Classic galactosemia

Valorization

The valorization of the acquired knowledge on the natural history of classic galactosemia, the underlying pathophysiological mechanisms and treatment approaches, will be further elaborated in this chapter.

Newborns with classic galactosemia present with life-threatening symptoms upon exposure to galactose-containing milk. These symptoms can be quickly resolved by early initiation of a galactose-restricted diet (1). Since 1935, a galactose-restricted diet has been the mainstream of treatment (2), and although it is lifesaving in the neonatal period, it fails to prevent the chronic complications. The long-term outcome is disappointing, many patients develop complications affecting brain, gonads and, to a lesser extent, bone. Importantly, this occurs irrespective of the severity of the illness in the newborn period and despite the early initiation of the diet (3). There is a broad phenotypic spectrum. Some patients experience only mild symptoms (and are able, e.g. to obtain an educational degree and to engage in society), while others are severely affected (e.g., need to live with their parents and are not able to build strong relationships outside family core). This phenotypic spectrum is also observed among siblings.

Despite decades of research, the complex pathophysiological mechanisms are not yet fully elucidated and there are currently no accurate biomarkers that can predict/monitor disease outcome or progression. The high costs of the disease monitoring and ineffective treatment represent a burden for the healthcare and social systems. There is a great unmet clinical need for a more effective therapy, and this therefore constitutes the ultimate aim in galactosemia research.

The first objective of this dissertation included describing the natural history of classic galactosemia based on a large cohort of patients. Classic galactosemia is a rare disorder with a worldwide prevalence of between 1:16,000 to 1:60,000 live-births (4, 5), affecting approximately thousands of patients worldwide. In the past, several studies have been conducted to gain more insight into the long-term complications and possible predictive factors, based on small cohorts. With the rare character of the disease, providing evidence based on a large dataset from one center or even one country can be difficult. Rare disease registries are a valuable tool to gather information from many patients with different genetic and ethnic backgrounds (6). Therefore, in 2014, the international network for galactosemia (GalNet) successfully established an international registry including information from patients with GALK, GALT, and GALE deficiency. This registry was founded and coordinated by our center and currently contains patient data from 15 countries and 32 centers. In this dissertation we presented the natural history of classic galactosemia (GALT deficiency) based on the hitherto largest patient cohort (n=509). We hereby described that most patient experience neonatal illness (79.8%) and despite the diet, developed complications affecting the brain (85.0%), female gonads (79.7%) and

bones (26.5%). These findings are in line with previous studies with smaller cohorts. Importantly, we identified that newborn screening, genetic variation, enzyme activity and diet are important predictive factors for the long-term outcome. Noteworthy, our study supports a more liberalized approach regarding dietary treatment, a lactose-restricted diet without limitations in intake in fruit and vegetables, which can tremendously affect patient's quality of life. It greatly contributes to patient's social integration and to mitigate the disease's burden, particularly in adolescence. Nevertheless, there are still nowadays countries that recommend a stricter diet. The evidence provided in this study is of utmost importance and distribution of the findings to healthcare professionals and patients/families is encouraged. The GalNet will continue to raise awareness for the findings from this study on the natural history of classic galactosemia. The findings from this study are currently being translated to other languages and are published on the GalNet website (https://www.galactosemianetwork.org/). Our ultimate aim is to provide the best possible care to all patients supported by current evidence.

Throughout the years, a myriad of research has taken place to unravel the pathophysiological mechanisms underlying classic galactosemia. Studies in different cellular and animal models have greatly contributed to increase our knowledge (7-11). This information is scattered throughout the different studies. Animal research has an important role in improving our knowledge of various diseases and the subsequent developments of new treatments. However, their use has been a subject of debate in many countries. To increase animal welfare, it is important that researchers maximize reduction of animals as much as possible. To achieve this, adequate knowledge on research performed in the past needs to be provided. We conducted a systematic review of the hitherto available literature to provide a comprehensive overview of the studied pathophysiological mechanisms underlying classic galactosemia, in cellular and animal models. This background information can be used by researchers in the field and can contribute to the reduction of the use of animals in future research. The conducted systematic review raised an important question regarding the pathophysiology. In the past, several groups have studied the levels of UDP-Glc and UDP-Gal in cell lines deriving from patients. Alterations in these levels were hypothesized to cause the observed glycosylation abnormalities and were considered as a key pathogenic factor. However, contradictory results have been presented, and, importantly, these levels have never been studied in the affected tissues, brain and gonads. To provide more information on the levels of UDP-Glc, UDP-Gal and other nucleotide sugars, we studied their levels in our *galt* knockout zebrafish model throughout development and in target tissues.

No abnormalities in UDP-Gal or UDP-Glc levels were found. This study showed stage- and tissue-specific profiles that have not been described earlier and that need to be taken into consideration in future research and interpretation of results.

