Predictive Medicine in Otitis Media

11

Raimundo José García-Matte, María José Herrera J, and Marcos V. Goycoolea

Introduction

Otitis media (OM) is a multifactorial, multifaceted disease that manifests as an inflammatory process in the middle ear (ME), mastoid, and Eustachian tube (ET). It is the result of prevailing aggression against the body's defense system, the degree of which depends on the interactions between these two opposing forces, i.e., the disease against the immunological defense system [1].

Selecting a rational therapy is essential to understand the anatomy, function, and pathology of the organs involved and the disease mechanisms. Understanding the mechanisms of disease allows for the most critical concept of timing. At the right time, insertion of a ventilating tube might be all that is needed, whereas, at the wrong time, a tube will not suffice. The ultimate goal is to prevent OM (e.g., through environmental factors, vaccines, innate immunity) and, if unsuccessful, treat it medically, reserving surgery only to restore function rather than to eradicate the disease [1].

Precision medicine (PM) is a relatively new concept to understand and face these disease mechanisms, which involves "treatments targeted to the needs of individual patients based on genetic, biomarker, phenotypic, or psychosocial characteristics that distinguish a given patient from other patients with similar clinical presentations. Inherent in this definition is the goal of improving clinical outcomes for individual patients and minimizing unnecessary side effects for those less likely to have a response to a particular treatment" [2].

R. J. García-Matte (✉)
Department of Otolaryngology, Clínica Universidad de Los Andes, and Hospital de la Florida, Santiago, Chile

M. J. Herrera J
Department of Otolaryngology, Clínica Universidad de Los Andes, and Hospital Luis Calvo Mackenna, Santiago, Chile

M. V. Goycoolea
Department of Otolaryngology, Clínica Universidad de Los Andes, Santiago, Chile

To provide each patient with the right treatment at the right dose and at the right time, considering all available information, is the philosophy behind PM. Predictive, preventive, personalized, and participatory (P4) are the four core values that guide its implementation and highlight the importance of overall individual wellness rather than the disease [3].

As "all the available information" can be a broad and unspecific term, it must be classified to retrieve such information in an orderly and helpful manner. Individual features are the result of the integration of internal and external information. Internal information corresponds to the genetic makeup or **genome**, and external information refers to the cumulative environmental exposures that individuals encounter throughout life or **exposome**. Interactions between the genome and the exposome result in each individual's phenotype "through **networks of biological pathways** that capture, transmit, and integrate signals and, finally, send instructions to the molecular machines that execute the functions of life" [3].

The same processes are valid in diseases, as the interactions between a genome and an exposome result in an observable clinical phenotype of a given disease. However, this concept of phenotype fell short under the PM prism as it did not acknowledge the mechanisms underlying the same phenotype with diagnostic and treatment implications. Accordingly, the phenotype definition was modified to "a single or combination of disease attributes that describe differences between individuals as they relate to clinically meaningful outcomes" [4], and the term "endophenotype" was resurfaced from the psychiatric literature, contracted to **endotype** and defined as "subtype of disease defined functionally and pathologically by a molecular mechanism or by treatment response" [5] Endotypes and phenotypes can be detected by appropriately validated **biomarkers**, which are objectively measurable indicators that can be evaluated to gauge a particular biological or pathogenic process or response to treatment (Biomarkers Definitions Working Group 2001) (see Fig. 11.1).

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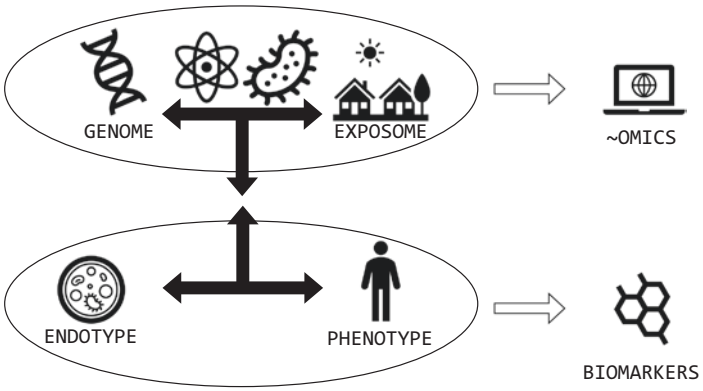

