Galactosemias

IMPACT PARAGRAPH

Hereditary galactosemias is a group of rare inherited disorders of galactose metabolism. Prevalences range from 1:10,000 – 1:60,000 in type 1 (classic galactosemia (CG)[1]), 1:1,000,000 in type 2 (galactokinase (GALK1) deficiency[2]), 1:6,700 – 1:60,000 in non-generalized galactose epimerase (GALE) deficiency and a ultrarare prevalence in generalized GALE deficiency[3]. Depending on the affected step in the galactose metabolism, patients have a broad phenotypic spectrum and experience a high burden of disease. Galactosemia type I, also known as CG, is the most well-studied type of the galactosemias. However, despite decades of research, the exact mechanism of disease remains challenging and the occurrence of long-term complications in the majority of CG patients require novel treatment options. On the other hand, galactosemia type II and III, respectively known as GALK1 and GALE deficiency, are not well characterized entities due to scarce data. There is need for more evidence-based guidelines to improve the standardized practice.

The first aim of this dissertation was to describe the natural history of galactosemia type II and III. Therefore, patients' data from different countries included through the galactosemia network (GalNet) and GalNet registry was used. For rare diseases, such as galactosemia, the implementation of an international web-based registry is key to acquire more data. Registries are powerful tools that fulfills multiple important purposes, such as better delineation of the natural history and phenotypic spectrum, investigating the impact of early diagnosis and treatment, as well as evaluating the current practices[4]. The GalNet registry enabled expansion of the existing knowledge on the phenotypic spectrum of GALK1 deficiency and GALE deficiency, allowing us to develop recommendations regarding diagnosis, treatment and follow-up. Initially, GALK1 deficiency was considered as a mild type of galactosemia, with bilateral cataract as the only consistent finding[5]. However, based on our gathered data from 53 GALK1 deficient patients from the GalNet registry, we found that in addition to cataract the phenotypic spectrum of GALK1 deficiency can include neonatal illness, such as elevation of transaminases (25.5%), bleeding diathesis (8.1%), and encephalopathy (2.0%). Moreover, in the majority of patients, periodical surveys to examine potential complications and additional work-up to exclude other genetic diseases, were not systematically performed. Therefore, we

recommended to include additional testing to exclude other genetic diseases in patients with complications beyond the neonatal period, as well as periodic clinical follow-up to examine the patients' symptoms. This is crucial to end the controversy that there are more symptoms than neonatal illness and bilateral cataract, which are most likely due to the presence of consanguinity in the described families.

In addition, the GalNet network and registry enabled us to describe the phenotypic spectrum of 22 patients with GALE deficiency, of whom 6 were classified as generalized. Since only 9 patients from 5 families were reported in the literature so far[6,7], the description of 6 more generalized GALE deficient patients is very valuable for further elaboration of the phenotypic spectrum of this entity. Moreover, due to lack of facilities to measure GALE enzyme activities in other cell types rather than red blood cells, we noticed that additional enzymatic or genetic testing to better classify the deficiency is not part of common practice in many centers. The implementation of GALE deficiency in the newborn screening (NBS) program for galactosemia urges a better classification into the different types and delineation of the phenotypes. In addition, better classification is important to decide whether or not to start with dietary restrictions and to provide proper clinical guidance. Currently, the malpractice of not categorizing the patient in 'generalized', 'intermediate' or 'peripheral' GALE deficiency leads to overtreatment, because even peripheral GALE deficient patients will be set on a galactose-restricted diet. In addition to genetic and enzymatic testing, the clinical picture should be more taken into account to classify the patient. Moreover, due to the lack of facilities, we suggested serum transferrin as diagnostic tool to help in the decision for dietary intervention. Thus, the GalNet registry allowed us to advance the knowledge of the existing gaps in the phenotypic spectrum as well as to gain insight in current non-standardized practices and allowed us to make recommendations for diagnosis, treatment and follow-up for galactosemia type II and III. This brings us one step closer to improving the standardized care for these patients.

The second objective of this dissertation was to review and identify pathophysiological mechanisms involved in primary ovarian insufficiency (POI) in CG. POI is considered as one of the most burdensome complication in CG[8]. This high burden of disease emphasizes the need and importance of adequate counseling and information on fertility preservation

