General introduction
Overview

The inner ear is an organ important for hearing and balance function. Inner ear disorders, for example hearing loss, tinnitus, and vestibular dysfunction, can have a negative impact on the quality of life of individuals. Hearing loss results in difficulty to communicate or participate in social activities, which in turn can lead to social isolation and depression. A condition associated with hearing loss is tinnitus, in which individuals experience ringing in the ears. Tinnitus can be experienced as distressing and can cause problems with sleeping and anxiety. Vestibular dysfunction can result in imbalance or coordination problems, which can negatively impact normal daily functioning. Inner ear disorders are age-related, and, therefore, negatively impact healthy aging. Besides individual impact, there are negative societal and economic impacts of inner ear disorders. Hearing loss causes difficulties in communication, and tinnitus can cause problems with concentration, both of which can lead to reduced job performance. Individuals with hearing loss require additional medical costs, including professional consultation, hearing aids or cochlear implants. The same is true for individuals that suffer from tinnitus who also are in need of professional consultation and treatment. Vestibular disorders and problems maintaining balance can increase the risk of accidents and falls, resulting in increase of healthcare costs, loss of job performance and increased morbidity and mortality. Current treatment options for inner ear disorders focus on rehabilitation and relief of symptoms. Restoration of hearing and balance disorders is unfortunately not possible yet. There is a need for new treatment options for inner ear disorders. Achieving this goal requires more knowledge about the underlying molecular mechanisms of inner ear disorders.

Inner ear disorders can be studied in both humans (Homo sapiens) or in model organisms, e.g. mouse (Mus musculus) or rat (Rattus norvegicus), with different models having their unique sets of benefits and limitations. Moreover, the inner ear is a small organ that contains structures important for both hearing (cochlea) and balance (vestibule) function. Most studies focus either on hearing or vestibular function; however, these two inner ear substructures show interesting overlap in cellular composition and physiology. This thesis aims to study the molecular mechanisms of inner ear disorders in both humans and animals, and provide novel starting points for both human and animal studies. Specifically, these novel insights might translate into novel treatment options for inner ear disorders. Furthermore, this thesis aims to study the auditory and vestibular comparatively to increase our understanding of inner ear disorders.

This introduction will give an overview of the prevalence and impact of inner ear disorders (starting at page 7). The anatomy, function and disease mechanisms of auditory and vestibular disorders is also presented (starting at page 7). This introduction will describe the main methods used to perform the studies described in this thesis (starting at page 14). Finally, an overview of the chapters of this thesis will be provided (starting at page 17).

Inner ear disorders: prevalence and impact

Hearing loss has become the world’s most prevalent sensory disorder, with a current estimated prevalence of 20%. Moreover, hearing loss accounts for approximately 1.3% of the total burden of disease in the elderly worldwide. The number of individuals with hearing loss will increase in the future because of population aging. The most common causes of hearing loss are noise-exposure, aging and exposure to ototoxic medication. Hearing loss can have a large negative impact on a patient’s quality of life, leading to social isolation and depression. More recent studies have shown that hearing loss is a risk factor for cognitive decline and dementia syndromes, such as Alzheimer’s disease.

Tinnitus is the perception of sound without the presence of a physical auditory stimulus. It is a hearing disorder with a large prevalence estimated between 4.1 and 37.2%, depending on the definition of tinnitus. Moreover, the prevalence of tinnitus increases with age. In the majority of the cases, tinnitus is subjective meaning it cannot be heard or measured by an examiner, and, therefore, diagnosing and phenotyping relies on subjective descriptions usually using questionnaires. In this thesis, “tinnitus” refers to subjective tinnitus. Hearing loss is the most important risk factor for tinnitus. Moreover, tinnitus is associated with depression and reduced health-related quality of life. It is important to note that only a small percentage of tinnitus patients have severe complaints that need consultation with an audiologist, otorhinolaryngologist or psychologist. This small group of patients with severe tinnitus utilize more healthcare, and therefore have higher overall healthcare costs compared to non-tinnitus patients. There are treatments available, such as cognitive behavioral therapy, that improve tinnitus severity scores. However, currently no evidence-based curative treatment options are available for tinnitus.

