a point mutation in the *Enpp1* gene, were also reported to have conductive hearing loss. At six weeks of age, the mutants exhibited effusion in the middle ear, thickened middle ear epithelium, impaired Eustachian tube function due to epithelia proliferation, fusion of the malleus and incus, and calcification of middle ear structures [39].

1 Mutations in *EDA*, *EDAR* and *EDARADD* genes have been described as triggering HED
2 (hypohidrotic ectodermal dysplasia). *Eda*- (Ectodysplasin a) and *Edar*- (Ectodysplasin a
3 receptor) mutant mice (Eda^{Ta} and $Edar^{dlJ/dlJ}$) were reported to develop OM, rhinitis and
4 nasopharyngitis. They also display reduced mucociliary clearance and the loss of glandular
5 secretions [53].
6 Studying the ENU mutant *edison* revealed that a point mutation in the *Nisch* (Nischarin)
7 gene results in the development of conductive hearing loss due to COM. Homozygous *edison*
8 mice spontaneously develop COM as early as three weeks of age. The mutants also demonstrate
9 mild craniofacial defects, middle ear cavities filled with fluid and lined with thickened
10 mucoperiosteum, polypoid growths into the middle ear cavity, and in the more severely affected
11 mice inflamed tympanic membranes. Furthermore, an investigation of the impact of the *edison*
12 mutation on pathways in which Nischarin is involved in has implicated LIMK1 and NF-κB
13 pathways in the development of COM [46].
14 BPIFA1 is a member of the bacterial permeability-increasing (BPI) fold containing
15 family of putative innate defense proteins and is one of the most abundant secretory proteins in
16 the upper respiratory tract. *Bpifa1*-knockout mice do not develop spontaneous OM up to six
17 months although BPIFA1 is highly expressed in the middle ear epithelium. However, deletion of
18 *Bpifa1* in *Junbo* mice, one of the first models of COM, results in significant exacerbation of the
19 phenotype including thickening of the middle ear mucosa and increased collagen deposition.
20 This finding indicates a role for BPIFA1 in mucosal protection [54].
21 Down syndrome is caused by an extra copy of some or all of the genes of human
22 chromosome 21 (Hsa21). The orthologs to the genes from Hsa21 are spread among three regions
23 on the mouse genome located on chromosomes 10 (Mmu10), 16 (Mmu16) and 17 (Mmu17).

Ongoing study at the MRC Harwell Institute on the phenotype of the DpTyb mice, which are mouse models of Down syndrome, revealed that mice with full duplication of the genes from Mmu16 have conductive hearing loss due to the development of middle ear inflammation. A detailed study on the OM phenotype of mouse strains with full duplication of the genes from Mmu10 and 17 and with duplication of smaller segments of the Dp1Tyb region will provide a better understanding of the genes and genetic pathways involved in the development of OM in Down syndrome.

3.3 Advances in Microbiome Analyses

Although viruses are known to play a role in OM, genomic studies on viruses in the middle ear were not conducted during the study period. Thus this section concentrates on bacterial genomics.

3.3.1 New technologies for 16S rRNA sequencing for OM bacterial pathogens

Recently an improved pan-bacterial domain molecular diagnostic (MDx) that uses PCR and circular consensus reads (CCS) of the entire bacterial 16S rRNA (FL16S) gene on Pacific Bioscience (PacBio) 3rd-4th generation sequencers has been developed [55,56]. The process incorporates both laboratory and informatic improvements over extant systems. Briefly, CCS provides for multiple polymerase-driven sequencing-read-passes of a single DNA molecule, where each ‘pass’ of the molecule corresponds to the sequence of sense or antisense strand. Each pass is used towards the creation of a consensus sequence, generating ultra-high-quality sequences (as individual PacBio errors are not sequence context dependent) providing for unambiguous species calls when paired with the companion informatics system, called MCSMRT. This pipeline for quantitatively and specifically profiling microbiota includes a

custom full-length 16S gene database. Beginning with PCR of the entire 16S rRNA gene the resultant amplimers are then sequenced on a PacBio sequencer. CCS read sequences are calculated based on average >15 passes resulting in expected error rates of ≤1 incorrectly called base per FL16S gene read in over half the data. MCSMRT has extended and improved upon previous applications of PacBio 16S sequencing [57-64], including: (A) incorporation of stringent filters eliminating sources of sequencing artifacts, effectively eliminating inflated operational taxonomic unit (OTU) counts, a pervasive problem in short read microbiome protocols [65]; (B) assignment of taxonomy and confidence values to OTUs using the custom-designed full-length 16S database which provides a uniform Linnaean hierarchy for each read. The combination of these factors resulted in a pan-domain MDx that is highly quantitative, even for complex microbiota. Analysis of the Critical Assessment of Metagenome Interpretation (CAMI) community (>250 species) demonstrated its ability to identify and determine abundances of hundreds of species over >3 logs of variance (Fig. 1) [55].

Two recent innovations that improve the accuracy and ability to discriminate among closely related species and strains have been added to MCSMRT: (A) upon ascertaining that ~5% of the 16S amplimers were hybrids of mismatched, closely related single-stranded DNAs (from closely related species, or different intragenomic copies), the ability to produce CCS reads derived from each strand was added as an option; and (B) a new, more accurate algorithm for calling sequences based on graph deconvolution, as opposed to a multiple sequence alignment strategy was added. This latter technique builds on the algorithm introduced in 2002 based on a directed acyclic graph [66]. Using these algorithmic improvements, the quality of even the *unfiltered* CCS reads substantially improved from an average of 8.0 to 2.2 expected errors, and resulting in improved post-filter yields, improving from 86% to 98% recovery.

1 Informatic analyses have seen additional recent advances. Significant challenges in
2 assessing differential abundance of microbial taxa exist due to unbalanced sampling, and
3 artifacts from both PCR and sequencing. Traditional methods (such as rarefaction) have been
4 shown to be untenable for accurate statistical comparisons within microbiome data [67]. New
5 analysis software has been designed that properly normalize OTU counts across samples using
6 Gaussian mixture models, and have been added to popular R microbiome analysis library
7 frameworks, such as phyloseq [68-70]. OTU representation of the microbiome (where sequences
8 are usually clustered together based on an identity threshold of 97%) have also been recently
9 challenged as inadequate to properly account for all microbial diversity within a sample [71].
10 Recent programmatic packages now exist that can be applied after OTU analysis (such as that
11 done in MCSMRT) to further examine diversity based on single nucleotide variants within the
12 OTU sequences [72,73].

13 Another recent advancement with strong implications for a FL16S microbiome pipeline
14 is the development of a kit by the company Shoreline Biome (Farmington, CT, USA), created
15 specifically to go from sample to prepared PacBio sequencing libraries in a series of predefined
16 laboratory steps. The advantages of this pipeline are a pre-designed system for cell lysis, PCR
17 amplification of FL16S, and sample pooling of up to 100 samples per run. This kit streamlines
18 the process resulting in a significant reduction of man hours required for sample library
19 preparation (estimated reduction from 16-25 hours to ~2 hours). Taken together, these exciting
20 new developments in microbial sequencing technologies are expected to have a major impact in
21 scientific discoveries and novel clinical applications in otopathogen detection and
22 characterization.
23

1 *3.3.2 Application of Novel Techniques in Microbiome Studies on COM with Effusion*

2 The above-mentioned microbiome MDx was used to characterize 20 bilateral COM with
3 effusion cases to investigate their polymicrobial nature (Fig. 2). Right and left middle ears
4 typically had similar communities though exceptions existed (patients 12, 27 and 32 in Fig. 2).
5 The major otopathogens causing AOM are NTHi, *Streptococcus pneumoniae* (Spn) and
6 *Moraxella catarrhalis* (Mcat). These OM pathogens were prevalent among the chronic OM
7 patients (6 *Mcat*, 4 *NTHi*, 3 Spn: 55% of cases with 1+), but under-recognized species were more
8 prevalent: 40% *Alloiococcus otitis*, 40% *Staphylococcus auricularis*, 30% *Turicella otitidis*, 30%
9 *Staphylococcus caprae,* and 10% *Aggregatibacter aphrophilus* [74]. The extremely high
10 abundances of these "atypical" species in all cases suggest that they are likely OM etiological
11 agents, and not simply contaminants from the external auditory canal.

12

13 ***3.4 Bacterial population genomics to dissect otopathogen-specific virulence genes and***
14 ***mechanisms***

15 Whole-genome shotgun (WGS) sequencing of clinical bacterial isolates is rapidly
16 revolutionizing clinical microbiology, not only by increasing the throughput and resolution of
17 strain typing but also enabling detection of associations between bacterial genetic variation and
18 clinical pathogenesis phenotypes [75-78]. Bacterial supra-GWAS (SGWAS) presents special
19 challenges [79-81], including inadequate or biased sampling (especially of carriage isolates),
20 limited clinical metadata availability, strong phylogenetic substructure and genomic (gene
21 content) diversity among strains, and a lack of consensus among varied bioinformatic analysis
22 pipelines. Nevertheless, increasingly large datasets and sophisticated tools [82-84] are now
23 detecting signatures of in-host bacterial evolution by identifying recurrent and parallel genomic

1 changes arising over the course of infections or between healthy and diseased states. Isolating
2 these *in situ* genomic signatures of bacterial transitions to pathogenesis ("pathoadaptations") in
3 turn informs our understanding of pathogenic mechanisms in the human host [85-87].
4 Combining comparative genomic analysis with transcriptomic and other -omic technologies to
5 investigate *in vivo* and *in situ* host-pathogen interactions will allow the integration of clinical and
6 molecular mechanistic studies of bacterial pathogenesis.

7 This emerging discipline of "genomic epidemiology" has been broadly applied to
8 bacterial pathogens, but has only begun to be used to dissect OM pathogenesis. The three main
9 bacterial otopathogens NTHi, Spn and Mcat are all amenable to large-scale SGWAS [88-105], a
10 technique that takes into account that the majority of bacterial species' genes are not found in all
11 strains of the species, but are rather distributed among the many strains. Indeed, all three
12 organisms have many publicly available WGS assemblies including complete reference genomes
13 at NCBI (as of June 2019: Sp=10,462 assemblies, NTHi=735, Mcat=114), and additional
14 genomes are continuing to accrue from diverse sources and disease states. In contrast to studies
15 of environmental opportunistic pathogens that typically colonize subjects as single clones that
16 subsequently evolve and diversify (*e.g. Pseudomonas* and *Burkholderia cenocepacea* in cystic
17 fibrosis infections) [106,107], population-level genomic studies in human-restricted normal
18 commensals—like the three main otopathogens—are complicated by the high diversity and
19 polyclonality of these species when isolated from diverse health and disease states. Below, we
20 summarize recent WGS surveys and association studies from NTHi. Although none have yet
21 been specifically focused on OM diseases, the findings suggest that our understanding of OM
22 pathogenesis could be greatly expanded by GWAS and other -omics approaches.

H. influenzae became an early genomic model system when the lab strain Rd became the first cellular organism to have its complete genome sequenced in 1995 [108], and the pace of WGS has continued to increase, with 735 assemblies publically available at NCBI (as of June 7, 2019) and >1,000 more expected in short order. Early and continuing comparative analysis of NTHi genomes were among the first to recognize the immense diversity in genic content seen in many bacterial species [88,89,99,100]. More recently, SGWAS studies identified gene presence/absence differences strongly associated with strains isolated from carriage or disease [98], and follow-up studies identified a novel multi-gene family associated with virulence, the vSLR genes, one of which was shown to increase survival in macrophages (*msf1*) and also enhancing the ability of OM that increases the severity of disease in the chinchilla model of OM [102]. Recent studies of NTHi in chronic obstructive pulmonary disease (COPD) have revealed recurrent genomic changes accumulating over time in different patients, particularly at phase variable genes encoding in lipooligosaccharide synthesis enzymes and outer membrane proteins [103,104]. This also identified recurrent loss-of-function mutations in the bifunctional *ompP1/fadL* gene as a specific NTHi patho-adaptation to the COPD lung, and also showed that this is a likely evolutionary trade-off between *ompP1* mutants' losing their ability to adhere and invade airway epithelial cells, but gaining resistance to arachidonic acid, an abundant inflammatory mediator in the COPD lung [103]. Similar work with a small number of strains in pediatric pulmonary infections found limited evidence for parallel evolution but nasal-lung isolate pairs also showed convergent transcriptional signatures by *in vitro* RNA-seq [105]. Additional SGWAS has been been performed for population-level and epidemiological analysis of the NTHi, including new invasive isolates from the US [106]and Portugal [107], as well as a detailed analysis of 265 strains collected in the UK that identified lineage-specific associations

1 with pneumococcus and shifts in NTHi population structure in carriage upon introduction of the
2 PCV13 vaccine [108]. These results all show the power of comparative analysis of bacterial
3 genomes.

4 Excitingly, recent technical and informatic advances in metagenomic sequencing together
5 with novel cultural protocols provide for the systematic capture of much deeper and more
6 complete views of highly complex polyclonal and polymicrobial populations while providing for
7 the near complete genome sequencing and assembly of both very rare strains and strains that are
8 recalcitrant to culture. These advances provide for deep, strain-traceable, metagenomic/WGS
9 characterization of polyclonal populations *in situ* – when combined with a multipartite specimen
10 collection approach to provide comprehensive strain-, gene-, and pan(supra)genome-based
11 analyses of any clinical sample -- and particularly useful for following over time the evolution of
12 clonal lineages and inter-strain population dynamics. A comprehensive approach to
13 understanding these issues with respect to the evolution of otopathogenesis at the population-
14 level, particularly with regard to horizontal gene transfer includes: (A) WGS of large numbers of
15 individual isolates recovered simultaneously from middle ear effusions and the nasopharynx; (B)
16 the metagenomic, and ligation-proximity-metagenomic sequencing of all recovered colonies
17 from each specimen that have been pooled to form a 'gimish' [109,110] to construct
18 genomes/clonal lineages of lower prevalence strains; (C) metagenomic sequencing of uncultured
19 clinical specimen DNA that has been selected using a species-specific capture reagent [98] to
20 provide coverage for unculturable strains; and (D) the use of gel microdroplets (GMD) to
21 encapsulate single cells for the production of sequestered clonal microcolonies grown in co-
22 culture which can provide sufficient genomic DNA for whole genome amplification (MDA) and
23 subsequent genomic assemblies [111,112]. Selection of individual GMD microcolonies for

sequencing is achieved via FACS using labeled probes made from metagenomically identified genes that are not present in the strains which underwent WGS.