options and unmet need of novel therapeutic strategies to prevent disease progression. In order to offer adequate fertility counseling and to develop new therapeutic strategies for POI, the exact underlying pathophysiological mechanisms need to be better elucidated. Therefore, we reviewed the current insights on the clinical picture, counseling paradigm, knowledge on the involved pathophysiological mechanisms, and current treatment options. We emphasized the significant psychological burden of POI and the need for adequate counseling and timely discussion of fertility preservation options. Clinicians should be aware of this high psychological burden and should emphasize the occurrence of spontaneous pregnancies in women with CG, so that patients feel supported in sharing their psychological distress and uncertainties concerning their desire to have children. Current insights on the underlying pathophysiological mechanisms of POI in CG at the molecular level in cellular models and a mouse model points to dysregulation of pathways crucial for normal folliculogenesis including phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), inositol pathway, mitogen-activated protein kinase, insulin-like growth factor-1 and transforming growth factor-β signaling. Impaired folliculogenesis leading to decreased ovarian function and severe POI seems essential in the development of POI in CG. We aimed to reproduce these findings in our zebrafish model by using new techniques available nowadays, such as transcriptomics. By analyzing the transcriptomic profile of female gonads of *galt* knockout (KO) and wildtype (WT) zebrafish, we were able to identify pathways involved in the pathogenesis of POI that are altered in *galt* KO zebrafish compared to WT zebrafish. We found two perturbed pathways in *galt* KO zebrafish, namely insulin signaling pathway and ubiquitin mediated proteolysis, both involved in proper folliculogenesis and oocyte maturation. These results support the hypothesis of impaired folliculogenesis as important pathophysiological mechanism in the development of POI in CG. However, the results of our transcriptomic pilot study are currently repeated in a larger study population of zebrafish and at different age stadia. Unraveling the underlying pathophysiological mechanisms are fundamental to develop diagnostic tools to predict disease progression and to find biomarkers that could open novel treatment avenues.

Because CG is the most well studied type of the different galactosemias, the third and last objective of this thesis was to explore new treatment options for CG. As described above, due to the high psychological burden and impaired quality of life, there is a need for new treatment options in CG. New treatment options could either be directed to the genetic/enzymatic defect directly, influence the cascade of events or focus on the clinical picture of patients with CG. In this dissertation, we investigated treatment strategies that either directly affect the enzymatic GALT deficiency (arginine) and that influence the clinical picture of CG patients (transcranial Alternating Current Stimulation (tACS)). Arginine, a chemical chaperone, showed a beneficial effect as protein stabilizer in a prokaryotic model of galactose sensitivity[9]. Therefore we investigated the therapeutic potential of arginine as chaperone in CG patients homozygous for c.563A>G (p.Gln188Arg). In our small study population of four patients, we did not find a significant therapeutic effect of arginine in these patients. Despite the negative outcome, these results are important in the journey to find new treatment options for CG patients. We have learned that CG patients homozygous for c.563A>G (p.Gln188Arg) will not benefit from arginine as chaperone, but this does not rule out that CG patients with other pathogenic *GALT* variants will. The pathogenic variant c.563A>G (p.Gln188Arg) is closely located to the active site and also affects the catalytic activity[10,11]. Pathogenic variants causing purely conformational changes may be more amenable to arginine. Moreover, the results could indicate that CG patients homozygous for c.563A>G (p.Gln188Arg) might benefit from other treatment strategies, such as mRNA therapy. There are promising results for mRNA therapy in CG[12], but further research is necessary to bring it to the patient.

In addition to arginine, we also explored therapeutic approaches that ameliorate the clinical consequences of CG. The brain is one of the major organs affected in CG, namely 85% of the patients suffer from cognitive and neurological complications including language and speech disorders[13]. Previous research has related these cognitive and language deficits to anatomical[14-18] and functional differences[19,20] in brains of CG patients compared to healthy controls. Studies using electroencephalograms (EEG) in CG patients and healthy controls during language production, found differences in the morphology of the event-related potential (ERP) components P100, P200 and P300[19]. Since theta

frequency plays an essential role in working memory[21] and executive control during language production[22], we conducted a case-control pilot study to investigate the effect of tACS in theta frequency on the language performance in CG patients compared to healthy controls. Our study showed promising results with a very specific impact of theta-tACS on accuracy and ERP amplitude in CG patients compared to healthy controls. Further research to stimulate other brain areas with other frequencies relevant for language production should be conducted by our research group. Currently, the duration of the stimulation effect and thus the practical use of this treatment option is still unknown, but the results seem promising. TACS could be a potential therapy to improve the language problems in CG patients, which are experienced as a high burden of disease[8].

In conclusion, the studies in this dissertation add to the existing knowledge of the different types of galactosemia. We elaborated the clinical phenotype and made recommendations for diagnosis, treatment and follow-up for galactosemia type II and III. In addition, our review will be of great value in understanding the perturbed signaling pathways in POI in CG. Awareness of the existing knowledge gaps is essential to develop new therapeutic strategies and to improve the current practices. The chemical chaperone arginine was not effective in CG patients homozygous for c.563A>G (p.Gln188Arg) but might be for other variants On the other hand, tACS could be a promising therapy for the language problems in CG patients. The studies presented in this dissertation bring us one step closer to better fundamental understanding and toward the development of better treatment, resulting in higher quality of life for this patient group.

