VALORISATION
Relevance of this thesis in patient care

The work presented in this thesis is not only relevant to improve our understanding of the genetic causes and pathophysiological mechanisms in mitochondrial disease from a research perspective, but it has also direct implications for the genetic diagnosis and in some cases opens novel options for personalized treatment of patients with mitochondrial disease in a clinical setting.

WES in establishing a genetic diagnosis

Until quite recently the genetic testing of a patient relied on the targeted sequencing of known disease genes based on the patient’s phenotype, where Sanger sequencing was considered the “gold standard” in diagnostic testing (1). Such approach required many time-consuming and expensive clinical and laboratory investigations in order to find a lead with previously reported gene defects, resulting in sequential sequencing of many candidate genes and a relatively low yield. As shown in this thesis, the development of next-generation sequencing (NGS) approaches, such as WES, has allowed the detection of genetic variants in all genes in parallel in the complete exome or genome of a patient. Current sequencing-by-synthesis platforms, such as the Illumina NovaSeq 6000 are, depending on the coverage depth and read-length, capable of sequencing up to 200 human exomes or 48 genomes in a single run. Both the costs and time for sequencing a complete exome or genome has rapidly declined over the last couple of years, making it a cost-effective strategy to apply in clinical diagnostics (2). Therefore, WES has revolutionized the identification of potential disease causing variants in rare genetic diseases, which despite their individual scarcity, affect approximately 6-8% of the population during life-time (3).

In both our experience and that of others (4), WES is a particularly powerful tool to identify the genetic cause in heterogeneous disorders, in which most often no evident candidate genes are available. Where conventional sequencing methods were reported to solve no more than 11% in a heterogeneous patient cohort (5), the work in this thesis shows that WES is performing much better in heterogeneous, mitochondrial disorders. Using a 2-step NGS approach, we could identify a genetic defect in the mtDNA of ~20%, and an exonic defect in ~50% of the patients, overall solving ~70% of our patient cohort (chapter 2). It should be stressed, that this is a carefully selected cohort and the diagnostic yield in clinical practice will be lower. Interestingly, we showed that ~30% of the gene defects in mitochondrial patients, covering both novel and known mitochondrial gene defects, were not included in the MitoCarta database, and therefore could missed when using targeted, gene panel based sequencing approaches. It is preferable to start directly with a complete exome and skip...
panel-based intermediate steps, as the choice for a panel is often arbitrary, never complete and genes and mutations are missed, causing unnecessary delay, when the complete exome needs to be analyzed as a second step. Based on our data, and that of others, we can conclude that WES is a comprehensive and effective method to find the genetic cause in mitochondrial disease, justifying the rapid introduction of WES in a diagnostic setting, and having direct implications for the establishment of a clinical genetic diagnosis in patients with mitochondrial disease.

In a clinical context, the complete exome should be analyzed completely, and one should not stop after the identification of a single candidate. This is not possible in gene panels, giving another argument for skipping this step in-between. As we show in chapter 3, patients with a multi-gene cause are not uncommon, especially in consanguineous families, and all exonic variants need to be evaluated for being disease causing, allowing the establishment of an accurate and complete genetic diagnosis in heterogeneous disorders that are caused by multiple gene defects. This is illustrated by the two patients with a broad variety of clinical symptoms, where the presence of multiple gene defects adds another level of complexity to the genetic diagnosis. In such cases, multiple gene defects (ACY1, ANTXR2, SERAC1) may appear as a single disease due to significantly overlapping (blended) phenotypic manifestations, or the individual gene defects (HPS1, BICD2) could present as non-overlapping (composite) disease phenotypes in the patient. We showed that WES is a powerful tool to resolve multi-gene disease, yet, one should ensure that analysis of the complete exome is being continued until each individual symptom can be genetically explained. It is important to understand the contribution of each individual gene defect to the disease phenotype, either in isolation or combination with the other defects, not only for prognosis and treatment, but also to avoid disease transmission. This especially applies to patients from consanguineous parents, who potential have a higher risk of inheriting multiple genetic defects than outbred families due to the presence of large homozygous genomic regions.

The improvements in identification of mtDNA and nuclear DNA defects, and the implementation of NGS based strategies in diagnostic setting allows is not only important for a rapid diagnosis. As illustrated by the patient with a genetic defect in the FAD transporter SLC25A32 (chapter 8), understanding the genetic basis of disease may directly result in a more effective treatment. In this case supportive treatment with riboflavin, a vitamin B2 precursor of FAD, will compensate for the lack of FAD, which functions as a crucial cofactor in the mitochondrial electron transport chain. A genetic diagnosis also allows the prevention of disease transmission by prenatal diagnosis (PND), as it gives parents the option to abort a pregnancy, or, if parents would opt for preimplantation genetic diagnosis (PGD), to select
for mutation free embryos (6). WES is a relatively fast strategy, as illustrated by the quick establishment of a genetic diagnosis in a pediatric patient with encephalomyopathy, in who an FBXL4 gene defect was identified during another pregnancy of his mother, allowing timely prenatal testing of the unborn child (7). WES based preconception carrier screening (PCS) is a next step to estimate this risk of transmitting a recessive genetic disorder, even prior to the first pregnancy, and is especially valuable in couples who are at risk of transmitting multiple diseases, for example due to consanguinity (8). PCS is currently made available to all healthy couples, as the method seems sensitive and cost-effective enough for this.