3.5 Epigenetic regulation in bacterial otopathogens

Many bacterial pathogens that are adapted to the human host display random, high-frequency on/off switching of gene expression, called phase variation [113]. This gene regulation strategy generates a diverse bacterial population that provides many possible solutions to the challenges posed by distinct immunological memories. In most cases, the phase variably expressed genes encode surface exposed antigens such as outer membrane proteins and variable oligosaccharide structures. Two of the major otopathogens *H. influenzae* [114] and Mcat [115] are archetypal examples of bacterial pathogens that exhibit phase variable gene expression and have many phase variable genes.

In 2005 a new type of epigenetic regulation system called a phase variable regulon (phasevarion), was described in *H. influenzae* [116]. Phasevarions are controlled by DNA methyltransferases that randomly switch on and off, leading to changes in DNA methylation at thousands of sites in the genome and consequent changes in global gene expression [117]. Both *H. influenzae* [118] and Mcat [119] contain multiple phasevarion systems that control virulence and immune evasion. In the case of *H. influenzae*, the impact of phasevarion switching in OM has been confirmed in the chinchilla model [118]. Spn also has a DNA methyltransferase that randomly switches between six different DNA methylation patterns by recombination that regulates virulence in a murine model system and immune evasion [120].

Studies in *in vitro* model systems indicate the potential importance of phasevarions in the pathogenesis of all major bacterial otopathogens causing OM [118-120]. Further studies on

human clinical samples are required to assess the impact of these systems in regulating the adaptation of the pathogen to the human host in distinct phases of disease and distinct host niches, e.g. to address the question of a potential role for phasevarion-mediated regulation of the transition from nasopharyngeal colonization to active OM infection.

The epigenetic regulation of phasevarions is mediated by changes in methylation at thousands of sites in the genome. The vast majority of these changes are neutral with respect to impact on gene expression. Thus, it is not possible at this time to conduct in silico prediction of which genes are subject to expression changes upon phasevarion switching. The inability to predict which genes will be subject to phasevarion regulation presents particular problems for genomics-based vaccine development [121] for these pathogens. Detailed studies of phasevarion-mediated gene expression are required in all three common AOM pathogens to define the repertoire of stably expressed immunological targets.

3.6 Precision Medicine for OM

Precision Medicine (PM), also called personalized medicine, is designed to treat the right patient, at the correct time, with the right treatment strategy. This takes into consideration the patients' genetic and environmental factors, including the molecular basis of disease. This strategy has been implemented in cancer treatment for some time [122-125]. Studies on primary immune deficiency have revealed several hundred genetic defects associated with various disorders, helped understand immunobiology and resulted in targeted new therapies [125]. For example, cystic fibrosis is a monogenic disease, but more than 2000 different mutations in the *CFTR* gene have been reported. Targeted therapies for some of these mutations, e.g. for carriers of the *CFTR* p.Phe508del variant, are already on the market [126].

1 Application of PM in infectious diseases is much more complex than in many other
2 medical fields and, combined with the lack of new efficacious antibiotics to deal with established
3 bacterial resistance to traditional antibiotics [127-130], makes for a very challenging
4 environment for the adoption of PM. For OM, the recognition that the expression of particular
5 allelic forms of the gel-forming mucins and aquaporins are specifically linked to pediatric
6 hearing loss [131] provides the potential for targeted therapeutic interventions. Similarly,the
7 recent identification of specific host microRNAs that are associated with OM [132] (mir-223)
8 and the hyperplastic response associated with COME [133] (mir-146) provide high value targets
9 for future interventional strategies Treatment strategies for PM in infectious diseases include
10 pathogen-targeted antibiotic treatment, adjuvant therapy to antibiotics, precision antimicrobial
11 therapies, and subclassification of patient groups according to intrinsic and extrinsic factors.
12 Bacterial culture and antimicrobial sensitivity tests are available but are slow to perform
13 and therefore microbial WGS to find resistant strains is evolving [130,134]. WGS can be used to
14 select an appropriate and efficacious medication, reducing side effects and excluding toxic drugs.
15 PM based on the bacterial strain is possible in OM with suppuration from tympanic membrane
16 perforation or tympanostomy tube discharge.
17 PM in infectious diseases also includes developing non-antibiotic strategies. Precision
18 antimicrobial treatment can target certain virulence factors in pathogens, whether a gene, a
19 cellular process, or a specific microbe. These strategies also leave the rest of the human
20 microbiota intact. An unbalanced microbiota is associated with a wide range of disorders, from
21 autoimmune to psychiatric diseases. Precision antibiotic treatments include lysins, nucleic acid-
22 based systems, synthetic peptides, and mannosides. One strategy in OM would be to identify

1 genes that favor colonization of OM pathogens in the middle ear and to develop small
2 antagonists against molecules produced by bacteria [127,128,135].
3 The term -omics comprises genomics, transcriptomics, proteomics, metabolomics, and
4 microbiomes, while translational -omics refers to clinical use of multi-omic data to treat disease
5 in a precise way. It enables the definition of different endotypes of a phenotype or trait. In a
6 Cochrane review of antibiotic treatment in OM, an example of an endotype that benefits most
7 from antibiotic treatment is defined as "children under two years of age with bilateral AOM, or
8 with both AOM and otorrhea" [136]. Such endotypes are used to identify patients who will
9 benefit from different treatment strategies with fewer side effects [127,137].
10
11 ***3.7 OM Therapeutics***
12 Understandably translation of –omics findings to therapeutic use takes a long time. Recent
13 studies on OM therapeutics have focused on steroids and antibiotics, which are not –omics-
14 guided and mostly with negative results. Francis et al. (2018) performed a randomized trial of
15 oral steroids for treatment of hearing loss in OM, and found no effect [138]. Ranakusuma et al.
16 (2018) reviewed studies of oral corticosteroids for AOM, and found no evidence of effectiveness
17 in reducing duration of symptoms [139]. Lewnard et al. (2018) found that long-term amoxicillin
18 treatment did not increase the carriage of resistant Spn strains [1140]. Ruohola et al. (2018)
19 noted that immediate amoxicillin treatment for AOM did not enhance clearance of later middle
20 ear effusion [141]. Hoberman et al. (2016) found that a shortened antibiotic regimen was not as
21 effective as a full course for the resolution of OM [142]. Te Molder et al. (2016) found that
22 antibiotic treatment for a first OM episode does not affect the probability of future recurrences
23 [143]. While these studies were negative, two other studies that had positive findings showed

that for children less than two years old, immediate amoxicillin was the most cost-effective treatment for AOM [1144], while immediate amoxicillin treatment was modestly more effective in treating OM than delayed antibiotics [145]. Outside of steroids and antibiotics, only one study by Kondura et al. (2016) found that circumin reduced inflammation in an animal model of OM [38].

Regarding delivery of drugs to the middle ear, Yang et al. (2016) found that delivery of ciprofloxacin and penetrants in a hydrogel to the external surface of the intact tympanic membrane resulted in delivery of therapeutic amounts of drug to the middle ear [146]. Kurabi et al. (2017) discovered rare peptides that support active transport of large particles across the intact eardrum, and enhanced transport by lengthening the peptides [147,148]. They also found that the peptides can transit the intact human tympanic membrane [149].

3.8 Vaccination for OM towards PM

3.8.1. Pneumococcal vaccination

At onset of AOM, Spn (58%) was the predominant organism in the nasopharynx followed by *Mca*t (55.1%) and then *H. influenzae* (38.2%) [150], which shows low prediction value of nasopharyngeal cultures to determine the etiology of middle ear bacterial pathogens and the importance of collection of middle ear fluid to identify vaccines for AOM prevention. The only current licensed vaccines targeting Spn are pneumococcal conjugate vaccines (PCVs). These vaccines contain 10 (PCV10) or 13 (PCV13) polysaccharide Spn serotypes conjugated to a protein carrier. PCVs are effective in reducing AOM caused by the serotypes contained within the vaccines [151-154]. In addition, administration of PCVs during infancy may reduce the risk of recurrent AOM and progression to more complex disease caused by non-vaccine serotypes

[155,156]. However, increases in both nasopharyngeal carriage and diseases caused by non-vaccine Spn capsule types are on the rise [157]. This has resulted in vaccine companies developing newer PCVs such as PCV15 (Merck) [158-160] and PCV20 (Pfizer). PCV15 contains the same 13 serotypes as in PCV13 plus 22F and 33F. PCV20 contains same serotypes as in PCV15 plus 8, 10A, 11A, 12F and 15B/C. Both PCV15 and PCV20 are in Phase 3 clinical studies in adults.

Although these newer PCVs will help to reduce the incidence of diseases caused by the additional vaccine serotypes, history tells us that non-vaccine serotype replacements will occur over time. Hence, there is a need to develop non-serotype dependent pneumococcal vaccines. Efforts have been made formulating pneumococcal protein-based vaccines either alone or in combination with PCVs [160,161]. However, none of these vaccines moved to Phase 3 clinical trials because they failed to reduce nasopharyngeal colonization similar to PCVs and the indirect herd immunity effect of PCVs is sought for overall public health benefit. Another approach being tested is a whole cell vaccine (WCV) that generates both antibody and Th1/Th17 cellular immunity thereby offering the potential to prevent Spn carriage [162,163]. A genetically engineered unencapsulated killed strain of Spn that elicits IL-17A production is in clinical trials [164,165]. Additional preclinical WCVs using different engineered strains and different routes of immunization (intraperitoneal or intranasal) are being studied [166,167].

3.8.2. Vaccination against H. influenzae

The only licensed vaccine is the *H. influenzae* serotype b (PRP) polysaccharide conjugate vaccine. This vaccine is not effective against other *H. influenzae* capsule types that cause invasive disease nor NTHi which, aside from being a major AOM pathogen, is a major pathogen

causing COPD and invasive diseases in infants and young children [168-170]. PCV10 vaccine contains protein D from *H. influenzae* conjugated to eight of the pneumococcal polysaccharides. In a pediatric clinical trial using an earlier version of PCV10 and PCV11 where all 11 polysaccharides were conjugated to protein D, a significant reduction in NTHi-caused AOM was measured [171]. Subsequent clinical and observational studies using PCV10 showed marginal efficacy against NTHi-caused AOM and had no impact on nasopharyngeal carriage of *H. influenzae* [172,173].

Current strategies for new vaccines to prevent *H. influenzae* infections are being explored and are focused on relatively conserved proteins expressed by the bacteria [174-176]. Two phase 1 clinical trials investigated a multi-component *H. influenzae* protein-based vaccine and a multi-component combination Spn and *H. influenzae* protein-based vaccine [177,178]. Another Phase 1 trial investigated a multi-component combination *H. influenzae* and Mcat protein-based vaccine to target subjects with acute exacerbations of COPD [179]. Oral vaccines using inactivated NTHi to prevent acute exacerbations of COPD have been in clinical studies [180-182]. A novel preclinical study using a transcutaneous immunization of a chimeric protein with a band aid prevented OM in chinchillas [183]. Animal studies mimicking the natural human mode of AOM as a result of nasal coinfection of virus and NTHi show promise in identifying vaccine candidates [184]. An outer membrane vesicle of NTHi has been shown to be protective against OM in a chinchilla model [185].

3.8.3. Vaccination for Mcat

There are no licensed vaccines against *Mcat*. One of the major issues in developing a vaccine against OM is lack of an animal model due to *Mcat* being a human-restricted pathogen [186].

Mouse pulmonary clearance and chinchilla nasopharyngeal colonization models have been used for testing Mcat vaccine candidates. A number of protein and lipooligosaccharide candidates have been studied preclinically [186,187]. Only one investigational clinical study of a vaccine containing three NTHi proteins and one Mcat protein has been reported targeting acute exacerbations of COPD [179].

3.8.3. Vaccination against Otopathogens within the Context of PM

From a PM perspective pneumococcal vaccines should be effective in the population which experience the greatest disease burden, e.g. otitis-prone children. Otitis-prone children have multiple dysfunctions in adaptive immunity including responses to vaccines [188-194]. In particular, otitis-prone children respond with significantly lower antibody levels and lower generation of memory B cells following PCV13 vaccination [188,194]. They respond to natural immunization induced by nasopharyngeal colonization and AOM infections with less antibody against pneumococcal proteins which are considered as vaccine candidates [192,195,196]. Otitis-prone children also have diminished Th1 and Th17 immune responses [193] and generally have poor response to most routine vaccination [188]. Similar to the response to pneumococcal vaccines, otitis-prone children respond less well to *H. influenzae* type b vaccine [188], as well as to several proteins expressed by *H. influenzae* and Mcat that are vaccine candidates [195,197,198].

