J. Inherit. Metab. Dis. 26 (2003) 309–318

X-linked creatine transporter defect: An overview

G. S. SALOMONS[1]*, S. J. M. VAN DOOREN[1], N. M. VERHOEVEN[1], D. MARSDEN[2], C. SCHWARTZ[3], K. M. CECIL[4], T. J. DEGRAUW[5] and C. JAKOBS[1]

[1]*Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands;* [2]*Department of Genetics and Metabolism, Children's Hospital Boston, Boston, Massachusetts;* [3]*J.C. Self Research Institute of Human Genetics, Greenwood, South Carolina; Divisions of* [4]*Radiology and* [5]*Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA*

**Correspondence: Department of Clinical Chemistry (Metabolic Unit 1 WBI 2), VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands. E-mail: g.salomons@vumc.nl*

Summary: In 2001 we identified a new inborn error of metabolism caused by a defect in the X-linked creatine transporter *SLC6A8* gene mapped at Xq28 (SLC6A8 deficiency, McKusick 300352). An X-linked creatine transporter defect was presumed because of (1) the absence of creatine in the brain as indicated by proton magnetic resonance spectroscopy (MRS); (2) the elevated creatine levels in urine and normal guanidinoacetate levels in plasma, ruling out a creatine biosynthesis defect; (3) the absence of an improvement on creatine supplementation; and (4) the fact that the pedigree suggested an X-linked disease. Our hypothesis was proved by the presence of a hemizygous nonsense mutation in the male index patient and by the impaired creatine uptake by cultured fibroblasts. Currently, at least 7 unrelated families (13 male patients and 13 carriers) with a SLC6A8 deficiency have been identified. Four families come from one metropolitan area. This suggests that SLC6A8 deficiency may have a relatively high incidence. The hallmarks of the disorder are X-linked mental retardation, expressive speech and language delay, epilepsy, developmental delay and autistic behaviour. In approximately 50% of the female carriers, learning disabilities of varying degrees have been noted.

CREATINE SYNTHESIS AND TRANSPORT

Creatine and phosphocreatine play essential roles in the storage and transmission of phosphate-bound energy (Walker 1979; Wyss and Kaddurah-Daouk 2000). Humans

maintain their creatine pool by biosynthesis and nutritional uptake. Two enzymes, arginine:glycine amidinotransferase (AGAT; EC 2.1.4.1) and guanidinoacetate methyltransferase (GAMT; EC 2.1.1.2) (Walker 1979) are involved in the biosynthesis of creatine. Creatine is synthesized primarily in the liver and also in kidney and pancreas. Creatine is transported via the blood. Cellular transport is of fundamental importance for creatine homeostasis in tissues void of creatine biosynthesis. The creatine transporter gene (*SLC6A8/CT1/CRTR;* McKusick 300036) has been mapped to Xq28 (Gregor et al 1995). This gene is expressed in most tissues, with highest levels in skeletal muscle and kidney, and somewhat lower levels in colon, brain, heart, testis and prostate (Nash et al 1994; Sora et al 1994). The first inborn error of creatine biosynthesis was identified as a result of GAMT deficiency (McKusick 601240, Schulze et al 1997; Stöckler et al 1996a,b; Stromberger et al 2003; van der Knaap et al 1999). We detected the second inborn error of creatine deficiency due to a defect in the creatine transporter (Salomons et al 2001, McKusick 300352). Recently, the third inborn error of metabolism was identified (Item et al 2001, McKusick 602360) due to a defect in AGAT, the first enzyme of creatine biosynthesis.

