SLC2A3: a new candidate gene for Attention-Deficit/Hyperactivity Disorder (ADHD)

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Introduction

- The SLC2A-family of glucose transporters (GLUTs) constitutes a group of carrier molecules which share the ability to convey glucose or other carbohydrates across the cell membrane by means of facilitated diffusion
- All GLUTs exhibit 12 transmembrane protein domains with both the amino- and carboxy-terminus located on the cytosolic side (Fig. 1)
- The isoform **GLUT3**, encoded by the gene ***SLC2A3***, is highly expressed in the brain (Fig. 2) but can also be found peripherally, e.g. in lymphocytes

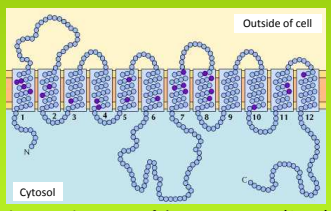

Fig. 1: Protein structure of glucose transporters (GLUTs) [adapted from www.ncbi.nlm.nih.gov/books/NBK9839]

- In cell culture, expression rate and intracellular localization of GLUT3 was shown to be influenced by several substances, such as insulin, haloperidol, brain-derived neurotrophic factor (BDNF) or 2,4-Dinitrophenol

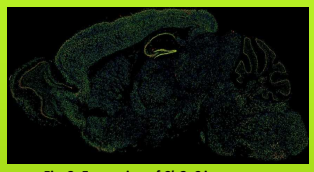
Fig. 2: Expression of *Slc2a3* in mouse brain [adapted from Allen Brain Atlas]

- Recently, *SLC2A3* appeared in the context of a genome-wide copy number variation analysis of attention-deficit/hyperactivity disorder (**ADHD**; Lesch *et al*., 2011)
- In a subsequent analysis of 840 ADHD patients vs. 764 controls, this duplication appeared significantly more often within the patient group (4.1% vs. 1.2%, p=0.024)
- For the present study, we intend to examine physiological and functional consequences of *SLC2A3* duplication in different human models
- The underlying aim is to elucidate whether metabolic dysfunctions leading to developmental deficits of the brain might be a pathogenic factor for ADHD

Methods

- Blood samples of human duplication carriers as well as controls were collected and leukocytes were isolated by means of ficoll density gradient centrifugation
- To obtain a propagating cell culture, we selectively infected lymphocytes (which are located within the mononuclear fraction) with Epstein-Barr Virus leading to immortalized cells → so-called lymphoblasts

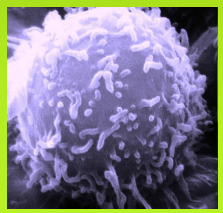
Fig. 5: Electron microscope image of a lymphocyte [adapted from www.iayork.com/Images/2008/3-31-08/SEM_Lymphocyte.jpg]

- These cells were cultivated for several weeks and quantified in hemocytometers for subsequent gene expression analysis
- In order to investigate **gene expression on RNA level**, we lysed cell samples and isolated total RNA
- RNA concentration was determined in order to normalize for subsequent reverse transcriptase-based cDNA synthesis
- Using these cDNA samples, we performed real-time quantitative PCR in a 384-well thermal cycler
- For examination of **gene expression on protein level**, cell samples were lysed in RIPA buffer and whole cell protein lysate concentrations were colorimetrically measured by means of BCA assay
- 10µg protein were loaded and electrophoretically separated in a reducing Bis-Tris SDS Gel
- Afterwards, protein bands were transferred onto a nitrocellulose membrane, followed by specific immunostaining and chemiluminescent detection of GLUT3 (Western blot). Protein bands were semi-quantitatively analyzed using GAPDH or β-actin as loading control

Preliminary Results

Gene expression analysis (mRNA level)

