Novel genetic causes and pathological mechanisms of neurological and mitochondrial disorders

Valorisation

SOCIAL AND CLINICAL RELEVANCE

Until a couple of years ago, a patient with a possible genetic disease was offered genetic testing of known disease genes based on the phenotype. Single gene testing was performed using Sanger sequencing, which was the technical 'gold standard' in clinical genetic testing for the past decade [1]. Candidate genes were mostly sequenced sequentially, which was time-consuming and expensive. As shown in my thesis, advances in new sequencing technologies, referred to as next-generation sequencing (NGS), enabled sequencing of many genes, large candidate regions, the whole exome and even the whole genome in a relative short period of time in an increasingly cost-effective manner. In parallel, rapid developments in bioinformatics have made analysis and interpretation of variants in both known and novel genes more effective and efficient.

The work described in this thesis, shows that neurological patients with likely or possible mitochondrial involvement are particularly suited for NGS sequencing, especially when, which is commonly the case, no evident candidate gene is available. We used a 2-step approach beginning with NGS screening for mtDNA defects followed by WES analysis of the unsolved cases. The NGS approach identified an mtDNA defect in 20% of our patients having characteristic mitochondrial disorders. The sensitivity and specificity were sufficiently high to test blood on patients. In a total of 50% of the patients a genetic cause was identified using WES, which is a major step forward, compared to conventional Sanger sequencing method, which only solved 11%. Interestingly, 32% of the disease-causing genes in these patients were at the date of genetic diagnosis not present in the MitoCarta database and therefore would have been missed with targeted sequencing approaches. Based on the success of our work and that of others we can conclude that WES is currently the best approach to unravel the cause in clinically and genetically heterogeneous disease, justifying a rapid introduction into molecular diagnostics, as has happened in the past 2 years. As our work has been performed on a very well-characterized cohort, both with respect to clinical manifestation and more importantly a highly likely genetic cause, it is obvious that in routine patient-care the diagnostic yield will be less. But reassuring is that in case there would be genetic cause the chances of identifying it are high.

Our work shows also that for the group of patients we have been investigating, a targeted gene-panel approach will fall short, as in about 1/3 of the patients, novel genes were involved, not present in the MitoCarta database and/or clinical exome at the date of discovery. WES allows simultaneous analysis of known genes while identifying novel genes linked to the patient phenotype at the same time. In literature, more than 180 novel rare-disease-causing genes have been discovered through WES and more than 130 were reported in 2012 only [2]. We have reported *CWF19L1*, *SLC25A46* as novel disease-causing genes, verified other reports which supported to the candidacy of the genes, and *COX18* as a new candidate gene in patients with isolated COX deficiency (chapter 2,3,4). Our work demonstrates that the most optimal diagnostic strategy to

identify the genetic cause in patients with neurological disorders and a possible mitochondrial involvement is an unbiased, complete exome analysis from the start. Although in diagnostics, mostly a step-wise procedure of a targeted panel followed by an open exome is being performed, this causes considerable delays in achieving a diagnosis for at least 1/3 of the patients.

Another group benefitting from WES analysis are patients with multiple genetic diseases. The presence of two or more separate diseases which may or may not conflate to appear as one adds to the complexity to the diagnosis, as illustrated by the two patients with a broad variety of clinical features in chapter 6. Three gene defects (*ACY1*, *SERAC1*, *ANTXR2*) in the first patient explained all symptoms, but manifestations were overlapping (blended phenotype). Two gene defects (*HPS1*, *BICD2*) in the second patient explained non-overlapping symptoms (composite phenotype) [3]. This is especially an issue in case of consanguinity among the parents. Also, the patient with mutations in *HKDC1* and *MED20* illustrates that is dangerous to stop when the first genetic defect, which could explain the disease, has been identified with a targeted analysis, as other, equally likely causes might be missed. Based on this data, again, we support an immediate start with WES, being a comprehensive and unbiased approach.

A genetic diagnosis allows a more accurate prognosis, follow-up investigations in families and the possibility to prevent further disease transmission by prenatal diagnosis or preimplantation genetic diagnosis. We have demonstrated that WES is also quick enough, enabling a genetic diagnosis in an affected boy, while his mother was pregnant again. In this case, the mitochondrial encephalomyopathy was caused by *FBXL4* mutations, which were tested prenatally for the unborn child [4]. For consanguineous couples in particular, but in future possibly for every couple with a child-wish, WES-based preconception carrier screening (PCS) will be available to detect couples at risk of transmitting recessive genetic disorders they were not aware of. PCS has been applied for centuries in populations with high carrier frequencies for certain diseases and significantly reduced the disease incidence. Currently, carrier screening is becoming available for all individuals, if desired, regardless of ancestry or geographic origin. [5].

While increasing the diagnostic yield, costs of WES are markedly lower than the average total traditional diagnostic costs. The clinical utility of whole-exome sequencing (WES) was investigated for complex pediatric neurology in terms of diagnostic yield and costs [6]. In a parallel study, all patients received both the standard diagnostic workup (e.g., cerebral imaging, muscle biopsies or lumbar punctures, and sequential gene-by-gene–based testing) and WES simultaneously. WES yielded significantly more conclusive diagnoses (29.3%) than the standard care pathway (7.3%) without incurring higher costs. Exploratory analysis of WES as a first-tier diagnostic test indicates that WES may even be cost-saving, depending on the extent of other tests being omitted [6]. This is in line with our studies. For 58 of 118 patients, WES led to a conclusive diagnosis, with no need for additional tests.

Identification of the genetic defect is not only important for diagnostics, but, unfortunately still in limited cases, also for therapeutic interventions. Patients with *SLC25A32*, *SLC19A3* and *TMEM126B* defects showed improvement upon treatment with respectively riboflavin, biotin/thiamine and high fat-diet. A successful treatment based on the gene defect which is identified by WES may guide treatment of other patients with the same gene defect. For example, immediate treatment with a high-dose of thiamine and, possibly, biotin for patients with *SLC19A3* mutations prevents fatal Leigh syndrome. Despite the significant improvements in the genetic diagnosis of mitochondrial disorders, the development of novel therapies has is lagging behind (chapter 6)

Our study and those of many others demonstrated the diagnostic utility of WES in patients with highly likely genetic, neurological and/or mitochondrial disorders. WES should be offered as the first diagnostic test to those patients. The early use of WES increases the diagnostic yield while reducing the time to diagnosis, the finance and burdens associated with prolonged investigations.

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