THE *SLC6A8* GENE IS A MEMBER OF A SOLUTE CARRIER FAMILY THAT TRANSPORTS NEUROTRANSMITTERS

The *SLC6A8* gene is a member of a superfamily of proteins that includes the family of Na^+- and Cl^--dependent transporters responsible for the uptake of certain neurotransmitters (e.g. dopamine, GABA, serotonin, and noradrenaline) and amino acids (e.g. glycine, proline, taurine) (Nash et al 1994). The creatine transporter gene spans approximately 8.4 kb and consists of 13 exons (GenBank accession no. Z66539) and encodes for a protein of 635 amino acids with a predicted molecular weight of 70 kDa (Sandoval et al 1996). The transcript has been reported to have a size of $\sim$4.3 kb, but additional size transcripts have been reported on northern blots (Nash et al 1994; Sora et al 1994). A paralogous gene has been mapped at chromosome 16, the *SLC6A10* gene (Eichler et al 1996; Xu et al 1997). This gene is 97.1% identical to the coding sequence of the *SLC6A8* gene and overall has a similarity of 94.6%. Most likely this gene represents a pseudogene, since (1) a premature stop codon occurs in exon 4 and (2) its mRNA expression has been associated with hypomethylation of the gene in testis. The testis-specific transcription may represent a side-product of the transient demethylation during spermatogenesis (Grunau et al 2000).

IDENTIFICATION OF THE FIRST INDEX PATIENT AND HIS FAMILY

Our index case, a caucasian boy, was diagnosed with mild mental retardation and severe delay in expressive speech and language function. Gross and fine motor functions were normal. He was slightly hypotonic. The epileptic seizures were well controlled. At age 6 years his head circumference increased from the 75th to the 95th centile. This prompted one of us (T.J.deG.) to request MRI and proton MRS. MRI showed a small focus of hyperintense signal in the right posterior periventricular white matter on T2-weighted images (similar to the MRIs taken at

the ages of 8 months and 2 years). The proton MRS of the brain revealed an almost complete absence of the creatine signal (Cecil et al 2001) as observed in GAMT-deficient and in AGAT-deficient patients (Item et al 2001; Schulze et al 1997; Stöckler et al 1996a,b; van der Knaap et al 1999). However, guanidinoacetate (GAA) in urine and plasma was normal, as measured by stable-isotope dilution GC-MS (Salomons et al 2001; Struys et al 1998). Creatine, however, was markedly present in urine and in plasma. Furthermore, supplementation of creatine monohydrate at 340 mg/kg during 3 months did not result in an improved clinical condition or in an increased creatine signal in the brain MRS (Cecil et al 2001). Thus, GAMT deficiency was ruled out. The index case is the only child of healthy parents. The mother and the maternal grandmother have a history of learning disability. The mother has two siblings, a severely retarded brother and an asymptomatic sister. The fact that the males were more severely affected than the females suggested an X-linked disorder.

We hypothesized that the patients suffered from a defect in the creatine transporter located at Xq28, as described above. Indeed, sequence analysis of amplified cDNA of *SLC6A8* from fibroblasts of the index patient revealed a nonsense mutation (Salomons et al 2001). The mutation, transition of c1540C>T of the *SLC6A8* (GenBank accession no. NM_005629) resulted in a substitution of arginine codon 514 by a termination codon (R514X). Genomic DNA sequence analyses confirmed this mutation. The three females were heterozygous for this mutation as determined by gDNA sequence analysis. In contrast to the grandmother and mother of the index case, the aunt is asymptomatic.

Skewed X-inactivation might explain this phenotypic difference from the other two female carriers. A half-sister of the index patient was born recently. At the age of 14 days her brain appeared to contain a reduced creatine signal as studied by proton MRS, when compared to an age-matched control (Cecil et al 2003). She is a carrier of the R514X mutation.

IDENTIFICATION OF 13 PATIENTS AND 13 CARRIERS WITH SLC6A8 DEFICIENCY

We have identified seven unrelated families with proven SLC6A8 deficiency. Most of the affected males were recognized owing to delays in expressive speech and language and the proton MRS of the brain (see Table 1) (Bizzi et al 2002; Cecil et al 2001; de Grauw et al 2002; D. Marsden, unpublished results). One family had X-linked mental retardation (XLMR) and had been linked to Xq28 (Hahn et al 2002; family 6, Table 1). Mutational analysis, fibroblast uptake studies and metabolic workup confirmed the diagnosis (see below: Tables 2–4).

