A pioneering young scientist with a background in biochemistry and medical genetics, Dr Erwin van Wijk, discusses his research into personalised therapeutic interventions to treat a devastating hereditary disorder known as Usher syndrome.

What sparked your interest in Usher syndrome (USH), a progressive genetic disorder leading to the complete loss of both vision and hearing?

I decided to study USH back in 2004 after my team and I discovered a novel isoform of the USH2A protein that was mutated in patients with the condition. Using a variety of proteomic tools – such as yeast two-hybrid and quantitative affinity purification – we effectively lifted the lid of Pandora’s Box and, excitingly, obtained novel insights into the pathogenic mechanisms underlying USH. Following the completion of my PhD in 2009, I obtained two personal research grants that enabled me to take my studies on USH to the next level – namely, the development of personalised therapeutic interventions for patients.

Are there details of your research project on USH2A-associated retina degeneration that you can share?

For my current project, I obtained a personal research grant from the Netherlands Organisation for Health Research and Development (ZonMW). This study is embedded in the Molecular Oto-genetics research theme of Professor Dr Hannie Kremer, who is based in the Ear-Nose-Throat Department at Radboud University Nijmegen Medical Centre (Radboudumc). In addition, we were able to hire two PhD students who are currently working alongside us on the project.

This project is multidisciplinary, situated at the confluence of human genetics, ophthalmic research and neuroscience. Have you found the multidisciplinary approach beneficial to the project? How have you brought these disciplines together?

The research is conducted at the crossroads of three different departments in Radboudumc: Human Genetics, Ear-Nose-Throat and Ophthalmology. Our close proximity to each of these departments allows us access to expertise in each of these disciplines, which in turn enables us to combine clinics with molecular diagnosis and develop preclinical therapeutic strategies that can be evaluated in clinical trials. The multidisciplinary approach is therefore highly beneficial to the project, providing us with important insights.

How has the European young investigators network for USH (Eur-USH) aided your research endeavours?

The Eur-USH consortium consists of three major components: improvement of clinical and genetic diagnostics; deciphering molecular pathogenesis; and therapeutic development for the retina. In order for a therapeutic development to be successful, a combination of all three components is essential. Moreover, the six partners within the Eur-USH consortium are embedded in leading research institutes and groups, bringing together a unique set of complementary expertise.

Can you summarise the project’s most significant achievements to date? Furthermore, what have been your personal highlights from this work?

This project has been particularly special to me because I have worked on it all the way from the initial identification of the gene to current therapeutic developments. As for the specific achievements themselves, the first has to be the generation of a zebrafish ush2a knockout. The second is the preclinical development of a promising antisense oligonucleotides-based therapy for a common intronic mutation in the USH2A gene, which results in the inclusion of a pseudo-exon that disturbs the open reading frame and leads to premature termination of the protein.

What challenges have you been presented with over the course of your study? Has the process of surmounting these obstacles provided you with any new research avenues?

In order to develop a successful therapeutic approach for hereditary disorders, two steps must be taken. First, it is essential to develop a therapeutic strategy, and second, it is important to have a model system that allows treatment efficiency to be monitored. Before we began our study, neither of these steps had been taken. Additionally, USH2A is one of the largest known genes within the human body, with a coding sequence of 15.6 kb. Gene augmentation therapy with previously published adeno-associated viruses or lentiviral vectors is not an option as the USH2A coding sequence exceeds the cargo capacity of these vectors. Also, the current Ush2a knockout mouse only shows very mild signs of retinal degeneration at a later age. These challenges have inspired us to develop alternative therapeutic strategies and explore the suitability of other model organisms than mice for our study. Excitingly, our zebrafish model has proven to be extremely effective.

What research have you found particularly inspiring? How has this inspired you to develop alternative therapeutic strategies?

The study of USH has been highly inspirational because it is a model for a new therapeutic strategy: first, develop a preclinical model organism that approaches the human age of onset of the disorder; second, identify the gene(s) that underlie the disorder; and third, perform preclinical studies to identify an effective therapeutic strategy. This project has definitely helped to fuel my own research endeavours.

Can you summarise the project’s most significant achievements to date? Furthermore, what have been your personal highlights from this work?

This project has been particularly special to me because I have worked on it all the way from the initial identification of the gene to current therapeutic developments. As for the specific achievements themselves, the first has to be the generation of a zebrafish ush2a knockout. The second is the preclinical development of a promising antisense oligonucleotides-based therapy for a common intronic mutation in the USH2A gene, which results in the inclusion of a pseudo-exon that disturbs the open reading frame and leads to premature termination of the protein.
Retinal insights

A multidisciplinary group based in the Department of Otorhinolaryngology at Radboud University Nijmegen Medical Centre is forging new insights into potential treatments for Usher syndrome – one of the leading causes of deaf-blindness.

IN A FAST-PACED world requiring high levels of mobility and instantaneous communication, individuals with sensory impairments often find it challenging to function at their full potential. While many are able to achieve an excellent quality of life, lack of access to the correct support can result in feelings of isolation from society. This is particularly pronounced among individuals with deaf-blindness, who must make sense of their surroundings using limited information.

USHER SYNDROME
Characterised by auditory loss and progressive visual degeneration, Usher syndrome (USH) is one of the leading causes of deaf-blindness worldwide, with recent studies suggesting that 15-18 per cent of deaf and hard-of-hearing children suffer from the syndrome. As a genetic disease, its degree of severity can vary. Although the hearing deficit can be compensated for by the use of hearing aids and cochlear implantation, physicians are unable to prevent retinal degeneration and the result is an eventual loss of peripheral vision.