Compared to the auditory system, the vestibular system has gotten less attention from the academic community. However, the sense of balance is important for a person’s daily functioning. The prevalence of vestibular...
dysfunction increases with age and ranges from 18.2%, between 40 and 49 years, to 61.9%, in people aged over 65 years. Age-related vestibular dysfunction can result in balance problems and increase the risk of traumatic falls. Furthermore, vestibular dysfunction can cause insecurity while walking, which might cause social isolation and depression. Some reports show that vestibular dysfunction is correlated with cognitive decline. Assessment of vestibular function is challenging, and there is not a good understanding of the mechanisms involved in age-related vestibular dysfunction. Improved knowledge regarding the age-related processes underlying vestibular dysfunction will give leads on how to detect and treat dysfunction.

Anatomy, physiology, and pathophysiology of the auditory and vestibular system

The inner ear processes both sounds and movements of the head. To achieve these dual functions the inner ear consists of two functionally distinct end organs, namely the cochlea and the vestibule. Most auditory and vestibular disorders arise from dysfunction and/or loss of the sensorineural and/or metabolic structures within these end organs. Thus, in this section a brief overview of the anatomy and physiology of these structures and their involvement in pathophysiology will be provided.

The auditory system

The auditory system is historically divided into two systems, the peripheral auditory system, consisting of the pinna, ear canal, middle ear, inner ear and auditory nerve, and the central auditory system, which includes auditory nuclei in the brainstem and cerebral regions.

Sounds are collected by the pinna of the outer ear. The shape of the helix directs sounds towards the ear canal, which funnels the sound waves to the tympanic membrane (Figure 1). As soon as the waves hit the tympanic membrane, the membrane starts to vibrate. The tympanic membrane connects to a chain of three auditory ossicles: malleus, incus and stapes. The main function of these ossicles is to provide efficient transfer of vibrations from the tympanic membrane to the fluid-filled inner ear. The last of the middle ear bones, the stapes, is attached to the oval window of the cochlea.

The cochlea is tightly encased in the mastoid bone, and inside it are three fluid-filled chambers that spiral upward and inwards (Figure 2A). These three chambers are separated by two fine membranes: Reisner’s membrane and the basilar membrane. From top to bottom the chambers are named scala vestibuli, scala media and scala tympani. The oval window, to which the stapes is connected, lies at the end of the scala vestibuli, whereas the round window is an opening to the scala tympani. The basilar membrane runs from the base to the apex of the cochlea. The mechanical properties of the basilar membrane are important for decomposing sounds into specific frequencies. On top of the basilar membrane lies the sensorineural structure named the organ of Corti.

When sound waves reach the oval window, displacement of the fluid inside the cochlea occurs, leading to oscillation of the basilar membrane.
and generation of a traveling wave. A traveling wave, for example produced by a pure tone, will travel from the base towards the apex of the cochlea. The point at which the traveling wave reaches a maximum amplitude corresponds with the location on the basilar membrane specific to that frequency. After this maximum amplitude, the traveling wave fades out. The basilar membrane is able to register specific frequencies along the membrane: higher frequencies are registered at the base, whereas lower frequencies are registered towards the apex. This location specific registration of frequencies is called tonotopy. The auditory pathway is tonotopically organized from the cochlea all the way to the auditory cortex.

![Figure 2: Anatomical overview of the cochlea. A) The cochlea with cross sectional opening along the spiraling duct. The top of the cochlea is named the apex, which tonotopically is associated with low frequencies. The modiolus is the center column of the cochlea and contains the cell bodies of the spiral ganglion neurons (primary afferent auditory neurons). B) Detailed cross section of the cochlear duct. The sensorineural structures are placed on top of the basilar membrane, which include the cochlear hair cells. The spiral ganglion neurons relay information to the cochlear nucleus. The lateral wall of the cochlea contains the endolymph generating organ, the stria vascularis. Abbreviations: LF: low frequency; HF: high frequency.](image)