Fig. 11.1 Graphical representation of the different components involved in the precision medicine disease model

PM requires and stimulates the creation of tons of data. As more information is generated exponentially every year, new complex forms and fields of study have surged, which are collectively referred to as "omics." Each "-omics" studies an "-ome" (e.g., proteomics studies the proteome), focusing on the collective characterization and quantification of large numbers of biological molecules that translate into the structure, function, and dynamics of an organism (CDC NIOSH2). Genomics studies the genome and exposomics the exposome, but there are many interrelated omics in between, such as epigenomics, transcriptomics, and proteomics. Moreover, omics that could be considered a part of the exposome are studied apart, like microbiomics.

The PM approach has been gaining traction, and recent models on cronic rhinosinusitis (CRS) and obstructive sleep apnea syndrome (OSAS) have been developed in otolaryngology [6, 7]. Although there have been efforts in OM toward PM, highlighting the importance of patterns in acute otitis media (AOM) to approximate individualized care [8, 9], there are no current formal models for this clinical entity. Despite the lack of OM precision models, its research has not been away from omics, and, so, in the next part, we review the different omics approaches to the study of OM.

Otitis Media Omics

Genomics

As the oldest and the most advanced omics, most OM studies focus on this area. Early observations of the heritability of OM prompted additional and specific studies to understand its pathophysiology and, ultimately, find new strategies for its prevention and treatment. There are many different approaches to clinical human genetic studies. However, all share the definition of a phenotype, in this case, OM, to which genetic data are compared. Therefore, it is crucial to carefully characterize the phenotypes in genetic studies to avoid biases induced by an inconsistent or poorly defined disease status. Regretfully, an accurate diagnosis is a known problem in OM, with frequent misdiagnosis, as addressed by the latest clinical practice guidelines [10, 11], so studies must acknowledge and avoid this problem.

Heritability Studies

Heritability is the proportion of observed variation in a particular trait that can be attributed to inherited genetic factors in contrast to environmental ones. Several studies have confirmed evidence for a heritable component in OM, some of which are exposed below.

AOM: One cohort study of 1279 Finnish children and their parents showed that the heritability to recurrent acute otitis media (RAOM) was 38.5% [12].

Otitis media with effusion (OME): Twin and triplet studies have shown robust evidence of genetic susceptibility to OME. In the prospective twin and triplet Pittsburgh study, the estimated heritability of OM at the 2-year end point was 0.79 in girls and 0.64 in boys [13]. The correlation between twins' middle ear effusion (MEE) duration was significantly higher in monozygotic than in dizygotic twins. A second study that extended the observation period estimated the same heritability to be 0.72 [14].

Chronic suppurative otitis media (CSOM): A 2013 study conducted among Australian aboriginal communities found that 12% of young children presented with CSOM, with a 50% and 37% prevalence of OME and AOM, respectively [15].

Approaches to Studying the Genetics of a Common Disease

Two general approaches have been widely used to study the genetics of OM that differ in terms of dependence on a previous pathophysiological hypothesis. First, candidate gene association studies evaluate genetic variations in a determined number of genes (candidates) that are selected based on the current knowledge of the disease's pathogenesis. In contrast, genome-wide studies do not require a prior hypothesis as they explore the whole genome of the researched group either in families through linkage studies or in large case–control population cohorts known as genome-wide association studies (GWASs).

Candidate Gene Association Studies in OM

As stated above, in candidate gene association studies, the frequencies of a determined genetic marker are compared between subjects presenting the phenotype of interest (cases) and control subjects that do not present such a phenotype. The control subjects can be unrelated healthy controls (case–control study) or healthy relatives (family study).

These candidate genes were previously determined mainly through animal models (generally mice) and assumed biology. In concordance with known pathophysiology, most candidate gene-association studies on OM have focused on inflammation and immunity genes, as has been highlighted by a recent, thorough review [16] (see Table 11.1).

Linkage Studies in Families

Linkage genome-wide family studies examine DNA from all the available family members (or series of families) and compare it with the phenotype of interest to assess the statistical linkage between them. A few chromosomes were associated with OM through this method, but the complex genetics and causative factors of this condition make linkage studies a not-so-effective tool.

