

A rare disease is defined as a disorder affecting less than 5 in 10,000 people. In Germany alone, about 4-5 million people are affected by a rare disease. As the diseases are an immense burden on affected individuals and on societies, drugs to treat these collectively common diseases are much needed. The development of effective therapeutic strategies requires fundamental knowledge about the involved genes and molecular pathomechanisms, in order to understand how genetics affect the phenotype of diverse rare disease subtypes. There is still a huge number of individuals affected by a rare disease with incorrect or missing diagnosis and an unknown genetic background. In these cases therapy is ineffective or simply not available. Research on rare diseases is therefore essential to provide a basis for the improvement of the life situation of affected individuals and their families. As about 80% of rare diseases have a genetic basis, I focused on diverse rare diseases including different types of autosomal recessive hearing loss, Kabuki syndrome, and otofaciocervical syndrome, used different methods for gene identification and performed diverse functional assays to elucidate the underlying pathogenesis of rare autosomal recessively inherited disorders.

By genome-wide linkage analysis we were able to identify the novel autosomal recessive non-syndromic hearing loss (ARNSHL) gene *TPRN* in two different families from Morocco and The Netherlands, which is expressed in human and mouse cochlea. Both deletions, c.42\_52del and c.1347delG, lead to a frameshift, a premature protein truncation, and most likely loss of protein function. Previously, our group was able to identify the first autosomal recessive aminoglycoside-induced hearing loss (AIHL) gene. *Eps8* knockout mice are deaf and have shorter and more numerous stereocilia. The p.L329P mutation in *EPS8*, a key regulator of actin dynamics, leads to a reduced interaction with whirlin. Compatible with the clinical phenotype of patients in a large consanguineous family from Turkey, primary fibroblasts of an affected family member responded with a dose-dependent increase in the rate of cell death on kanamycin treatment when compared to homozygous wild-type cells. Further analysis of the inner ear of different mouse models led to the hypothesis that MyoXVa, whirlin, and *Eps8* form a complex at stereocilia tips, which is essential for stereocilia elongation. On this basis, I performed various experiments and could show that MyoXVa is able to compensate the weakened *EPS8*<sup>L329P</sup>-whirlin interaction. Aminoglycoside treatment had an additive disruptive effect on complex interactions, which led to the degradation of the tip complex, most likely resulting in the depolymerization of the stabilizing actin core and the disruption of stereocilia *in vivo*. Additionally, I was able to identify the second autosomal recessive AIHL gene, *SLC26A5*. The donor splice site mutation c.971+2T>C causes skipping of exon 9 of *SLC26A5 in vitro*, which leads to a frameshift, a premature protein truncation, and most likely to a loss of protein function.

Moreover, I elucidated the molecular pathogenesis of Kabuki syndrome caused by a homozygous mutation in the novel Kabuki gene *RAP1A*. *RAP1A* is a regulator of both

convergent extension (CE) and MAPK signaling by BRAF activation and RAF1 repression, two opposing functions most likely depending on the tissue and cell-specific context. *Rap1* knockdown in zebrafish embryos resulted in CE defects. The hypofunctional RAP1A mutation p.R163T caused MAPK signaling defects in patient fibroblasts, which I also observed in different *KMT2D*-defective cellular systems, as mutations in *KMT2D* are a frequent cause of Kabuki syndrome. I further show that *RAP1* interacts genetically with *KMT2D* and that *RAP1B* expression is downregulated in *KMT2D*-defective patient fibroblasts due to reduced *KMT2D*-dependent H3K4 trimethylation of the *RAP1B* promoter.

By performing whole-exome sequencing on a single pooled DNA sample with DNAs of four affected individuals, I was able to identify the c.497G>T (p.G166V) variant in *PAX1* in a family with otofaciocervical syndrome (OFCS), representing another rare autosomal recessive disorder characterized by ear anomalies, protruding shoulders, winging scapula and mild intellectual disability. As the p.G166V mutation is located within the highly conserved DNA-binding paired-box domain of the transcription factor *PAX1*, I performed a dual luciferase reporter assay and observed a reduced transactivation of a regulatory sequence in the *Nkx3-2* promoter region as a direct target of mouse *Pax1* transcriptional regulation *in vitro*. Beside *EYA1*, *PAX1* is a novel disease-causing gene for OFCS, which is supported by its critical role in pattern formation during vertebrate embryogenesis and the development of vertebral structures.

Taken together, my results provide novel insights into the pathophysiology of different autosomal recessively inherited disorders and might be the basis for the development of therapeutic strategies for the treatment of rare diseases. The identification of a homozygous *EPS8* variant predisposing to ototoxicity upon aminoglycoside administration links this pathological drug response to actin dynamics and might help in developing strategies to counteract and prevent this severe side effect of aminoglycosides. Moreover, my data show that defective MEK-ERK signaling is a common molecular driver for Kabuki syndrome, identifying the disorder as belonging to the disease group of the RASopathies and providing a new interesting direction for the treatment of Kabuki syndrome.