5.1 SUMMARY

In 2001 X-linked creatine transporter deficiency (CRTR-D) was discovered as a cause of X-linked intellectual disability characterized by severely reduced creatine signal on proton MR spectroscopy (^{1}H-MRS), increased creatine/creatinine ratio in urine, deficient creatine uptake in cultured skin fibroblasts, and mutations in the *SLC6A8* gene, encoding the creatine transporter.

To obtain an overview of this newly discovered condition, we performed a retrospective study of clinical, biochemical and molecular genetic data of 101 males with X-linked creatine transporter deficiency (CRTR-D) from 85 families with a pathogenic mutation in *SLC6A8* (**chapter 2.1**). Most patients developed moderate to severe intellectual disability; mild intellectual disability was rare in adult patients. Speech language development was especially delayed but almost a third of the patients was able to speak in sentences. Other common symptoms were behavior problems, mainly attention deficit, hyperactivity, and autistic features, and seizures. In addition, mild to moderate motor dysfunction, including extrapyramidal movement disorder, occurred frequently. Feeding difficulties, vomiting, and failure to thrive were early and sometimes first presenting symptoms while severe constipation and ileus developed later in life. Evaluation of the biochemical phenotype revealed unexpected normal to slightly elevated creatine levels in cerebrospinal fluid. Urinary creatine/creatinine ratio proved a reliable screening method and diagnostic guidelines were defined which also include MR spectroscopy, molecular genetic testing, and creatine uptake studies. Two common *SLC6A8* mutations accounted for 14% of the families. However, most patients had unique mutations, missense mutations, and one-amino acid deletions being the most common. A third of patients had a de novo mutation. No clear-cut genotype-phenotype correlation was observed, but missense mutations with residual activity might be associated with a milder phenotype.

In **chapter 2.2** an unusual severe presentation of CRTR-D with multi-exon deletions of *SLC6A8* is discussed. We studied the genotype-phenotype correlations in nine patients with (contiguous) gene deletions involving *SLC6A8*, *BCAP31* and/or *ABCD1*. Mutations in *ABCD1* are associated with X-linked adrenoleukodystrophy and, coincidently just during the process of this study, isolated defects of *BCAP31* were found to be associated with profound developmental delay, dystonia, deafness and childhood mortality. The deletion of two CRTR-D patients included the *BCAP31* gene and their severe phenotype can largely be explained by the *BCAP31* defect. However, another CRTR-D patient with an isolated

deletion of the 3'end of *SLC6A8* had a very similar phenotype with childhood death but without deafness. Thus, also isolated deletions of *SLC6A8* extending beyond its 3'end appear associated with a severe phenotype which might be caused by disturbance of a regulatory element between *SLC6A8* and *BCAP31*. In addition we found that only patients with deletions involving both *BCAP31* and *ABCD1* developed hepatic cholestasis and died in the first year of life. This might be explained by synergistic effects of loss of *BCAP31* and *ABCD1*.

In **chapter 2.3** we discuss the treatment of CRTR-D. While patients with cerebral creatine deficiency due to defects in the creatine synthesis (AGAT and GAMT deficiency) profit from creatine supplementation, no effective treatment exists for CRTR-D. Since brain cells are capable of endogenous creatine synthesis, supplementation with creatine precursors L-arginine and glycine was considered a good option to increase cerebral creatine synthesis. Treatment with creatine monohydrate, L-arginine, and glycine was started in the Erasmus MC in Rotterdam in nine Dutch boys between 8 months and 10 years old with molecularly confirmed CRTR-D. The effects were followed with repeated ^{1}H-MRS and neuropsychological assessments during 4-6 years. The treatment did not lead to a significant increase in cerebral creatine content as observed on ^{1}H-MRS. After an initial improvement in locomotor and personal-social IQ subscales, no lasting clinical improvement was recorded. Additionally, we noticed an age-related decline in IQ subscales in boys affected with CRTR-D.

We investigated the clinical features and pattern of X-inactivation in a Dutch cohort of eight female heterozygotes (**chapter 3.1**). Because CRTR-D is an X-linked condition, X-inactivation will play an important role in the phenotype in female heterozygotes. We found that symptoms of CRTR-D (intellectual disability, learning difficulties, and constipation) can be present in female heterozygotes. However, the diagnosis in females was not straightforward: (i) The creatine/creatinine ratio in urine was elevated only in three of eight females. (ii) Although as a group the females had a significantly decreased cerebral creatine concentration measured with 1H-MRS, individual females had creatine concentrations overlapping with normal controls. (iii) Skewed X-inactivation was found in the cultured fibroblasts, in favor of either the mutated or the wild-type allele, leading to either deficient or normal results in the creatine uptake studies in fibroblasts. Thus, in females screening by these tests is unreliable and screening by DNA analysis is recommended. We found no consistent skewing of the X-inactivation in peripheral tissues indicating that there is no selection against the *SLC6A8* mutation.

In **chapter 3.2** we describe somatic mosaicism in a mother of two sons with CRTR-D. A pathogenic mutation in *SLC6A8* was detected in both boys but direct DNA sequencing failed to detect the mutation in blood of the mother. However, denaturing high-performance liquid chromatography (DHPLC) indeed revealed low-level somatic mosaicism in maternal blood of about 6%. In the cohort of 101 males from 85 families with CRTR-D (**chapter 2.1**) we found, in addition to this family, three more mothers with low level somatic mosaicism for the *SLC6A8* mutation. The occurrence of mosaicism has important consequences for the counseling of parents with an affected son with a presumed de novo mutation. They should be counseled about a recurrence risk in further pregnancies due to the possibility of low level somatic or germline mosaicism.

Chapter 4 concerns the pathophysiology of CRTR-D. We need to understand why the brain, capable of creatine synthesis, becomes creatine depleted in CRTR-D and does not respond to arginine and glycine supplementation. This is of paramount importance in the development of an effective treatment. We found normal to slightly elevated creatine levels cerebrospinal fluid (CSF) in patients with CRTR-D (**chapter 2.1**) whereas a reduction (as observed in GAMT deficiency) would be expected. In **chapter 4.1** we hypothesize that the brain synthesizes creatine, but that in CRTR-D creatine is lost in CSF due to reuptake failure and that the cerebral creatine deficiency derives from defective creatine recycling. Mouse models of neurotransmitter transporter defects underline the importance of reuptake for maintenance of intracellular neurotransmitter stores. This model supports a role of creatine as a neuromodulator or even neurotransmitter as previously suggested. **Chapter 4.2** provides a comprehensive overview of the current clinical and pathophysiological insights based on the results described in this thesis and review of the literature.