The C57BL/6 mouse exhibits sporadic congenital portosystemic shunts

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Introduction:

C57BL/6 mice are the most widely used strain of laboratory mice. Using *in vivo* proton Magnetic Resonance Spectroscopy (^{1}H MRS), we have repeatedly observed an abnormal neurochemical profile in the brains of both wild-type and genetically modified mice derived from the C57BL/6J strain, consisting of a several fold increase in cerebral glutamine (Gln) and two fold decrease in myo-inositol (Ins) ("High Gln" mice). This strikingly abnormal neurochemical "phenotype" resembles that observed in chronic liver disease or portosystemic shunting [1-4]. The aim of the present study was to determine if the C57BL/6J mice with the observed abnormal neurochemical profile showed evidence of portosystemic (PS) shunting, a congenital anomaly well described in other mammalian species [5-6].

Methods: All our mice (C57BL/6J) were provided by official suppliers or different animal facilities. We are presenting only some of the "High Gln" mice identified in our laboratory (total of n=23 mice). *In vivo* serial ^{1}H MRS spectra were measured during post natal day (P) 10, 20, 30, 60, 90 in the cortex of "Normal Gln" mice (n=11) and "High Gln" mice (n=11) to control for any developmental effect. In adult animals (4 and 12 months (n=12)), striatum, hippocampus and cortex were measured individually. All data were acquired on a 14.1T/26cm MRI system using the SPECIAL spectroscopy sequence (TE=2.8ms, TR=4s) [7]. First and second order shims were adjusted using FASTMAP. Concentrations of metabolites were calculated by LCModel using water as internal reference. Portal angiographies were performed on a digital subtraction angiography system equipped with a 40 × 48-cm flat panel detector (Allura Xper FD 20; Philips Medical System). Standard postero-anterior angiograms were obtained during continuous injection of iodinated contrast media (350 mg iodine/ml of iohexol, flow rate of 150 mL/h, n=5 for "Normal Gln" mice and n=5 for "High Gln" mice).

Results and discussion: High Gln (3 fold increase, p<0.001) and low Ins concentrations (significant 30% decrease) were apparent in all brain regions in the "High Gln" mice (Fig 1A and B), and during brain development (data not shown). As many as 25% of ^{1}H MRS scanned C57BL/6J mice in any given study showed the abnormal neurochemical profile. Findings were independent of transgene, type of investigation, chow or origin and were not associated with liver failure. 100% of the mice "High Gln" mice displayed portosystemic shunting by concomitant portal angiography while all mice with normal brain glutamine did not (Fig 2). The shunt was visible as a short segment that ran perpendicular to both the portal vein and inferior vena cava within the left side of the liver, consistent with a patent ductus venosus.

To our knowledge, this is the first report showing that a high fraction of C57BL/6J mice present an abnormal neurochemical profile and that these changes are associated with the presence of congenital PS shunts. It is interesting to note that the same abnormal neurochemical profile was reported in a significant proportion of C57BL/6 mice [8], although the underlying cause was not identified.

The commonly accepted pathophysiology of central nervous changes in liver disease and PS shunting is that portal blood bypasses the liver. Thus, ammonia enters the systemic circulation directly. In the CNS, ammonia is detoxified into Gln via the astrocyte-specific enzyme glutamine synthethase [4]. The end-result of CNS ammonia detoxification is elevated Gln concentration in astrocytes and efflux of other osmolytes [1-4]. We therefore conclude that the abnormal neurochemical profile detected in the C57BL/6J mice is most likely due to portosystemic shunting-induced CNS ammonia detoxification.

The implications of the current findings are likely far-reaching: in humans, PS shunting is associated with many systemic changes, namely cardiac, renal, hepatic and pulmonary. Additionally, portosystemic shunting is known to cause alterations in gene expression in many organs including the brain [9-11]. **We conclude** that portosystemic shunting may be the most significant problem associated with C57BL/6 inbreeding both for its effect on the central nervous system and for its systemic repercussions.