4. Implications for Practice

Despite the considerable morbidity and health care costs for OM treatment, a barrier that prevents development of new vaccines and treatments for OM including those arising from

1 knowledge brought forth by new genomic technologies is the lack of appreciation of OM as a
2 major public health threat, particularly by industry. Genetic diversity among OM pathogens is
3 another major challenge that must be addressed. From a public health perspective vaccines
4 targeting otopathogens would be most cost-effective if they not only reduced OM but also
5 reduced nasopharyngeal carriage to produce an indirect herd immunity effect. However, for any
6 vaccine, is total elimination of nasopharyngeal carriage risky? Might elimination of potential
7 otopathogens open a niche for other pathogenic organisms? Some scientists recommend that
8 elimination of nasopharyngeal colonization is not desirable, and rather enhancing the host
9 immunity to prevent bacteria from reaching a pathogenic threshold should be the goal [160].
10 Eliciting mucosal responses by formulating vaccines to enhance Th17 responses or by mucosal
11 immunization is a strategy under investigation. Combination vaccines targeting Spn, NTHi and
12 Mcat would be the preferred formulation to target OM and other diseases caused by these
13 pathogens. Modulating the microbiome by monitoring bacterial abundances not just of OM
14 pathogens but also of the commensal middle ear bacteria is another potential goal for OM
15 prevention.
16 The increasing pace of large-scale WGS of clinical bacterial isolates and novel insights
17 into bacterial population dynamics and pathogenesis offer great promise to dissect how bacteria
18 adapt during pathogenesis, pointing to the molecular mechanisms important within the human
19 host. The methods and tools are now available to gain direct insights into the specific bacterial
20 genomic variation that impacts OM. In combination with carefully collected and curated clinical
21 isolate collections, applying these analyses to bacterial otopathogens will not only improve
22 epidemiological tracking but also dissecting bacterial pathoadaptation and virulence
23 mechanisms. Increasing use of functional approaches, like transcriptome and metabolome

analyses [199,1200] —applied in a comparative context—will help inform new disease management strategies, identify new diagnostic biomarkers, and suggest new routes for therapeutic intervention.

In the near future, human GWAS using next-generation sequence data will further illuminate how coding and non-coding variants with a wide MAF spectrum, i.e. both common and rare variants, play a role in OM susceptibility. Further studies in non-European populations are also needed to elucidate OM susceptibility variants that are important in various human ethnic groups. In addition there continue to be many advances in the identification of genes that play a role in OM from animal studies, virtually all in the mouse. These include natural and ENU-induced mutations, as well as studies of knockout and other gene-modified mice. The categories that influence OM include genes related to immunity, inflammation, secretory activity, morphology, and tissue growth. This diversity of OM-related pathways suggests that many more such OM-related genes will be discovered in human and mouse studies, which are important to understand mechanism of disease in OM and to determine pathways that may be targeted for treatment and prevention. Collaboration between disciplines and across populations is mandatory in the development of PM, where research and development in translational -omics will be the cornerstone.

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References

1. Subcommittee on Management of Acute Otitis Media, Diagnosis and Management of Acute Otitis Media, Pediatrics 113 (2004) 1451-1465.
2. A.S. Lieberthal, A.E. Carroll, T. Chonmaitree, T.G. Ganiats, A. Hoberman, M.A. Jackson, M.D. Joffe, D.T. Miller, R.M. Rosenfeld, X.D. Sevilla, R.H. Schwartz, P.A. Thomas, D.E. Tunkel, The diagnosis and management of acute otitis media, Pediatrics 131 (2013) e964-e999.
3. M.K. Laine, P.A. Tahtinen, O. Ruuskanen, P. Huovinen, A. Ruohola, Symptoms or symptom-based scores cannot predict acute otitis media at otitis-prone age, Pediatrics 125 (2010) e1154-e1161.
4. M.E. Pichichero, M.D. Poole, Assessing diagnostic accuracy and tympanocentesis skills in the management of otitis media, Arch. Pediatr. Adolesc. Med. 155 (2001) 1137-1142.
5. N. Balasundaram, D. Phan, D. Mazzoni, E. Duong, A. Sweeny, C. Del Mar, G. Keijzers, Acute otitis media in children presenting to the emergency department: Is it diagnosed and managed appropriately? J. Paediatr. Child Health (2019). doi: 10.1111/jpc.14414.

6. M.E. Pichichero, M.D. Poole, Comparison of performance by otolaryngologists, pediatricians, and general practioners on an otoendoscopic diagnostic video examination, Int. J. Pediatr. Otorhinolaryngol. 69 (2005) 361-366.
7. C.R. Paul, C.L. Gjerde, G. McIntosh, L.S. Weber, Teaching the pediatric ear exam and diagnosis of Acute Otitis Media: a teaching and assessment model in three groups, BMC Med. Educ. 17 (2017) 146.
8. M.E. Pichichero, Diagnostic accuracy of otitis media and tympanocentesis skills assessment among pediatricians, Eur. J. Clin. Microbiol. Infect. Dis. 22 (2003) 519-524.
9. J.M. Legros, H. Hitoto, F. Garnier, C. Dagorne, E. Parot-Schinkel, S. Fanello, Clinical qualitative evaluation of the diagnosis of acute otitis media in general practice, Int. J. Pediatr. Otorhinolaryngol. 72 (2008) 23-30.
10. M.E. Pichichero, J.R. Casey, A. Almudevar, Reducing the Frequency of Acute Otitis Media by Individualized Care, Pediatr. Infect. Dis. J. 32 (2013) 473-478.
11. E. Einarsdottir, L. Hafrén, E. Leinonen, M.F. Bhutta, E. Kentala, J. Kere, P.S. Mattila, Genome-wide association analysis reveals variants on chromosome 19 that contribute to childhood risk of chronic otitis media with effusion, Sci. Rep. 6 (2016) 33240.
12. J.K. Pickrell, T. Berisa, J.Z. Liu, L. Ségurel, J.Y. Tung, D.A. Hinds, Detection and interpretation of shared genetic influences on 42 human traits, Nat. Genet. 48 (2016) 709-717.
13. C. Tian, B.S. Hromatka, A.K. Kiefer, N. Eriksson, S.M. Noble, J.Y. Tung, D.A. Hinds, Genome-wide association and HLA region fine-mapping studies identify susceptibility loci for multiple common infections, Nat. Commun. 8 (2017) 599.

1 14. E.K. Allen, W.M. Chen, D.E. Weeks, F. Chen, X. Hou, J.L. Mattos, J.C. Mychaleckyj, F.
2 Segade, M.L. Casselbrant, E.M. Mandel, R.E. Ferrell, S.S. Rich, K.A. Daly, M.M. Sale, A
3 genome-wide association study of chronic otitis media with effusion and recurrent otitis
4 media identified a novel susceptibility locus on chromosome 2, J. Assoc. Res. Otolaryngol.
5 14 (2013) 791-800.
6 15. G. van Ingen, J. Li, A. Goedegebure, R. Pandey, Y.R. Li, M.E. March, V.W. Jaddoe, M.
7 Bakay, F.D. Mentch, K. Thomas, Z. Wei, X. Chang, A.G. Uitterlinden, H.A. Moll, C.M.
8 van Duijn, F. Rivadeneira, H. Raat, R.J. Baatenburg de Jong, P.M. Sleiman, M.P. van der
9 Schroeff, H. Hakonarson, Genome-wide association study for acute otitis media in children
10 identifies FNDC1 as disease contributing gene, Nat. Commun. 7 (2016) 12792.
11 16. K. Tefs, M. Gueorguieva, J. Klammt, C.M. Allen, D. Aktas, F.Y. Anlar, S.D. Aydogdu, D.
12 Brown, E. Ciftci, P. Contarini, C.E. Dempfle, M. Dostalek, S. Eisert, A. Gökbuget, O.
13 Günhan, A.A. Hidayat, B. Hügle, M. Isikoglu, M. Irkec, S.K. Joss, S. Klebe, C. Kneppo, I.
14 Kurtulus, R.P. Mehta, K. Ornek, R. Schneppenheim, S. Seregard, E. Sweeney, S. Turtschi,
15 G. Veres, P. Zeitler, M. Ziegler, V. Schuster, Molecular and clinical spectrum of type I
16 plasminogen deficiency: a series of 50 patients, Blood 108 (2006) 3021-3026.
17 17. P.O. Eriksson, J. Li, T. Ny, S. Hellström S, Spontaneous development of otitis media in
18 plasminogen-deficient mice, Int. J. Med. Microbiol. 296 (2006) 501-509.
19 18. R.L.P. Santos-Cortez, C.M. Chiong, D.N. Frank, A.F. Ryan, A.P.J. Giese, T.C. Bootpetch
20 Roberts, K.A. Daly, M.J. Steritz, W. Szeremeta, M. Pedro, H. Pine, T.K.L. Yarza, M.A.
21 Scholes, E.G.d.V. Llanes, S. Yousaf, N. Friedman, M.L.C. Tantoco, T.M. Wine, P.J. Labra,
22 J. Benoit, A.G. Ruiz, R.A.R. de la Cruz, C. Greenlee, A. Yousaf, J. Cardwell, R.M.A.
23 Nonato, D.C. Ray, K.M.C. Ong, E. So, C.E. Robertson, J. Dinwiddie, S.M. Lagrana-

Villagracia, University of Washington Center for Mendelian Genomics, S.P. Gubbels, R.S. Shaikh, S.P. Cass, E. Einarsdottir, N.R. Lee, D.A. Schwartz, T.L.I. Gloria-Cruz, M.J. Bamshad, I.V. Yang, J. Kere, G.T. Abes, J.D. Prager, S. Riazuddin, A.L. Chan, P.J. Yoon, D.A. Nickerson, E.M.C. Cutiongco-de la Paz, S.O. Streubel, M.R.T. Reyes-Quintos, H.A. Jenkins, P.S. Mattila, K.H. Chan, K.L. Mohlke, S.M. Leal, L. Hafrén, T. Chonmaitree, M.M. Sale, Z.M. Ahmed, FUT2 variants confer susceptibility to familial otitis media, Am. J. Hum. Genet. 103 (2018) 679-690.

19. M. Thorven, A. Grahn, K.O. Hedlund, H. Johansson, C. Wahlfrid, G. Larson, L. Svensson, A homozygous nonsense mutation (428G->A) in the human secretor (FUT2) gene provides resistance to symptomatic norovirus (GGII) infections, J. Virol. 79 (2005) 15351-15355.

20. A. Hazra, P. Kraft, J. Selhub, E.L. Giovannucci, G. Thomas, R.N. Hoover, S.J. Chanock, D.J. Hunter, Common variants of FUT2 are associated with plasma vitamin B12 levels, Nat. Genet. 40 (2008) 1160-1162.

21. M. Takeuchi, N. Mizuki, A. Meguro, M.J. Ombrello, Y. Kirino, C. Satorius, J. Le, M. Blake, B. Erer, T. Kawagoe, D. Ustek, I. Tugal-Tutkun, E. Seyahi, Y. Ozyazgan, I. Sousa, F. Davatchi, V. Francisco, F. Shahram, B.S. Abdollahi, A. Nadji, N.M. Shafiee, F. Ghaderibarmi, S. Ohno, A. Ueda, Y. Ishigatsubo, M. Gadina, S.A. Oliveira, A. Gül, D.L. Kastner, E.F. Remmers, Dense genotyping of immune-related loci implicates host responses to microbial exposure in Behcet's disease susceptibility, Nat. Genet. 449 (2017) 438-443.

22. J. Chen, X. Zhang, J. Li, C. Song, Y. Jia, W. Xiong, Identification of a Novel ENU-Induced Mutation in Mouse Tbx1 Linked to Human DiGeorge Syndrome, Neural Plast. (2016) 5836143.

1 23. J.C. Fuchs, J.F. Linden, A. Baldini, A.S. Tucker AS, A defect in early myogenesis causes
2 Otitis media in two mouse models of 22q11.2 Deletion Syndrome, Hum. Mol. Genet. 24
3 (2015) 1869-1882.
4 24. K. Bønnelykke, P. Sleiman, K. Nielsen, E. Kreiner-Møller, J.M. Mercader, D. Belgrave,
5 H.T. den Dekker, A. Husby, A. Sevelsted, G. Faura-Tellez, L.J. Mortensen, L. Paternoster,
6 R. Flaaten, A. Mølgaard, D.E. Smart, P.F. Thomsen, M.A. Rasmussen, S. Bonàs-Guarch,
7 C. Holst, E.A. Nohr, R. Yadav, M.E. March, T. Blicher, P.M. Lackie, V.W. Jaddoe, A.
8 Simpson, J.W. Holloway, L. Duijts, A. Custovic, D.E. Davies, D. Torrents, R. Gupta, M.V.
9 Hollegaard, D.M. Hougaard, H. Hakonarson, H. Bisgaard, A genome-wide association
10 study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe
11 exacerbations, Nat. Genet. 46 (2014) 51-55.
12 25. Y.A. Bochkov, K. Watters, S. Ashraf, T.F. Griggs, M.K. Devries, D.J. Jackson, A.C.
13 Palmenberg, J.E. Gern, Cadherin-related family member 3, a childhood asthma
14 susceptibility gene product, mediates rhinovirus C binding and replication, Proc. Natl.
15 Acad. Sci. U S A 112 (2015) 5485-5490.
16 26. K. Bønnelykke, A.T. Coleman, M.D. Evans, J. Thorsen, J. Waage, N.H. Vissing, C.J.
17 Carlsson, J. Stokholm, B.L. Chawes, L.E. Jessen, T.K. Fischer, Y.A. Bochkov, C. Ober,
18 R.F. Lemanske Jr, D.J. Jackson, J.E. Gern, H. Bisgaard, CDHR3 Genetics and Rhinovirus
19 C Respiratory Illnesses, Am. J. Respir. Crit. Care Med. 197 (2018) 589-594.
20 27. E.H. Mortensen, T. Lildholdt, N.P. Gammelgard, P.H. Christensen, Distribution of ABO
21 blood groups in secretory otitis media and cholesteatoma, Clin. Otolaryngol. Allied Sci. 8
22 (1983) 263-265.

