Valorization
The work presented in this dissertation aimed to further investigate the chronic impairments of classic galactosemia using a combined approach that encompasses both basic and clinical research. In this addendum the valorization potential of this thesis is presented.

Upon exposure to galactose in dairy milk, neonates with classic galactosemia develop a potentially lethal syndrome that resolves when galactose-restriction is initiated timely. Notwithstanding its irrefutable life-saving role in the newborn phase, the current standard of therapy – a lifelong galactose-restricted diet – is unsatisfactory, since it fails to prevent damage to ovaries, brain and bones. Importantly, even those patients who were never exposed to galactose develop long-term impairments (1, 2), suggesting a more sophisticated therapy is needed to overcome the complex disease pathogenesis. The phenotypic spectrum is diverse, ranging from patients who are severely affected and unable to live independently to patients who experience only mild symptoms. In general, the chronic debilitations negatively influence quality of life in the galactosemia population, and they form a major concern for the patients’ parents as well (3). Furthermore, educational levels and employment rates are lower in patients, leading to impaired societal functioning (4). Due to the high costs of disease monitoring and treatment, the complications of classic galactosemia also form a burden for the healthcare system. As a result, the ultimate aim of galactosemia research worldwide is the development of new therapies that can prevent these long-term impairments and their negative impacts on patients and their families, society and the healthcare system.

Thus far, the timing of onset of organ damage in this disorder – more specifically the presence or absence of prenatal toxicity – remains to be elucidated. The existing disease models, the mouse and Drosophila melanogaster models (5, 6), showed their importance for disease pathogenesis studies (7-11), yet fail to meet the features demanded to evaluate whether damage has a prenatal and/or postnatal origin. Furthermore, these models are less amenable to testing pharmacologic compounds through a high-throughput screening approach. Therefore, the first objective of this dissertation was to develop a novel disease model for classic galactosemia. For this purpose, we developed a galt knockout zebrafish model. The model mimics the human phenotype at both biochemical and clinical levels, making it suitable to study the onset of organ damage and to develop new therapeutic strategies.

Throughout the process of this thesis, we developed the galt knockout zebrafish model and crossed our model to reporter lines. Future studies in this disease model will focus on the organ-specific onset of damage (gonads and brain) and time-specific extent of damage, in order to answer the long-standing open question if, and to what extent, prenatal organ damage occurs in this disorder. The many advantageous features of the zebrafish allow the application of innovative study designs and analysis techniques. Transgenic zebrafish lines that carry tissue-specific promoters driving green fluorescent protein (GFP) expression provide rapid, real-time in vivo developmental systems for analyzing tissue and organ development (12). Accordingly, to study damage
to gonads and brain, we crossed our *galt* knockout transgenic zebrafish line to two reporter lines: a reporter line with fluorescent primordial germ cells (PGCs) (*vasa*:GFP) (13), and a reporter line with fluorescent myelin (*mbp*:GFP) (14). By generating a *galt* knockout line with labeled PGCs and a *galt* knockout line with fluorescent myelin, we established two excellent models to study the damage to female gonads and brain from embryonic stage to adulthood.

Secondly, our zebrafish model is amenable to a high-throughput screening approach, enabling rapid and efficient testing of pharmacologic compounds of interest. As a result, the zebrafish is increasingly used as a disease model in pharmacologic studies, with experience rapidly evolving (12). Hitherto, several potential therapeutic agents have been proposed for classic galactosemia treatment, including galactokinase inhibitors and pharmacologic chaperones (15-18). Previous studies demonstrated that enzymatic impairment of the most important GALT variants results from altered protein folding, followed by aggregation and rapid destruction of the misfolded GALT protein (18-20). Pharmacologic chaperones - molecules that have the ability to stabilize (misfolded) proteins (21-23) - are therefore suggested as potential therapeutic agents for this disorder (18, 19). By changing protein conformation, the stability and activity of the protein is increased. Since individuals with a GALT activity of >10% are generally not considered patients (24), only a slight elevation of residual enzyme activity might be sufficient to prevent chronic impairments. One of the compounds of interest is arginine, an amino acid with high therapeutic potential as a pharmacologic chaperone and outstanding *in vitro* results (15). Future studies are planned to assess arginine’s potential as a chaperone treatment for classic galactosemia in our zebrafish model. For this, a *galt* p.Q188R knock-in model will be developed.

Since the establishment of well-validated therapies is a long-lasting process, it is highly important that current patient care is optimized and that interventions are developed to maximize patient functioning despite impairments. Previous research showed that many differences exist with regard to diagnosis, treatment and follow-up of patients with classic galactosemia (25). The second aim of this thesis was to further investigate the extent and clinical implications of the damage to ovaries, brain and bones, thereby providing recommendations for improvement of patient care.

Ever since the report on a remarkably high prevalence of hypergonadotropic hypogonadism in women with classic galactosemia in 1979 (26), female patients were counseled for infertility from an early age on. Current findings from our international, epidemiological study on pregnancy chance in women with classic galactosemia and primary ovarian insufficiency indicate this counseling approach is incorrect. We showed that, despite ovarian damage, these women have a considerable chance of spontaneous conception. This shifting paradigm has significant implications for fertility counseling and the potential application of fertility preservation techniques. In the future, female patients and their families/parents should be counseled for reduced fertility rather than for infertility, which is the current practice.
Physicians are often asked about possible options to preserve fertility in female patients. However, due to the lack of guidelines on fertility preservation in this population, there is a great diversity of approaches around the world. Therefore, we evaluated the risks and benefits of fertility preservation in females with this disorder and made recommendations on how to address this important matter. With the support of a multidisciplinary expert team, we suggested a conservative approach towards fertility preservation through invasive techniques, given the significant chance of spontaneous conception. If preservation of an individual’s fertility is truly wished for, ovarian tissue cryopreservation at an early age in a research setting seems the best option, due to the early depletion of the follicle pool.

In addition, we were the first to investigate functional connectivity during rest in this population, using functional magnetic resonance imaging. We evaluated resting-state functional brain networks in patients and matched controls. Our results point towards several substantial group differences, with repeatedly altered connectivity of some brain regions across analyses. Importantly, the affected networks are known to be involved in motor (speech) planning, visuospatial processing, working memory and language processing, which is in line with the neurocognitive profile of these patients. These findings can form the basis for future in-depth studies and the development of new interventions, targeting the affected cognitive functions (speech and language, working memory, sensory-motor integration, visuospatial capacities). Our data suggest patients might benefit from visuospatial training, which has not yet been implicated before, but clinical studies are needed to further explore this hypothesis.

Our meta-analysis on bone mineral density was the first study to provide adequate evidence for mild bone mass reduction in classic galactosemia. Previous studies were underpowered due to small sample sizes and results varied across studies (27-30), hampering solid conclusions on severity and clinical relevance of bone mass reduction in this disorder. Our findings warrant clinical awareness and monitoring of bone health, since 10-25% of patients are at risk of developing low bone mass. We also developed recommendations on diagnosis, treatment and follow-up of bone health in order to encourage consensual and accurate patient care worldwide. Implementation of these data on bone mass and bone health monitoring in future international guidelines is of importance.
REFERENCES