Our data illustrates the power of WES as a tool to characterize the genetic defects underlying a patient’s disease manifestations, but much more valuable information can be extracted from a patient’s exome dataset. As WES detects all variations in the patient’s exome, it will also include variants, which might lead to treatable disease or which define side-effects or efficacy of drugs. One might argue that one is obliged to provide the patient with all clinically-actionable or potentially clinically actionable information from the WES as this is not so much different from the heel pricking test. Obviously, this is more complex for variants, causing late-onset disease, risk factors or variants of unknown significance. This will require adequate counselling, informed consent and strict guidelines in terms of the genetic information that is reported back to the patient. But eventually, genome data will not be available for patients only, but will become standard information for every person in our society, entering the era of personalized, genome-based medicine.

WES will also and always result in the identification of novel, unknown, variants or variants of unknown significance in known or novel genes, complicating the interpretation of WES data. The development of novel bioinformatics tools to predict variant pathogenicity and gene function, for example based on gene conservation, 3D protein structure, or co-expression data (WeGET tool, chapter 8), is therefore crucial for interpreting WES variants, just as for the more recent WGS applications, which rely on the estimation of intronic- and splice-variants. As a result, validation and eventually implementation of novel bioinformatics tools, laboratory assays or genetic (animal) models to interpret WES variants, has direct impact on the diagnostic yield in a patient cohort. In chapter 5, we showed that lentiviral complementation in patient fibroblasts can be used for testing novel gene defects in OXPHOS deficient patients. Also in vivo models have a prominent role in establishing a relation between gene and phenotype or in understanding the transmission of mitochondrial disease (9). In chapter 7 we show that the zebrafish is a reliable model to study mtDNA mutations and their transmission. Still, implementation of such models in diagnostic setting will require standardization, validation and a sufficient throughput and speed to establish the genetic model in order to keep up with the fast generation of NGS data.
Compound testing in patient cell-lines

As described in chapter 5, patient cell-line based assays can be a powerful tool to study the responsiveness of a patient to specific drugs or compounds in the patient’s genetic context. The latter is crucial, as other mutations, but also polymorphisms, in the nuclear DNA and mtDNA can influence the pharmacological responsiveness and toxicity to a specific compound. As shown in chapter 5, despite the fact that TMEM126B and ACAD9 function in the same complex I assembly complex, the TMEM126B patient significantly responded to palmitic acid treatment by an increase in mitochondrial respiration, whereas the ACAD9 patient did not. The latter illustrates that individual patients with a similar disease, biochemical defect, or even similar gene defect or mutation, might respond differently to a specific treatment. Responsiveness to a certain compound should therefore be evaluated individually. Our patient-cell line assay, based on the Seahorse XF96, answers to this need for personalized medicine, as it allows the testing of different compounds, concentrations, and treatment durations in patient cells based on mitochondrial respiration parameters. Whereas in clinical practice, different diets or drugs are often subsequently tested in the patient, without knowing the effective components, our cell-line assay could overcome these limitations as a tool to identify patient responsiveness to specific FFAs, nutrients or drugs. Although, cultured fibroblasts are relatively easy to test in this assay, as patient skin biopsies can easily be taken and fibroblasts are often readily available in diagnostic centers, these cells might not always display a mitochondrial biochemical defect. Therefore, studying mitochondrial respiration parameters in muscle precursors or other cell types might be an interesting alternative. Although, our assay allows the identifying of compounds that improve mitochondrial respiration in the patient, it does not reveal clinical efficacy or toxicity, and eventually a complete pathway based analysis is needed to judge the potential for treatment and estimate possible toxic side-effects. In chapter 8, we report an example of possible pathway-based intervention in patient cells with an MTFMT defect, where targeting PDF (enzyme responsible for deformylation of methionine) with antibiotics and compound inhibitors should shift the substrate towards an increased formylated state and rescue mitochondrial translation. Assessment of this predicted rescue pathway should be performed at different molecular levels (e.g. formylation state, enzyme activity, mitochondrial translation) to judge the effectiveness of such compounds. Besides, negative or toxic effects of a compound, such as ROS production, lipid peroxidation, altered mitochondrial membrane potential and integrity, should be examined, preferably in the patient’s own cells, as also toxicity levels dependent on the unique genetic background of the patient. This is illustrated by the observation that low levels of ROS might already become toxic in some patients with an OXPHOS defect, whereas others can cope with much higher ROS levels as they are able to upregulate their natural anti-oxidant defense system.
(10). For the future, it will therefore be important to assess all aspects of mitochondrial functioning and toxicity in these patient cell-lines, implementing parameters for all these mitochondrial processes in a single patient cell-line based assay to identify promising compounds or therapies for an individual patient.
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References