1 28. K. Apostopoulos, E. Labropoulou, B. Konstantinos, S. Rhageed, E. Ferekidis, Blood group
2 in otitis media with effusion, ORL J. Otorhinolaryngol. Relat. Spec. 64 (2002) 433-435.
3 29. Q. Zhou, O. Soufan, J. Ewald, R.E.W. Hancock, N. Basu, J. Xia, NetworkAnalyst 3.0: a
4 visual analytics platform for comprehensive gene expression profiling and meta-analysis,
5 Nucleic Acids Res. 2019. doi:10.1093/nar/gkz240.
6 30. Aul, J.J., Anderson, K.W., Wadowsky, R., Doyle, W.J., Kingsley, L.A., Post, J.C., **Ehrlich, G.D.^**
7 Comparative evaluation of culture and PCR for the detection and determination of persistence of
8 bacterial strains and DNAs in the *Chinchilla laniger* model of otitis media. *Annals of Otology,*
9 *Rhinology and Laryngology* 107:508-513, 1998.
10 31. Buchinsky, F. J., Forbes, M., Hayes, J., Hu, F.Z., Greenberg, P., Post J.C., and **Ehrlich, G.D.^**
11 Phenotypic Plurality among Clinical Strains of nontypeable *Haemophilus influenzae* determined by
12 symptom severity in the Chinchilla model of Otitis Media. *BMC Microbiology* 14;7:56, 2007.
13 32. Shimoyama M, Hong W, Khampang P, Ehrlich GD, Bakaletz L, Kerschner, JE. The
14 Chinchilla Research Resource Database: resource for an otolaryngology disease model.
15 Database – The Journal of Biological Databases and Curation 2016 May 12 Epub pii:
16 baw073 doi:10.1093.
17 33. J.A. Bartlett, D.K. Meyerholz, C.L. Wohlford-Lenane, P.W. Naumann, N.H. Salzman, P.B.
18 McCray, Increased susceptibility to otitis media in a Splunc1-deficient mouse model, Dis.
19 Model Mech. 8 (2015) 501-508.
20 34. A. Kurabi, J. Lee, C. Wong, K. Pak, H. Hoffman, A.F. Ryan, S. Wasserman, The
21 inflammasome adaptor ASC contributes to multiple innate immune processes in the
22 resolution of otitis media, Innate Immun. 21 (2015) 203-214.

1 35. D. Deniffel, B. Nuyen, K. Pak, K. Suzukawa, J. Hung, A. Kurabi, S.I. Wasserman, A.F.
2 Ryan, Otitis Media and Nasopharyngeal Colonization in ccl3(-/-) Mice, Infect Immun. 85
3 (2017). doi: 10.1128/IAI.00148-17.
4 36. H.W. Lim, K. Pak, A. Kurabi, A.F. Ryan, Lack of the hyaluronan receptor CD44 affects
5 the course of bacterial otitis media and reduces leukocyte recruitment to the middle ear,
6 BMC Immunol. 20 (2019) 20.
7 37. D. Punwani, B. Pelz, J. Yu, N.C. Arva, K. Schafernak, K. Kondratowicz, M. Makhija, J.M.
8 Puck, Coronin-1A: immune deficiency in humans and mice, J. Clin. Immunol. 35 (2015)
9 100-107.
10 38. A.S. Konduru, S. Matsuyama, B.C. Lee, K. Komatsu, J.D. Li, Curcumin Inhibits NTHi-
11 Induced MUC5AC Mucin Overproduction in Otitis Media via Upregulation of MAPK
12 Phosphatase MKP-1, Int. J. Inflam. 2017 (2017) 4525309.
13 39. C. Tian, B. Harris, K. Johnson, Ectopic mineralization and conductive hearing loss in
14 enpp1/asj mutant mice, a new model for otitis media and tympanosclerosis, PLoS One 11
15 (2016) e0168159.
16 40. J. Del-Pozo, N. MacIntyre, A. Azar, J. Glover, E. Milne, M. Cheeseman, Chronic otitis
17 media is initiated by a bulla cavitation defect in the FBXO11 mouse model, Dis. Model
18 Mech. 12 (2019). doi: 10.1242/dmm.037804.
19 41. M.R. Carpinelli, E.A. Kruse, B.D. Arhatari, M.A. Debrincat, J.M. Ogier, J.C. Bories, B.T.
20 Kile, R.A. Burt, Mice haploinsufficient for Ets1 and Fli1 display middle ear abnormalities
21 and model aspects of Jacobsen syndrome, Am. J. Pathol. 185 (2015) 1867-1876.

1 42. Z. Wang, Q. He, X. Zhang, Y. Ma, F. Fan, Y. Dong, W. Xu, Y. Yin, Y. He, Innate anti-
2 microbial and anti-chemotaxis properties of progranulin in an acute otitis media mouse
3 model, Front. Immunol. 9 (2018) 2952.
4 43. K. Suzukawa, J. Tomlin, K. Pak, E. Chavez, A. Kurabi, A. Baird, S.I. Wasserman, A.F.
5 Ryan, A mouse model of otitis media identifies HB-EGF as a mediator of inflammation-
6 induced mucosal proliferation, PLoS One 9 (2014) e102739.
7 44. W. Yao, M. Frei, J. Pan, K. Pak, N. Webster, S.I. Wasserman, A.F. Ryan, C-Jun N-terminal
8 Kinase (JNK) isoforms play differing roles in otitis media, BMC Immunol. 15 (2014) 46.
9 45. J. Zhang, M. Xu, Q. Zheng, Y. Zhang, W. Ma, Z. Zhang, Blocking macrophage migration
10 inhibitory factor activity alleviates mouse acute otitis media in vivo, Immunol. Lett. 162
11 (2014) 101-108.
12 46. M. Crompton, T. Purnell, H.E. Tyrer, A. Parker, G. Ball, R.E. Hardisty-Hughes, R. Gale,
13 D. Williams, C.H. Dean, M.M. Simon, A.M. Mallon, S. Wells, M.F. Bhutta, M.J. Burton,
14 H. Tateossian, S.D.M. Brown, A mutation in Nischarin causes otitis media via LIMK1 and
15 NF-κB pathways, PLoS Genet. 13 (2017) e1006969.
16 47. J.I. Woo, S. Oh, P. Webster, Y.J. Lee, D.J. Lim, S.K. Moon, NOD2/RICK-dependent β-
17 defensin 2 regulation is protective for nontypeable Haemophilus influenzae-induced middle
18 ear infection, PLoS One 9 (2014) e90933.
19 48. S.G. Shin, S.H. Koh, C.H. Woo, J.H. Lim, PAI-1 inhibits development of chronic otitis
20 media and tympanosclerosis in a mouse model of otitis media, Acta Otolaryngol. 134
21 (2014) 1231-1238.
22 49. S. Maguire, J. Estabel, N. Ingham, S. Pearson, E. Ryder, D.M. Carragher, N. Walker,
23 Sanger MGP Slc25a21 Project Team, J. Bussell, W.I. Chan, T.M. Keane, D.J. Adams, C.L.

1 Scudamore, C.J. Lelliott, R. Ramírez-Solis, N.A. Karp, K.P. Steel, J.K. White, A.K.
2 Gerdin, Targeting of Slc25a21 is associated with orofacial defects and otitis media due to
3 disrupted expression of a neighbouring gene, PLoS One 9 (2014) e91807.
4 50. X. Li, L. Xu, J. Li, B. Li, X. Bai, J.F. Strauss 3rd, Z. Zhang Z, H. Wang, Otitis media in
5 sperm-associated antigen 6 (Spag6)-deficient mice, PLoS One 9 (2014) e112879.
6 51. Y. Huang, Z. Wang, C. Jin, L. Wang, X. Zhang, W. Xu, Y. Xiang, W. Wang, X. He, Y.
7 Yin, Y. He, TLR2 promotes macrophage recruitment and Streptococcus pneumoniae
8 clearance during mouse otitis media, Pediatr Res. 80 (2016) 886-893.
9 52. S.D. Brown, R.E. Hardisty-Hughes, P. Mburu P, Quiet as a mouse: dissecting the
10 molecular and genetic basis of hearing, Nat. Rev. Genet. 9 (2008) 277-290.
11 53. A. Azar, C. Piccinelli, H. Brown, D. Headon, M. Cheeseman, Ectodysplasin signalling
12 deficiency in mouse models of hypohidrotic ectodermal dysplasia leads to middle ear and
13 nasal pathology, Hum. Mol. Genet. 25 (2016) 3564-3577.
14 54. A. Mulay, D.W. Hood, D. Williams, C. Russell, S.D.M. Brown, L. Bingle, M. Cheeseman,
15 C.D. Bingle, Loss of the homeostatic protein BPIFA1, leads to exacerbation of otitis media
16 severity in the Junbo mouse model, Sci Rep. 8 (2018) 3128.
17 55. J.P. Earl, N.D. Adappa, J. Krol, A.S. Bhat, S. Balashov, R.L. Ehrlich, J.N. Palmer, A.D.
18 Workman, M. Blasetti, B. Sen, J. Hammond, N.A. Cohen, G.D. Ehrlich, J.C. Mell, Species-
19 level bacterial community profiling of the healthy sinonasal microbiome using Pacific
20 Biosciences sequencing of full-length 16A rRNA genes, Microbiome 23 (2018) 190.
21 56. K.L. Greathouse, J.R. White, A.J. Vargas, V.V. Bliskovsky, J.A. Beck, N. von Muhlinen,
22 E.C. Polley, E.D. Bowman, M.A. Khan, A.I. Robles, T. Cooks, B.M. Ryan, N. Padgett,
23 A.H. Dzutsev, G. Trinchieri, M.A. Pineda, S. Bilke, P.S. Meltzer, A.N. Hokenstad, T.M.

1 Stickrod, M.R. Walther-Antonio, J.P. Earl, J.C. Mell, J.E. Krol, S.V. Balashov, A.S. Bhat,
2 G.D. Ehrlich, A. Valm, C. Deming, S. Conlan, J. Oh, J.A. Segre, C.C. Harris, Interaction
3 between the microbiome and TP53 in human lung cancer, Genome Biol. 19 (2018) 123.
4 57. J.T. Babauta, E. Atci, P.T. Ha, S.R. Lindemann, T. Ewing, D.R. Call, J.K. Frederickson, H.
5 Beyenal, Localized electron transfer rates and microelectrode-based enrichment of
6 microbial communities within a phototrophic microbial mat, Front. Microbiol. 5 (2014) 11.
7 58. R. D'Amore, U.Z. Ijaz, M. Schirmer, J.G. Kenny, R. Gregory, A.C. Darby, M. Shakya, M.
8 Podar, C. Quince, N. Hall, A comprehensive benchmarking study of protocols and
9 sequencing platforms for 16S rRNA community profiling, BMC Genomics 17 (2016) 55.
10 59. E.B. Fichot, R.S. Norman, Microbial phylogenetic profiling with the Pacific Biosciences
11 sequencing platform, Microbiome 1 (2013) 10.
12 60. J.J. Mosher, E.L. Bernberg, O. Shevchenko, J. Kan, L.A. Kaplan, Efficacy of a 3rd
13 generation high-throughput sequencing platform for analyses of 16S rRNA genes from
14 environmental samples, J. Microbiol. Methods 95 (2013) 175-181.
15 61. J.J. Mosher, B. Bowman, E.L. Bernberg, O. Shevchenko, J. Kan, J. Korlach, L.A. Kaplan,
16 Improved performance of the PacBio SMRT technology for 16S rDNA sequencing, J.
17 Microbiol. Methods 104 (2014) 59-60.
18 62. P.D. Schloss, M.L. Jenior, C.C. Koumpouras, S.L. Westcott, S.K. Highlander, Sequencing
19 16S rRNA gene fragments using the PacBio SMRT DNA sequencing system, PeerJ 4
20 (2016) e1869.
21 63. E. Singer, B. Bushnell, D. Coleman-Derr, B. Bowman, R.M. Bowers, A. Levy, E.A. Gies,
22 J.F. Cheng, A. Copeland, H.P. Klenk, S.J. Hallam, P. Hugenholtz, S.G. Tringe, T. Woyke,

1 High-resolution phylogenetic microbial community profiling, ISME J. 10 (2016) 2020-
2 2032.
3 64. J. Wagner, P. Coupland, H.P. Browne, T.D. Lawley, S.C. Francis, J. Parkhill, Evaluation of
4 PacBio sequencing for full-length bacterial 16S rRNA gene classification, BMC Microbiol.
5 16 (2016) 274.
6 65. V. Kunin, A. Engelbrektson, H. Ochman, P. Hugenholtz, Wrinkles in the rare biosphere:
7 pyrosequencing errors can lead to artificial inflation of diversity estimates, Environ.
8 Microbiol. 12 (2010) 118-123.
9 66. C. Lee, C. Grasso, M.F. Sharlow, Multiple sequence alignment using partial order graphs,
10 Bioinformatics 18 (2002) 452-464.
11 67. P.J. McMurdie, S. Holmes, Waste not, want not: why rarefying microbiome data is
12 inadmissible, PLoS Comput. Biol. 10 (2014) e1003531.
13 68. N.T. Ho, F. Li, S. Wang, L. Kuhn, metamicrobiomeR: an R package for analysis of
14 microbiome relative abundance data using zero-inflated beta GAMLSS and meta-analysis
15 across studies using random effects models, BMC Bioinformatics 20 (2019) 188.
16 69. P.J. McMurdie, S. Holmes, phyloseq: an R package for reproducible interactive analysis
17 and graphics of microbiome census data, PLoS One 8 (2013) e61217.
18 70. P.J. McMurdie, S. Holmes, Shiny-phyloseq: Web application for interactive microbiome
19 analysis with provenance tracking, Bioinformatics 31 (2015) 282-283.
20 71. A.M. Eren, L. Maignien, W.J. Sul, L.G. Murphy, S.L. Grim, H.G. Morrison, M.L. Sogin,
21 Oligotyping: Differentiating between closely related microbial taxa using 16S rRNA gene
22 data, Methods Ecol. Evol. 4 (2013).