CLINICAL PRESENTATION OF MALES WITH SLC6A8 DEFICIENCY

The group of patients diagnosed with SLC6A8 deficiency is relatively large for the time of awareness of the disorder. However, the group is too small for a definite description of the clinical presentation. In addition, as with most newly recognized

Table 1 General overview of the first seven families identified with SLC6A8 deficiency

Family	*Origin*	*Studied because of*	*No. of generations studied*	*No. of affected males*	*No. of female carriers (symptomatic/asymptomatic)*
1[a]	Cincinnati, OH, USA	H-MRS § Metabolic work-up	3	2	4(3/1)
2[b]	Cincinnati, OH, USA	Dysphasia	2	2	2(1/1)
3[b]	Cincinnati, OH, USA	H-MRS §	2	1	1(0/1)
4[b]	Cincinnati, OH, USA	H-MRS §	2	1	0
5[c]	Milan, Italy	Clinical presentation and H-MRS, initially creatine biosynthesis deficiency presumed	2	1	1(0/1)
6[d]	Greenwood, SC, USA	XLMR family linked to Xq28	3	5	5(2/2 and 1 unknown)
7[e]	United Arab Emirates (UAE)/MA, USA	H-MRS	2	1	0
				13	13 (6/6 and 1 unknown)

H-MRS, proton magnetic resonance spectroscopy; XLMR, X-linked mental retardation
[a]Cecil et al (2001), Salmons et al (2001)
[b]DeGrauw et al (2002)
[c]Bizzi et al (2002)
[d]Hahn et al (2002)
[e]D. Marsden, unpublished results

Table 2 Overview of the clinical and laboratory findings[a]

Clinical or laboratory sign	*Type or characteristic*	*No. out of 7*
Developmental delay	Mild	5
	Severe	2
Dysphasia		7
Seizures		5
Autistiform behaviour		7
Poor motor development		2
Head circumference	Macrocephalic	1
	Microcephalic	2
	Normocephalic	4
Reduced height		3

[a]Based on the presentation of the index male within the first 7 families with proven SLC6A8 deficiency

Table 3 H-MRS of brain and metabolic workup

Family no.	*H-MRS Cr signal?*	*Age* (y)	*Urine Cr/Crn*	*Plasma* (μmol/L)		*CSF* (μmol/L)	
				Cr	*Crn*	*Cr*	*Crn*
1	Absent	7	1.5	75	35	62[a]	15
2	Absent	20	3.1	95	37	56	14
3	Reduced	4	3.1	68	38	ND	ND
4	Reduced	2	5.5	96	27	ND	ND
5	Absent	4	4.5	83	ND	ND	ND
6	ND[b]	66	2.5	ND	ND	ND	ND
7	Absent	2	3.8	69	ND	ND	ND
Controls			<4 y: 0–1.2 >4 y: 0–0.7 >12 y: 0–0.3	<10 y: 17–109 >10 y: 6–50	<10 y: 18–62 >10 y: 50–110	24–53 ($n = 34$)	29–41[c]

H-MRS, proton magnetic resonance spectroscopy; Cr, creatine; Crn, creatinine; ND, not done
[a]CSF taken during creatine supplementation of 340 mg/kg per day
[b]Large X-linked mental retardation family (Hahn et al 2002) that showed linkage at Xq28
[c]Stöckler et al (1997)

Table 4 ***SLC6A8*** **mutational analysis and creatine uptake in fibroblasts of 7 index males**

Family	*Description of mutation*	*Type of mutation*	*Exon*	*Creatine uptake*
1	R514X	Nonsense	11	Impaired
2	delF107	Deletion of one amino acid	2	Impaired
3	delF408	Deletion of one amino acid	7	Impaired
4	Y262X	Nonsense	5	Impaired
5	delF408	Deletion of one amino acid	7	ND
6	G381R	Missense leading to splice site mutation	7/8	Impaired
7	deletion of 3′ end of gene	Large deletion	8–13	Impaired