Genome-Wide Association Studies

A GWAS compares the general population's genetic profiles to individuals with phenotypic traits of interest to identify genetic variants that may be related to said traits. A catalog of the most published GWASs (https://www.ebi.ac.uk/gwas) shows that 5 studies and 11 associations are related to OM. The most recent study, an independent GWAS on European ancestry individuals, has studied genetic association with childhood ear infections and myringotomy in 121,810 and 89,227 subjects, respectively. They reported a significant ($p < 5 \times 10^{-8}$) association in 13 genomic regions for infections and in 1 for myringotomy. The strongest associations for ear infections were FUT2 and TBX1, with the latter also being related to myringotomy [17].

Table 11.1 The principal genes involved in OM pathogenesis according to the immune pathway and their roles

Immunity	Pathway	Gene	Gene function
Innate	Immune response and inflammation	MBL2	COMPLEMENT ACTIVATION
		TLR2	PRRR WIDE
		TLR4	PRRR LPS
		CD14	TLR4 CORECEPTOR
	Tissue clearance	SFPTA	GOBLET CELL SURFACTANT
		SFTPA1	GOBLET CELL SURFACTANT
		SFTPD	GOBLET CELL SURFACTANT
		SLC11A1	PATHOGEN CLEARANCE
		MUC2	GOBLET CELL MUCUS
		MUC5AC	GOBLET CELL MUCUS
		MUC5B	GOBLET CELL MUCUS
	Microbe adhesion	ABO	BACTERIAL ADHESION
		FUT2	BACTERIAL ADHESION
	Anatomy	TBX1	ET FUNCTION
Adaptative	Cytokines	IL6	CYTOKIN
		IL10	CYTOKIN
		IL1A	CYTOKIN
		IL1B	CYTOKIN
		TNFA	CYTOKIN
		IFNG	CYTOKIN
		TGFB1	ANTIGEN BINDING
	Transcriptional modulation	SMAD2	TRANSCRIPTION MODULATOR
		SMAD4	TRANSCRIPTION MODULATOR
	Extracellular matrix	A2ML1	PROTEASE INHIBITOR
		PAI1	PROTEASE REGULATOR
	Protein modification	CPT1A	FATTY ACID OXIDATION
		FBXO11	PROTEIN UBIQUITINATION
	Channel activity	SCN1B	ION CHANNEL BINDING

Epigenomics

Epigenomics refers to the study of epigenetics, the biochemical and functionally relevant changes of DNA without altering its sequence. Epigenetic mechanisms include DNA methylation, a gene expression suppressor, and histone modification, considered a protein production modifier. These processes can result from intrinsic regulation factors or extrinsic stimuli (exposures), such as tobacco smoke and viral infections, and are tissue-type- and time-specific, although hindering the epigenetic study.

The fibronectin type III domain-containing protein 1 gene (*FNDC1*), believed to be an activator of G protein, was significantly associated with AOM through a GWAS. In the same study, *FNDC1* variants were positively correlated with *FNDC1* expression levels but negatively correlated with the methylation status of *FNDC1*, indicating the epigenetic nature of the alteration [18].

Histone modifications, which control T-cell differentiation and memory formation, have also been associated with OM. One study showed that UTX, a histone demethylase, played a role in antibody generation in chronic infections and presented reduced expression in the immune cells of patients with Turner syndrome, a genetic disorder of partial

or complete loss of chromosome X in females [19]. Previous studies described a greater prevalence and longer duration of middle ear pathologies in Turner syndrome [16]. Although the immunological status has been studied in these patients, the results are contradictory.

Transcriptomics

The study of gene expression through RNA sequencing (RNA-Seq) is known as transcriptomics. As gene expression is highly dynamic and has multiple influences, its study must account for the specific conditions and cell/tissue types on which it is performed. RNA will not have the same status in a healthy tissue as in a sick tissue (what transcriptomics wants to portray). The transcriptome has mainly been described using microarrays, but with the recent technological advances that allow whole transcriptome analysis, next-generation RNA sequencing (RNA-Seq) studies have flourished. Given the transcriptome's variability, tissue selection is of the highest importance to accurately describe how the disease affects gene expression. Cells directly affected by disease must be studied, and, in the case of OM, transcriptomes from the middle ear epithelium (MEE) and middle ear effusions (MEEFs) have been described.

