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REVIEW ARTICLE

Cellular effects and clinical implications of *SLC2A3* copy number variation

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Funding information

EU Seventh Framework Program, Grant/Award Number: 602805 (Aggressotype); EU Horizon 2020 Framework Program: Grant/Award Numbers: 643051 (MIND), 728018 (Eat2beNICE), 01EW1902, (ERA-Net DECODE), 01EW1602B, (ERA-Net NEURON/RESPOND); Russian Academic Excellence Project, Grant/Award Number: 5-100; Deutsche Forschungsgemeinschaft, Grant/Award Numbers: CRC TRR 58 A1/A5, 413657723 (Clinician Scientist-Programm UNION CVD)

Abstract

SLC2A3 encodes the predominantly neuronal glucose transporter 3 (GLUT3), which facilitates diffusion of glucose across plasma membranes. The human brain depends on a steady glucose supply for ATP generation, which consequently fuels critical biochemical processes, such as axonal transport and neurotransmitter release. Besides its role in the central nervous system, GLUT3 is also expressed in nonneural organs, such as the heart and white blood cells, where it is equally involved in energy metabolism. In cancer cells, GLUT3 overexpression contributes to the Warburg effect by answering the cell's increased glycolytic demands. The *SLC2A3* gene locus at chromosome 12p13.31 is unstable and prone to non-allelic homologous recombination events, generating multiple copy number variants (CNVs) of *SLC2A3* which account for alterations in *SLC2A3* expression. Recent associations of *SLC2A3* CNVs with different clinical phenotypes warrant investigation of the potential influence of these structural variants on pathomechanisms of neuropsychiatric, cardiovascular, and immune diseases. In this review, we accumulate and discuss the evidence how *SLC2A3* gene dosage may exert diverse protective or detrimental effects depending on the pathological condition. Cellular states which lead to increased energetic demand, such as organ development, proliferation, and cellular degeneration, appear particularly susceptible to alterations in *SLC2A3* copy number. We conclude that better understanding of the impact of *SLC2A3* variation on disease etiology may potentially provide novel therapeutic approaches specifically targeting this GLUT.

KEYWORDS

copy number variation, energy metabolism, glucose transporter, GLUT3, neurodegeneration, neurodevelopment, *SLC2A3*

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1 | INTRODUCTION

1.1 | Carbohydrate metabolism

Carbohydrates are the major source of energy in humans. Carbohydrate intake, digestion, absorption and eventual distribution requires a well-balanced interplay between glycolytic and gluconeogenic hormones. For healthy individuals, this results in a relatively narrow range of plasma glucose levels, in order to protect cells from the toxic effects of hyperglycemia and life-threatening hypoglycemia. Before glucose can be used as an electron donor in glycolysis, it must be carried across the plasma membrane. The highly hydrophilic glucose molecule cannot enter cells passively, and therefore specific transmembrane glucose transporters (GLUTs) have evolved in all metazoan species, with a high grade of genetic conservation (Jia, Yuan, Lan, Xuan, & Jeon, 2019). Sodium glucose transporters (SGLTs) exhibit a secondary active transport mechanism. However, transport via GLUTs utilizes the electrochemical gradient of glucose to facilitate diffusion in an energy-independent manner. Whereas SGLTs are expressed for the sole purpose of glucose absorption and reabsorption, particularly in intestinal cells and renal proximal tubular cells, GLUTs are ubiquitously expressed throughout the body, with single members of the GLUT-family showing differential tissue expression patterns. GLUTs are essential for glucose homeostasis as they are involved in the control of cellular glucose uptake and consumption, glucose storage, and hormonal regulation. Besides their role as cellular gatekeepers, GLUTs have also been suggested to play a role in glucose sensing, with implications for fasting glucose levels and diabetes risk (Dupuis et al., 2010).

1.2 | Towards cellular and clinical implications of SLC2A3 CNVs

Haploinsufficiency of GLUT1 leads to developmental delay, microcephaly, and neurological symptoms, such as epileptic seizures, spasticity, hypotonia, and complex motor symptoms (De Giorgis & Veggiotti, 2013). Rare loss-of-function mutations in GLUT2, GLUT9, and GLUT10 are associated with Fanconi-Bickel syndrome, renal hypouricemia, and arterial tortuosity syndrome, respectively. These diseases all follow an autosomal recessive inheritance pattern, and are due to protein truncation and/or impaired membrane trafficking of the affected GLUT (Coucke et al., 2006; Matsuo et al., 2008; Santer et al., 1997). Mutations in the other GLUTs have not been observed to cause disorders by a Mendelian inheritance mode. However, in recent studies copy number variants (CNVs) of the SLC2A3 (GLUT3) gene were associated with the risk for various clinical phenotypes, such as attention-deficit/hyperactivity disorder (ADHD), rheumatoid arthritis (RA), chorea huntington, and congenital heart defects (Lesch et al., 2011; Mlynarski et al., 2015; Monteiro et al., 2017; Prakash et al., 2016; Veal et al., 2014; Vittori et al., 2014). This raises the question of whether there exists a common disease mechanism in different tissue types, that is dependent on SLC2A3 gene dosage.

In this context, we will first illustrate the unique characteristics of GLUT3, with emphasis on expression data and experimental manipulation of SLC2A3 gene dosage. Second, we will highlight the role of GLUT3 in carcinogenesis, and discuss whether research findings from metabolic cancer research can be translated into other clinical fields. We will then portray the potential impact of structural SLC2A3 variation on clinical phenotypes, focusing on neuropsychiatric, cardiovascular, and immune diseases. Finally, we will suggest future research to determine the effects of structural SLC2A3 variation, and propose hypotheses regarding protective and detrimental consequences of SLC2A3 duplication and deletion events.

2 | THE GLUCOSE TRANSPORTER GLUT3

The SLC2A family of GLUTs comprises 14 members, designated SLC2A1-SLC2A12, the myoinositol transporter HMIT (SLC2A13), and SLC2A14. GLUT3 is encoded by the SLC2A3 gene which is located at chromosome 12p13.31. Cloning of SLC2A3 was achieved by Kayano et al. (1988) using a complementary DNA library derived from fetal skeletal muscle. Consistent with other GLUTs, GLUT3 contains 12 transmembrane domains with both the amino- and the carboxy-terminus located intracellularly, and carries a glycosylation site at the first extracellular loop (Augustin, 2010). The transmembrane protein comprises 496 amino acids accounting for a molecular weight of 54 kDa. GLUT3 has a high affinity ($K_m = 1.4$) for its main substrate D-glucose (Colville, Seatter, Jess, Gould, & Thomas, 1993), but also has the capacity to transport galactose, mannose and ascorbic acid in vitro (Gould, Thomas, Jess, & Bell, 1991; Rumsey et al., 1997). A general overview of GLUT3 structure, kinetics and expression patterns is given by Simpson et al. (2008).

2.1 | SLC2A3 copy number variation

It is thought that several duplication events took place during the evolution of the SLC2A family, creating novel GLUT genes with distinct properties regarding tissue and substrate specificity; however, several functional motifs have been highly conserved (Wilson-O'Brien, Patron, & Rogers, 2010). The detection of copy number variants among the GLUT genes (including SLC2A3) suggests that these recombination events are still occurring, accounting for both gene duplications and deletions. The detected structural variants around the SLC2A3 locus span 122–142 kb (range: chr.12:7,987,761–8,129,708, hg19, own data) and comprise the entire SLC2A3 gene, the pseudogene NANOGP1, and the first two exons of SLC2A14 according to the gene structure published by Wu and Freeze (2002). As previously reported (Merker et al., 2017), our comparative genomic hybridization array data showed that chromosomal breakpoints were located in segmental duplications with high sequence similarity (first duplcon at chr12:7995630–7998390, second duplcon at chr12:8124315–8128199; fraction matching

0.92). It therefore appears that non-allelic homologous recombination (NAHR) is the most likely cause of *SLC2A3* CNVs.