ND, not done

disorders, most likely we have encountered the more severely affected patients. Nevertheless, the clinical presentation of SLC6A8 disorder showed marked differences among the patients (Table 2). So far we have identified 13 males from 7 unrelated families with this disorder (Bizzi et al 2002; Cecil et al 2001; deGrauw et al 2002, 2003; Hahn et al 2002; Salomons et al 2001; D. Marsden, unpublished results). The clinical signs of the index patient have been scored for each family. In the families in whom more than one patient was encountered, it appears that the clinical phenotypes differ less within the family than compared within the group of 13 patients. Whether these differences are due to a genotype–phenotype relationship or are due to other genetic defects and/or environmental factors is currently unknown. Furthermore, it should be noted that not all patients have been seen by the same physician. The current patient population suggests that the most important clinical hallmarks are mental retardation, severe delay in expressive speech and language function, epilepsy in 50% of the cases and autistic behaviour.

METABOLIC WORKUP: REDUCED CREATINE IN BRAIN, INCREASED CREATINE IN URINE

Creatine and guanidinoacetate in urine, plasma and, if available, in CSF were determined by GC-MS using stable-isotope-labelled creatine and/or guanidinoacetate as internal standards (Salomons et al 2001; Struys et al 1998). Creatinine was determined by the Jaffe method in the routine clinical chemistry department. Urine samples were obtained as random samples, not by 24 h collection. So far, in all male patients, creatine excretion appears to be a valuable marker. It should be noted that the values are expressed as a ratio of creatine to creatinine. Absolute values of creatine were not symbolic of increased creatine excretion. Creatine/creatinine measurement in urine is therefore the choice for a metabolite screening protocol. It is not known whether some

patients with milder mutations in the *SLC6A8* gene may be missed if creatine excretion is the only marker used to detect creatine transporter deficiency. For the AGAT biosynthesis defect, measurement of plasma creatine (decreased) and guanidinoacetate (decreased) is preferred by the authors. A GAMT defect can be detected by increased levels of guanidinoacetate and reduced levels of creatine and creatinine in both urine and plasma.

In all cases that were examined by H-MRS ($n = 6$), the complete absence or marked reduction of creatine was observed (Bizzi et al 2002; Cecil et al 2001; de Grauw et al 2002; D. Marsden, unpublished results).

ABSENCE OF HOTSPOT MUTATIONS

Mutational analysis of the *SLC6A8* gene by DNA sequence analysis resulted in the identification of two nonsense mutations, two single-amino-acid deletions of which one occurred twice, a large deletion and one missense mutation resulting in the use of at least one alternative splice site. The mutations were encountered in exons 2, 5, 7, 11 and 8–13. As yet no hotspot mutation or hot spot region of the gene has been identified. All mutations resulted in impaired creatine uptake in fibroblasts when they were cultured at physiological levels of creatine (25–125 μmol/L) as described (de Grauw et al 2002; Salomons et al 2001; unpublished results).

TREATMENT

Treatment of this disorder for affected males is not possible by straightforward oral creatine supplementation. However, preliminary results indicate that female carriers can have their creatine pool in the brain restored by creatine supplementation. This finding is in favour of the possible (partial) reduction/reversion and even prevention of clinical manifestation of adolescent carriers and/or newborn/unborn carriers, respectively.

FINAL REMARKS

Within the last 2 years, a novel creatine deficiency syndrome has been recognized and more than 13 male patients and 13 carriers have now been identified. The fact that four families from one metropolitan area were found in a relatively short time suggests that this disorder may have a relatively high incidence. Large screening protocols are needed to elucidate the prevalence of this disorder. Therefore, a collaboration has been initiated with the European X-linked mental retardation (XLMR) consortium. Families are enrolled in the XLMR studies if at least two males in a family have mental retardation. The consortium has archived DNA samples of 290 unrelated index males. These samples will be analysed by direct sequence analysis of genomic PCR products. If mutations are identified, the nature of the mutation will need to be investigated. The creatine uptake in fibroblasts, metabolic workup, proton

MRS of the brain and segregation of the mutation within the family could indicate the pathogenicity as well as screening of specific mutations in a large series of non-mentally-retarded males.