The cellular structures inside the scala media are essential for hearing function, and can be divided into a sensorineural substructure and a metabolic substructure (Figure 2B). The structure directly important for transmission of acoustic signals to the brain is the organ of Corti, which contains the apical stereocilia of the hair cell, and this deflection opens mechanotransduction channels. It is believed that the outer hair cells, via the protein prestin, mediate cochlear amplification by changing the length of the cell via the prestin protein. The inner hair cells send sound information to the brain via the release of the neurotransmitter glutamate when basilar membrane oscillations are detected. The stria vascularis is the metabolic structure of the cochlea, which lies against the outer wall of the scala media and is essential for maintaining the ionic concentration of the endolymph. The endolymph contains high K+ and low Na+ concentrations, which results in a positive endocochlear potential. This potential is essential for normal function of the hair cells. The perilymph has higher concentrations of Na+ compared to K+. After opening of the mechanotransduction channels, caused by deflection of the stereocilia, the positive endocochlear potential allows for rapid K+ influx into the IHCs. The rapid influx of K+ causes depolarization of the cell, which is followed by activation of voltage-gated Ca2+ channels, which in turn results in release of the neurotransmitter glutamate in the synaptic cleft.

On the neural side of the synaptic cleft, the dendrites of the spiral ganglion neurons (SGNs) are the first relay point for auditory information. SGNs are bipolar neurons with long projections on both sides of the soma (cell bodies). The soma are located in Rosenthal’s canal, which spirals along the modiolus of the cochlea. Originally, two types of spiral ganglion neurons were described, the type I spiral ganglion neurons, which innervate the inner hair cells, and the type II spiral ganglion neurons, which innervate outer hair cells. In mice, the vast majority of these spiral ganglion neurons are of the type I variant (90-95%), and each inner hair cell is innervated by 10-20 type I spiral ganglion neurons. Type I neurons generate action potentials in response to glutamate released by the inner hair cells and transmit these action potentials to the cochlear nucleus in the brainstem. The type II spiral ganglion neurons transmit signals from the outer hair cells towards the brainstem; however, since these axons are unmyelinated, therefore the contribution of their signaling to the temporal processing of the acoustic signals is not completely understood. Recent literature also suggests that type II spiral ganglion neurons might be involved in auditory nociception, which could be an evolutionary benefit to avoid painfully loud sounds.

Over the last decade much more has been discovered about these type I spiral ganglion neurons. Work by Charles Liberman already suggested that there are multiple subpopulations of the type I spiral ganglion neurons. This work showed that individual spiral ganglion neurons are functionally different in terms of their spontaneous activity, dynamic range and characteristic frequency. Functional heterogeneity of spiral ganglion neurons allows the spiral ganglion neurons to decode complex acoustic signals.
and encode these in terms of frequency, temporal, intensity information. Type I spiral ganglion neurons contain a diversity of ion channel types and neurotransmitter receptor distributions contribute to spiral ganglion neuron heterogeneity. Moreover, different spiral ganglion neurons are differently vulnerable for degradation and cell death in response to noise exposure and aging. Single cell RNA-sequencing has also recently identified three subtypes of type I spiral ganglion neurons based on their differential patterns of gene expression. These different subtypes likely have different physiological characteristics and might have different innervation patterns. Moreover, follow-up studies need to investigate whether specific subtypes of type I spiral ganglion neurons are more vulnerable to aging, auditory overexposure or ototoxic exposure.

The central auditory system (Figure 3) starts at the cochlear nuclei (dorsal and ventral), and these nuclei receive input from the spiral ganglion neurons. It is important to know that the auditory periphery and central auditory system are connected to each other bidirectionally, which allows for feedforward and feedback mechanisms to finetune the sense of hearing. Input received at the cochlear nuclei is forwarded to the cochlear nucleus. Hereafter, the responses are being forwarded to the superior olivary complex, inferior colliculus, medial geniculate body, and finally to the auditory cortex. Moreover, auditory and non-auditory networks are integrated and shape auditory signals that reach the auditory cortex. It is therefore important to consider that loss of auditory input from the cochlea causes changes in neuronal firing rate in higher order auditory regions of the brain, which might underlie the generation of tinnitus. Sounds in the general environment are complex, in which perceived sounds are often a combination of speech or target sounds and environmental noise, and, therefore, the auditory system needs to decode different temporal and intensity patterns and break these down into separate auditory information streams our brain understands. Temporal decoding is also important for detecting signals in the spatial domain, for the localization of sounds. Therefore, the auditory pathway consists of these different hubs where auditory signals are processed and decoded in order to understand the meaning of these signals.