It is widely accepted that *SLC2A14* evolved from a duplication of *SLC2A3*, and is therefore the youngest member of the *SLC2A*-family of GLUTs (Augustin, 2010; Mueckler & Thorens, 2013; Scheepers, Joost, & Schurmann, 2004; Wu & Freeze, 2002). However, a recent BLAST search has led us to conclude that, it is actually the other way around; *SLC2A3* and *NANOGP1* evolved by a NAHR event that led to duplication of *SLC2A14* and *NANOG* approximately 29 million years ago, in the common ancestor Old World monkeys (Figure 1). The newly evolved *SLC2A3* gene then has assumed the role of a high-affinity transporter for tissues with elevated glucose demand, whereas the phylogenetically older *SLC2A14* gene appears to play a specialized role in the testis (Wu & Freeze, 2002). This view is supported by extensive sequence similarities between the *NANOG* gene and its numerous pseudogenes, amongst which *NANOGP1* is one of the youngest (Fairbanks & Maughan, 2006). Under the assumption of NAHR with

subsequent tandem duplications, only *NANOG/SLC2A14* or *SLC2A3/NANOGP1* can represent the true ancestral gene combination (Figure 1). It is therefore highly unlikely that *SLC2A14* and *NANOGP1* originated from the same duplication event. As *SLC2A14* expression is limited to a few specific tissues (Shaghghi, Murphy, & Eck, 2016), and *NANOGP1* is classified as a pseudogene, the *SLC2A3* gene is of main clinical interest.

A meta-analysis containing 37,661 control subjects from different populations observed a *SLC2A3* duplication frequency of 3.44% and deletion frequency of 0.79% (Table 1), suggesting that the *SLC2A3* duplication is a common structural variant (>1%) whereas the *SLC2A3* deletion is a rare variant (<1%). The different frequencies between duplication and deletion variants is likely due to a lower survival rate of embryos carrying a heterozygous deletion, as seen in rodents (Ganguly et al., 2007). This supports the hypothesis that duplications are generally under less selection pressure than deletions (Zarrei, MacDonald, Merico, & Scherer, 2015). To date, the highest *SLC2A3* gene copy number ourselves and others have

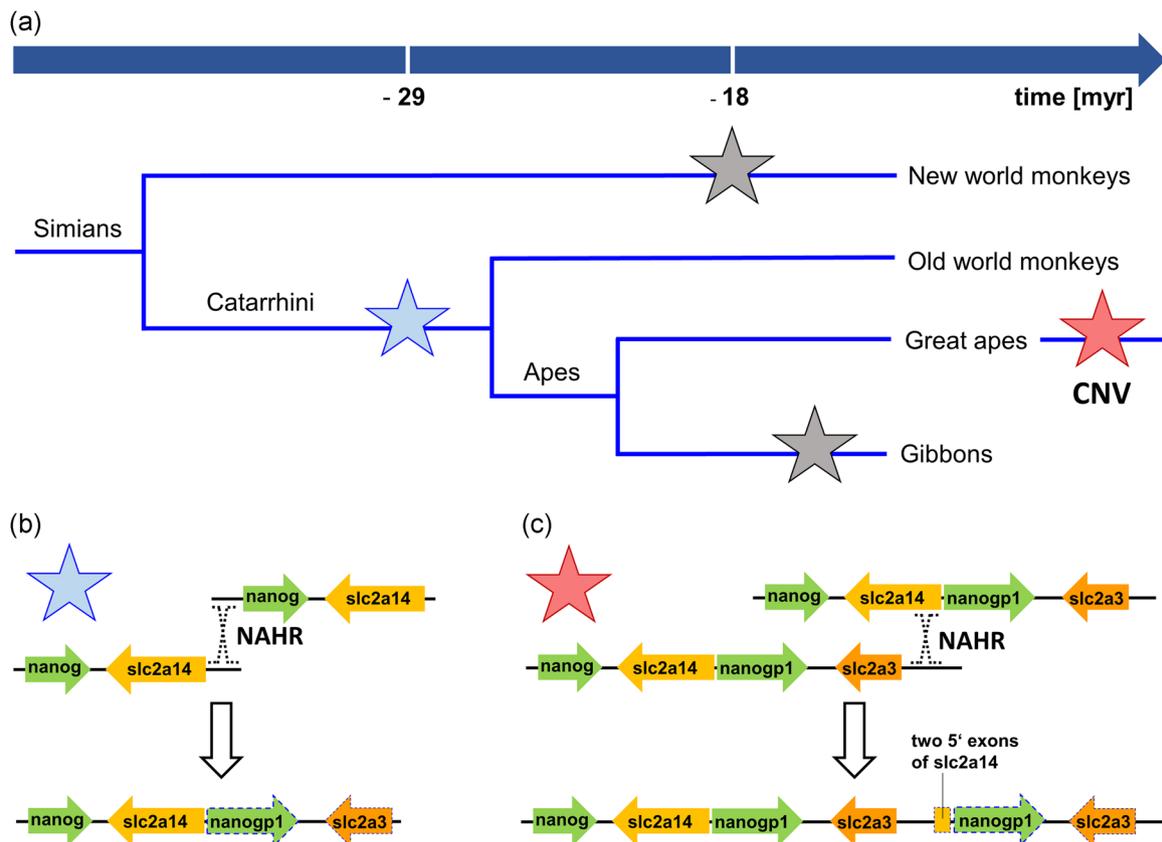


FIGURE 1 Evolution of *SLC2A3* and *SLC2A3* CNVs. (a) Genealogical tree of *SLC2A3* evolution in human ancestors. (b) Evolution of *SLC2A3* by NAHR. (c) Emergence of *SLC2A3* CNVs. In contrast to the common hypothesis that *SLC2A14* evolved from *SLC2A3* by a duplication event, and is the phylogenetically youngest member of the GLUT family, *SLC2A3* together with *NANOGP1* can be considered as the more recent sequences. A NAHR duplication event in the common ancestor (Catarrhini) of old world monkeys created a copy of *SLC2A14* approximately 29 million years ago (blue star). This copy became expressed in brain and other structures and received the name *SLC2A3*. Due to the segment order, the new sequence appears more prone to duplication events as can be seen in the gibbon genome, where the duplication of *SLC2A3* was integrated permanently (lower gray star), or in humans with the CNV (red star). Independent of the old world lineage, new world monkeys developed a duplication of the original *SLC2A3* circa 18 million years ago (upper gray star). Some species also developed further duplications. CNV, copy number variant; GLUT, glucose transporter; NAHR, non-allelic homologous recombination

TABLE 1 Meta-analysis of *SLC2A3* duplication (gain) and deletion (loss) frequency

	<i>n</i>	Gain	Gain (%)	Loss	Loss (%)	Population
Pinto et al. (2007)	776	15	1.93	4	0.52	Mixed
Jakobsson et al. (2008)	485	14	2.89	2	0.41	Mixed
Shaikh et al. (2009)	2,026	56	2.76	9	0.44	Mixed
Vogler et al. (2010)	1,109	31	2.80	5	0.45	Mixed
Suktitipat et al. (2014)	3,017	75	2.49	8	0.27	Asian
Cooper et al. (2011)	8,329	143	1.72	62	0.74	Mixed
Veal et al. (2014)	1,269	55	4.33	33	2.60	Caucasian
Merker et al. (2017)	1,721	68	3.95	19	1.10	Caucasian
Simpfendorfer et al. (2019)	18,929	839	4.39	155	0.86	Mixed
	37,661	1296	3.44	297	0.79	

SLC2A3 CNV data from different cohorts of control individuals from mixed populations were gained from the Database of Genomic Variants (<http://dgv.tcag.ca/dgv/app/home>). Only studies investigating both duplication and deletion variants were included in the meta-analysis. In 37,661 individuals from different populations the mean duplication frequency was 3.44% whereas the mean deletion frequency was 0.79%.

detected is four (Vittori et al., 2014). It is likely that heterozygous loss-of-function mutations in *SLC2A3* are generally tolerable, as approximately 1 in 130 people carry only one copy of the gene. This is supported by statistical evidence demonstrating a low probability of being loss-of-function intolerant (probability of being loss-of-function intolerant [pLI] score = 0.01, gnomad.broadinstitute.org). Considering the embryonal abortion of *Glut3* null mice (Schmidt et al., 2009), and that no humans with zero *SLC2A3* copies have been identified so far, we can infer that homozygous LOF mutations of *SLC2A3*, though, are incompatible with life.