The fact that both the creatine biosynthesis defects and the transporter defect exhibit prominent brain involvement indicates the pivotal importance of creatine for normal brain function. The well-known function of creatine is storage and shuttling of energy, which may explain the prominent brain damage. Creatine supplementation has also been associated with protection of the brain in patients with stroke and several neurological syndromes (Tarnopolsky and Beal 2001; Wyss and Schulze 2002). The high homology between the superfamily of neurotransmitters at the molecular level could indicate that creatine may have an additional role as neurotransmitter.

Knockout mouse models and clinical trials will hopefully result in a better understanding of the disorder and in appropriate treatment protocols for both affected males and females.

The authors suggest that male patients with mental retardation, autistic behaviour, epilepsy and/or expressive speech and language delay should be tested for creatine deficiency disorders. Screening could be based on metabolite measurement of both urine and plasma, H-MRS of brain and/or mutational analysis by direct sequencing of the gene(s). Functional tests could prove the biochemical defect and should include tests for AGAT and GAMT enzyme activity or creatine uptake into lymphoblasts or fibroblasts, respectively.

ACKNOWLEDGEMENTS

Roos deGrauw and Dana Bunea are greatly appreciated for their assistance with DNA sequence analysis, and Efraim Rosenberg for critical reading of the manuscript. Birthe Roos, Wjera V. Wickenhagen, Ulbe Holwerda and Erwin E. Jansen are very much appreciated for their continuing assistance with regard to metabolic workup and GCMS analysis (all at Metabolic Laboratory of the VU University Medical Center of Amsterdam). Gail Chuck has been and is very valuable for her efforts in collecting specimens of the patients at the Department of Neurology, Cincinnati Children's Hospital Medical Center, USA. The European XLMR consortium and Dr Helger Yntema (Department of Human Genetics, University Medical Centre Nijmegen) are acknowledged for making the DNA samples of their consortium available for our collaborative study in XLMR males.

REFERENCES

Bizzi A, Bugiani M, Salomons GS, et al (2002) X-linked creatine deficiency syndrome: a novel mutation in creatine transporter gene SLC6A8. *Ann Neurol* **52**: 227–231.

Cecil KM, Salomons GS, Ball WS, et al (2001) Irreversible brain creatine deficiency with elevated serum and urine creatine: a creatine transporter defect? *Ann Neurol* **49**: 401–404.

Cecil KM, DeGrauw TJ, Salomons GS, Jakobs C, Egelhoff JC, Clark JF (2003) Magnetic resonance spectroscopy in a 9-day-old heterozygous female child with creatine transporter deficiency. *J Comput Assist Tomogr* **27**: 44–47.

deGrauw TJ, Salomons GS, Cecil KM, et al (2002) Congenital creatine transporter deficiency. *Neuropediatrics* **33**: 232–238.

deGrauw TJ, Cecil KM, Byars AW, Ball WS, Jakobs C (2003) The clinical syndrome of creatine transporter deficiency. *Mol Cell Biochem* **244**: 45–48.

Eichler EE, Lu F, Shen Y (1996) Duplication of a gene-rich cluster between 16p11.1 and Xq28: a novel pericentromeric-directed mechanism for paralogous genome evolution. *Hum Mol Genet* **5**: 899–912.

Gregor P, Nash SR, Caron MG, Seldin MF, Warren ST (1995) Assignment of the creatine transporter gene (SLC6A8) to human chromosome Xq28 telomeric to G6PD. *Genomics* **25**: 332–333.

Grunau C, Hindermann W, Rosenthal A (2000) Large-scale methylation analysis of human genomic DNA reveals tissue-specific differences between the methylation profiles of genes and pseudogenes. *Hum Mol Genet* **9**: 2651–2663.

