Creatine metabolism disorder leads to structural and physiological abnormalities in the brain of Creatine Transporter KO mice.

Devashish Das[1], Chi-Un Choe[2], Malte Stockebrand[2], Andor Veltien[1], Houshang AmiriDoumari[1], Dirk Isbrandt[2], and Arend Heerschap[1]
[1]*Radboud University Medical Center, Nijmegen, Nijmegen, Netherlands,* [2]*Center for Molecular Neurobiology Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

Introduction: X-linked creatine metabolism defect is a result of mutations in Slc6a8 gene (1). This gene encodes a transmembrane creatine transporter protein (CrT), which is highly expressed in brain and skeletal muscle cells with fluctuating energy demands. In male carriers creatine transporter deficiency severely affects brain structure and function. These patients experience growth and developmental delay, mental retardation, severe cognitive impairment and often they encounter epileptic seizures (1). Recently, we engineered conditional CrT-KO mice, which require validation (unpublished Choe,C.U *et.al*). Functional MRI experiments like ASL (arterial spin labeling), and DTI can measure cerebral blood flow (CBF) and Fractional Anisotropy (FA), which are indirect but reliable biomarkers for diagnosis of diseased micro-vascular structure and altered neurogenesis in human cohorts (2,3). Diagnosis of sporadic CrT patients is a challenging task. Here we study male CrT-KO mice with the aim to potentially identify MR guided biomarkers, and thereby explore phenotypic similarities between CrT-KO mice and CrT-patients.

Method: For this study, we used transgenic Creatine Transporter (CrT) mice. All MR experiments were performed at 7T on a Clinscan MR system (Bruker), and at 11.7T on a Biospec MR system (Bruker). Animals were anesthetized by inhalation of isoflurane/air/oxygen mixture. For ^{31}P-MRSI we used a pulse acquire sequence incorporating a BIR-45 pulse for ^{31}P spin excitation and 100µl (4x5x5) voxel selection in three orthogonal planes, coronal, sagital and transversal, of mouse brain (TR=1.5sec, FOV=40x32x40 mm, Matrix= 8x8x8 NA= 64). For metabolite quantification we used LC-model approach as described before (4). For control and CrT-KO mice ^{31}P-spectra of hindleg were acquired as described before (4). Next we performed ASL (FAIR) and DTI experiments on a Biospec (11.7T) Bruker MR system. For data analysis we used Bruker image analysis toolbox.

Results and Discussion: In this study, we found that the brain and hind leg skeletal muscle of the male transgenic CrT-KO mice is deficient in PCr (phosphocreatine) and Cr (creatine) pools compare to control littermates. In the brain of CrT-KO mice we also found lower ATP/Pi ratio, clearly suggesting their brain cells synthesize ATP at a rate lower than that of control mice. Reduced intracellular ATP concentration is indicative of energy-deprived CrT-KO brain leading to structural and physiological abnormalities (5,6). In contrast, measured PCr/ATP ratio in the whole brain of control mice is consistent with earlier reports (4,6). Next by LC-Model quantification approach we derived proton metabolite concentrations in CrT-KO and control brain (4). In particular, taurine (Tau) and myoinositol (Im) concentrations are lower in CrT-KO brain. Suggesting their brain cells is under osmotic stress. Notably, osmotic deregulation results in membrane potential breakdown, and is commonly linked to leaky membrane junctions, causing buffer capacity of cells to fail. Finally, our functional MRI studies reveal reduced cerebral blood flow (CBF) (data not shown), and increased fractional anisotropy (FA) in the whole brain of CrT-KO mice than control mice. These two distinct parameters again relate osmotic deregulation and energy deprivation with cells response to micro-vascular adaption as seen in CrT-KO brain. It is tempting to speculate that the brain of CrT-KO is under controlled vasoconstriction (2,7), which is anticipated to inhibit synapses by lowering the degrees of neural branching (3). Interestingly, in human age associated brain disorders similar adaptation of brain micro-vascular structure is well documented (2). In conjunction increased FA is connected with reduced neural branching, and neurological deficits in ADHD patients (3). In conclusion we show Creatine transporter defect is prevalent in the brain and skeletal muscle of transgenic CrT-KO mice, which is consistent with that seen in human patients. Depleted PCr pool is a hallmark of metabolic stress, which may trigger a plethora of events; at first perturb cAMPK signaling, which then can impede mitochondrial complex-1 function in the brain and skeletal muscle of transgenic CrT-KO mice (5,6).

Acknowledgement: This work is supported by funding from the CSBB (Centre for Systems Biology and Bioenergetics), Radboud University Nijmegen. Authors are thankful to Sjaak van Asten for software and technical assistance, and the supporting staff of PRIME facility for administrative and experimental support.

References [11] Salomons, G.S. (2003) *J Inherit Metab Dis*, **26**, 309-318; [2] Chen, J.J. (2011) *Neuroimage*, **55**, 468-478; [3] Silk, T.J. (2009) *Hum Brain Mapp*, **30**, 2757-2765; [4] Nabuurs, C.I. (2012) *J Physiol*, **591**, 571-592; [5] Skelton, M.R. (2011) *PLoS One*, **6**, e16187; [6] Kurosawa, Y. (2012) *J Clin Invest*, **122**, 2837-2846; [7] Attwell, D. (2002) *Trends Neurosci*, **25**, 621-625;

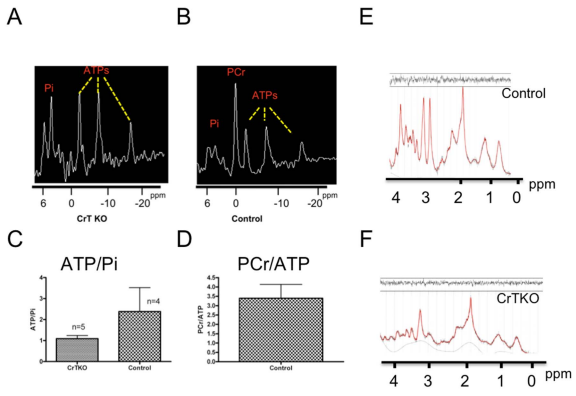

Figure 1 CrT KO mice are deficient in cerebral PCr and Cr, while cerebral ATP level is also lower than control mice. Repesentative (A) ^{31}P-Brain CrT-KO (B) ^{31}P-Brain control spectra (C) ATP/Pi ratio in the CrT KO and control brain (D) PCr/ATP ration in the control brain (E) ^{1}H-MRSI brain spectra of the control (F) ^{1}H-MRSI brain spectra of CrT KO. Creatine (Cr) is depleted in the brain of CrT KO, whereas Taurine (Tau) and Myoinositol (mI) levels are significantly reduced .

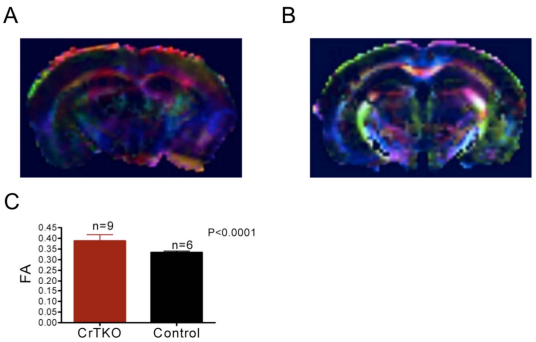

Figure 2 Functional MRI data. Representative (A) Brain eigenvector map of CrT KO (B) Brain eigenvector map of control (C) Increased FA (Fractional Anisotropy) value seen in the brain of CrT KO.