2.2 | Physiological regulation of GLUT3 expression and function

SLC2A3 is most highly expressed in the brain, leading to its unofficial title of “neuronal glucose transporter”. However, *SLC2A3* is also expressed in other human tissues, such as cardiomyocytes, testis, spermatozoa, placenta, and white blood cells (Haber, Weinstein, O’Boyle, & Morgello, 1993; Kipmen-Korgun, Bilmen-Sarikcioglu, Altunbas, Demir, & Korgun, 2009; Shepherd et al., 1992). In the human brain, GLUT3 seems to be mainly localized to axonal and dendritic processes both in the neocortex and in deeper cortical structures (Mantych, James, Chung, & Devaskar, 1992), suggesting an important role for GLUT3 in ATP-dependent axonal transport processes and synaptic plasticity. In ultrastructural studies of rodent brain tissue, subcellular localization of GLUT3 to cellular processes of the neuropil but also cell bodies has been observed (Fields, Rinaman, & Devaskar, 1999; Leino, Gerhart, van Bueren, McCall, & Drewes, 1997). Both in the mouse and rat brain, highest GLUT3 expression can be found in hippocampal regions, Purkinje cells of the cerebellum, nuclear regions of the brain stem, and the neocortex (Bondy, Lee, & Zhou, 1992; Nagamatsu et al., 1992). *SLC2A3* expression is not only tissue-specific, but also inducible. Metabolic,

hormonal, medicinal and toxic factors can trigger an induction and/or suppression of *SLC2A3* expression, which has been particularly observed under hypoglycemic and hypoxic conditions (Korgun et al., 2002; Wood, Wang, Lorente-Cebrian, & Trayhurn, 2007). Such regulation can enable potent upregulation or downregulation of gene expression, depending on the specific conditions and consequent energy demand. For example, the transcription factor hypoxia-inducible factor 1 α (HIF1 α) can upregulate *SLC2A3* gene expression during hypoxia to facilitate sufficient increased glucose supply (Baumann, Zamudio, & Illsley, 2007; Thamotharan, Raychaudhuri, Tomi, Shin, & Devaskar, 2013). Additionally, HIF1 α can be activated by phosphoinositide 3-kinase (PI3K)/Akt signaling upon binding of epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) at their respective receptors (Figure 2; Kamei et al., 1999; Yu et al., 2012). The K_m of GLUT3 is relatively low, leading to rapid saturation of its transport capacity in normoglycemic conditions, yet preserving transporter function in hypoglycemic conditions with a half-maximum reaction rate at 25 mg/dl blood glucose (Colville et al., 1993).

Energy-dependent glucose uptake can be fine-tuned by translocation of GLUT3 protein from intracellular vesicles to the plasma membrane. Although GLUT3 is generally considered an insulin-independent GLUT, multiple studies have suggested that insulin can trigger GLUT3 translocation in human leukocytes and rat neurons (Piatkiewicz, Czech, Taton, & Gorski, 2010; Uemura & Greenlee, 2006). GLUT3 protein has also been identified in the mitochondrial membrane of neuronal cell lines and PC12-cells (Leino et al., 1997; Thodis et al., 1999), as well as in platelet α -granules, which migrate to the plasma membrane following thrombin stimulation (Heijnen, Oorschot, Sixma, Slot, & James, 1997). A possible translocatory mechanism for GLUT3 in cortical and hippocampal neurons has been proposed; after NMDA receptor binding calcium influx leads to activation of the NO-synthase pathway, with consecutive cGMP production followed by fusion of GLUT3 containing

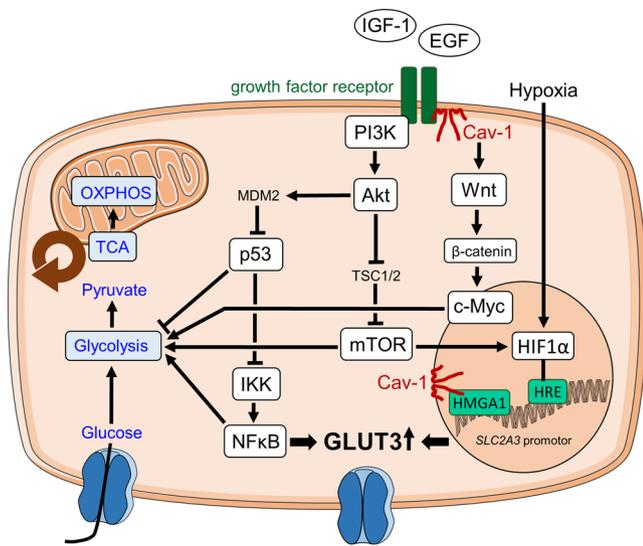


FIGURE 2 Signaling pathways for *SLC2A3* and glycolytic regulation. PI3K/Akt signaling plays a key role for the regulation of both *SLC2A3* expression levels and glycolytic activity by disinhibition of mTOR via TSC1/2. Consecutively, mTOR activates HIF1 α , which binds hypoxia-responsive elements (HRE) in the *SLC2A3* promoter region. The PI3K/Akt pathway can be activated by both extrinsic growth hormones (such as EGF and IGF-1), and by intrinsic factors (such as the oncogene Caveolin-1; Cav-1) which interacts with growth hormone receptors. Additionally, Cav-1 can increase glycolytic activity via Wnt pathway targeting of the oncogene c-MYC, and enhance *SLC2A3* transcription by direct nuclear interaction with the transcription factor HMGA1. Loss of the tumor suppressor gene *p53* during oncogenic transformation leads to increased glycolytic activity and enhanced *SLC2A3* expression levels via NF κ B signaling. This figure was designed in part using Servier Medical ART (<https://smart.servier.com>). EGF, epidermal growth factor; HIF1 α , hypoxia-inducible factor 1 α ; IGF-1, insulin-like growth factor; IKK, I κ B kinase; mTOR, mammalian target of rapamycin; NF κ B, nuclear factor κ B; OXPHOS, oxidative phosphorylation; PI3K, phosphoinositide 3-kinase; TCA, tricarboxic acid cycle

vesicles with the plasma membrane (Ferreira, Burnett, & Rameau, 2011). Additionally, AMPK activation upon removal of Ca²⁺ by ATPases can trigger GLUT3 translocation to the plasma membrane (Weisova, Concannon, Devocelle, Prehn, & Ward, 2009). This implies a bidirectional relationship between GLUT3 surface expression and neurotransmitter release, allowing activity-dependent regulation of neuronal glucose transport, potentially aiding synaptic formation, and excitatory neurotransmission (Figure 3).