Two recent studies of MEE transcriptomes have been conducted. In one, RNA-Seq demonstrated differential gene expression in MEE cells between pediatric OME patients and children with a healthy ME. Genes with differences in expression were involved in inflammation, immune responses to bacterial OM pathogens, mucociliary clearance, regulation of proliferation and transformation, and auditory cell differentiation. Pathway analysis revealed an association with auditory development nicotine degradation genes [20]. In the other study, the RNA expression of six "candidate" molecules (tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1B, IL-6, IL-8, IL-10, and mucin 5B (MUC5B)) was assessed in MEE cell cultures from children with RAOM, OME, and no ME pathology and an immortalized MEE adult cell line (HMEEC-1). The reported expressions were frequently higher in all pediatric lines compared to the adult line, and, within the pediatric lines, OME lines were often more responsive than were RAOM lines. Noting the difference among age groups, the researchers concluded that pediatric MEE cultures are needed to improve the OM research [21].

Other human investigations regarding the OM transcriptome have focused on MEEFs, reporting a hypoxic inflammatory environment in the genome-wide transcript of white blood cells in the effusions of OME [22]. Furthermore, the study of MEEF microRNAs in exosomes as a distant cell genetic communication system opens the door to new pathways in the intricate OM pathophysiology [23]. MicroRNAs are small RNA molecules that can negatively control their target gene expression posttranscriptionally. There are two types relevant to OM, miR-378 and miR-146, associated with mucogenic responses and mucosal inflammation, respectively [24, 25].

Proteomics, Lipidomics, and Glycomics

Although genes contain "life's instructions," life's actual building blocks are three other molecule groups: proteins, lipids, and carbohydrates (glycans). Moreover, though disease research has mainly concentrated on genomics, new technologies, and more significant data processing capacity, studies on these molecules have gained terrain.

The omics approach to these molecules can be defined as the qualitative and quantitative analysis of the collection of protein, lipid, or glycan constituents in a biological sample. Polyacrylamide gel electrophoresis (PAGE), matrix-assisted laser desorption/ionization (MALDI), and liquid chromatography-tandem mass spectrometry (LC-MS) are some of the techniques used to study proteins and lipids. These methods provide measures of their types and abundance in biological samples.

So far, proteomics is the only one of these omics approaches with promising publications. In 2010, LC-MS allowed the identification of the MUC5B protein as the predominant mucin in mucoid MEEF [26]. A more extensive analysis of mucoid samples from MEEFs of children undergoing grommet surgery showed the presence of abundant innate immunity products, leukocytes, neutrophil extracellular traps (NETs), epithelial/glandular antimicrobial proteins, and mucins such as MUC5B [27].

Proteomic analysis has also differentiated MEEF compositions and biological signatures between mucoid and serous effusions. For example, mucoid MEEF showed a neutrophilic signature associated with MUC5B presence and extracellular DNA, confirming the implication of NETs in OME. On the other hand, serous MEEFs contained a much lower number of mucins and neutrophil markers but a higher amount of early innate immunity markers (complement and immunoglobulin proteins) and serum proteins. It can be suggested that this difference could be due to the MEE propensity to remodel in patients exhibiting mucoid MEEFs [24].

Microbiomics

Microbiomics, the characterization of the microbes that reside in an individual (or a determined anatomical site), is another omics type that has exponentially increased in the last two decades, especially since the National Institutes of

Health (NIH) launched the Human Microbiome Project (HMP) in 2008. There are ten times more bacteria than human cells in our bodies, so studying them seems appropriate. Moreover, the human microbiome is dynamic and changeable, making it an attractive target for therapy in highly diverse pathologies such as cancer, autism, inflammatory bowel disease, and infectious diseases.

The microbiota plays a beneficial role in the host's immunity. It protects the host from pathogenic bacterial colonization by different methods like competing for adherence to epithelial cells and by immune response regulation. There is also evidence of an interaction between the upper airway microbiome and the host's genes and innate mucosal immunity [28], with a role in the modulation of inflammatory processes [29].

