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REVIEW

Structure of, and functional insight into the GLUT family of membrane transporters

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Abstract: This review examines the development of structure and function of the human GLUT proteins, gene family hSLC2A. These proteins are essential for moving the key metabolites, glucose, galactose, and fructose in and out of cells, as well as a number of other important substrates. Despite over five decades of research, it is still not fully understood how they work at the molecular level, although the recent publication of a crystal structure of GLUT1 suggests this may be resolved soon. The GLUT family is divided into three classes based on their sequence homology. The physiological roles of Class I GLUTs, ie, 1, 2, 3 (14), and 4 have been extensively studied for their contributions to metabolism and development. However, the other two classes have received far less attention. Genetic diseases associated with GLUTs are very rare, emphasizing their critical roles, but it is likely that as our understanding of these transporters increases, there may be more clinical conditions found to be associated with subtle changes in their activity. Another promising area of investigation is the changes in expression levels of GLUTs associated with, and likely in consequence of, disease processes. It has long been known that GLUT1 expression increases significantly in cancer cells, but it is now becoming appreciated that other GLUTs may also be involved. Determination of alterations in expression patterns may prove to be a useful diagnostic tool, and in some cases these are already being taken advantage of for the imaging of cancers. Finally, as we better understand how GLUTs bind and transport their substrates, it may be possible to design drugs that can be delivered into target cells for the treatment of a number of diseases.

Keywords: GLUT proteins, Facilitated hexose transporters, protein trafficking, cancer imaging, genetic diseases

Introduction

Hexoses are an essential carbon and energy source for the cells of the human body, and their entry and exit across the plasma membrane is critical for life.[1] There are now known to be at least two distinct transporter gene families responsible for the handling of hexoses in mammalian cells. The first is the sodium/glucose co-transporters (SGLTs), which are secondary active transporters that use the sodium gradient across the cell membrane to drive glucose and galactose uptake.[2,3] In contrast, GLUTs are passive transporters that use either chemical or electrochemical gradients to transport hexoses or other substrates.[4,5]

There are 14 mammalian facilitative glucose transporters (Table 1), and they belong to the sugar porter family in the major facilitator superfamily (MFS).[4,5] They are encoded by the solute linked carrier family 2, subfamily A gene family, *SLC2A*, and the original name glucose transporter is somewhat misleading as many have substrates other than hexoses.[6–8] They are subdivided into three classes based on

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their protein sequence and structural similarity. Class I, the "glucose transporters", includes GLUT1, GLUT2, GLUT3, GLUT4, and GLUT14. Class II glucose transporters include GLUT5, GLUT7, GLUT9, and GLUT11, and are known to transport fructose as well. Class III is made up of GLUT6, GLUT8, GLUT10, GLUT12, and GLUT13 (HMIT1); this class is believed to be the most ancient group, with largely as yet unknown substrates.

Molecular characterization of Class I, II, and III GLUTs

Class I GLUTs were all cloned in the 1980s. Human GLUT1 protein was purified by Kasahara and Hinkle in 1977 from human erythrocytes, in which GLUT1 has very high expression levels. The molecular weight of the transporter protein was identified as a 55,000 Dalton band on sodium dodecyl sulfate-polyacrylamide gel, sometimes referred to as band 3.[9,10] After reconstitution into liposomes, this protein was able to show the transport activities for D-glucose but not L-glucose. Furthermore, 90% of the D-glucose transport activity was inhibited by low concentrations of cytochalasin B, a cell-permeable mycotoxin. In 1985, Mueckler et al obtained the complete gene and protein sequence of GLUT1 from the human HepG2 hepatoma cell line.[11] Northern blot analysis demonstrated that GLUT1 is expressed in most tissues and cell types in mammals.[6,11] Consisting of 492 amino acids, it was predicted to have 12 putative membrane spanning domains with both the C-terminus and N-terminus in the cytoplasm, and a putative glycosylation site Asn45 on the first extracellular loop.[11]

