Chapter 10

Summary and discussion

Creatine deficiency syndromes (CDS), GAMT, AGAT and CRTR deficiencies, are disorders of creatine biosynthesis and transport and have been described in the last two decades [1-3]. The GAMT and AGAT deficiencies are autosomal recessive disorders and CRTR deficiency is an X-linked disorder. More than 200 patients with CDS have been reported or diagnosed since first description of these disorders (chapter 2) [4].

Seven patients with GAMT deficiency were reported in the literature between 1994 and 2003 [5-10]. In 2004, we reported 7 new patients and 7 new mutations in the *GAMT* gene together with clinical phenotype ranging from mild to severe (chapter 3) [11]. The first 27 patients with GAMT deficiency were summarized in 2006 as an international retrospective data collection study using a questionnaire including patients from Egypt, UK, Portugal, The Netherlands, Italy, Germany and Spain (chapter 4) [12]. This study was the largest patient cohort for this very rare neurometabolic disease. There was no correlation between clinical phenotype and GAA levels in body fluids. The most common panethnic mutation (c.327G>A) and the most common Mediterranean mutation (c.59G>C) were found in patients with a phenotype ranging from mild to severe. There was no correlation between clinical phenotype and biochemical phenotype and genotype (chapter 4) [12].

The first long term treatment outcome of the 22 patients with GAMT deficiency was reported in 2006 (chapter 4) [12]. Forty percent of the patients showed improvements in epilepsy and/or movement disorder; however 22% showed no clinical improvement on creatine therapy alone. One patient on combined therapy had improvements in intractable epilepsy and movement disorder with no response to creatine therapy alone [13]. None of the patients achieved normal neurodevelopmental outcome or cognitive functions on creatine alone or on combined treatment (chapter 4) [12]. This was attributed to late diagnosis and delays in initiation of the disease specific treatment.

A new patient with GAMT deficiency, who was diagnosed and treated from 22 months of age with a moderate clinical phenotype, was reported in 2012 (chapter 5) [14]. Despite strict treatment for two years, she had 1.5 year delay in her gross motor, social and adaptive skills and two year delay in her fine motor skills and about three year delay in her language skills compared to her chronological age. The main reason of therapy failure was attributed to the chronic progressive central nervous system damage secondary to creatine deficiency and the effect of neurotoxic GAA accumulation, which occurred prior to diagnosis. Even after strict

combined therapy (creatine and ornithine supplementation and arginine restricted diet) for two years, this patient still had mild cerebral creatine deficiency in cMRS (the intensity ratios of creatine to choline (Cr/Cho) increased approximately 82% of normal values in basal ganglia and 88% in white matter) and elevated GAA levels in the CNS (CSF GAA decreased 88%, but still 11 times higher than normal control levels) (chapter 5) [14]. It was disappointing that neither neurodevelopmental nor biochemical outcome was reversible despite moderate phenotype, early diagnosis and treatment initiation. However, currently available treatment was able to stop disease progression and further disease related complications such as seizures and movement disorder in this young child. This warrants us to search for new treatment options to normalize creatine deficiency and neurotoxic GAA accumulation in the central nervous system to achieve normal or close to normal neurodevelopmental outcome.

Given the effectiveness of early intervention [15] and severe neurodevelopmental outcome in untreated patients [4], GAMT deficiency seems to be an excellent candidate for newborn screening. However, it appears to be a rare disorder with less than 70 patients having been described since its first identification in 1994 [2]. To assess carrier frequency of GAMT deficiency, we performed a pilot study for the two most common GAMT mutations (c.59G>C and c.327G>A) by the QIAxcel system and GAA measurement by a novel two-tier biochemical method in 3000 anonymized newborn blood spot cards (chapter 5) [9]. Two novel heterozygous variants (c.283_285dupGTC; p.Val95dup and c.278_283delinsCTCGATGCAC; p.Asp93AlafsX35) were identified, that should be considered due to their truncating nature. Carrier frequency for these insertion/deletion types of *GAMT* mutations was 1/1475 in this small cohort of newborns. False positive rate for the first-tier biochemical test was 0.1%. Using a chromatographic second-tier test, the false positive rate was decreased from 0.1% to 0%. No GAMT mutations were identified in 4 of the newborns with elevated GAA levels in the first tier testing (chapter 6) [16]. Next step would be a pilot newborn screening by two-tier GAA and creatine measurement in blood spot cards and molecular genetic testing by direct Sanger sequencing of the *GAMT* gene for two years to be able to identify through carrier frequency of the GAMT deficiency in the newborn population.

The CK is a critical enzyme for neurotransmitter release, maintenance of membrane potentials and restoration of ion gradients in conjunction with membrane bound Na–K-ATPase in the central nervous system [17,18]. Cytosolic and mitochondrial brain-CK transcripts were down-

regulated in patients with bipolar disorder which was attributed to reduced phosphocreatine in c-MRS caused by neuronal cell loss [19]. Additionally in vivo intrastriatal GAA administration in rat was shown to inhibit CK and Na–K-ATPase causing apoptosis and neuronal loss in the central nervous system (CNS) [20]. We detected low serum total creatine kinase (tCK) in two patients with CDS. Low peripheral tCK in both patients might be explained by down-regulated CK transcription secondary to creatine and phosphocreatine deficiency and accumulation of GAA in CDS (chapter 7) [21]. Low serum tCK might be a potential screening biomarker with low specificity and sensitivity in CDS. Not only cerebral creatine deficiency and accumulation of GAA, but also inhibition of tCK and Na-K-ATPase might play an important role in the neuropathogenesis of CDS. A prospective study to investigate patients with low CK and with global developmental delay and/or seizures, movement disorder and behavioural problems have been started to evaluate value of low CK in CDS.

The reported frequency of CRTR deficiency is between 0.8-5.4% in males with intellectual disability [22-27]. We developed a new high throughput method for simultaneous measurement of urinary creatine and creatinine by tandem mass spectrometry. We used random urine samples from 975 individuals (557 males and 418 females) between 0 and 18 years (median 19.5 months), (0–1 year: n = 537; 1–3 years: n = 148; 3–8 years: n = 162; 8–18 years: n = 128) sent to the laboratory for selective metabolic screening. We identified two new patients with CRTR deficiency in 87 males older than 1 year of age with global developmental delay and/or behavioural problems and seizures. The prevalence of CRTR deficiency was 2.3% in symptomatic males. Additionally we developed age related normal values for creatine and creatinine in 975 individuals (chapter 8) [28].

Creatine supplementation alone was not sufficient to replenish cerebral creatine deficiency in males with CRTR deficiency [2]. Arginine and glycine supplementation to increase intracranial creatine synthesis was reported in various patients with no prominent clinical and biochemical improvement [29-32]. We reported a female with CRTR deficiency who had intractable epilepsy and mild intellectual disability at the age of 6.5 years. She had clinical phenotype comparing to males with no skewed X-inactivation in peripheral blood DNA. She was treated with l-arginine and l-glycine supplementation therapy and showed complete resolution of her intractable epilepsy after 10^{th} month of therapy (chapter 9) [33]. This is the only case with successful treatment outcome.

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