Abstract

Epilepsy is characterized by recurrent and unprovoked seizures and represents one of the most common neurological disorders worldwide. Epilepsy is clinically heterogeneous and is divided into four major types according to the International League Against Epilepsy classification: Focal epilepsy (FE), genetic generalized epilepsy (GGE), combined generalized and focal epilepsy, and unknown epilepsy (UE). Seizures can also occur outside the epilepsy spectrum, such as in the case of the so-called psychogenic nonepileptic seizures (PNES). These seizures are considered nonepileptic since they display normal EEG signatures, in contrast to epileptic seizures. Genetic markers for PNES remain to be identified. In contrast, epilepsy is in many cases considered genetic and heritable. However, the majority of epilepsy patients who undergo genetic testing have a negative result. This implies that the genetic contribution to epilepsies is not yet fully deciphered. Therefore, the aim of this dissertation is to characterize and discover rare genetic factors associated with subtypes of all major epilepsy types and PNES. Specifically, we performed four major analyses:

In my first study, genes and rare genetic variants previously classified as disease-associated in patients with different types of lesional focal epilepsies (LFE) were re-evaluated using state-of-the-art guidelines for variant interpretation. Misclassified genes or variants can lead to false or missed diagnosis, and ultimately to misdirected treatment. We classified only 63.6% of the previously classified genes to be likely disease-associated. Similarly, only 39.6% of the variants were classified as being pathogenic according to American College of Medical Genetics and Genomics (ACMG) guidelines. To address the challenge of variant interpretation, we proposed improved variant interpretation guidelines, including novel bioinformatic approaches, such as our self-developed bioinformatic variant score ranking.

In my second study, we assessed rare genetic variant burden and genotype-phenotype correlations in LFE. We performed targeted next-generation sequencing of brain tissue from patients with hippocampal sclerosis, ganglioglioma, dysembryoplastic neuroepithelial tumors, and focal cortical dysplasia. The highest number of likely pathogenic variants according to ACMG guidelines was observed in patients with ganglioglioma (43.75%; all somatic). We observed likely pathogenic variants in 37.5% (all somatic) of patients with dysembryoplastic neuroepithelial tumors and in 20% of cases with focal cortical dysplasia (13.33% somatic, 6.67% germline). Furthermore, we identified genetic variants potentially involved in the disease etiology of hippocampal sclerosis. This study represents the first comparative

reference for the genetic variant burden across the four major lesion entities in focal epilepsy patients.

In my third study, a genome-wide structural variant analysis in a case-control cohort of 11,246 epilepsy patients and 7,318 controls was performed to characterize common (frequency > 1%) and rare genetic risk factors in all major epilepsy types. For the first time, we analyzed lesional and non-lesional focal epilepsy separately. We identified novel epilepsy-associated copy number variant (CNV) loci at 9p11.2 - 9q21.11 in GGE, LFE, and UE. Furthermore, we identified a significant deletion burden outside of known genomic rearrangement hotspots previously associated with epilepsy. Our results help to refine the list of promising candidate CNVs associated with specific epilepsy types and extend the phenotypic spectrum for identified loci.

Finally, in the fourth study, we analyzed genetic variant burden in a subset of patients diagnosed with PNES. In order to determine the pathogenic CNV and single nucleotide variant burden of PNES patients compared to patients with common epilepsy, we genotyped and sequenced 102 patients with PNES and 448 patients with focal or generalized epilepsy. We inspected the overall pathogenic variant burden and observed a similar variant burden of likely pathogenic variants that affect genes associated with epilepsy, neurological disorders, or psychiatric disorders among the study groups (PNES: 9.8%; FE: 6.36%; GE: 1.82%; 3x2 $\chi 2 = 3.37$, $P=0.19$). Our results show for the first time that genetic factors are likely to play a role also in PNES or/and its comorbidities.

Overall, in the projects I led during my PhD, we generated and analyzed large-scale genetic data from more than 10,000 patients and performed extensive statistical analysis to decipher an epilepsy type-specific variant or risk burden. We show that not only disease-gene discovery but also variant interpretation are fast evolving domains in epilepsy and genetic disorder research. Clinical genetic testing is almost routine practice for pediatric epilepsies in modern hospitals. The increasing use of genetic testing and correct variant interpretation will foster our understanding of disease etiologies and, eventually, aid in developing biomarkers for outcome measures and clinical decision-making. Future epilepsy research will incorporate genetic data with deep phenome data available through electronic health records.