1 72. B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J. Johnson, S.P. Holmes, DADA2:
2 High-resolution sample inference from Illumina amplicon data, Nat. Methods 13 (2016)
3 581-583.
4 73. A.M. Eren, O.C. Esen, C. Quince, J.H. Vineis, H.G. Morrison, M.L. Sogin, T.O. Delmont,
5 Anvi'o: an advanced analysis and visualization platform for 'omics data, PeerJ 3 (2015)
6 e1319.
7 74. N. Norskov-Lauritsen, M. Kilian, Reclassification of actinomycetemcomitans,
8 Haemophilus aphrophilus, Haemophilus paraphrophilus and Haemophilus segnis as
9 Aggregatibacter actinomycetemcomitans gen. nov., comb. nov., Aggregatibacter
10 aphrophilus comb. nov. and Aggregatibacter segnis comb. nov., and emended description
11 of Aggregatibacter aphrophilus to include V factor-dependent and V factor-independent
12 isolates, Int. J. Syst. Evol. Microbiol. 56 (2006) 2135-2146.
13 75. X. Didelot, R. Bowden, D.J. Wilson, T.E.A. Peto, D.W. Crook, Transforming clinical
14 microbiology with bacterial genome sequencing, Nat. Rev. Genet. 13 (2012) 601-612.
15 76. X. Didelot, A.S. Walker, T.E. Peto, D.W. Crook, D.J. Wilson, Within-host evolution of
16 bacterial pathogens, Nat. Rev. Microbiol. 14 (2016) 150-162.
17 77. E. Klemm, G. Dougan, Advances in Understanding Bacterial Pathogenesis Gained from
18 Whole-Genome Sequencing and Phylogenetics, Cell Host Microbe 19 (2016) 599-610.
19 78. S.K. Sheppard, D.S. Guttman, J.R. Fitzgerald, Population genomics of bacterial host
20 adaptation, Nat. Rev. Genet. 19 (2018) 549-565.
21 79. P.E. Chen, B.J. Shapiro, The advent of genome-wide association studies for bacteria, Curr.
22 Opin. Microbiol. 25 (2015) 17-24.

1 80. J.A. Lees, S.D. Bentley, Bacterial GWAS: not just gilding the lily, Nat. Rev. Microbiol. 14
2 (2016) 406.
3 81. R.A. Power, J. Parkhill, T. de Oliveira, Microbial genome-wide association studies: lessons
4 from human GWAS, Nat. Rev. Genet. 18 (2017) 41.
5 82. E. Aun, A. Brauer, V. Kisand, T. Tenson, M. Remm, A k-mer-based method for the
6 identification of phenotype-associated genomic biomarkers and predicting phenotypes of
7 sequenced bacteria, PLoS Comput. Biol. 14 (2018) e1006434.
8 83. C. Collins, X. Didelot, A phylogenetic method to perform genome-wide association studies
9 in microbes that accounts for population structure and recombination, PLoS Comput. Biol.
10 14 (2018) e1005958.
11 84. M. Jaillard, L. Lima, M. Tournoud, P. Mahé, A. Van Belkum, V. Lacroix, L. Jacob, A fast
12 and agnostic method for bacterial genome-wide association studies: Bridging the gap
13 between k-mers and genetic events, PLoS Genet. 14 (2018) e1007758.
14 85. J.D. Caballero, S.T. Clark, B. Coburn, Y. Zhang, P.W. Wang, S.L. Donaldson, D.E. Tullis,
15 Y.C. Yau, V.J. Waters, D.M. Hwang, Selective sweeps and parallel pathoadaptation drive
16 Pseudomonas aeruginosa evolution in the cystic fibrosis lung, MBio 6 (2015) e00981-15.
17 86. E.V. Sokurenko, D.L. Hasty, D.E. Dykhuizen, Pathoadaptive mutations: gene loss and
18 variation in bacterial pathogens, Trends Microbiol 7 (1999) 191-195.
19 87. B.C. Young, C.H. Wu, N.C. Gordon, K. Cole, J.R. Price, E. Liu, A.E. Sheppard, S. Perera,
20 J. Charlesworth, T. Golubchik, Severe infections emerge from commensal bacteria by
21 adaptive evolution, eLife 6 (2017). doi: 10.7554/eLife.30637.

1 88. K. Shen, P. Antalis, J. Gladitz, S. Sayeed, A. Ahmed, S. Yu, J. Hayes, S. Johnson, B. Dice,
2 R. Dopico, Identification, distribution, and expression of novel genes in 10 clinical isolates
3 of nontypeable Haemophilus influenzae, Infect. Immun. 73 (2005) 3479-3491.
4 89. J.S. Hogg, F.Z. Hu, B. Janto, R. Boissy, J. Hayes, R. Keefe, J.C. Post, G.D. Ehrlich,
5 Characterization and modeling of the Haemophilus influenzae core and supragenomes
6 based on the complete genomic sequences of Rd and 12 clinical nontypeable strains,
7 Genome Biol. 8 (2007) R103.
8 90. K. Shen, P. Antalis, J. Gladitz, B. Dice, B. Janto, R. Keefe, J. Hayes, A. Ahmed, R. Dopico,
9 N. Ehrlich, J. Jocz, J. Kropp, S. Yu, L. Nistico, D.P. Greenberg, K. Barbadora, J.C. Post,
10 G.D. Ehrlich, F.Z. Hu, Characterization, Distribution and Expression of Novel Genes Among
11 Eight Clinical Isolates of Streptococcus pneumoniae, Infect. Immun. 74 (2006) 321-330.
12 91. N.L. Hiller, B. Janto, J.S. Hogg, R. Boissy, S. Yu, E. Powell, R. Keefe, N.E. Ehrlich, K.
13 Shen, J. Hayes, K. Barbadora, W. Klimke, D. Dernovoy, T. Tatusova, J. Parkhill, S.D.
14 Bentley, J.C. Post, G.D. Ehrlich, F.Z. Hu, Comparative Genomic Analyses of Seventeen
15 Streptococcus pneumoniae Strains: Insights into the Pneumococcal Supragenome, J.
16 Bacteriol. 189 (2007) 8186-8195.
17 92. C. Donati, N.L. Hiller, H. Tettelin, A. Muzzi, N.J. Croucher, S.V. Angiuoli, M. Oggioni, D.
18 Riley, A. Covacci, S.D. Bentley, M. Kilian, G.D. Ehrlich, F.Z. Hu, R. Rappuoli, E.R. Moxon,
19 V. Masignani, Structure and dynamics of the pan-genome of Streptococcus pneumoniae and
20 closely related species, Genome Biol. 11 (2010) R107.
21 93. R. Boissy, A. Ahmed, B. Janto, J. Earl, B.J. Hall, J. Hogg, G.D. Pusch, N.L. Hiller, E.
22 Powell, J. Hayes, S. Yu, S. Kathju, P. Stoodley, J.C. Post, G.D. Ehrlich, F.Z. Hu,
23 Comparative Supragenomic Analyses among the Pathogens Staphylococcus aureus,

1 Streptococcus pneumoniae, and Haemophilus influenzae Using a Modification of the Finite
2 Supragenome Model, BMC Genomics 12 (2011) 187.
3 94. J.J. Davie, J. Earl, S.P.W. de Vries, A. Ahmed, F.Z. Hu, H.J. Bootsma, K. Stol, P.W.M.
4 Hermans, R.M. Wadowsky, G.D. Ehrlich, J. Hays, A.A. Campagnari, Comparative Analysis
5 and Supragenome Modeling of Twelve Moraxella catarrhalis Clinical Isolates, BMC
6 Genomics 12 (2011) 70.
7 95. J.P. Earl, S.P.W. de Vries, A. Ahmed, E. Powell, M.P. Schultz, P.W.M. Hermans, D.J. Hill,
8 C.I. Constantinidou, F.Z. Hu, H.J. Bootsma, G.D. Ehrlich, Comparative Genomic Analyses
9 of the Moraxella Catarrhalis Serosensitive and Seroresistant Lineages Demonstrates Their
10 Independent Evolution, Genome Biol. Evol. 8 (2016) 955-974.
11 96. R.H. Rau, R.L. Marvig, G.D. Ehrlich, S. Molin, L. Jelsbak, Deletion and acquisition of
12 genomic content during early stage adaptation of Pseudomonas aeruginosa to a human host
13 environment, Environ. Microbiol. 14 (2012) 2200-2211.
14 97. A.H.Y. Lee, S. Flibotte, S. Sinha, A. Paiero, R.L. Ehrlich, S. Balashov, G.D. Ehrlich, J.E.A.
15 Zlosnik, J.C. Mell, C. Nislow, Phenotypic diversity and genotypic flexibility of Burkholderia
16 cenocepacia during long-term infection of cystic fibrosis lungs, Genome Res. 27 (2017) 1-14.
17 98. R.D. Fleischmann, M.D. Adams, O. White, R.A. Clayton, E.F. Kirkness, A.R. Kerlavage,
18 C.J. Bult, J.F. Tomb, B.A. Dougherty, J.M. Merrick, Whole-genome random sequencing
19 and assembly of Haemophilus influenzae Rd, Science 269 (1995) 496-512.
20 99. A. Harrison, D.W. Dyer, A. Gillaspy, W.C. Ray, R. Mungur, M.B. Carson, H. Zhong, J.
21 Gipson, M. Gipson, L.S. Johnson, Genomic sequence of an otitis media isolate of
22 nontypeable Haemophilus influenzae: comparative study with H. influenzae serotype d,
23 strain KW20, J. Bacteriol. 187 (2005) 4627-4636.

100. M. De Chiara, D. Hood, A. Muzzi, D.J. Pickard, T. Perkins, M. Pizza, G. Dougan, R. Rappuoli, E.R. Moxon, M. Soriani, Genome sequencing of disease and carriage isolates of nontypeable Haemophilus influenzae identifies discrete population structure, Proc. Natl. Acad. Sci. U S A 111 (2014) 5439-5444.

101. R.A. Eutsey, N.L. Hiller, J.P. Earl, B.A. Janto, M.E. Dahlgren, A. Ahmed, E. Powell, M.P. Schultz, J.R. Gilsdorf, L. Zhang, Design and validation of a supragenome array for determination of the genomic content of Haemophilus influenzae isolates, BMC Genomics 14 (2013) 484.

102. J.M. Kress-Bennett, N.L. Hiller, R.A. Eutsey, E. Powell, M.J. Longwell, T. Hillman, T. Blackwell, B. Byers, J.C. Mell, J.C. Post, Identification and characterization of msf, a novel virulence factor in Haemophilus influenzae, PloS One 11 (2016) e0149891.

103. J. Moleres, A. Fernández-Calvet, R.L. Ehrlich, S. Martí, L. Pérez-Regidor, B. Euba, I. Rodríguez-Arce, S. Balashov, E. Cuevas, J. Liñares, Antagonistic Pleiotropy in the Bifunctional Surface Protein FadL (OmpP1) during Adaptation of Haemophilus influenzae to Chronic Lung Infection Associated with Chronic Obstructive Pulmonary Disease, mBio 9 (2018) e01176-01118.

104. M.M. Pettigrew, C.P. Ahearn, J.F. Gent, Y. Kong, M.C. Gallo, J.B. Munro, A. D'Mello, S. Sethi, H. Tettelin, T.F. Murphy, Haemophilus influenzae genome evolution during persistence in the human airways in chronic obstructive pulmonary disease, Proc. Natl. Acad. Sci. U S A 115 (2018) E3256-E3265.

105. A. Aziz, D.S. Sarovich, E. Nosworthy, J. Beissbarth, A.B. Chang, H. Smith-Vaughan, E.P. Price, T.M. Harris, Molecular signatures of non-typeable Haemophilus influenzae lung

adaptation in paediatric chronic lung disease, bioRxiv (2019) 614214. https://doi.org/10.1101/614214.

106. N. Topaz, D. Boxrud, A.C. Retchless, M. Nichols, H.Y. Chang, F. Hu, X. Wang, BMScan: using whole genome similarity to rapidly and accurately identify bacterial meningitis causing species, BMC Infect. Dis. 18 (2018) 405.

107. M. Pinto, A. González-Díaz, M. Machado, S. Duarte, L. Vieira, J. Carriço, S. Marti, M. Bajanca-Lavado, J. Gomes, Insights into the population structure and pan-genome of Haemophilus influenzae, Infect. Genet. Evol. 67 (2019) 126-135.

108. D. Cleary, V. Devine, D. Morris, K. Osman, R. Gladstone, S. Bentley, S. Faust, S. Clarke, Pneumococcal vaccine impacts on the population genomics of non-typeable Haemophilus influenzae, Microb. Genom. 4 (2018). doi: 10.1099/mgen.0.000209.

109. R.D. Stewart, M.D. Auffret, A. Warr, A.H. Wiser, M.O. Press, K.W. Langford, I. Liachko, T.J. Snelling, R.J. Dewhurst, A.W. Walker, R. Roehe, M. Watson, Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen, Nat. Commun. 9 (2018) 870.

110. D.M. Bickhart, M. Watson, S. Koren, K. Panke-Buisse, L.M. Cersosimo, M.O. Press, C.P. Van Tassell, J.A.S. Van Kessel, B.J. Haley, S.W. Kim, C. Heiner, G. Suen, K. Bakshee, I. Liachko, S.T. Sullivan, P.R. Myer, J. Ghurye. M. Pop, P.J. Weimer, A.M. Phillippy, T.P.L. Smith, Assignment of virus and antimicrobial resistance genes to microbial hosts in a complex microbial community by combined long-read assembly and proximity ligation, Genome Biol. 153 (2019). doi: https://doi.org/10.1101/491175.