In rodents, changes in GLUT3 expression occur concurrently with crucial steps of organ maturation in the central nervous system (CNS; Vannucci et al., 1998) and the heart (Grover-McKay, Walsh, & Thompson, 1999). These observations support the hypothesis that adaptive mechanisms of GLUT3 expression may be advantageous during pre- and postnatal developmental stages, when organ growth coincides with increased energy demand.

2.3 | Regulation of GLUT3 expression in cancer cells

It is well-known that malign dedifferentiation is accompanied by changes in cellular respiration, as originally demonstrated by Warburg who performed pioneering measurements of oxygen consumption in cancer cells (Warburg, 1956). Healthy cells produce the bulk of ATP by oxidative phosphorylation (OXPHOS), gaining 36 mol of ATP from only 1 mol of glucose. However, cancer cells preferentially bypass OXPHOS and use glycolysis only, even when enough oxygen is available as a terminal electron acceptor in the respiratory chain (aerobic glycolysis; Warburg, 1956). It is still unknown why cancer cells utilize this less effective method of energy production, which generates only 2 mol of ATP from 1 mol of glucose (Hsu & Sabatini, 2008). Warburg postulated that the metabolic shift from OXPHOS to aerobic glycolysis in cells was the main mechanism of cancer development (Warburg, 1956).

Currently, glycolytic cancer metabolism is considered an adaptation to intracellular alterations, including mitochondrial dysfunction due to oncogene activation or tumor suppressor loss (King, Selak, & Gottlieb, 2006). Moreover, rapid proliferation can exceed the tissue's vascular capacity, meaning that cancer cells often have to cope with a hypoxic tumor microenvironment in which glycolysis remains the only feasible option for energy production (Hsu & Sabatini, 2008). Due to inefficient ATP synthesis from glycolysis, the cancer cell's glucose demand is considerably elevated.

In support of this, GLUT1 and GLUT3 are overexpressed in many cancer cells, including tumors of the digestive system (Yamamoto et al., 1990), extracranial head and neck tumors (Mellanen, Minn, Grenman, & Harkonen, 1994), and gliomas (Boado, Black, & Pardridge, 1994). GLUT3 overexpression has additionally been associated with carcinoma grade and prognosis in non-small cell lung cancer (Younes, Brown, Stephenson, Gondo, & Cagle, 1997), laryngeal carcinoma (Baer, Casaubon, Schwartz, Marcogliese, & Younes, 2002), and oral squamous cell carcinoma (Ayala et al., 2010). Flavahan et al. (2013) conducted a database search (www.oncomine.org) which confirmed the correlation between tumor GLUT3 expression and prognosis in several breast cancer, colorectal carcinoma, ovarian cancer, and lung cancer samples. Moreover, the authors demonstrated the dependence of glioblastoma-initiating cells on GLUT3-mediated glucose uptake, and observed reduced tumor growth after GLUT3 knockdown (Flavahan et al., 2013). Metastases of breast and lung cancer exhibit even higher GLUT3 expression than the underlying primary tumor, further highlighting the potential impact of GLUT3 expression on tumor progression (Kuo et al., 2019; Kurata, Oguri, Isobe, Ishioka, & Yamakido, 1999). Consequently, the association of GLUT overexpression with tumor proliferation and negative survival outcomes has suggested that novel treatment strategies which aim at starving out tumors by blockade of GLUT-mediated glucose uptake may be feasible. Supporting this, there has been increasing evidence showing antiproliferative and apoptotic effects of GLUT inhibition in cancer cells (Barron, Bilan, Tsakiridis, & Tsiani, 2016).

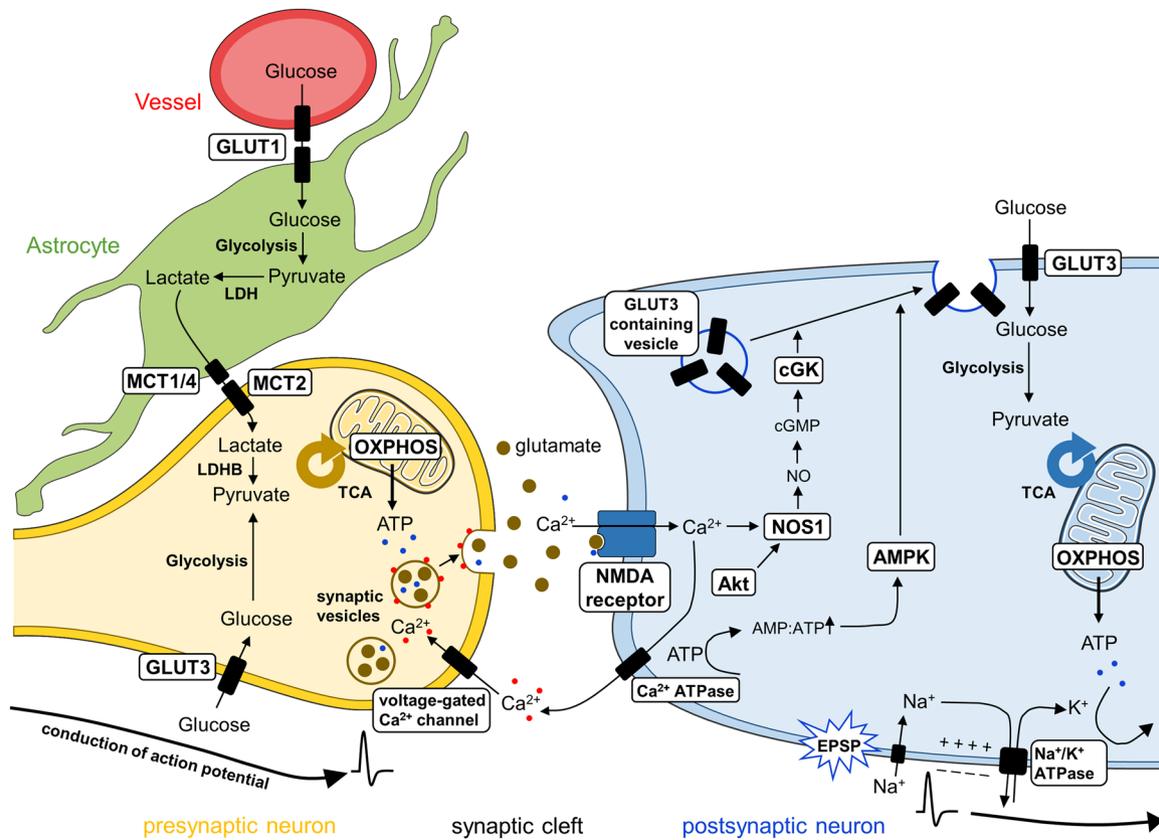


FIGURE 3 The role of GLUT3 for excitatory neurotransmission. Glucose crosses the blood-brain barrier via GLUT1. Lactate, generated by anaerobic glycolysis in astrocytes, enters neurons with the help of the monocarboxylate transporter MCT2. Neurons can use both lactate and glucose (which enters the cell by facilitated diffusion via GLUT3) as substrates for oxidative phosphorylation for mitochondrial ATP generation. ATP is necessary for energy-dependent assembly of synaptic vesicles in the presynaptic terminal and is additionally used as cotransmitter. Upon arrival of action potentials at the terminal, Ca^{2+} influx leads to fusion of synaptic vesicles with the presynaptic membrane, and neurotransmitter release into the synaptic cleft. Glutamate binding at NMDA receptors triggers Ca^{2+} influx into the postsynaptic neuron. Ca^{2+} and Akt lead to phosphorylation of NOS1, evoking translocation of GLUT3-containing vesicles to the plasma membrane, induced by cyclic GMP-dependent protein kinases (cGK). AMP-dependent kinases (AMPK), which are activated by increased AMP:ATP ratio, can additionally provoke GLUT3 translocation to the plasma membrane. GLUT3-mediated glucose supply is crucial for the generation of ATP, which is needed for restoration of resting membrane potential after depolarization during action potential conduction. This figure was designed in part using Servier Medical ART (<https://smart.servier.com>). EPSP, excitatory postsynaptic potential; GLUT3, glucose transporter 3

The proposed gene-dose effect of *SLC2A3* CNVs on cellular glucose uptake, and the increased mutation rate in cancer cells (Jackson, 1998), suggest that *SLC2A3* duplications could be enriched in tumor tissue thereby helping to cover elevated glucose demands. The observation of increased *SLC2A3* duplications in human embryonic stem cell lines (9 out of 69 cell lines; Laurent et al., 2011) additionally infers an increase of underlying NAHR events in highly proliferative tissue. Conversely, *SLC2A3* deletions may disrupt the cancer cell's need for glucose, and thereby decelerate tumor growth. However, to the authors' knowledge, there are currently no studies which have investigated an association between *SLC2A3* copy number (intratumoral or inherited) and specific cancers or cancer prognosis. There are also no positive genome-wide association study (GWAS) findings in this regard.