Figure 3: Overview central auditory system. Along the auditory pathway signals are relayed and processed across different brain nuclei. Some nuclei receive bilateral input which allow comparison of time differences between the signals, which encodes spatial information. Along the auditory pathway neural signals from other brain areas are integrated.

Hearing loss is considered a complex disease caused by environmental factors and genetic factors that contribute to the risk and severity of the disease. Genetic forms of hearing loss are generally more severe but relatively rare in comparison with other forms of hearing loss. The most common form of acquired hearing loss in Western countries is age-related hearing loss or presbycusis, in which hearing starts to deteriorate with age at the higher frequencies. Based on early post-mortem histopathological studies, it is suggested that acquired hearing loss can be divided into four broad categories: 1) sensory hearing loss caused by loss of hair cells; 2) neural hearing loss resulting from the loss of spiral ganglion neurons; 3) metabolic hearing loss caused by deterioration of the stria vascularis; and 4) cochlear conductive hearing loss, which is caused by changes in mechanical properties of the cochlea and the basilar membrane. It is possible that patients fall into multiple categories at the same time. Moreover, based on the current diagnostic tools available it is often difficult to classify patients with hearing loss based on the Schuknecht classification.

Recent literature also describes an additional category of hearing loss, in which the synaptic contacts between hair cells and spiral ganglion neurons are lost, which leads to a form hearing loss without auditory threshold
shifts and is named ‘hidden hearing loss’. This form of hearing loss, or cochlear synaptopathy, which has only been diagnosed in animal studies and a few human post-mortem temporal bone studies, might be an early form of hearing loss. It is hypothesized that patients with hidden hearing loss might have trouble with speech in noise, which makes this pathology similar to Schuknecht’s neural hearing loss category. Pure tone audiometry is presumably normal in patients with hidden hearing loss and, therefore, detecting hidden hearing loss in humans remains difficult. Moreover, more has to be known about the prevalence, cause-effect relation and pathological mechanisms to really define hidden hearing loss as a form of hearing loss.

One of the potential consequences of hearing loss is tinnitus. The pathophysiology underlying tinnitus remains elusive. Several theories have been proposed to explain tinnitus. The central auditory gain theory states that reduced neural input from the cochlea results in compensatory neural gain in the central auditory areas, which in turn causes the perception of tinnitus. An alternative theory, namely reduced gain theory, suggests that tinnitus is caused by reduced gain and connectivity along the auditory pathway, which increases central noise. However, research in both humans and animals have not revealed which underlying mechanisms are occurring in tinnitus.

Several twin and family studies revealed that tinnitus is partly heritable, which suggests that genetic mechanisms are involved in tinnitus pathophysiology. Early genetic studies did not identify specific genes or pathways to be associated with tinnitus. Only recently, by utilizing the power of large population-based studies, the first genome-wide association studies on tinnitus have been published. This study identified genetic risk factors for tinnitus and also showed that tinnitus was genetically correlated with both hearing loss and neuropsychiatric disorders. Follow-up studies in both humans and animal models are needed to investigate which molecular mechanisms are affected by these genetic risk factors. More insight in the molecular mechanisms of tinnitus might yield drug targets for the treatment of tinnitus.