1 111. A.E.K. Dichosa, A.R. Daughton, K.G. Reitenga, M.S. Fitzsimons, C.S. Han, Capturing and
2 cultivating single bacterial cells in gel microdroplets to obtain near-complete genomes, Nat.
3 Protoc. 9 (2014) 610.
4 112. A.E.K. Dichosa, D.W. Davenport, P.E. Li, S.A. Ahmed, H. Daligault, C.D. Gleasner, Y.
5 Kunde, K. McMurry, C.C. Lo, K.G. Reitenga, A.R. Daughton, C. Shen, S. Frietze, D.
6 Wang, S.L. Johnson, D.I. Drautz-Moses, S. Schuster, P.S. Chain, C. Han, Draft Genome
7 Sequence of Thauera sp. Strain SWB20, Isolated from a Singapore Wastewater Treatment
8 Facility Using Gel Microdroplets, Genome Announc, 3 (2015) e00132-15.
9 113. R. Moxon, C. Bayliss, D. Hood, Bacterial contingency loci: the role of simple sequence
10 DNA repeats in bacterial adaptation, Annu. Rev. Genet. 40 (2006) 307-333.
11 114. P.M. Power, W.A. Sweetman, N.J. Gallacher, M.R. Woodhall, G.A. Kumar, E.R. Moxon,
12 D.W. Hood, Simple sequence repeats in *Haemophilus influenzae,* Infect. Genet. Evol. 9
13 (2009) 216-28.
14 115. L.V. Blakeway, A. Tan, I.R.A. Peak, K.L. Seib, Virulence determinants of Moraxella
15 catarrhalis: distribution and considerations for vaccine development, Microbiology 163
16 (2017) 1371-1384.
17 116. Y.N. Srikhanta, T.L. Maguire, K.J. Stacey, S.M. Grimmond, M.P. Jennings, The
18 phasevarion: a genetic system controlling coordinated, random switching of expression of
19 multiple genes, Proc. Natl. Acad. Sci. U S A 102 (2005) 5547-5551.
20 117. Y.N. Srikhanta, K.L. Fox, M.P. Jennings, The phasevarion: phase variation of type III
21 DNA methyltransferases controls coordinated switching in multiple genes, Nat. Rev.
22 Microbiol. 8 (2010) 196-206.

1 118. J.M. Atack, Y.N. Srikhanta, K.L. Fox, J.A. Jurcisek, K.L. Brockman, T.A. Clark, M.
2 Boitano, P.M. Power, F.E. Jen, A.G. McEwan, S.M. Grimmond, A.L. Smith, S.J.
3 Barenkamp, J. Korlach, L.O. Bakaletz, M.P. Jennings, A biphasic epigenetic switch
4 controls immunoevasion, virulence and niche adaptation in non-typeable Haemophilus
5 influenzae, Nat. Commun. 6 (2015) 7828.
6 119. L.V. Blakeway, P.M. Power, F.E. Jen, S.R. Worboys, M. Boitano, T.A. Clark, J. Korlach,
7 L.O. Bakaletz, M.P. Jennings, I.R. Peak, K.L. Seib, ModM DNA methyltransferase
8 methylome analysis reveals a potential role for Moraxella catarrhalis phasevarions in otitis
9 media, FASEB J. 28 (2014) 5197-5207.
10 120. A.S. Manso, M.H. Chai, J.M. Atack, L. Furi, M. De Ste Croix, R. Haigh, C. Trappetti, A.D.
11 Ogunniyi, L.K. Shewell, M. Boitano, T.A. Clark, J. Korlach, M. Blades, E. Mirkes, A.N.
12 Gorban, J.C. Paton, M.P. Jennings, M.R. Oggioni, A random six-phase switch regulates
13 pneumococcal virulence via global epigenetic changes, Nat. Commun. 5 (2014) 5055.
14 121. Capecchi B, Serruto D, Adu-Bobie J, Rappuoli R, Pizza M, The genome revolution in
15 vaccine research, Curr. Issues Mol. Biol. 6 (2004) 17-27.
16 122. F.V. Filipp, Precision medicine driven by cancer systems biology, Cancer Metastasis Rev.
17 36 (2017) 91-108.
18 123. G. Rossi, M. Ignatiadis, Promises and pitfalls of using liquid biopsy for precision medicine,
19 Cancer Res. 79 (2019) 2798-2804.
20 124. S. Morganti, P. Tarantino, E. Ferraro, P. D'Amico, G. Viale, D. Trapani, B.A. Duso, G.
21 Curigliano, Complexity of genome sequencing and reporting: Next generation sequencing
22 (NGS) technologies and implementation of precision medicine in real life, Crit. Rev.
23 Oncol. Hematol. 133 (2019) 171-182.

1 125. J.W. Leiding, M. Ballow, Precision medicine in the treatment of primary
2 immunodeficiency diseases, Curr. Opin. Allergy Clin. Immunol. 18 (2018) 159-166.
3 126. M. Skov, C.R. Hansen, T. Pressler, Cystic fibrosis - an example of personalized and
4 precision medicine, APMIS 127 (2019) 352-360.
5 127. M.W. Pletz, M. Bauer, A.A. Brakhage, One step closer to precision medicine for infectious
6 diseases, Lancet Infect. Dis. 19 (2019) 564-565.
7 128. C.N. Spaulding, R.D. Klein, H.L. Schreiber 4th, J.W. Janetka, S.J. Hultgren, Precision
8 antimicrobial therapeutics: The path of least resistance? NPJ Biofilms Microbiomes 4
9 (2018) 4.
10 129. M.R. Atreya, H.R. Wong, Precision medicine in pediatric sepsis, Curr. Opin. Pediatr. 31
11 (2019) 322-327.
12 130. S. Mahomed, N. Padayatchi, J. Singh, K. Naidoo, Precision medicine in resistant
13 tuberculosis: Treat the correct patient, at the correct time, with the correct drug, J. Infect.
14 78 (2019) 261-268.
15 131. Samuels TL, Yan JC, Khampang P, Dettmar PW, MacKinnon A, Hong W, Johnston N,
16 Papsin BC, Chun RH, McCormick ME, Kerschner JE. Association of gel-forming mucins
17 and aquaporin gene expression with hearing loss, effusion viscosity and inflammation in
18 otitis media with effusion. JAMA Otolaryngology-Head & Neck Surgery 2017;143(8):810-
19 817
20 132. Val S, Jeong S, Poley M, Krueger A, Nino G, Brown K, Preciado D. Purification and
21 characterization of microRNAs within middle ear fluid exosomes: implication in otitis
22 media pathophysiology. Pediatric Research 2017;81(6):911-918.

1 133. Samuels TL, Yan J, Khampang P, MacKinnon A, Hong W, Johnston N, Kerschner JE.
2 Association of microRNA 146 with middle ear hyperplasia in pediatric otitis media.
3 International Journal of Pediatric Otorhinolaryngology 2016;88(9);104-108.
4 134. C.G. Park, S. Kim, E.J. Lee, H.S. Jeon, S. Han, Clinical relevance of point mutations in the
5 23S rRNA gene in helicobacter pylori eradication: A prospective, observational study,
6 Medicine (Baltimore) 97 (2018) e11835.
7 135. C. de la Fuente-Nunez, M.D. Torres, F.J. Mojica, T.K. Lu, Next-generation precision
8 antimicrobials: Towards personalized treatment of infectious diseases, Curr. Opin.
9 Microbiol. 37 (2017) 95-102.
10 136. R.P. Venekamp, S.L. Sanders, P.P. Glasziou PP, C.C. Del Mar, M.M. Rovers, Antibiotics
11 for acute otitis media in children, Cochrane Database Syst. Rev. 6 (2015) CD000219.
12 137. A. Wafi, R. Mirnezami, Translational -omics: Future potential and current challenges in
13 precision medicine, Methods 151 (2018) 3-11.
14 138. N.A. Francis, C.A. Waldron, R. Cannings-John, E. Thomas-Jones, T. Winfield, V.
15 Shepherd, D. Harris, K. Hood, D. Fitzsimmons, A. Roberts, C.V. Powell, M. Gal, S. Jones,
16 C.C. Butler, Oral steroids for hearing loss associated with otitis media with effusion in
17 children aged 2-8 years: the OSTRICH RCT, Health Technol. Assess. 22 (2018) 1-114.
18 139. R.W. Ranakusuma, Y. Pitoyo, E.D. Safitri, S. Thorning, E.M. Beller, S. Sastroasmoro,
19 C.B. Del Mar, Systemic corticosteroids for acute otitis media in children, Cochrane
20 Database Syst. Rev. 3 (2018) CD012289.
21 140. J.A. Lewnard, P.A. Tähtinen, M.K. Laine, L. Lindholm, J. Jalava, P. Huovinen, M.
22 Lipsitch, A. Ruohola, Impact of Antimicrobial Treatment for Acute Otitis Media on

1 Carriage Dynamics of Penicillin-Susceptible and Penicillin-Nonsusceptible Streptococcus
2 pneumoniae, J. Infect. Dis. 218 (2018) 1356-1366.
3 141. A. Ruohola, M.K. Laine, P.A. Tähtinen, Effect of Antimicrobial Treatment on the
4 Resolution of Middle-Ear Effusion After Acute Otitis Media, J. Pediatric Infect. Dis. Soc. 7
5 (2018) 64-70.
6 142. A. Hoberman, J.L. Paradise, H.E. Rockette, D.H. Kearney, S. Bhatnagar, T.R. Shope, J.M.
7 Martin, M. Kurs-Lasky, S.J. Copelli, D.K. Colborn, S.L. Block, J.J. Labella, T.G. Lynch,
8 N.L. Cohen, M. Haralam, M.A. Pope, J.P. Nagg, M.D. Green, N. Shaikh, Shortened
9 Antimicrobial Treatment for Acute Otitis Media in Young Children, N. Engl. J. Med. 375
10 (2016) 2446-2456.
11 143. M. Te Molder, M.L. de Hoog, C.S. Uiterwaal, C.K. van der Ent, H.A. Smit, A.G. Schilder,
12 R.A. Damoiseaux, R.P. Venekamp, Antibiotic Treatment for First Episode of Acute Otitis
13 Media Is Not Associated with Future Recurrences, PLoS One 11 (2016) e0160560.
14 144. N. Shaikh, E.E. Dando, M.L. Dunleavy, D.L. Curran, J.M. Martin, A. Hoberman, K.J.
15 Smith, A Cost-Utility Analysis of 5 Strategies for the Management of Acute Otitis Media
16 in Children, J. Pediatr. 189 (2017) 54-60.e3.
17 145. G.K. Spurling, C.B. Del Mar, L. Dooley, R. Foxlee, R. Farley, Delayed antibiotic
18 prescriptions for respiratory infections, Cochrane Database Syst. Rev. 9 (2017) CD004417.
19 146. R. Yang, V. Sabharwal, O.S. Okonkwo, N. Shlykova, R. Tong, L.Y. Lin, W. Wang, S.
20 Guo, J.J. Rosowski, S.I. Pelton, D.S. Kohane, Treatment of otitis media by transtympanic
21 delivery of antibiotics, Sci. Transl. Med. 8 (2016) 356ra120.

1 147. A. Kurabi, K.A. Beasley, L. Chang, J. McCann, K. Pak, A.F. Ryan, Peptides actively
2 transported across the tympanic membrane: Functional and structural properties, PLoS One
3 12 (2017) e0172158.
4 148. A. Kurabi, D. Schaerer, L. Chang, K. Pak, A.F. Ryan, Optimisation of peptides that
5 actively cross the tympanic membrane by random amino acid extension: a phage display
6 study, J. Drug Target 26 (2018) 127-134.
7 149. A. Kurabi, D. Schaerer, V. Noack, M. Bernhardt, K. Pak, T. Alexander, J. Husseman, Q.
8 Nguyen, J.P. Harris, A.F. Ryan, Active Transport of Peptides Across the Intact Human
9 Tympanic Membrane, Sci. Rep. 8 (2018) 11815.
10 150. Xu Q, Gill S, Xu L, Gonzalez E, Pichichero ME. Comparative Analysis of Microbiome in
11 Nasopharynx and Middle Ear in Young Children With Acute otitis Media. Front Genet.
12 2019 Nov 19;10:1176.
13 151. T. Mrkvan, S.I. Pelton, J. Ruiz-Guinazu, A.A. Palmu, D. Borys, Effectiveness and impact
14 of the 10-valent pneumococcal conjugate vaccine, PHiD-CV: review of clinical trials and
15 post-marketing experience, Expert Rev. Vaccines 17 (2018) 797-818.
16 152. X. Zhou, C. de Luise, M. Gaffney, C.W. Burt, D.A. Scott, N. Gatto, K.J. Center, National
17 impact of 13-valent pneumococcal conjugate vaccine on ambulatory care visits for otitis
18 media in children under 5 years in the United States, Int. J. Pediatr. Otorhinolaryngol. 119
19 (2019) 96-102.
20 153. A.C. Fortanier, R.P. Venekamp, C.W. Boonacker, E. hak, A.G. Schilder, E.A. Sanders,
21 R.A. Damoiseaux, Pneumococcal conjugate vaccines for preventing acute otitis media in
22 children, Cochrane Database Syst. Rev. 5 (2019) CD001480.

154. M. Pichichero, R. Kaur, D.A. Scott, W.C. Gruber, J. Trammel, A. Almudevar, K.J. Center, Effectiveness of 13-valent pneumococcal conjugate vaccination for protection against acute otitis media caused by Streptococcus pneumoniae in healthy young children: a prospective observational study, Lancet Child Adolesc. Health 2 (2018) 561-568.
155. R. Dagan, S. Pelton, L. Bakaletz, R. Cohen, Prevention of early episodes of otitis media by pneumococcal vaccines might reduce progression to complex disease, Lancet Infect. Dis. 16 (2016) 480-492.
156. R. Dagan, S. Pelton, L. Bakaletz, R. Cohen, First Otitis Media and Pneumococcal Conjugate Vaccine Serotypes in Infants, Pediatr. Infect. Dis. J. 37 (2018) e351-e352.
157. R. Kaur, J.R. Casey, M.E. Pichichero, Emerging Streptococcus pneumoniae Strains Colonizing the Nasopharynx in Children After 13-valent Pneumococcal Conjugate Vaccination in Comparison to the 7-valent Era, 2006-2015, Pediatr. Infect. Dis. J. 35 (2016) 901-906.
158. D. Greenberg, P.A. Hoover, T. Vesikari, C. Peltier, D.C. Hurley, R.D. McFetridge, M. Dallas, J. Hartzel, R.D. Marchese, B.G. Coller, J.E. Stek, C. Abeygunawardana, M.A. Winters, J.E. MacNair, N.S. Pujar, L. Musey, Safety and immunogenicity of 15-valent pneumococcal conjugate vaccine (PCV15) in healthy infants, Vaccine 36 (2018) 6883-6891.
159. H.L. Stacey, J. Rosen, J.T. Peterson, A. Williams-Diaz, V. Gakhar, T.M. Sterling, C.J. Acosta, K.M. Nolan, J. Li, A. Pedley, P. Benner, C. Abeygunawardana, M. Kosinski, W.J. Smith, H. Pujar, L.K. Musey, Safety and immunogenicity of 15-valent pneumococcal conjugate vaccine (PCV-15) compared to PCV-13 in healthy older adults. Hum. Vaccin. Immunother. 15 (2019) 530-539.