Cancer research has provided a multitude of data on the intracellular signaling cascades which control both *SLC2A3* expression levels and activity of glycolytic genes. Converging evidence

points to a direct interaction of oncogenes and tumor suppressor genes with glucometabolic pathways. The oncogene Caveolin-1 (Cav-1) activates the canonical Wnt-pathway, which in turn targets the oncogene c-Myc with a consecutive increase in glycolytic activity (Tahir et al., 2013). Nuclear Cav-1 additionally leads to enhanced *SLC2A3* expression by facilitating HMGA1 binding in the *SLC2A3* promoter region (Ha & Chi, 2012). Furthermore, Cav-1 directly interacts with IGF-1 and insulin receptors, with consecutive activation of the PI3K/Akt pathway which boosts the expression of *SLC2A3* and glycolytic genes via mammalian target of rapamycin (mTOR; Tahir et al., 2013). Loss of the tumor suppressor p53 also leads to heightened glycolytic activity and *SLC2A3* overexpression via nuclear factor κB (Figure 2; Kawachi, Araki, Tobiume, & Tanaka, 2008). Thus, the loss of tumor suppressor genes and activation of oncogenes directly regulate the cancer cell's glucose supply and glycolytic utilization, supporting the Warburg effect.

Even though there is presently no evidence for an association between *SLC2A3* CNVs and cancer or cancer prognosis, the investigation of glucose metabolism and GLUTs in cancer research has led to valuable knowledge about the expressional regulation of GLUT3, which can inform further research on the cellular consequences of *SLC2A3* CNVs. Cellular signaling upstream of GLUT3 transcriptional regulation has been well-characterized for the above reviewed pathways. However, it is currently unclear whether *SLC2A3* CNVs can have gene dosage-dependent consequences for downstream effectors beyond the established glucometabolic processes of glycolysis and tricarboxic acid cycle (TCA). Therefore, the following sections are devoted to the consequences of *SLC2A3* CNV in cellular models, and disease associations are discussed.

2.4 | Functional consequences of *SLC2A3* expression changes on the cellular level

Studies from mouse and cell culture models have suggested that *SLC2A3* CNVs may have an impact on the cellular level. Heterozygous *SLC2A3* knockout (KO) mice showed a decrease in *Slc2a3* messenger RNA (mRNA) and protein expression in whole brain lysates compared with wild-type (WT), yet only a small decrease in glucose uptake, which coincided with increased GLUT1 and MCT2 protein (Zhao et al., 2010). Two further studies using heterozygous KO models did not find any differences in brain glucose uptake between WT and *Slc2a3*^{+/-} mice, despite a 50% decrease in protein expression (Schmidt et al., 2008; Stuart et al., 2011). One possible explanation could be compensatory changes in other GLUT expression, as observed above, but the discrepancy may also potentially be explained by posttranslational translocation of GLUT3. In the case of reduced whole cell GLUT3, vesicular stores may be depleted first, leaving functional GLUT3 located at the plasma membrane mostly unaffected. However, in the studies described only whole brain glucose uptake and glucose uptake in astrocytes were measured, which are both highly dependent on GLUT1 as the major GLUT in astrocytes and the blood-brain barrier (Morgello, Uson, Schwartz, & Haber, 1995). When measuring glucose uptake in cell models of *SLC2A3* CNV in future studies, inhibition of other GLUTs should be considered, with an inhibitor for GLUT1 already available (Siebeneicher et al., 2016).

Previous reports have suggested that *SLC2A3* duplication increases *SLC2A3* mRNA expression by more than 50%; Yang et al. (2009) described increased *SLC2A3* expression of approximately 75% in human fibroblasts, and in our studies, we found expression increased by 73% in human lymphoblasts and 220% in human leukocytes (Merker et al., 2017). Whether GLUT3 protein expression reflects this increased transcription in duplication carriers remains controversial. To date, only a single study by Vittori et al. (2014) found increased protein expression in cell lines from duplication carriers, whereas we and others did not observe such an effect in similar experimental settings (Merker et al., 2017; Simpfordorfer et al., 2019). Furthermore, the duplication variant did not affect

glucose uptake levels in lymphoblastoid cells under basal conditions (Merker et al., 2017). This discrepancy between mRNA and protein expression/transport capacity in *SLC2A3* duplication could be due to posttranscriptional or posttranslational modifications that may be necessary for the organism to flexibly adapt to changes in glucose supply and demand. An increase in GLUT3 protein half-life was described as a posttranslational mechanism in episodes of prolonged energy demand (Khayat, McCall, & Klip, 1998). Further studies, preferably in neuronal cell lines, should aim to elucidate whether *SLC2A3* gene duplication can also influence protein expression.

There are not many studies which have investigated whether *SLC2A3* CNVs can alter downstream metabolic pathways, and systematic analyses determining whether compensatory/transregulatory expression changes in non-GLUT genes may occur are lacking. An initial study has reported reduced glycolytic activity in the immune cells of deletion carriers (Simpfordorfer et al., 2019), which could potentially lead to cellular growth restriction. Synaptic outgrowth has also been observed reduced in murine neurons with *SLC2A3* knockdown in early postnatal development, supporting the role of GLUT3 as an important factor in neuronal maturation (Segarra-Mondejar et al., 2018).

2.5 | Functional consequences of *SLC2A3* expression changes in animal models

Slc2a3 KO mice models have been created by several working groups. Heterozygosity leads to early pregnancy loss in approximately 25% of affected mouse embryos (Ganguly et al., 2007), whereas a bi-allelic deletion of *Slc2a3* is deleterious, leading to abortion at Day 12 p.c. It has therefore been suggested that GLUT3-mediated glucose uptake is pivotal for organogenesis (Schmidt et al., 2009). *Slc2a3*^{+/-} mice do not differ from WT animals in feeding behavior, body weight, blood-glucose, or insulin concentrations. However, electrophysiology has demonstrated that *Slc2a3*^{+/-} mice exhibit overall increased cerebrocortical activity, and an increased startle response to acoustic stimuli (Schmidt et al., 2008). Zhao et al. (2010) additionally described reduced vocalization, motor stereotypies, increased epileptic activity in electroencephalography (EEG) recordings with rare tonic posturing and motor arrests, and impaired spatial learning and working memory. These features caused the authors to classify the symptoms as an autism-like phenotype.