References:[1] H.Kostler, Metab Brain Dis (1998);[2] R.F.Butterworth, J Hepatol (2003);[3] J.Cordoba, et al, Hepatology (1996); [4]O.Braissant, et al, Journal of Inherited Metabolic Disease (2012);[5]A.C.Berent, et al, Vet Clin North Am Small Anim Pract (2009);[6] F.G.vanSteenbeek, et al , Mamm Genome (2012); [7]; Mlynárik V, et al, MRM (2006)[8]Tkac I, et al, ISMRM (2011) [9]P.Desjardins, et al, Metab Brain Dis (1999);[10]A.Agusti, et al, Gut (2011),[11]G.Q.Song, et al, Journal of Neuroscience Research (2002) **Acknowledgements**. Supported by CIBM of the UNIL, UNIGE, HUG, CHUV, EPFL, the Leenaards and Jeantet Foundations; SNF grant 131087, SCORE grant 3232230-126233.

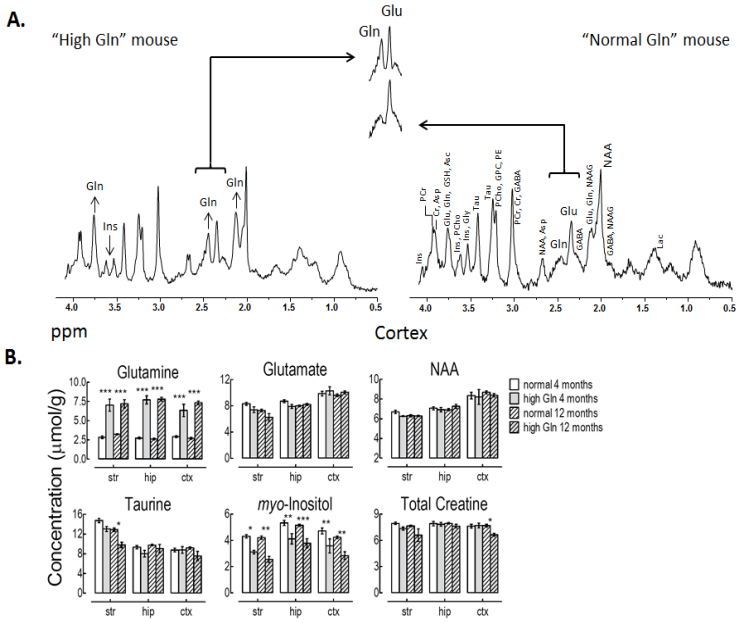

Fig. 1: A) I*n vivo* spectra acquired at 14.1T in the cortex (VOI=0.8×4×1.2mm^3) of "High Gln" and "Normal Gln" C57BL/6J mice.B) Metabolite concentrations in the striatum (str), hippocampus (hip) and cortex (ctx) of "High Gln" and "Normal Gln" C57BL/6 mice at 4 and 12 months of age. Statistically significant differences are marked *(p<0.05), **(p<0.01) and ***(p<0.001).

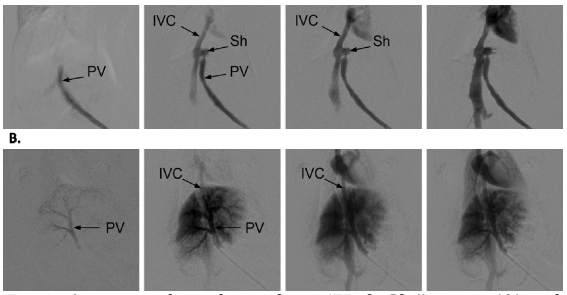

Fig. 2: Aangiographies obtained in a "High Gln" mouse (A) and in a "Normal Gln" (B) C57BL/6J mouse. Normal filling of the portal tree is visible in B. In A, injection in the superior mesenteric vein leads to immediate filling of the inferior vena cava. Inferior vena cava (IVC), portal vein (PV), shunt (Sh).