The vestibular system
The vestibule, the organ important for the sense of balance, is situated right next to the cochlea, and also resides in the inner ear. The vestibule contains five end organs to detect head movements, these include the three semicircular canals–horizontal, superior and posterior canals–and two otolith organs–the sacculus and utriculus. The semicircular canals are important for angular rotations, whereas the otolith organs are sensitive to linear accelerations. Interestingly, the sensory structures of the vestibular system show similarities with the sensorineural machinery of the cochlea, in particular with respect to the vestibular ganglion cells and hair cells. Vestibular end organs contain type I and type II hair cells that relay information to three types of vestibular neurons. These primary vestibular neurons show distinct morphological differences and are distributed differently across the vestibular neuroepithelium. Accelerations in the otolith structures are detected by the movement of otoconia, calcium carbonate crystal, over the neuroepithelium, which results in deflections of the hair cells and release of neurotransmitters, which in turn initiates generation of action potentials in the afferent vestibular ganglion cells. The semicircular canals do not contain otoconia. In the case of rotational acceleration the movement of the fluid relative to a gelatinous structure on top of the hair cells, named the cupula, causes deflection of the hairs which in turn generates action potentials. The signals that originate from the vestibular system are integrated with proprioceptive signals and input from the vestibular system to become the sense of balance.
The function of the vestibular end organs declines with increasing age; however, not all end organs show the same age-related rate of decline. The semicircular canals decline first and are followed by the saccus and utriculus. The physiological decline in the vestibular end organs contributes to loss of vestibular function associated with various vestibular disorders and dysfunctions. Vertigo is the sensation of motion in the absence of true motion. The most commonly diagnosed disorder in patients with vertigo is benign paroxysmal vertigo (BPPV), in which patients experience intermittent episodes of vertigo in specific positions.

Recent literature shows that dysfunction of the different functional structures of the inner ear might be related. This tight relation between the cochlea and the vestibule is exemplified by a disease that involves both systems, namely Menière’s disease. In Menière’s disease, patients experience episodes of vertigo that last from several minutes to several hours. Patients concurrently experience aural pressure, sensorineural hearing and tinnitus. Other patients report only vestibular or auditory symptoms, classified as atypical Menière’s disease. The believed pathophysiological mechanism that causes Menière’s disease is based on endolymphatic hydrops, in which there is a buildup of endolymph in the inner ear causing a temporary leak which results in the mixing of endolymph and perilymph. Age-related decline in vestibular function might be correlated with age-related hearing loss. In another study, hearing loss and dizziness seem to be associated. The exact mechanisms underlying the age-related decline of vestibular and cochlear function still remain elusive. However, there might be similar molecular mechanisms involved in vestibular and cochlear dysfunction.

Methodology used in this thesis: Clinical to pre-clinical and molecular to epidemiological approaches

Assessment of inner ear function in animals and humans
Inner ear function can be assessed both in humans and in animals. It is important to assess end organ function and to correlate function to other types of assessments, for example histological or genetic data. In humans, hearing function is typically assessed using pure-tone audiometry (PTA). To test the hearing function of animals a different technique is typically used, namely auditory brainstem responses (ABR), which assesses the evoked activity in the brainstem in response to pure tone or click sounds. Both PTA and ABR allow for reliable measurement of auditory thresholds, and, therefore, both are excellent methods to assess cochlear function.

Vestibular function is tested differently in humans and animals. Since the movements registered by the vestibule are complex, a battery of tests are needed to get a complete assessment of the vestibular system. In clinical settings, most tests assess the vestibular-ocular reflex (VOR) to examine whether the vestibular end organs function accordingly. For example, a delayed response of the VOR to a rotation of the head might indicate dysfunction of the horizontal canal. In animal studies both VOR and vestibular evoked potentials (VSEP). VSEP are the electrophysiological response to a change in linear acceleration of the head. The responses can be measured to assess whether the vestibule functions accordingly.

Besides behavioral and physiological testing of inner ear function, it is possible to assess changes in the structure or molecular composition of the inner ear using tissue samples of end organs. Animal models are typically used for these studies, since it is generally not possible to obtain inner ear tissue samples of humans. In excised tissues it is possible to assess changes in protein expression and the innervation of the inner ear end organs using molecular markers. These techniques are especially useful in disease models, for example to assess changes following acoustic overexposure. A combination of assessments of inner ear function will be used throughout this thesis to better understand the pathophysiolog of inner ear disorders.

Genome-wide association studies
A genome-wide association study (GWAS) is a powerful hypothesis-free method to detect genetic risk factors for diseases. It allows investigation of the genetics associated with a disease in a large human population. Results of GWAS, such as specific genes or pathways associated with a disease, are great starting points to further investigate the molecular mechanisms, for example using gene knockout models. The GWAS study design is an excellent approach to identify novel disease mechanisms for inner ear disorders.