160. J.T. Peterson, H.L. Stacey, J.E. MacNair, J. Li, J.S. Hartzel, T.M. Sterling, P. Benner, G.M. Tamms, L.K. Musey, Safety and immunogenicity of 15-valent pneumococcal conjugate vaccine compared to 13-valent pneumococcal conjugate vaccine in adults ≥65 years of age previously vaccinated with 23-valent pneumococcal polysaccharide vaccine, Hum. Vaccin. Immunother. 15 (2019) 540-548.

161. M.E. Pichichero, Pneumococcal whole-cell and protein-based vaccines: changing the paradigm, Expert Rev. Vaccines 16 (2017) 1181-1190.

162. M.R. Alderson, Status of research and development of pediatric vaccines for Streptococcus pneumoniae, Vaccine 34 (2016) 2959-2961.

163. G. Gabutti, C. Azzari, P. Bonanni, R. Prato, A.E. Tozzi, A. Zanetti, G. Zuccotti, Pertussis. Hum. Vaccin. Immunother. 11 (2015) 108-117.

164. E. Hoe, L.K. Boelsen, Z.Q. Toh, G.W. Sun, G.C. Koo, A. Balloch, R. Marimla, E.M. Dunne, L. Tikoduadua, F.M. Russell, C. Satzke, E.K. Mulholland, P.V. Licciardi, Reduced IL-17A Secretion Is Associated with High Levels of Pneumococcal Nasopharyngeal Carriage in Fijian Children, PLoS One 10 (2015) e0129199.

165. J.J. Campo, T.Q. Le, J.V. Pablo, C. Hung, A.A. Teng, H. Tettelin, A. Tate, W.P. Hanage, M.R. Alderson, X. Liang, R. Malley, M. Lipsitch, N.J. Croucher, Panproteome-wide analysis of antibody responses to whole cell pneumococcal vaccination, Elife (2018) 7. doi: 10.7554/eLife.37015.

166. K.L. Moffitt, P. Yadav, D.M. Weinberger, P.W. Anderson, R. Malley, Broad antibody and T cell reactivity induced by a pneumococcal whole-cell vaccine, Vaccine 30 (2012) 4316-4322.

1 167. S.C. David, Z. Laan, V. Minhas, A.Y. Chen, J. Davies, T.R. Hirst, S.R. McColl, M.
2 Alsharifi, J.C. Paton, Enhanced safety and immunogenicity of a pneumococcal surface
3 antigen A mutant whole-cell inactivated pneumococcal vaccine, Immunol. Cell Biol.
4 (2019). doi: 10.1111/imcb.12257.
5 168. M. Giufre, M. Fabiani, R. Cardines, F. Riccardo, M.G. Caporali, F. D'Ancona, P. Pezzotti,
6 M. Cerquetti, Increasing trend in invasive non-typeable Haemophilus influenzae disease
7 and molecular characterization of the isolates, Italy, 2012-2016, Vaccine 36 (2018) 6615-
8 6622.
9 169. S. Naito, N. Takeuchi, M. Ohkusu, A. Takahashi-Nakaguchi, H. Takahashi, N. Imuta, J.
10 Nishi, K. Shibayama, M. Matsuoka, Y. Sasaki, N. Ishiwada, Clinical and Bacteriologic
11 Analysis of Nontypeable Haemophilus influenzae Strains Isolated from Children with
12 Invasive Diseases in Japan from 2008 to 2015, J. Clin. Microbiol. 56 (2018). doi:
13 10.1128/JCM.00141-18.
14 170. M.M. Pettigrew, C.P. Ahearn, J.F. Gent, Y. Kong, M.C. Gallo, J.B. Munro, A. D'Mello, S.
15 Sethi, H. Tettelin, T.F. Murphy, Haemophilus influenzae genome evolution during
16 persistence in the human airways in chronic obstructive pulmonary disease, Proc. Natl.
17 Acad. Sci. U S A 115 (2018) E3256-E3265.
18 171. R. Prymula, P. Peeters, V. Chrobok, P. Kriz, E. Novakova, E. Kaliskova, I. Kohl, P.
19 Lommel, J. Poolman, J.P. Prieels, L. Schuerman, Pneumococcal capsular polysaccharides
20 conjugated to protein D for prevention of acute otitis media caused by both Streptococcus
21 pneumoniae and non-typable Haemophilus influenzae: a randomised double-blind efficacy
22 study, Lancet 367 (2006) 740-748.

1 172. C. Clarke, L.O. Bakaletz, J. Ruiz-Guinazu, D. Borys, T. Mrkvan, Impact of protein D-
2 containing pneumococcal conjugate vaccines on non-typeable Haemophilus influenzae
3 acute otitis media and carriage, Expert Rev. Vaccines 16 (2017) 1-14.
4 173. M.R. van den Bergh, J. Spijkerman, K.M. Swinnen, N.A. Francois, T.G. Pascal, D. Borys,
5 L. Schuerman, E.P. Ijzerman, J.P. Bruin, A. van der Ende, R.H. Veenhoven, E.A. Sanders,
6 Effects of the 10-valent pneumococcal nontypeable Haemophilus influenzae protein D-
7 conjugate vaccine on nasopharyngeal bacterial colonization in young children: a
8 randomized controlled trial, Clin. Infect. Dis. 56 (2013) e30-39.
9 174. F. Jalalvand, K. Riesbeck, Update on non-typeable Haemophilus influenzae-mediated
10 disease and vaccine development, Expert Rev. Vaccines 17 (2018) 503-512.
11 175. L.A. Novotny, K.L. Brockman, E.M. Mokrzan, J.A. Jurcisek, L.O. Bakaletz, Biofilm
12 biology and vaccine strategies for otitis media due to nontypeable Haemophilus influenzae,
13 J. Pediatr. Infect. Dis. 14 (2019) 69-77.
14 176. M.N. Khan, D. Ren, R. Kaur, S. Basha, R. Zagursky, M.E. Pichichero, Developing a
15 vaccine to prevent otitis media caused by nontypeable Haemophilus influenzae, Expert
16 Rev. Vaccines 15 (2016) 863-878.
17 177. G. Leroux-Roels, P. Van Damme, W. Haazen, S. Shakib, M. Caubet, E. Aris, J.M.
18 Devaster, M. Peeters, Phase I, randomized, observer-blind, placebo-controlled studies to
19 evaluate the safety, reactogenicity and immunogenicity of an investigational non-typeable
20 Haemophilus influenzae (NTHi) protein vaccine in adults, Vaccine 34 (2016) 3156-3163.
21 178. J. Berglund, P. Vink, F. Tavares Da Silva, P. Lestrate, D. Boutriau, Safety,
22 immunogenicity, and antibody persistence following an investigational Streptococcus

pneumoniae and Haemophilus influenzae triple-protein vaccine in a phase 1 randomized controlled study in healthy adults, Clin. Vaccine Immunol. 21 (2014) 56-65.

179. P. Van Damme, G. Leroux-Roels, C. Vandermeulen, I. De Ryck, A. Tasciotti, M. Dozot, L. Moraschini, M. Testa, A.K. Arora, Safety and immunogenicity of non-typeable Haemophilus influenzae-Moraxella catarrhalis vaccine, Vaccine 37 (2019) 3113-3122.

180. M.K. Tandon, M. Phillips, G. Waterer, M. Dunkley, P. Comans, R. Clancy, Oral immunotherapy with inactivated nontypeable Haemophilus influenzae reduces severity of acute exacerbations in severe COPD, Chest 137 (2010) 805-811.

181. R.L. Clancy, M.L. Dunkley, J. Sockler, C.F. McDonald, Multi-site placebo-controlled randomised clinical trial to assess protection following oral immunisation with inactivated non-typeable Haemophilus influenzae in chronic obstructive pulmonary disease, Intern. Med. J. 46 (2016) 684-693.

182. E. Teo, K. Lockhart, S.N. Purchuri, J. Pushparajah, A.W. Cripps, M.L. van Driel, Haemophilus influenzae oral vaccination for preventing acute exacerbations of chronic bronchitis and chronic obstructive pulmonary disease, Cochrane Database Syst. Rev. 6 (2017) CD010010.

183. L.A. Novotny, J.D. Clements, S.D. Goodman, L.O. Bakaletz, Transcutaneous Immunization with a Band-Aid Prevents Experimental Otitis Media in a Polymicrobial Model, Clin. Vaccine Immunol. 24 (2017) e00563.

184. L.V. Michel, R. Kaur, M. Zavorin, K. Pryharski, M.N. Khan, C. LaClair, M. O'Neil, Q. Xu, M.E. Pichichero, Intranasal coinfection model allows for assessment of protein vaccines against nontypeable Haemophilus influenzae in mice, J. Med. Microbiol. 67 (2018) 1527-1532.

185. L.E. Winter, S.J. Barenkamp, Immunogenicity of Nontypeable Haemophilus influenzae Outer Membrane Vesicles and Protective Ability in the Chinchilla Model of Otitis Media, Clin. Vaccine Immunol. 24 (2017).
186. A.C. Perez, T.F. Murphy, A Moraxella catarrhalis vaccine to protect against otitis media and exacerbations of COPD: An update on current progress and challenges, Hum. Vaccin. Immunother. 13 (2017) 2322-2331.
187. D. Ren, M.E. Pichichero, Vaccine targets against Moraxella catarrhalis, Expert Opin. Ther. Targets 20 (2016) 19-33.
188. M.E. Pichichero, J.R. Casey, A. Almudevar, Nonprotective responses to pediatric vaccines occur in children who are otitis prone, Pediatr. Infect. Dis. J. 32 (2013) 1163-1168.
189. M.E. Pichichero, Ten-Year Study of the Stringently Defined Otitis-prone Child in Rochester, NY, Pediatr. Infect. Dis. J. 35 (2016) 1033-1039.
190. S. Basha, M.E. Pichichero, Poor memory B cell generation contributes to non-protective responses to DTaP vaccine antigens in otitis-prone children, Clin. Exp. Immunol. 182 (2015) 314-322.
191. S.K. Sharma, J.R. Casey, M.E. Pichichero, Reduced memory CD4+ T-cell generation in the circulation of young children may contribute to the otitis-prone condition, J. Infect. Dis. 204 (2011) 645-653.
192. S.K. Sharma, J.R. Casey, M.E. Pichichero, Reduced serum IgG responses to pneumococcal antigens in otitis-prone children may be due to poor memory B-cell generation, J. Infect. Dis. 205 (2012) 1225-1229.

193. S. Basha, R. Kaur, T.R. Mosmann, M.E. Pichichero, Reduced T-Helper 17 Responses to Streptococcus pneumoniae in Infection-Prone Children Can Be Rescued by Addition of Innate Cytokines, J. Infect. Dis. 215 (2017) 1321-1330.

194. S. Basha, M.E. Pichichero, Decreased TNF family receptor expression on B-cells is associated with reduced humoral responses to Streptococcus pneumoniae infections in young children, Cell Immunol. 320 (2017) 11-19.

195. R. Kaur, J.R. Casey, M.E. Pichichero, Serum antibody response to five Streptococcus pneumoniae proteins during acute otitis media in otitis-prone and non-otitis-prone children, Pediatr. Infect. Dis. J. 30 (2011) 645-650.

196. Q. Xu, J.R. Casey, E. Newman, M.E. Pichichero, Otitis-Prone Children Have Immunologic Deficiencies in Naturally Acquired Nasopharyngeal Mucosal Antibody Response after Streptococcus pneumoniae Colonization, Pediatr. Infect. Dis. J. 35 (2016) 54-60.

197. D. Ren, T.F. Murphy, E.R. Lafontaine, M.E. Pichichero, Stringently Defined Otitis Prone Children Demonstrate Deficient Naturally Induced Mucosal Antibody Response to Moraxella catarrhalis Proteins, Front. Immunol. 8 (2017) 953.

198. D. Ren, A.L. Almudevar, T.F. Murphy, E.R. Lafontaine, A.A. Campagnari, N. Luke-Marshall, M.E. Pichichero, Serum antibody response to Moraxella catarrhalis proteins in stringently defined otitis prone children, Vaccine 37 (2017) 4637-4645.

199. A. Harrison, L.G. Dubois, L.S. John-Williams, M.A. Moseley, R.L. Hardison, D.R. Heimlich, A. Stoddard, J.E. Kerschner, S.S. Justice, J.W. Thompson, Comprehensive proteomic and metabolomic signatures of nontypeable Haemophilus influenzae-induced acute otitis media reveal bacterial aerobic respiration in an immunosuppressed environment, Mol. Cell. Proteomics, 15 (2016) 1117-1138.

200. B. Baddal, A. Muzzi, S. Censini, R.A. Calogero, G. Torricelli, S. Guidotti, A.R. Taddei, A. Covacci, M. Pizza, R. Rappuoli, Dual RNA-seq of nontypeable Haemophilus influenzae and host cell transcriptomes reveals novel insights into host-pathogen cross talk, MBio 6 (2015) e01765-15.