References

1. Berry GT. Classic Galactosemia and Clinical Variant Galactosemia. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. GeneReviews((R)). Seattle (WA)1993.
2. Kalaydjieva L, Perez-Lezaun A, Angelicheva D, et al. A founder mutation in the GK1 gene is responsible for galactokinase deficiency in Roma (Gypsies). Am J Hum Genet. 1999;65(5):1299-1307.
3. Openo KK, Schulz JM, Vargas CA, et al. Epimerase-deficiency galactosemia is not a binary condition. Am J Hum Genet. 2006;78(1):89-102.
4. Kolker S, Gleich F, Mutze U, Opladen T. Rare Disease Registries Are Key to Evidence-Based Personalized Medicine: Highlighting the European Experience. Front Endocrinol (Lausanne). 2022;13:832063.
5. Demirbas D, Coelho AI, Rubio-Gozalbo ME, Berry GT. Hereditary galactosemia. Metabolism. 2018;83:188-196.
6. Dias Costa F, Ferdinandusse S, Pinto C, et al. Galactose Epimerase Deficiency: Expanding the Phenotype. JIMD Rep. 2017;37:19-25.
7. Walter JH, Roberts RE, Besley GT, et al. Generalised uridine diphosphate galactose-4-epimerase deficiency. Arch Dis Child. 1999;80(4):374-376.
8. Randall JA, Sutter C, Wang S, et al. Qualitative interviews with adults with Classic Galactosemia and their caregivers: disease burden and challenges with daily living. Orphanet J Rare Dis. 2022;17(1):138.
9. Coelho AI, Trabuco M, Silva MJ, et al. Arginine Functionally Improves Clinically Relevant Human Galactose-1-Phosphate Uridylyltransferase (GALT) Variants Expressed in a Prokaryotic Model. JIMD Rep. 2015;23:1-6.
10. Coelho AI, Trabuco M, Ramos R, et al. Functional and structural impact of the most prevalent missense mutations in classic galactosemia. Mol Genet Genomic Med. 2014;2(6):484-496.
11. McCorvie TJ, Kopec J, Pey AL, et al. Molecular basis of classic galactosemia from the structure of human galactose 1-phosphate uridylyltransferase. Hum Mol Genet. 2016;25(11):2234-2244.
12. Delnoy B, Haskovic M, Vanoevelen J, et al. Novel mRNA therapy restores GALT protein and enzyme activity in a zebrafish model of classic galactosemia. J Inherit Metab Dis. 2022;45(4):748-758.
13. Rubio-Gozalbo ME, Haskovic M, Bosch AM, et al. The natural history of classic galactosemia: lessons from the GalNet registry. Orphanet J Rare Dis. 2019;14(1):86.
14. Kaufman FR, McBride-Chang C, Manis FR, Wolff JA, Nelson MD. Cognitive functioning, neurologic status and brain imaging in classical galactosemia. Eur J Pediatr. 1995;154(7 Suppl 2):S2-5.
15. Timmers I, Zhang H, Bastiani M, Jansma BM, Roebroeck A, Rubio-Gozalbo ME. White matter microstructure pathology in classic galactosemia revealed by neurite orientation dispersion and density imaging. J Inherit Metab Dis. 2015;38(2):295-304.
16. Timmers I, van der Korput LD, Jansma BM, Rubio-Gozalbo ME. Grey matter density decreases as well as increases in patients with classic galactosemia: A voxel-based morphometry study. Brain Res. 2016;1648(Pt A):339-344.
17. Timmers I, Roebroeck A, Bastiani M, Jansma B, Rubio-Gozalbo E, Zhang H. Assessing Microstructural Substrates of White Matter Abnormalities: A Comparative Study Using DTI and NODDI. PLoS One. 2016;11(12):e0167884.
18. Dubroff JG, Ficicioglu C, Segal S, Wintering NA, Alavi A, Newberg AB. FDG-PET findings in patients with galactosaemia. J Inherit Metab Dis. 2008;31(4):533-539.
19. Timmers I, Jansma BM, Rubio-Gozalbo ME. From mind to mouth: event related potentials of sentence production in classic galactosemia. PLoS One. 2012;7(12):e52826.
20. Timmers I, van den Hurk J, Hofman PA, et al. Affected functional networks associated with sentence production in classic galactosemia. Brain Res. 2015;1616:166-176.
21. Fuster JM, Bressler SL. Cognit activation: a mechanism enabling temporal integration in working memory. Trends Cogn Sci. 2012;16(4):207-218.
22. Jasper H. The 10-20 electrode system of the International Federation. Electroencephalogr Clin Neuropysiol. 1958;10:370-375.