The microbiome of the upper respiratory tract (URT) is present since birth, varies over time, and depends on several factors. The delivery and feeding modes are the most critical factors in the initial months of life. In addition, environmental and host factors can modify the microbiota from a balanced and resilient one (remains healthy even when exposed to stress) to an unstable community that predisposes the host to an infection or inflammation. Currently, the identified factors are host genetics, seasons, siblings, antibiotic use, daycare attendance, and tobacco exposure. There is also evidence of the beneficial role of vaccines and probiotics [30, 31].

Microbiome study was initially conducted through culture, a slow and inefficient method. However, the development of new sequencing techniques, such as 16S ribosomal RNA (16S rRNA), has led to more extensive population studies and the discovery of new microorganisms that did not appear in previous culture research.

OM microbiomics focuses on studying the middle ear (ME) and nasopharynx (NP) microbiota, communicated through the Eustachian tube (ET). According to the pathogen reservoir hypothesis (PRH), the adenoids, a lymphatic tissue mass in the NP, serve as a source of pathogens that can grow in this region and further spread to the respiratory system and ME, thus resulting in different infections like OM.

Healthy microbiome: Healthy (without OM) human NP and ME microbiomes are diverse [32] and change with age. Some bacterial genera, namely, *Corynebacterium* and *Dolosigranulum*, can be considered commensals of a healthy nasopharynx [33].

AOM

The ME: The primary bacterial pathogens contributing to AOM are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. However, newer studies suggest a pathogenic role for other taxa, such as *Turicella* (*T. otitidis*) and *Alloiococcus* (*A. otitidis*) [34].

Regarding these new bacteria, a 2020 review found that there was currently insufficient evidence available to determine whether these organisms are pathogens, commensals, or contribute indirectly to the pathogenesis of OM. They also remarked that their closest relatives are the NP commensals *Dolosigranulum* and *Corynebacterium*, thus proposing that they may have a commensal, homologous role in the ME, generated by developing specialized and highly different interactions with the dominant pathogens in their respective niches [33].

The NP: As discussed above, evidence is available on the commensal role of *Dolosigranulum* and *Corynebacterium* in the NP. They have been associated with a healthy status and a lower colonization rate of otopathogens such as *S. pneumoniae* [35]. Moreover, the risk of developing AOM after a viral URT infection has been related to the number of otopathogens colonizing the nasopharynx. Half of the children carrying all the three primary AOM pathogens develop AOM after a viral URT infection, compared to only 10% if none of these pathogens is present [36].

A different global microbiome profile and reduced alpha diversity were observed in the NP microbiome of otitis-prone children compared to healthy controls at 6 months of age. This difference was resolved when both groups were compared at 12 months of age. The same study showed that dysbiosis occurs in the NP microbiome of otitis-prone children at an early age, even when healthy [37].

OME

The ME: The dominant bacteria in the MEEFs of OME patients appears to be *H. influenzae*, as different systematic reviews have reported. The addition of PCR techniques increases the detection of known otopathogens (mostly *M. catarrhalis*). The newest sequencing techniques have added several bacteria to the OME microbiome, with *A. otitidis* standing out [34, 38, 39]. Many of these new MEEF bacteria are usually found in the external auditory canal (EAC) but not in the NP. By definition, OME occurs with an intact tympanic membrane (TM), which has led to consider the EAC microbiome's role in OM. This colonization could occur in previous asymptomatic perforations or through inflammation-mediated TM microlesions that allow bacterial translocation to the ME. Both theories are plausible as OME frequently occurs after a suppurated AOM and TM substance transport has been previously demonstrated [11, 40].

Biofilms also seem to play a role in OME pathogenesis, as a case–control study reported a significant difference regarding biofilm presence in the middle ear mucosa (84% in OME patients and 0% in controls (p-value <0.001*)) [41].

The NP

There are differences between the NP microbiome of patients with and without OME. In addition, the NP microbiome is less diverse in children suffering from OME than in controls [42, 43].