Towards the ultimate aim to develop new, effective treatment strategies, we evaluated two approaches: messenger RNA (mRNA) therapy and a chemical chaperone.

The mRNA approach is emerging as a treatment modality that can treat a variety of diseases by restoring the respective defective protein. Notably, in opposition to gene therapy, the risk of insertional mutagenesis is low, since mRNA does not transit to the nucleus. Clinical translation seems extremely promising. The potential of mRNA-based therapy was evaluated in our *galt* knockout zebrafish model. We showed that delivery of naked and lipid nanoparticle (LNP)-packaged human *GALT* mRNA to *galt* knockout zebrafish rescues GALT enzyme activity. This is a very promising finding with great potential impact on the treatment of this disease. Future studies will assess the time course of the restoration of GALT activity, as well as evaluate the effect on biochemical and clinical outcomes in out *galt* knockout zebrafish. mRNA strategies that are able to target tissues of damage, brain and gonads, will be developed. Additionally, studies assessing the time of onset of damage will contribute to ascertain the window of opportunity for treatment.

Pharmacological and chemical chaperones, aiming to stabilize variant GALT, have been suggested as a potential therapeutic approach in this disorder (12, 13). Studies in a prokaryotic model suggested therapeutic potential of the chemical chaperone arginine (14). We evaluated arginine's potential in patients homozygous for the most common variant, p.Gln188Arg. Arginine was not beneficial in patients carrying this variant. It is important that its effect has been evaluated in patients, since the hitherto studies evaluating pharmacological/chemical chaperones had only been performed *in vitro*. Although we did not find promising results in patient carrying the most common variant, this does not preclude that arginine might be beneficial in patients with other genetic variations. Patients homozygous for p.Gln188Arg might benefit from a different treatment such as the mRNA-based approach, which is non-mutation-specific.

The gonads are one of the main target organs affected by classic galactosemia, leading to primary ovarian insufficiency (POI) and subsequent subfertility in many female patients. Despite the fertility issues, spontaneous pregnancies have been described (15). Nevertheless, POI with subsequent subfertility represents a great burden for patients and families (16) and treating physicians are often consulted for fertility preservation options. Recommendations from a multidisciplinary team regarding this topic were published aiming to improve patient care (17). One of the options often brought up by patients and their families is intra-familial oocyte donation, most likely from a mother or sister to the galactosemia patient. Noteworthy, in case mother-to-daughter oocyte donation is considered, obtaining oocytes from the mother may be necessary when patients are still very young. An important aspect in this disorder is a varying spectrum in cognitive impairments, where some patients are not

able to live on their own without support and the wide range in intelligence quotient (IQ, 47-122) (18). The cognitive impairments may not be fully clear at the time that mother-to-daughter oocyte donation is considered, in view of the young age of the patient. Furthermore, possible role confusion, pressure on both donor and recipient are important point to consider. Intra-familial oocyte donation raises medical, ethical and societal questions and health care providers might be reluctant mentioning this option. To provide a tool that can be implemented into patient care, we, with a multidisciplinary team, conducted a qualitative study assessing the most important aspects to consider when discussing this topic with patients and their families. Relevant points emerged from the conducted interviews, where for example possible role confusion created by intra-familial oocyte donation seems not to be a point of concern in patients, in contrary to the point of view of family members and professionals. The important topics to be considered when discussing intra-familial oocyte donation include family relations, medical impact, patient's cognitive level, organization of counseling, disclosure to the child and need for follow-up. Following these recommendations will be of great benefit to patients and society, by addressing this topic carefully, and while being aware of the clinical spectrum of classic galactosemia.

The studies described in this dissertation add value to the already existing knowledge on galactosemia. We have successfully developed an international registry for the different types of galactosemia and described the natural history of classic galactosemia in the hitherto largest cohort of patients. This is of great value for patients, their families and healthcare professionals involved in patient care. Our systematic review on the studied pathophysiological mechanisms in cellular and animal models so far will be of great value for future studies and may help reduce the use of more animals in research. In addition, we provided relevant information regarding nucleotide sugar levels in classic galactosemia that shed light on the pathophysiology underlying glycosylation abnormalities observed in this disease. Towards our ultimate aim to develop new treatment strategies and provide the best possible care to the patients, we evaluated two treatment modalities. The non-mutation-specific mRNA-based approach holds great promise for future research, and eventually, clinical implementation. Our pilot study evaluating the therapeutic potential of arginine showed not to be beneficial in patients homozygous for p.Gln188Arg. The research presented in this dissertation will contribute to alleviate the burden on the healthcare and social systems, and to improve the psycho-social outcomes and quality of life for all galactosemia patients.

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