Recently, a conditional tissue-specific postnatal *Slc2a3*^{-/-} KO mouse was designed, permitting normal embryonal development (Shin et al., 2018). In the neural-specific KO, the animals died between Day 15 and 31 after genetic manipulation. They showed diminished brain weight, reduced cortical thickness, and reduction of dendritic spines, as well as fewer ultrasonic vocalizations. Electrophysiological recordings also suggested cortical hyperexcitability. When KO was restricted to the adult male limbic system normal survival was observed, but coincided with reduced locomotor activity, reduced grip strength and body tone, reduced coordination skills, and impaired spatial learning. Fear conditioning tests and the open-field test also implied reduced fear responses and less caution in social interactions when testing social novelty (Shin et al., 2018).

Taken together, characterization of the different *Slc2a3* KO mouse models supports the notion that GLUT3 plays an important role in brain electrochemical balance. Loss-of-function may inhibit synaptic plasticity in critical developmental stages, evoking cortical hyperexcitability with increased risk for epileptic seizures, perturbed social interactions, and impaired learning abilities (Shin et al., 2018; Zhao et al., 2010). The novel findings of cortical hyperexcitability both in constitutive and conditional *SLC2A3* KO models (Shin et al., 2018; Zhao et al., 2010) highlight the capacity of GLUT3 to modulate excitatory synaptic plasticity, which is additionally indicated by the close interaction of GLUT3-mediated glucose supply and neurotransmission in glutamatergic cell culture models (Figure 3) (Ferreira et al., 2011; Weisova et al., 2009). *Slc2a3* KO mice have so far only been investigated for alterations in the CNS and the metabolic system. However, other organ systems should also be assessed in future studies. The development of a GLUT3 overexpression mouse model would also further elucidate the potential molecular and behavioral consequences of *SLC2A3* duplication events, complementing the already achieved insights from current KO-mouse data.

3 | *SLC2A3* COPY NUMBER VARIATION AND ASSOCIATED CLINICAL PHENOTYPES

3.1 | Neuropsychiatric disorders

In humans, the brain has the highest relative energy demand of any organ, consuming up to 20% of the body's oxygen and glucose intake, yet only accounting for 2% of the total bodily mass (Rolfe & Brown, 1997). The majority of the brain's energy is needed for neurotransmission; specifically, to restore the resting membrane potential, which collapses when neurons fire (Harris, Jolivet, & Attwell, 2012). Fatty acids cannot pass the blood-brain barrier, and therefore fatty acid oxidation cannot be used for ATP production in the brain. However, during prolonged fasting periods, ketone bodies can be used as alternative electron donors (Morris, 2005; Owen, 2005). Additionally, a lactate-shuttle from astrocytes to neurons has been extensively discussed as an additional potential source of neuronal energy supply. This mechanism enables transport of lactate from anaerobic glycolysis of astrocytes to neurons via monocarboxylate transporters MCT1/2/4 (Figure 3). Consequently, neurons use this lactate for pyruvate synthesis to fuel TCA in aerobic respiration (Mason, 2017; Pellerin, Pellegrini, Bittar, Martin, & Magistretti, 1998). Despite these other possible energy sources, it is still widely accepted that under non-fasting conditions the brain's energy demand is primarily provided by glucose (Clarke & Sokoloff, 1999; Lundgaard et al., 2015). Therefore alterations in GLUT3-mediated neuronal glucose uptake may have a large impact on the brain, and warrant investigation as a potential pathomechanism in neuropsychiatric disorders.

We first identified the *SLC2A3* duplication in a genome-wide copy number variation analysis in an extended pedigree of patients

with ADHD as an inherited variant (Lesch et al., 2011). In a consecutive case-control study we found a significant overrepresentation of the duplication in our German child and adult ADHD sample, but not in the Spanish and Dutch replication samples. In a meta-analysis of all three samples, a significant additive effect of *SLC2A3* copy number and a single nucleotide polymorphism (SNP) in the *SLC2A3* 3'-untranslated region was identified, suggesting a synthetic association of the common SNP variant with the more rare CNVs. We therefore hypothesized that *SLC2A3* duplication-associated ADHD risk is dependent on genetic background, in a population-specific manner. On the endophenotypic level, duplication carriers underestimated the amount of calories in high-caloric food. Furthermore, we found an influence of the duplication variant on EEG-measured event-related potentials in neurocognitive tasks. These tasks comprised a continuous performance test (Go-NoGo paradigm) and an n-back task, which represent measures of cognitive response control as a frontal capacity and working memory performance, respectively. Our results suggest that *SLC2A3* duplication variants may alter neural processing in tasks requesting cognitive efforts (Merker et al., 2017). The potential mechanisms behind this finding are currently unclear, but hypothetically could be related to impaired emotional decision-making as a prefrontal function of altered reward-related circuits, in concordance with the frontostriatal network hypothesis of ADHD (for review, see Cubillo, Halari, Smith, Taylor, & Rubia, 2012). Considering the neurodevelopmental nature of ADHD, fluctuating *SLC2A3* expression that correlates with axonal sprouting and pruning (Khan, Rajakumar, McKnight, Devaskar, & Devaskar, 1999; Vannucci, 1994), and the severe stunting observed in homozygous KO mice (Shin et al., 2018), it is a plausible option that alterations in *SLC2A3* gene dosage interfere with neurodevelopment. This may not only apply to ADHD, but also to other neuropsychiatric disorders with a significant genetic contribution, such as schizophrenic psychosis and bipolar disorder. For the former, altered glucose metabolism was found in the striatum with reduced TCA and consequently enhanced anaerobic glycolytic activity (Dean, Thomas, Scarr, & Udawela, 2016). Supporting the latter, in an Amish pedigree family a diagnosis of bipolar disorder was observed in approximately 50% of *SLC2A3* gene duplication carriers (Yang et al., 2009).

Besides the potential impact of GLUT3-mediated glucose uptake for neurodevelopmental disorders, alterations in GLUT3 expression have also been associated with neurodegenerative disorders, such as Alzheimer's (AD) and Huntington's disease (HD). Decreased GLUT3 expression levels in post-mortem brain tissue was observed for both diseases (Covarrubias-Pinto et al., 2015; Gamberino & Brennan, 1994; Liu, Liu, Iqbal, Grundke-Iqbal, & Gong, 2008). Decreased GLUT3 and GLUT1 protein levels have been associated with Tau hyperphosphorylation and neurofibrillary tangle load, implying a possible link between impaired brain glucose metabolism and AD (Liu et al., 2008; Simpson, Chundu, Davies-Hill, Honer, & Davies, 1994). Reduced GLUT expression has been associated with a decrease in HIF1 α in the brain of patients with AD (Liu et al., 2008). Conversely, mounting evidence suggests a crucial role of the PI3K/Akt pathway with mTOR hyperactivation for the formation of senile plaques and

neurofibrillary tangles (Tramutola et al., 2015). However, increased mTOR signaling would result in enhanced glycolytic activity and GLUT3 expression via HIF1 α (Figure 2). This apparent contradiction might be explained by an as yet unknown mechanism of *SLC2A3* downregulation despite HIF1 α activation, or a general deficit in protein synthesis caused by oxidative stress in late-stage neurodegenerative processes (Dasuri, Zhang, & Keller, 2013). Overexpression of p53 was also been repeatedly found in various neurodegenerative disorders (Morrison & Kinoshita, 2000), and could possibly be responsible for decreased GLUT3 levels, if p53 can evade negative regulation via the PI3K/Akt pathway (Figure 2).

