Chapter 1

Objectives and outline of the thesis
Guanidinoacetate methyltransferase (GAMT) deficiency (MIM #612736), the first recognized disorder of creatine biosynthesis, was described by Stockler et al in 1994 [1]. In 2001, the first patient with creatine transporter (CRTR) deficiency (MIM #300352), was reported by Salomons et al [2] and the second disorder of creatine biosynthesis, arginine glycine amidinotransferase (AGAT) deficiency (MIM #612718), was described by Item et al also in 2001 [3]. The GAMT and AGAT deficiencies are autosomal recessive disorders and CRTR deficiency is an X-linked disorder. About 70 patients with GAMT deficiency, 9 patients with AGAT deficiency and 150 patients with CRTR deficiency have been reported or diagnosed since the first description of these disorders (chapter 2 [4]). The cerebral creatine deficiency in cranial magnetic resonance spectroscopy (cMRS) is the most common biomarker to all three disorders except females with CRTR deficiency who can have normal creatine levels in the cMRS. Guanidinoacetate (GAA), precursor of creatine, is elevated in GAMT and decreased in AGAT deficiencies, which can be measured in urine, plasma and cerebral spinal fluid (CSF) as disease specific biomarker (chapter 2) [4]. Increased creatine excretion in urine is the disease specific biomarker in males with CRTR deficiency, which however is often within the reference range in both symptomatic and asymptomatic females with heterozygous mutations in the SLC6A8 gene [chapter 2] [5]. Creatine supplementation is the treatment of choice for the replenishment of cerebral creatine deficiency in GAMT and AGAT deficiencies (chapter 2) [6]. Additionally in GAMT deficiency to normalize accumulation of GAA in the central nervous system (CNS), patients are treated with ornithine supplementation and arginine-restricted diet [7].

The research of this thesis was initiated by reporting 7 new patients with GAMT deficiency identifying seven new mutations in the GAMT gene (chapter 3) [8]. This study was the foundation of another study involving the first 27 patients with GAMT deficiency (chapter 4) [9]. The phenotype ranged from mild (intellectual disability) to severe (intellectual disability, epilepsy and movement disorder). We also reported long-term treatment outcome in 22 out of 27 patients for the first time in this paper (chapter 4) [9]. Most of the patients were treated with creatine therapy alone with minimal clinical or no response. Only 27% of the patients were treated with combined therapy (creatine and ornithine supplementation and arginine-restricted diet) with favourable clinical and biochemical outcome. Mean age of diagnosis was 12.3 years (ranging 2-29 years) which was a rather late diagnosis in many patients for a treatable disease (chapter 4) [9].
This paper raised the question, that when we can identify patients affected with GAMT deficiency within first two years of life prior to completion of brain development and treat them early with combined therapy (creatine and ornithine supplementation and arginine-restricted diet), can we prevent neurodevelopmental delay? Perhaps a more favourable clinical outcome due to decreased exposure time to the neurotoxic GAA accumulation and creatine deficiency in the brain could be obtained. Indeed this question could be partly answered by diagnosing and treating a new patient with moderate (global developmental delay and autism) GAMT deficiency at the age of 21 months. (chapter 5) [10].

As we know now, there are no disease specific clinical features and age of onset ranges from 3 months to 2 years. Due to these factors the diagnosis will not be possible until late second year of life even in the centers with creatine deficiency syndromes (CDS) experts and infrastructure for the diagnosis such as cMRS and urinary GAA and creatine measurements. The report of a normal neurodevelopmental outcome in a newborn with GAMT deficiency [11] highlights the importance of newborn screening for this disease. Despite many Newborn Screening Programs have introduced GAA measurements into their routine expanded newborn screening panels by tandem mass spectrometry since the early 2000s, there are no peer reviewed publications reporting the findings of these GAMT screening programs. To our knowledge none of the centers have diagnosed a newborn with GAMT deficiency through newborn screening. One patient was diagnosed with GAMT deficiency at the age of 22 months in Austria who had elevated GAA level in the first blood spot card on the fifth day of life in the Austrian National Newborn Screening Program. The urinary GAA level was marginally elevated in the first urine sample by retrieval, but was within normal range in a repeat urine sample. Newborn screening was not able to identify this patient [12]. Furthermore GAMT deficiency appears to be a very rare disorder with less than 70 patients having been described since its first identification in 1994 [1]. We therefore decided to perform a pilot study to assess carrier frequency of GAMT deficiency in the newborn population to establish an evidence base for newborn screening for GAMT deficiency and compared the results with GAA measurements. In 3000 anonymized newborn blood spots GAA was measured by tandem mass spectrometry and two most common mutations (c.59G>C in exon 1 and c.327G>A in exon 2) covering about 46.5% of all alleles reported in the literature chosen for targeted mutation analysis (chapter 6) [13].

Despite CDS have been known for more than 10 years, the number of patients identified is still low. It is still not known whether we are not able to diagnose patients while cMRS and urinary metabolite measurements are not widely available or indeed these are very rare disorders. To investigate if other more available biomarkers could be used, we reviewed patients laboratory data with confirmed diagnosis of CDS (chapter 7) [14].
Urinary creatine and creatinine are usually determined with two separate methods and normal age dependent values for both metabolites were reported only in a limited number of controls. We developed a new method allowing rapid simultaneous determination of creatine and creatinine in urine by tandem mass spectrometry (chapter 8) [15]. Interestingly, during establishment of this method, we identified two new patients with CRTR deficiency. This clinical research also allowed us to study the prevalence of CRTR deficiency in males with global developmental delay and behavioural problems (chapter 8) [15].

In CRTR deficiency, creatine supplementation alone was not able to reverse cerebral creatine deficiency in males [2]. There were no reports regarding treatment outcome in females with CRTR. We diagnosed a female with severe CRTR deficiency who presented with intractable epilepsy and failed to respond to 8 anti-epileptic medications and ketogenic diet. She was first treated with creatine supplementation following her diagnosis with seizure freedom for two weeks. This was the proof for us that her intractable epilepsy was caused by cerebral creatine deficiency. At that time arginine supplementation to increase intracranial creatine synthesis was reported in few patients with no major clinical and biochemical improvement, but none of these patients presented with seizures [16-17]. We treated our patient with L-arginine and L-glycine supplementation (chapter 9) [18].

References