The initial sign of retinal degeneration is night blindness, which occurs as a result of damaged rod cells. Usually emerging in early adulthood, the condition gradually progresses to tunnel vision over the course of a couple of decades, ending in complete blindness around the age of 40 or 50. The time between disease onset and the culmination of blindness represents a significant window of opportunity for clinical intervention, but to date no therapeutic strategies have been developed that can prevent or slow the progression of the disease.

MAPPING THE MUTATIONS
Scientists have identified a notable overlap in the molecular pathogenesis of USH and non-syndromic retinitis pigmentosa (RP), with mutations in the USH2A gene accounting for an estimated 50 per cent of USH cases and between 12 and 25 per cent of cases for autosomal recessive non-syndromic RP. Indeed, current estimations project that over 400,000 people worldwide suffer from progressive retinal degeneration as a result of RP caused by mutations in USH2A. Interestingly, in these cases, the retina appears to be far more prone to degeneration than the inner ear. Until now, however, researchers have been unable to develop clear genotype-phenotype correlations that adequately explain the difference in phenotypic outcome of the described mutations.

In view of the knowledge gaps and absence of effective treatments for retinal degeneration, a multidisciplinary research group based in the Department of Otorhinolaryngology at Radboud University Nijmegen Medical Centre in the Netherlands is attempting to elucidate USH2A-associated retinal disease. Together, in a study entitled ‘USH2A-Associated Retina Degeneration: Towards a View on Vision’, the researchers aim to develop a therapeutic approach that slows down or completely stops the progression of retinal degeneration. “To reach this goal, we are exploring the therapeutic potential of two complementary approaches: exon skipping using antisense oligonucleotides (AONs) and intervention with molecular pathogenesis using small compounds,” discloses Dr Erwin van Wijk, the study’s coordinator. “These approaches will be evaluated through the use of a zebrafish model.” With expertise in zebrafish handling and a comprehensive understanding of the USH protein complex, van Wijk is particularly well placed to lead this project.

THERAPEUTIC ADVANCES
As small RNA molecules, AONs can interfere with splicing because their genetic sequence complements that of the target pre-mRNA molecules. Specifically, when an AON is bound, the targeted region of the pre-mRNA is no longer available for splicing and results in the skipping of that particular exon. As a successful therapeutic method in several other genetic disorders, exon skipping with AONs can either be used to redirect the normal splicing of genes or to skip exons that carry protein-truncating mutations in a way that ensures that the mRNA reading frame remains intact.

In the context of van Wijk’s study, the researchers are using AONs in their zebrafish and cellular models to induce skipping of the most commonly mutated exons and pseudo-exons in the human USH2A gene. With their minigene splice assay, they are analysing the therapeutic effect of such AON exon skipping – and have found that skipping of the most common pseudo-exon, which leads to the expression of the WT protein, could help save residual vision in patients with USH2A-associated retinal degeneration.

Van Wijk and his team are also exploring the potential of interfering with the pathogenic mechanisms that underpin the syndrome. This has necessitated a thorough analysis of the molecular mechanisms behind USH2A-associated retinal degeneration. With preliminary data from protein interactions suggesting USH protein complexes play both a structural role and a dynamic role in vesicle transport and signalling, the group is investigating the outcomes.

TYPES OF USHER SYNDROME

TYPE 1 – children with this type are profoundly deaf at birth and have severe balance problems. Many of these children obtain little or no benefit from hearing aids and are therefore often provided with cochlear implants. They begin to develop vision problems in early childhood, almost always by the time they reach age 10, and also have significant balance problems.

TYPE 2 – children with type 2 Usher syndrome are born with normal balance, but moderate to severe hearing impairment; however, they usually benefit from hearing aids. Vision problems progress more slowly than for type 1, with no apparent signs until the teenage years.

TYPE 3 – hearing, sight and balance worsen over time, but the rate at which they decline can vary, with hearing and sight problems often not presenting until puberty. By midlife, most affected individuals are profoundly deaf.
OBJECTIVES

• To investigate the therapeutic potential of exon skipping by antisense oligonucleotides in zebrafish for two of the most common mutations in the USH2A gene to partially restore the retinal function of the USH2A protein

• To inhibit or completely prevent USH2A-associated retinal degeneration by disrupting Wnt and mTOR/AKT1 signalling in zebrafish. Additionally, novel handles for therapeutic intervention will be obtained through the isolation of native Usher syndrome-associated protein complexes from the retina.

FORWARD FACING

Looking ahead to the next five years, van Wijk and his team plan to launch phase I clinical trials for the therapeutic approach they are currently developing. Indeed, saving or partially restoring visual function in patients with RP will greatly improve the quality of individual lives, as well as result in wider socioeconomic benefits by relieving the burden that visual impairments place on healthcare systems worldwide.

“From a broader perspective, we anticipate that the results obtained from our study will be extrapolated to other large genes involved in the aetiology of USH,” concludes van Wijk. “While our present challenge centres on saving residual visual function in patients with progressive retinal disorders, the key for the future will be the restoration of retinas that have already degenerated.”

For more information on USH, please visit:

FACING CHALLENGES

A number of challenges are associated with research into USH. These include:

• Existing databases and rare material collections are local, small scale and not standardised or readily accessible

• The natural history of the ophthalmic component of the syndrome is not fully understood

• Its heterogeneous nature makes USH very difficult to treat

“From a broader perspective, we anticipate that the results obtained from our study will be extrapolated to other large genes involved in the aetiology of USH,” concludes van Wijk. “While our present challenge centres on saving residual visual function in patients with progressive retinal disorders, the key for the future will be the restoration of retinas that have already degenerated.”

For more information on USH, please visit:

INTELLIGENCE