GWAS focuses on identifying common variations (present in >1% of the population) in the genome and associating these variations with the disease or traits of interest. GWAS are typically case-control studies in which participants or patients are genotyped using microarrays. In microarrays, DNA libraries, obtained from samples, are hybridized with complementary probes to detect several hundred thousand genetic variations in the genome, or detect gene expression levels in a much lower number of genes. Using this method, variation in nucleotide pairs at specific locations (locus) in the DNA can be detected. A substitution of a single nucleotide at a locus is named single-nucleotide polymorphisms.
SNPs can either fall in DNA regions that code for a gene, or fall in the non-coding regions. Variations in both the coding and non-coding DNA can have consequences for normal cellular function, and might be involved in disease. Because of the high number of SNPs detected in microarrays, and, therefore, the high number of statistical comparisons, very large sample sizes, like population cohorts, are needed to have enough statistical power.

In recent years, several large general-population cohort studies have been initiated, including the Lifelines cohort study and the UK Biobank, both of which include over one hundred thousand participants from the general population. These studies collect information on demographics, diseases, lifestyle factors and also genotype these participants, and thereby allow researchers from around the world to perform studies otherwise not possible for individual research groups. The genetics of inner ear disorders have not been studied extensively in these population cohorts, and there are, therefore, interesting opportunities to identify novel disease mechanisms of these disorders.

**Next-generation sequencing**

Next-generation sequencing is a technique that allows dentification of the exact nucleotide sequence of DNA or RNA. RNA-sequencing (RNA-seq), or whole transcriptome sequencing (Figure 5B), reveals the quantity of all RNA transcripts in a tissue or cell at a moment in time, which allows for an unbiased analysis of the expression of genes. Since the molecular mechanisms of inner ear disorders, including changes in response to aging, are largely unknown, RNA-seq is an excellent technique to explore the pathophysiology of these disorders.

**Figure 5: Molecular analyses used: GWAS and RNA-sequencing.** A) A representation of a genome-wide association study. In a case-control study, single nucleotide polymorphisms are compared between cases and controls using a regression analysis. This analysis allows single nucleotide polymorphisms to be associated with the disease or trait. B) RNA-sequencing allows for the detection of gene expression, or mRNA transcript levels, at a specific time point in a tissue or cell. This technique allows quantification of gene expression levels of all expressed mRNA transcripts.

Maybe as important as next-generation sequencing is the development and improved accessibility of complex analyses of these large genetic datasets. Moreover, open access databases containing gene annotations and curated gene sets associated with specific biological mechanisms improves the understanding of the transcriptomic signal that is found in
these experiments. RNA-seq and bioinformatic analyses are now being used abundantly to investigate the biological mechanisms of development, aging and the effect of treatment or disease.

This thesis

This thesis aims to study the mechanisms underlying tinnitus, hearing loss and vestibular dysfunction. Specifically for tinnitus, the goal is to study disease mechanisms of tinnitus in the general population and to identify hypotheses for testing in both humans as well as animal models. Moreover, studies in this thesis simultaneously investigate changes in the vestibular and auditory end organs of the inner ear and aim to investigate the unique and shared mechanisms of dysfunction within the inner ear. Furthermore, the goal of this thesis is to translate findings from the animal studies to pre-clinical and clinical studies to work towards novel treatment options for inner ear disorders. The combined approaches presented in this thesis should be of interest to both basic scientists, including biologists and neuroscientists, and clinicians, including medical doctors, audiologists and psychologists.

This thesis consists of three parts. Part I includes chapters 2 to 4 and aims to characterize tinnitus in a general population cohort study and to identify pathophysiological pathways. Chapters 5 and 6 in part II of this thesis aims to identify new and common pathways that underlie age-related vestibular and auditory dysfunction. Part III contains chapters 7 and 8 and aims to identify the molecular mechanisms of acquired hearing loss in mouse models. The final chapter, chapter 9, presents a summary and implications of the results of the studies presented in this thesis. In summary, this thesis integrates clinical and preclinical data and diverse techniques and, in so doing, contributes to our knowledge of the molecular mechanisms involved in hearing loss, tinnitus and vestibular disorders. These insights are necessary to identify, develop, and bring to the clinic novel treatment options in the future.