There is no strong correlation between the NP microbiome and the MEEFs in patients with OME. Several studies have shown that OME patients' MEEF microbiome is dissimilar to the NP with diversity analyses [44–46]. One case–control study showed that although the three main otopathogens were highly prevalent in the NP of children, only *S. pneumoniae* and *M. catarrhalis* were significantly related to OME [47].

The NP microbiome undoubtedly plays a role in OME, but it seems more complex than just a pathogen reservoir.

CSOM

The ME: There is scarce recent evidence regarding the ME microbiome and CSOM. A 2017 study characterized the microbiomes with cultures and 16S rRNA sequence of 155 subjects with no ME pathology, dry CSOM, and wet CSOM. The main findings were a significant change in the normal ME microbiota with age. The healthy ME and dry CSOM microbiome did not present significant differences but did differ from wet CSOM [48].

The penetration of microorganisms residing in the EAC into the ME has been considered in the pathogenesis of active inflammation in CSOM, but further studies are needed to define this aspect better.

The NP: The NP microbiome has not been studied in CSOM patients to the best of our knowledge.

Exposomics

The exposome is the sum of exposures that an individual encounters over a period of time. These may include nutrients, foods, toxins, stresses, exercise, vaccinations, medications, and other exposures. The exposome is highly dynamic and malleable over an individual's life.

The exposome attempts to measure, integrate, and interpret the complex exposures faced throughout life. Furthermore, it measures how these complex exposures impact our biological systems and provides a connection to health and disease outcomes.

High-resolution metabolomics (HRM), which uses gas or liquid chromatography with ultrahigh-accuracy mass spectrometry, is the most promising analytical technology for an exposome platform for precision medicine.

A recent meta-analysis of OM risk factors has shown that passive smoke and low social status significantly increased the risk of chronic otitis media/recurrent otitis media (COM/ROM) in children [49]. Similar results regarding social status were reported in a Latin American study of adults with CSOM, in which a higher socioeconomic status was found to be a protective factor [50].

Regarding air pollution and OM, it has been determined that an increase in the concentration of air particulate matter is directly associated with the incidence of AOM [51].

Other exposome risk factors for OM in children include day-care attendance for AOM [52–54] and the use of pacifiers for AOM and RAOM if used after 6 months of age [55, 56].

On the other hand, breastfeeding has been proven to protect children against AOM until 2 years of age, with a more significant effect observed in exclusive and more prolonged-duration breastfeeding [57].

Otitis Media Impact

While the omics approach can help us understand OM risks factors, disease severity, biological activity, and treatment response, an essential part of the PM domain is the personalized approach to the impact of the disease on the patient's life.

There are many instruments designed to measure this impact on the quality of life (QoL) of the patient or the family, with the Otitis Media 6 (OM-6) being one of the most used ones. This validated questionnaire consists of six items that assess physical suffering, hearing loss, speech impairment, emotional distress, activity limitations, and caregiver concerns [58].

As OM encompasses different diseases with their own clinical manifestations, it is only logical to think that the burden of each disease is also specific to it. AOM negatively affected the QoL of the caregivers and children in varying degrees. When AOM occurs in younger patients, the episode is more severe (as perceived by the parents), or in cases of RAOM, the caregivers' QoL is more affected [59]. Parents of children with RAOM also have a poorer QoL when compared with parents of children with OME and those without OM [60–62]. In COM/OME patients, it seems that hearing level improvement is correlated with QoL after surgery (tympanomastoidectomy/grommets) [60, 63].

Clinical Application

Current

PM can help us deliver better medicine to our patients, but there is a risk of getting lost within the vast amount of growing available information. As there is no point in collecting millions of gigabytes of OM omics data if we cannot use it with our patients, we need to develop PM application instruments. These instruments must allow us to apply the PM principles in our daily activity without