Hahn KA, Salomons GS, Tackels-Horne D (2002) X-linked mental retardation with seizure and carrier manifestations is caused by a mutation in the creatine-transporter gene (SLC6A8) located in Xq28. *Am J Hum Genet* **70**: 1349–1356.

Item CB, Stöckler-Ipsiroglu S, Stromberger C, et al (2001) Arginine:glycine amidinotransferase deficiency: the third inborn error of creatine metabolism in humans. *Am J Hum Genet* **69**: 1127–1133.

Nash SR, Giros B, Kingsmore SF (1994) Cloning, pharmacological characterization, and genomic localization of the human creatine transporter. *Receptors Channels* **2**: 165–174.

Salomons GS, Dooren JM, Verhoeven NM, et al (2001) X-linked creatine-transporter gene (SLC6A8) defect: a new creatine deficiency sydrome. *Am J Hum Genet* **68**: 1497–1500.

Sandoval N, Bauer D, Brenner V (1996) The genomic organization of a human creatine transporter (CRTR) gene located in Xq28. *Genomics* **35**: 383–385.

Schulze A, Hess T, Wevers R (1997) Creatine deficiency syndrome caused by guanidinoacetate methyltransferase deficiency: diagnostic tools for a new inborn error of metabolism. *J Pediatr* **131**: 626–631.

Sora I, Richman J, Santoro G (1994) The cloning and expression of a human creatine transporter. *Biochem Biophys Res Commun* **204**: 419–427.

Stöckler S, Isbrandt D, Hanefeld F, Schmidt B, von Figura K (1996a) Guanidinoacetate methyltransferase deficiency: the first inborn error of creatine metabolism in man. *Am J Hum Genet* **58**: 914–922.

Stöckler S, Hanefeld F, Frahm J (1996b) Creatine replacement therapy in guanidinoacetate methyltransferase deficiency, a novel inborn error of metabolism. *Lancet* **348**: 789–790.

Stöckler S, Marescau B, De Deyn PP, Trijbels JM, Hanefeld F (1997) Guanidino compounds in guanidinoacetate methyltransferase deficiency, a new inborn error of creatine synthesis. *Metabolism* **46**: 1189–1193.

Stromberger C, Bodamer OA, Stöckler-Ipsiroglu S (2003) Clinical characteristics and diagnostic clues in inborn errors of creatine metabolism. *J Inherit Metab Dis* **26**: 299–308.

Struys EA, Jansen EE, ten Brink HJ, Verhoeven NM, van der Knaap MS, Jakobs C (1998) An accurate stable isotope dilution gas chromatographic–mass spectrometric approach to the diagnosis of guanidinoacetate methyltransferase deficiency. *J Pharm Biomed Anal* **18**: 659–665.

Tarnopolsky MA, Beal MF (2001) Potential for creatine and other therapies targetting cellular energy dysfunction in neurological disorders. *Ann Neurol* **49**: 561–574.

van der Knaap MS, Verhoeven NM, Maaswinkel-Mooij P, et al (2000) Mental retardation and behavioral problems as presenting signs of creatine synthesis defect. *Ann Neurol* **47**: 540–543.

Walker JB (1979) Creatine: biosynthesis, regulation and function. *Adv Enzymol Relat Areas Mol Biol* **50**: 177–242.

Wyss M, Kaddurah-Daouk R (2000) Creatine and creatinine metabolism. *Physiol Rev* **80:** 1107–1213.

Wyss M, Schulze A (2002) Health implications of creatine: can oral creatine supplementation protect against neurological and atherosclerotic disease? *Neuroscience* **112**: 243–260.
Xu W, Liu L, Gorman PA, Sheer D, Emson PC (1997) Assignment of the human creatine transporter type 2 (SLC6A10) to chromosome band 16p11.2 by *in situ* hybridization. *Cytogenet Cell Genet* **76**: 19.