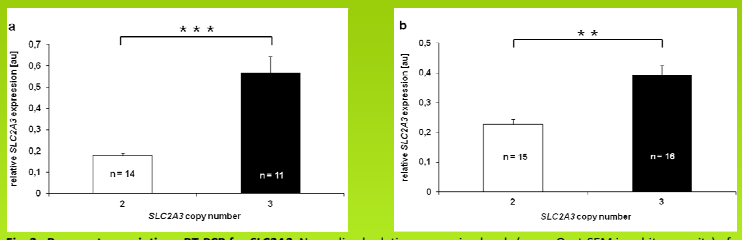

Fig. 3: Reverse transcription qRT-PCR for *SLC2A3*: Normalized relative expression levels (mean Qn ± SEM in arbitrary units) of duplication carriers (copy number: 3) and control subjects (copy number: 2) are shown. Figure **a** refers to peripheral blood mononuclear cell samples and figure **b** to lymphoblast cell samples (Mann-Whitney U-test: ** p < 0.01, *** p < 0.001, n: sample size)

- As shown in Fig. 3, relative expression of *SLC2A3* was notably higher in duplication group (black columns) than in control group (white columns) as well in native mononuclear cells (220% difference) as in immortalized lymphoblasts (70% difference)
- Additionally, we measured expression of other selected genes which we assumed to be affected by elevated *SLC2A3* levels. Among these were *SLC2A1* (**GLUT1**), *SLC2A4* (**GLUT4**), *SLC2A14* (**GLUT14**), *SLC6A2* (**NET**) *SLC6A3* (**DAT**) and *SLC6A4* (**SERT**). However, the only genes showing significant effects were *SLC2A1* and *SLC2A14* which both were upregulated in samples of our duplication group

Gene expression analysis (protein level)

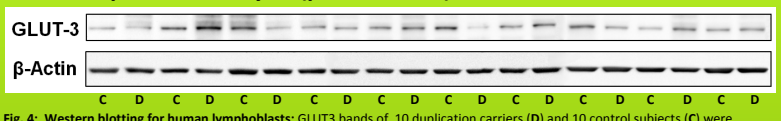

Fig. 4: Western blotting for human lymphoblasts: GLUT3 bands of 10 duplication carriers (**D**) and 10 control subjects (**C**) were compared using β-actin as loading control. No significant group effect could be found.

- Neither in immortalized lymphoblasts (p=0.219) nor in native mononuclear cells (p=0.265), **GLUT3 protein** amounts were notably different between the two groups. Thus, these findings did not comply with above-mentioned mRNA results (Fig. 3), suggesting regulatory effects at the post-transcriptional/translational level

Future Work

- Our future goals involve functional assays in our lymphoblast cell model in order to verify if (insulin-dependent) glucose uptake is affected by the duplication, even though absolute GLUT3 protein levels were found to be unremarkable
- As Fig. 6 illustrates, we will also focus on other models which together may enable a comprehensive view

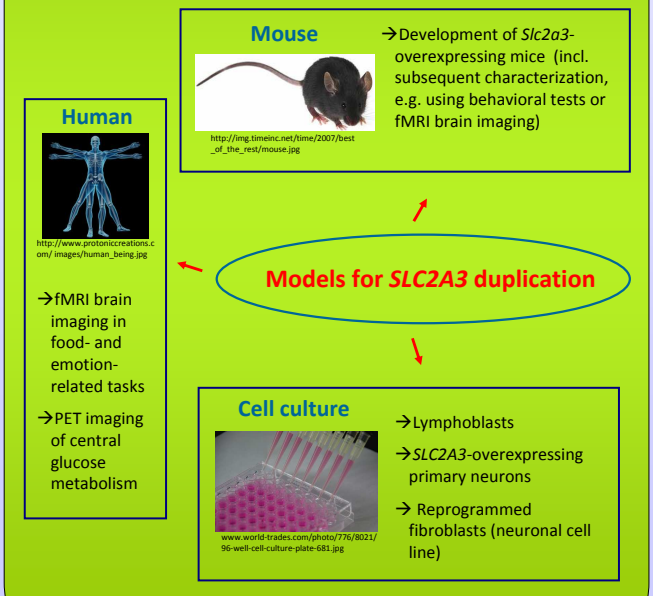

Fig. 6: Present and projected models for *SLC2A3* duplication

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