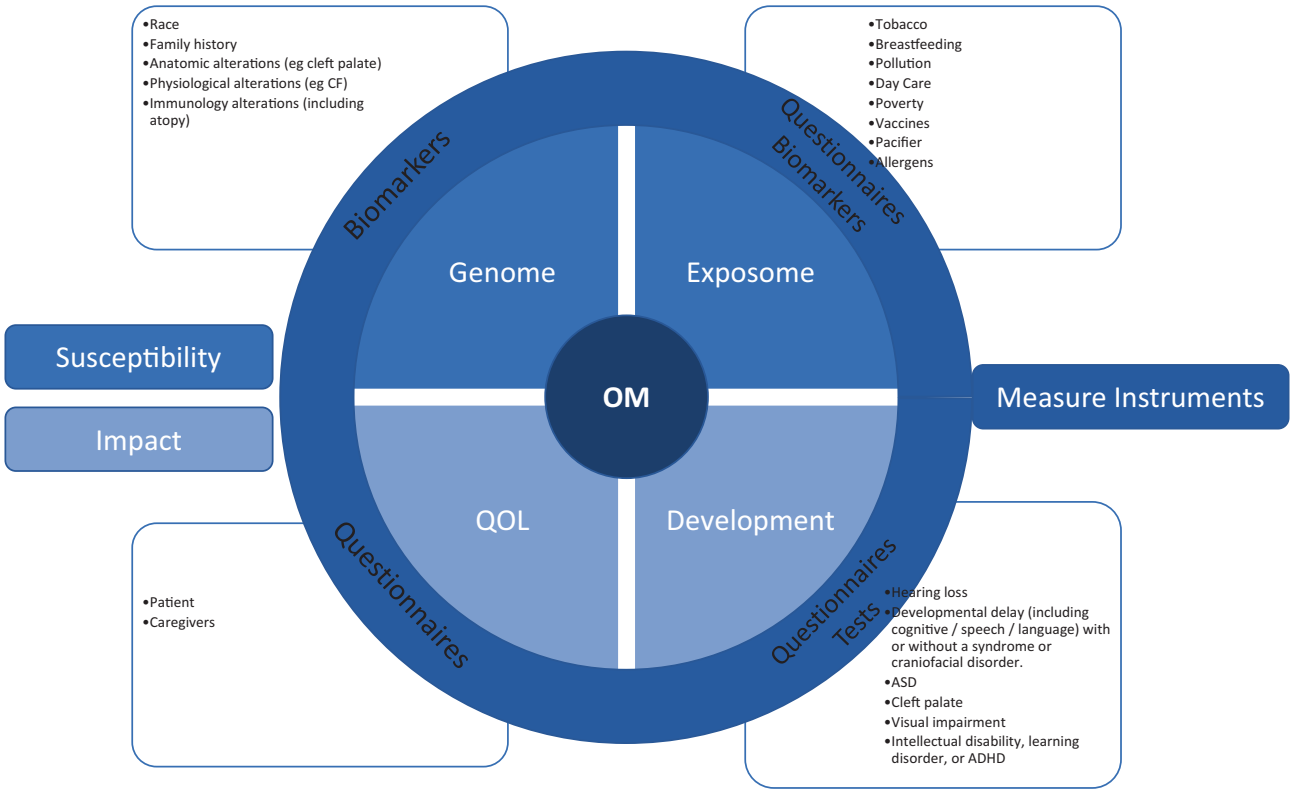

Fig. 11.2 The proposed model for clinical application of precision medicine on otitis media. *OM* otitis media, *QoL* quality of life, *ASD* autistic spectrum disorder, *ADHD* attention-deficit/hyperactivity disorder, *CF* cystic fibrosis

interfering with patient care. The model proposed by Ruben [8] is a step in the right direction under the PM prism but needs to be updated as it was created more than 10 years ago. Acknowledging the current information reviewed here, we propose this update, as can be seen in detail in Fig. 11.2.

Future

Using the PM approach in OM could help us identify different endotypes, with their respective biomarkers linked to the existing phenotypes. For example, RAOM, one of the OM phenotypes, has multiple causes, including anatomical alterations, NP otopathogens, and immune deficiencies. These causes could represent a different endotype with a specific biomarker waiting to be discovered. Taken to practice, when faced with a patient with RAOM, some tests should be conducted (biomarkers) to determine its endotype with the corresponding best treatment strategy. For example, children with NP-altered microbiome RAOM would have a different therapeutic approach than would children with innate immune deficiency RAOM. One could hypothesize that the former could be treated with probiotics, whereas the latter with biological therapy.

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