In HD the *SLC2A3* duplication has been associated with a later age of onset (Vittori et al., 2014). Glucose hypometabolism precedes cell decline and is already detectable in premanifest patients with HD (Ciarmiello et al., 2006), supporting the view that alterations in neuronal glucose metabolism are not only a result of advanced neurodegenerative cell damage but rather contribute to the pathophysiology in some way. Hence, a reduction in GLUT3-mediated neuronal glucose uptake seems to be a common feature of neurodegenerative diseases, and initial evidence suggests a protective effect of the *SLC2A3* duplication variant for HD (Vittori et al., 2014). This is possibly due to a larger glucose transport capacity, conferring metabolic resistance against oxidative stress. However, the potential role of glucometabolic changes in neurodegeneration should not be overestimated, as these changes may just be a side-effect of causative pathophysiological changes. Current CNV GWAS have so far not associated *SLC2A3* CNVs with AD (Swaminathan et al., 2012) or age of onset of AD (Szigeti et al., 2014). However, the data has indirectly suggested a genetic association between *SLC2A3* and AD; an intronic SNP rs10845990 in the neighboring *SLC2A14* gene was among the 12 top hits (though not genome-wide significant), with the G-allele conferring higher risk for AD (Shulman et al., 2011). This finding was replicated in a subsequent case-control study within a different population (Wang et al., 2012). A database search on expression quantitative trait loci derived from whole blood (www.eqtngen.org) shows that the rs10845990 G-allele is not only associated with lower *SLC2A14* expression, but also with lower *SLC2A3* and higher *C3AR* expression levels (Vösa et al., 2018). *C3AR* encodes a complement receptor whose brain expression correlates with cognitive decline in humans, and which has been associated with Tau pathology in a mouse model (Litvinchuk et al., 2018). Therefore, the functionality of the *SLC2A14* Alzheimer risk SNP might be due to cis-regulation of *SLC2A3* and *C3AR*, thereby connecting possible glucometabolic dysregulation and complement-mediated neuroinflammation in neuronal cell damage.

Taken together, current findings indicate the relevance of GLUT3 for neurodevelopmental and neurodegenerative processes. Future research should focus on consequences of *SLC2A3* overexpression and deletion for neuronal glucose uptake, and determine whether there are downstream cellular signaling targets which depend on *SLC2A3* gene dosage. It should be emphasized that no human studies have so far associated *SLC2A3* deletion variants with neuropsychiatric disorders, and our own control cohorts include several

adult patients without any obvious symptoms of a neuropsychiatric disorder that are also carriers of heterozygous *SLC2A3* deletion.

3.2 | Cardiovascular diseases

The human heart is versatile in its ability to oxidize different energy substrates, including glucose, fatty acids, ketone bodies, lactate and amino acids. It is therefore less dependent on glucose metabolism than the brain. Due to its vital hemodynamic role and scarce ATP storage capacity, which only provides enough ATP for several beats, the heart depends on a steady supply of substrate fuels (Grynberg, 2005). This energy supply is mainly provided by fatty acid oxidation, accounting for up to 70% total energy under normal physiological conditions in healthy adults (Opie, 1991). Although GLUT4 and GLUT1 are thought to be the primary cardiac GLUTs in humans and rodents (Grover-McKay et al., 1999; Kraegen et al., 1993), GLUT3 is also expressed in the human heart: In fetal cardiomyocytes, GLUT3 expression increases from Week 10–15, and then subsequently decreases until Week 21 gestational age (Grover-McKay et al., 1999). This suggests an important role for GLUT3 in cardiogenesis. Supporting this, a recent study found that *SLC2A3* mRNA expression in fetal myocardium was 15.6-fold higher than in newborns (Kong, Liu, & Lu, 2008). These changes in expression may reflect the switch from prenatal anaerobic glycolysis to postnatal fatty acid oxidation as a preferential source of ATP generation (Ascutto & RossAscutto, 1996).

SLC2A3 duplication has been repeatedly associated with congenital syndromic heart defects, including in Turner syndrome (Prakash et al., 2016) and 22q11.2 deletion syndrome (Mlynarski et al., 2015). In the latter, it was suggested that increased gene dosage might interact with genes in the 22q11.2 region, increasing risk in a “two hit model” (Mlynarski et al., 2015). These findings are supported by the increased maternal serum glucose levels observed in tetralogy of Fallot (Priest, Yang, Reaven, Knowles, & Shaw, 2015). These data, combined with the knowledge that mature heart formation requires strict regulation of energy for normal cell guidance and proliferation (Kloesel, DiNardo, & Body, 2016), suggest a path from impaired glucose metabolism to cardiac misdevelopment. An alternative hypothesis for the markedly increased frequency of *SLC2A3* duplication in congenital heart defects would be a gain-of-function effect due to improved energetic supply, perhaps promoting survival in the face of otherwise lethal cardiac development impairments.

There are currently no studies demonstrating an association of *SLC2A3* CNVs with coronary heart disease, postischemic outcomes, or cardiac remodeling resulting in ventricular hypertrophy. However, overexpression of GLUTs has been demonstrated to have cardioprotective effects post-ischemia in mice (Luptak et al., 2007). Furthermore, chronic hypertrophic stress has been observed to cause a switch from fatty acid oxidation back to glycolysis as the preferential source of ATP supply in cardiomyocytes, similar to in the fetus (Barger & Kelly, 1999). A beneficial effect of *SLC2A3* duplication

in cardiodegeneration therefore seems feasible, but is currently lacking evidential support. Lastly, GLUT3 is also essential for the secretory and aggregatory activity of platelets, and therefore may potentially influence the development of atherosclerotic lesions (Fidler, Middleton et al., 2017). In a mouse conditional double KO of GLUT3 and GLUT1, reduced platelet activation and thrombogenic potential was observed, suggesting a role of GLUT3 in thrombus formation (Fidler, Campbell et al., 2017).

Taken together, the data suggests that *SLC2A3* CNVs may have an impact on cardiac development in syndromal disorders but systematic efforts to disentangle the role of glucometabolic alterations in cardiovascular diseases are lacking. Therefore, current data warrants further investigation of the potential role of GLUT3 in cardiovascular diseases, particularly in cardiogenesis and ischemic heart failure. As the heart relies on glycolytic energy exploitation only under certain conditions, such as during cardiogenesis and hypertrophic stress, this might be a promising starting point for researching the role of GLUT3-mediated glucose supply in developmental and degenerative heart disease.

3.3 | Immunodisorders

Activation of both the innate and adaptive immune systems is associated with increased proliferation, increased protein synthesis (for antibody and chemokine production), cytoskeletal rearrangements (for targeted cell migration), and enhanced secretory activity. These cellular changes cause a rapid increase in energy demand, as demonstrated by elevated glucose utilization in activated immune-competent cells (Calder, Dimitriadis, & Newsholme, 2007; Newsholme, Costa Rosa, Newsholme, & Curi, 1996). In response to this increased energy demand, immune cells can upregulate their GLUT expression (including GLUT3) by up to 6-fold (Fu, Maianu, Melbert, & Garvey, 2004; Maratou et al., 2007). This has also been demonstrated in CD4⁺ T-cells, which showed increased GLUT1 and GLUT3 expression followed by a concomitant elevation of glycolytic rate, promoting T-cell activation, survival and expansion of T-effector cells (Macintyre et al., 2014). Increased GLUT expression may represent a general adaptive mechanism to high energetic demands, indirectly reflecting disease activity under certain conditions. For example, in autoimmune hepatitis disease activity appears to correlate with enhanced glucose metabolism and strong GLUT3 expression in inflammatory IgG4⁺ cells (Araki et al., 2018). However, chronic demyelinated lesions in multiple sclerosis conversely exhibit a reduction in axonal GLUT3 (Nijland et al., 2014), although this may represent adaptation to a decreased energy demand following neurodegenerative damage, rather than a direct response to a pathogenic catalyst. It is currently unclear whether inflammatory activity could potentially be decreased by reducing GLUT3-mediated glucose uptake in leukocytes. Further studies should aim to elucidate this possibility, as it could potentially represent a novel therapeutic approach for the treatment for immune system disease by GLUT inhibition.