Part I: Studies investigating the impact of tinnitus, disease associations and genetics using population-based studies.

Tinnitus has mainly been studied in clinical populations, which are often biased by more severe forms of tinnitus. General population studies allow investigation of broad aspects of disorders, including individuals with mild to severe forms of a disease. Since these cohort studies include very large amounts of participants and are broadly phenotyped, general population cohort studies are excellent opportunities to investigate these broad aspects of disorders. In chapter 2, self-rated health and predictors for self-rated health in tinnitus have been assessed. Besides identifying potential modifiable factors to improve self-rated health in tinnitus, these predictors could also aid in identifying common neurobiological pathways that affect tinnitus. Chapter 3 aimed to investigate the prevalence of tinnitus in the general population and identify comorbidities of tinnitus. Comorbidities might have overlapping etiologies or common pathophysiological pathways that can aid in identifying mechanisms that underlie tinnitus. Previous genetic studies on tinnitus were generally underpowered. As a result, genetic loci associated with tinnitus have been identified only recently. To provide more insight into potential genetic loci associated with tinnitus, Chapter 4 describes a study on the heritability of tinnitus and a genome-wide association study of tinnitus.

Part II: Identifying common pathways for age-related vestibular and auditory dysfunction.

Recent studies suggest correlations between loss of auditory function with vestibular dysfunction. Age-related mechanisms that result in dysfunction might, therefore, overlap in the different end organs of the inner ear. Investigating the association between these two end organs could aid in understanding the pathophysiology of age-related hearing loss and age-related vestibular dysfunction. Overlapping molecular mechanisms underlying auditory and vestibular diseases remain elusive and require a systematic literature approach to reveal the current state to direct future research. Chapter 5 provides a literature review on age-related vestibular dysfunction and age-related hearing loss. In this review, these two diseases are compared based on their prevalence, physiological measures, and molecular mechanisms to provide avenues for future research. Many studies do not directly investigate vestibular function but instead use balance assessments or questionnaires to assess vestibular dysfunction. In the case of correlated functional decline of auditory and vestibular function, regular auditory assessments could be used as screening tools for vestibular dysfunction. Early detection of vestibular dysfunction could help to prevent traumatic falls in the elderly. Therefore, in chapter 6, a study is presented which aims to identify commonly used audiometric assessments in vestibular schwannoma patients to predict age-related vestibular decline.

Part III: Investigating molecular mechanisms of acquired hearing loss.

Molecular mechanisms of acquired hearing loss have largely remained elusive. Data-driven methods such as whole transcriptome sequencing are needed to further understand the molecular mechanisms of hearing
loss. Recent work by our lab shows that deletion of the sodium-activated potassium channels involved in spiral ganglion activity (K$_{Na}$1.1 and K$_{Na}$1.2), results in a hidden hearing loss phenotype in mice$^{39}$. In chapter 7, we utilize K$_{Na}$1.1 and K$_{Na}$1.2 double knockout mouse model to identify the molecular mechanisms that might underlie this phenotype of hidden hearing loss using whole transcriptome sequencing. Moreover, in this project the effects of deletion of K$_{Na}$1.1 and K$_{Na}$1.2 on age-related changes in auditory function and cochlear synaptic innervation have been investigated. Because of the close anatomical relation between the cochlea and the vestibule, and the presence of voltage-gated potassium channels in the vestibule, we also investigated whether deletion of K$_{Na}$1.1 and K$_{Na}$1.2 showed physiological or transcriptomic changes in the vestibule. Finally, it is important to translate findings of basic research back towards the clinical setting. The project described in chapter 8 had several goals. The first goal was to compare transcriptional differences in the sensorineural and metabolic substructures of the cochlea, again utilizing a whole transcriptome approach. The second goal was to identify the changes in gene expression of these substructures with aging. Finally, we tried to identify potential drug targets for age-related hearing loss by identifying currently approved drugs that could target genes that change during aging in the cochlea. This study aimed to accelerate the drug discovery process for age-related hearing loss.

Finally, in chapter 9 the results of the studies presented in this thesis are summarized and discussed. Implications of these results for future research are also discussed.
PART I

Mechanisms of tinnitus in the general population