Legend to Figures

Fig. 1. Analysis of the Critical Assessment of Metagenome Interpretation community (>250 species) demonstrated the ability of MDx to identify and determine abundances of hundreds of species over >3 logs of variance. Reproduced from reference 52 which is an author owned paper

Fig. 2. Twenty bilateral microbiomes for chronic otitis media with effusion. The top 18 most prevalent species are shown as colored bars. Reproduced from reference 52 which is an author owned paper

Table 1. Genome-wide significant[a] loci from genome-wide association studies on otitis media

Ref	*dbSNP ID*	*Gene*	*Variant[b]*	*All MAF*	*NFE MAF*	*Lat MAF*	*Afr MAF*
11	rs2406176	*TMPRSS15*	c.1172-3049A>C	0.72	0.75	0.80	0.61
12,13	rs681343	*FUT2*[c]	c.249C>T (p.Tyr83=)	0.39	0.47	0.27	0.50
11	rs4825724	*C1GALT1C1—[]—CT47B1*	Intergenic	0.47	0.40	0.45	0.63
12,13	rs1978060[c]	*TBX1*	c.410+722A>G	0.64	0.61	0.46	0.75
12,13	rs2808290	*RAB18--[]—MKX*	Intergenic	0.38	0.49	0.40	0.18
12	rs7174062	*SPATA8--[]—ARRDC4*	Intergenic	0.78	0.72	0.81	0.89
12,13	rs4329147[d]	*HLA-DRB5--[]--HLA-DQA1*	Intergenic	0.84	0.85	0.88	0.83
12,13	rs8176643	*ABO* [c]	c.28+869delG	0.14	0.20	0.13	0.04
12,13	rs1802575	*EFEMP1*	c.*1004C>G	0.07	0.11	0.05	0.03
12,13	rs5829676	*NT5C1B-RDH14--[]--OSR1*	Intergenic	0.31	0.40	0.39	0.19
11	rs885932	*HLA-G—[]—HLA-H*	Intergenic	0.11	0.15	0.08	0.05
11	rs3821170	*ADAM23*	c.1852+432C>T	0.14	0.10	0.14	0.23
12,13	rs72931768	*FGF3--[]--ANO1*	Intergenic	0.10	0.12	0.11	0.05
12,13	rs35213789	*AUTS2*	c.310-96260C>T	0.25	0.26	0.19	0.23
12,13	rs114947103	*CDHR3*	c.1653+409T>C	0.21	0.17	0.17	0.27
12,13	rs13281988	*NIPAL2--[]--KCNS2*	Intergenic	0.31	0.29	0.33	0.32
14	rs10497394[c]	*CDCA7--[]—SP3*	Intergenic	0.23	0.26	0.34	0.12
12,13	rs67035515	*BSN*	c.225-5877_225-5874delTGAA	0.86	0.83	0.89	0.87
15	rs2932989[c]	*FNDC1*	~6kb from 3'UTR	0.88	0.87	0.91	0.87
12,13	rs73015965	*PLG*	c.112A>G (p.Lys38Glu)	0.003	0.005	0.003	0.001
12	rs151208372	*DCBLD2--[]--COL8A1*	Intergenic	0.09	0.08	0.15	0.13

Abbreviations: Ref, reference; MAF, minor allele frequencies from gnomAD database; NFE, non-Finnish European; Lat, Latino, Afr, African.

[a]$p<5x10^{-8}$; Variants are listed in order of increasing p-value.

[b]RefSeq accession numbers for coding and intronic variants: *TMPRSS15,* NM_002772.2; *FUT2,* NM_000511.5; *TBX1,* NM_005992.1; *ABO,* NM_020469.2; *EFEMP1,* NM_001039348.2; *ADAM23,* NM_003812.3; *AUTS2,* NM_015570.3; *CDHR3,* NM_152750.4; *BSN,* NM_003458.3; *PLG,* NM_000301.3.

[c]Only these variants/genes have been replicated in human subjects with otitis media using an independent dataset.

[d]This variant was associated with childhood ear infections in the initial GWAS; however reanalysis with conditioning for the *HLA-DRB1* Gln96 allele resulted in loss of association with the rs4329147 variant.

Table 2. Single-tissue expression quantitative trait loci (eQTL) in the Genotype-Tissue Expression (GTEx) database among genome-wide significant loci for otitis media

Variant (Chromosomal Band)	***Gene***	***Tissue***	***eQTL p-value***
rs681343 (19q13.33)	*FUT2*	Esophageal mucosa	$1.4x10^{-102}$
		Unexposed skin	$5.0x10^{-30}$
		Exposed skin	$1.5x10^{-25}$
		Transverse colon	$2.7x10^{-15}$
		Small intestine (ileum)	$2.9x10^{-10}$
		Lung	$7.4x10^{-06}$
	NTN5	Transverse colon	$2.7x10^{-15}$
		Unexposed skin	$2.0x10^{-13}$
	RASIP1	Esophageal mucosa	$4.6x10^{-16}$
		Exposed skin	$4.9x10^{-14}$
		Unexposed skin	$3.1x10^{-10}$
		Sigmoid colon	$2.0x10^{-07}$
	IZUMO1	Lung	$1.2x10^{-06}$
	FAM83E	Exposed skin	$3.8x10^{-06}$
rs1978060 (22q11.21)	*TBX1*	Prostate[b]	$5.2x10^{-12}$
rs2808290 (10p12.1)	*PTCHD3*	Testis[b]	$9.7x10^{-06}$
rs4329147 (6p21.32)	*HLA-DRB5*	Lung	$6.4x10^{-86}$
		Exposed skin	$7.0x10^{-76}$
		Esophageal mucosa	$3.8x10^{-73}$
		Unexposed skin	$1.2x10^{-64}$
		Stomach	$5.8x10^{-43}$
		Sigmoid colon	$2.6x10^{-33}$
		Vagina	$1.1x10^{-21}$
		Uterus	$1.8x10^{-19}$
		Small intestine (ileum)	$2.3x10^{-18}$
	HLA-DRB6	Exposed skin	$2.6x10^{-45}$
		Lung	$2.8x10^{-42}$
		Esophageal mucosa	$3.7x10^{-39}$
		Unexposed skin	$3.5x10^{-32}$
		Transverse colon	$6.8x10^{-28}$
		Stomach	$6.0x10^{-27}$
		Small intestine (ileum)	$9.9x10^{-16}$
		Sigmoid colon	$4.8x10^{-15}$
		Uterus	$1.2x10^{-10}$
		Vagina	$3.2x10^{-10}$
	HLA-DQB1	Lung	$1.2x10^{-22}$
		Esophageal mucosa	$1.1x10^{-20}$
		Exposed skin	$7.2x10^{-20}$
		Stomach	$6.5x10^{-15}$
		Transverse colon	$4.9x10^{-13}$
		Sigmoid colon	$2.0x10^{-11}$
		Small intestine (ileum)	$8.8x10^{-07}$
	HLA-DRB9	Lung	$1.0x10^{-18}$
		Small intestine (ileum)	$9.1x10^{-10}$
		Unexposed skin	$1.6x10^{-08}$
		Vagina	$1.3x10^{-05}$
		Exposed skin	$3.1x10^{-05}$

	HLA-DRB1	Lung	9.6×10^{-14}
		Exposed skin	1.9×10^{-12}
		Esophageal mucosa	2.2×10^{-09}
		Unexposed skin	2.9×10^{-07}
		Transverse colon	2.4×10^{-05}
	HLA-DQA2	Esophageal mucosa	2.4×10^{-13}
		Lung	5.7×10^{-12}
		Exposed skin	5.0×10^{-11}
		Transverse colon	1.1×10^{-09}
		Sigmoid colon	2.8×10^{-06}
		Unexposed skin	4.8×10^{-06}
		Stomach	5.5×10^{-06}
	PRRT1	Esophageal mucosa	6.1×10^{-11}
		Exposed skin	2.8×10^{-06}
		Transverse colon	2.1×10^{-05}
	HLA-DQB2	Lung	5.1×10^{-10}
		Transverse colon	4.4×10^{-09}
		Sigmoid colon	4.8×10^{-07}
		Stomach	1.8×10^{-06}
	HLA-DQA1	Lung	2.8×10^{-09}
		Exposed skin	7.3×10^{-06}
		Esophageal mucosa	2.5×10^{-05}
	NOTCH4	Unexposed skin	3.1×10^{-09}
	CYP21A2	Exposed skin	4.3×10^{-05}
		Unexposed skin	6.6×10^{-05}
	C4A	Exposed skin	5.1×10^{-05}
rs8176643 (9q34.2)	*ABO*	Esophageal mucosa	1.2×10^{-06}
	SURF1	Exposed skin	2.4×10^{-06}
		Transverse colon	2.8×10^{-06}
		Lung	1.1×10^{-05}
rs1802575 (2p16.1)	*EFEMP1*	Unexposed skin	8.0×10^{-07}
		Exposed skin	2.0×10^{-06}
rs885932 (6p22.1)	*ZFP57*	Exposed skin	6.0×10^{-35}
		Esophageal mucosa	1.6×10^{-32}
		Lung	1.4×10^{-29}
		Unexposed skin	1.2×10^{-28}
		Transverse colon	4.0×10^{-24}
		Small intestine (ileum)	5.7×10^{-13}
		Sigmoid colon	5.7×10^{-11}
		Vagina	2.4×10^{-08}
	HLA-K	Unexposed skin	2.4×10^{-15}
		Transverse colon	3.0×10^{-13}
		Esophageal mucosa	6.4×10^{-13}
		Lung	5.0×10^{-12}
		Small intestine (ileum)	2.0×10^{-06}
		Sigmoid colon	1.1×10^{-05}
	HLA-A	Exposed skin	1.4×10^{-13}
		Unexposed skin	3.4×10^{-12}
		Esophageal mucosa	6.0×10^{-11}
	TRIM31	Unexposed skin	8.2×10^{-12}
		Lung	1.1×10^{-06}

		Exposed skin	4.5×10^{-06}
	HLA-J	Lung	5.4×10^{-11}
		Exposed skin	3.9×10^{-09}
		Transverse colon	5.6×10^{-09}
		Unexposed skin	2.0×10^{-07}
		Esophageal mucosa	7.0×10^{-07}
		Sigmoid colon	1.4×10^{-06}
	HLA-V	Lung	2.7×10^{-08}
		Esophageal mucosa	7.4×10^{-05}
	HLA-T	Lung	9.0×10^{-05}
	HLA-U	Unexposed skin	2.1×10^{-05}
		Transverse colon	4.9×10^{-05}
		Esophageal mucosa	1.1×10^{-04}
	IER3	Esophageal mucosa	3.5×10^{-05}
	TRIM27	Exposed skin	1.1×10^{-04}
	RNF39	Exposed skin	1.2×10^{-04}
rs3821170 (2q33.3)	*LOC200726*	Heart (left ventricle)[b]	1.2×10^{-07}
rs10497394 (2q31.1)	*CDCA7*	Lung	9.0×10^{-05}
rs2932989 (6q25.3)	*FNDC1*	Esophageal muscle[b]	1.6×10^{-09}

[a]No significant single-tissue eQTLs in the GTEx database were identified for variants rs2406176 (21q21.1), rs4825724 (Xq24), rs7174062 (15q26.2), rs72931768 (11q13.3), rs35213789 (7q11.22), rs114947103 (7q22.3), rs13281988 (8q22.2), rs67035515 (3p21.31), rs73015965 (6q26) and rs151208372 (3q12.1). Variant rs5829676 (2p24.1) was not in the GTEx database.
[b]No other mucosal or epithelial tissue identified for single-tissue eQTLs.

Table 3. Otitis media (OM)-related genes: animal studies 2014-2019

Gene	*Protein product*	*Known role in OM*	*Ref.*
Bpifa1	Antibacterial	Deletion increases OM susceptibility	30
Casp4	Inflammasome effector	Inflammasome deficiency enhances OM	31
Ccl3	Chemotactic chemokine	Deletion enhances OM	32
Ccr2	Chemokine receptor	Deletion enhances OM	U
Cd44	Hyaluronin receptor, multifunctional	Deletion alters leukocyte recruitment in OM	33
Celsr1	Intercellular organization	Mutation causes OM	J
Coro1a	Lytic granule secretion	Mutation causes immunodeficiency and chronic OM	34
Dusp1	MAP kinase phosphatase	Inhibits MUC5AC production in response to NTHi	35
Enpp1	Transmembrane glycoprotein	Mutations cause OM with ectopic bone formation	36
Entpd1	ATP hydrolase	Predicted to increase chronic OM	A
Fbxo11	Transcription factor	Mutation causes a middle ear cavitation	37
Fli1	Transcription factor	Haploinsufficiency causes OM	38
Grn	Granulin growth factor precursor	Deletion reduces bacterial clearance in OM	39
Hbegf	Epithelial growth factor	Stimulates middle ear epithelial growth	40
Il1rn	IL1 receptor antagonist	Deletion exacerbates OM	U
Mapk9	JNK isoform 2, gene regulation	JNK2 mutation exacerbates OM	41
Mif	Pro-inflammatory mediator	Blocking MIF alleviates OM	42
Mkp1	MAPK phosphatase	Upregulation reduces MUC5AB production in OM	35
Ncf2	Superoxide source in neutrophils	Predicted to increase chronic OM	A
Nfkb1	Immune, growth signaling	Deletion enhances OM	U
Nisch	Cell signaling	Mutation causes OM	43
Nlrp3	Pathogen receptor, inflammasome	Inflammasome deficiency enhances OM	31
Nod1	Pathogen receptor	Mutation exacerbates OM	U
Nod2	Pathogen receptor	Mutation reduces bacterial clearance in OM	44
Pai1	Plasminogen inhibitor	Deletion exacerbates OM	45
Pax9	Growth-related transcription factor	Down-regulation leads to OM	46
Pycard	ASC inflammasome component	Deletion enhances OM	31
Ripk2	Pathogen receptor signaling	Deletion disables OM recovery	U
Spag6	Cilia gene	Deletion causes OM	47
Tbx1	Transcription factor	Mutation alters facial morphology, increases OM incidence	22
Tlr2	Pathogen receptor	Deletion exacerbates OM	48

Abbreviations: U, unpublished observations from D.G. Hur, B. Nuyen and A. Kurabi; J, phenotype from Jackson Labs; NTHi, non-typeable *Haemophilus influenzae;* A, ARCHS4 database

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