SLC2A3 CNV, specifically deletion, has been suggested to play a role in autoimmune diseases. Frequency of *SLC2A3* deletion was found significantly lower in patients with RA than healthy controls (odds ratio = 0.5) in a large study of a Swedish cohort, a finding which could be replicated in two other samples, bringing the total cohort number to 6124 cases (deletion frequency 0.78%) and 9511 controls (deletion frequency 1.13%; Veal et al., 2014). These data suggest that *SLC2A3* may provide a protective effect against RA. However, in a higher-powered independent replication study, this association was not observed (Simpfendorfer et al., 2019). Although initial results may have been a false positive, it is also possible that there is a population-specific effect of *SLC2A3* CNVs on different disease phenotypes (Merker et al., 2017). Rare SNP variants, which have been enriched in certain populations due to specific evolutionary pressures, may explain this discrepancy in results and should be further explored.

RA is caused by an incorrect response of both the innate and adaptive immune systems, where proliferation of fibroblast-like synoviocytes and various immune cells (such as macrophages, mast cells, lymphocytes, and neutrophils) results in inflammation of the synovial membrane (Smolen, Aletaha, & McInnes, 2016). Garcia-Carbonell et al. (2016) demonstrated that these fibroblast-like synoviocytes from patients with RA are metabolically shifted towards glycolysis, and that glucose deprivation could inhibit cytokine secretion, cell proliferation and migration. Moreover, in a mouse model of inflammatory arthritis, inhibition of glycolysis led to reduction in arthritic inflammation.

These data strongly suggest that there are adaptive metabolic changes in activated immune cells, to answer increased energy demands. Furthermore, the *SLC2A3* deletion variant may even act to inhibit pathologic cell proliferation in RA via limitation of GLUT3-mediated glucose uptake, though evidence supporting a protective effect of diminished glucose uptake in immunodisorders is sparse. Further research determining the potential effect of *SLC2A3* CNVs in other immune system cell types, and additional functional studies (e.g., inducing arthritis in GLUT3-KO versus WT mice (Monach, Mathis, & Benoist, 2008)) are necessary to support the hypothesis of an *SLC2A3* protective loss-of-function in immune diseases.

4 | CONCLUSION: HOW MANY *SLC2A3* GENE COPIES ARE "IDEAL"?

As a high affinity glucose transporter GLUT3 plays an essential role in providing energetic fuel for both neurons and several other cell types, which require either a consistently high or rapidly alternating ATP supply due to specific functions. Cellular functions potentially affected by GLUT3-dependent energy supply include the reconstitution of resting membrane potentials in neurons, cardiomyocytes, and the continuous cytoskeletal rearrangements for lymphocyte migration in immune reactions. Furthermore, GLUT3 contributes to the Warburg effect, as it helps to answer the cancer cell's increased glucose demand. Even though *SLC2A3* CNVs may not cause disease in

a mendelian inheritance pattern, the combined data suggests that they likely contribute to pathological activity in several clinical disorders. Pathological conditions with mitochondrial dysfunction and alterations in ATP demand are therefore of particular interest. Due to the increased energy demand during organogenesis and altered GLUT expression profiles in cell decline, we suggest putting a focus both on developmental and degenerative diseases in upcoming investigations. Future studies assessing the association between *SLC2A3* CNVs and disease should be well-powered and independently replicated, especially as the frequency of *SLC2A3* copy number alterations is low. Possible additive effects of *SLC2A3* CNVs and polygenic risk load of common SNPs should also be considered, to include the impact of any gene-gene interactions.

Alterations in *SLC2A3* gene dosage appear to have the ability to be beneficial or deleterious depending on the specific situation, influencing disease risk, progression, and prognosis. Distinct examples are the delayed age of onset of HD in *SLC2A3* duplication carriers, and the worse survival outcome of cancer patients with GLUT3 overexpressing tumors. Whereas the former highlights the degenerating neuron's vital dependence on GLUT3-mediated glucose supply, the latter illustrates the cancer cell's high glucose requirements, with GLUT overexpression facilitating invasive tumor growth and metastases. These examples highlight the potential cellular consequences of *SLC2A3* CNVs for clinical outcomes, depending on tissue-specificity and individual pathological predisposition, reflecting a modifying rather than a causative role of *SLC2A3* CNVs for disease activity. This is supported by the data which suggests that the *SLC2A3* gene is not particularly sensitive to loss-of-function mutations ((pLI score = 0.01, gnomad.broadinstitute.org). There is therefore no general "ideal" *SLC2A3* copy number. We suggest that dynamic cellular processes, which rely on a high ATP supply, are especially susceptible to alterations in *SLC2A3* copy number. This susceptibility may become apparent when energy demands alter due to normal aging or pathological activity.

Additional *SLC2A3* copies may convey advantageous effects in disease conditions where energy metabolism becomes insufficient, such as cellular degradation and hypertrophy, by increasing overall glucose uptake capacity. In neurodevelopmental disorders, such as ADHD or autism however, *SLC2A3* CNVs could disturb highly energy-dependent axonal organization and pruning during the formation of neural networks. Similarly, *SLC2A3* deletion may improve disease states that are characterized by uncontrolled cell proliferation (such as autoimmune diseases and neoplasms), yet potentially intensify neurodegeneration. Embryonal differentiation, organogenesis, maturation, cancer formation, and degenerative processes in a broad range of organ systems, such as the CNS, heart, and immune-competent cells are therefore important targets for future investigations.

To date, the majority of data is only correlational, with no definitive cause-and-effect relationship between *SLC2A3* gene dosage and clinical phenotypes reported. Future studies should aim to follow a multidimensional approach in an attempt to elucidate this potential relationship. Such an approach requires the integration

of data from basic molecular biology, electrophysiology, neuropsychology and imaging, to provide a clearer picture of the possible impacts of structural *SLC2A3* variation. With development of an *SLC2A3* overexpression mouse and the advent of induced pluripotent stem cells (Jansch et al., 2018) there will be two new models available to study the influence of *SLC2A3* CNVs on both the molecular and behavioral level.

Finally, the designation of GLUT3 as a "neuronal glucose transporter" may have led to its role in peripheral tissues being overlooked. However, newly identified associations between *SLC2A3* CNVs and various clinical disorders demonstrate that GLUT3-mediated bioenergetic imbalances may be important in a range of tissue types, reflecting a broad spectrum of diseases. This research avenue is of high relevance not only due to general interest in glucometabolic pathomechanisms, but also due to the potential of GLUTs as promising novel therapeutic targets.

ACKNOWLEDGMENTS

Sincere thanks go to Johanna Zöller for assisting with the design of Figure 3. This work is supported by the Deutsche Forschungsgemeinschaft (DFG): CRC TRR 58 A1/A5 and Project No. 413657723 Clinician Scientist-Program UNION CVD), the European Union's Seventh Framework Program (FP7/2007–2013) under Grant No. 602805 (Aggressotype), the Horizon 2020 Research and Innovation Program under Grant No. 728018 (Eat2beNICE) and Grant No. 643051 (MiND), ERA-Net NEURON/RESPOND, No. 01EW1602B and ERA-Net DECODE, No. 01EW1902 and 5-100 Russian Academic Excellence Project.

CONFLICT OF INTERESTS

None of the authors declare any financial conflicts of interest.

AUTHOR CONTRIBUTION

GCZ wrote the first draft of the manuscript. PA performed BLAST searches and found that *SLC2A3* descends from *SLC2A14*. All authors participated in literature research, conception, design, and discussion of the manuscript, and approved its final version.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Ziegler GC, Almos P, McNeill RV, Jansch C, Lesch K-P. Cellular effects and clinical implications of SLC2A3 copy number variation. *J Cell Physiol*. 2020;1–16. <https://doi.org/10.1002/jcp.29753>