Human GLUT2 was cloned from rat and human liver cDNA libraries in 1988[12,13] having 524 amino acids and an 80% homology and 55% identity with GLUT1. It is found mainly in the intestine, liver, kidney, and islet cells, and has been shown to transport both glucose and fructose with relatively low affinities, an important feature of liver hexose transport.[14–16] Kayano et al identified human GLUT3 in 1988.[15] GLUT3 is a 496 amino acid protein and was cloned from a human fetal skeletal muscle cDNA library.[15] It has 64.4% and 51.6% identity with GLUT1 and GLUT2, respectively, and is expressed mostly in the brain, in particular in the frontal lobe of the cerebrum.[15,17–19] Its primary substrate is also glucose.[15] GLUT14 was identified by Wu and Freeze as a duplicon of GLUT3 with 94.5% identity.[20] It appears to

Table 1 Summary of the GLUT family proteins and their characteristics

GLUT classes	GLUT isoforms	Gene name	Tissue distribution	Substrate specificity	Trans-acceleration	Crystal structure/ computer model
I	GLUTI	SLC2A1	Red blood cells, Ubiquitous	Glucose/Galactose/ Dehydroacetic Acid	Yes	Crystal structure
I	GLUT3 (GLUT14)	SLC2A3 (SLC2A14)	Neurons (Testis)	Glucose/Galatose/ Dehydroacetic Acid	Yes	Computer model
I	GLUT4	SLC2A4	Muscle cells, Fat cells (Adipocytes)	Glucose/Dehydroacetic Acid	No	Computer model
I	GLUT2	SLC2A2	Intestine, Liver, Kidney, Beta cells	Glucose/Fructose/ Galatose/Glucosamine/ Dehydroacetic Acid	No	N/A
II	GLUT5	SLC2A5	Intestine, Kidney Muscle, Sperm, Brain	Fructose	N/A	Computer model
II	GLUT7	SLC2A7	Intestine, Colon	Fructose/Glucose	N/A	Computer model
II	GLUT9	SLC2A9	Kidney, Liver, Placenta, Colon	Urate/Fructose/Glucose	Yes	Computer model
II	GLUT11	SLC2A11	Muscle, Heart, Placenta, Kidney, Pancreas, Fat	Glucose	N/A	N/A
III	GLUT6	SLC2A6	Brain, Spleen	Glucose	N/A	N/A
III	GLUT8	SLC2A8	Testes, Brain, Fat, Liver, Spleen	Glucose/Fructose	N/A	N/A
III	GLUT10	SLC2A10	Heart, Lung	Glucose	N/A	N/A
III	GLUT12	SLC2A12	Insulin-sensitive tissues	Glucose/Fructose	N/A	N/A
III	GLUT13 (HMIT)	SLC2A13	Brain	Myo-inositol	N/A	N/A

Note: GLUT14 is grouped together with GLUT3 because GLUT14 has 94.5% identity with GLUT3.
Abbreviation: N/A, no data available.

be exclusively expressed in the human testis. Human GLUT4 was cloned from the human small intestine and skeletal muscle by Fukumoto et al in 1989.[13] It is a 509 amino acid protein that has 65.3%, 54.3%, and 57.5% identities with GLUT1, GLUT2, and GLUT3, respectively. The majority of human GLUT4 is expressed in cardiac and skeletal muscle and in adipose tissue, and glucose transport activity is highly insulin-sensitive.[21]

Class II GLUTs comprise GLUT5, GLUT7, GLUT9, and GLUT11, and they all transport fructose and to some degree glucose. Human GLUT5 was cloned from small intestinal cDNA, and was proposed as a second hexose transport system in the brush border (apical) membrane of intestinal epithelial cells along with SGLT1.[22] GLUT5 is a 501 amino acid protein expressed mostly in the human small intestine and at low levels in kidney, skeletal muscle, and adipose tissues. GLUT5 has 41.7%, 40.0%, 38.7%, and 41.6% identities with GLUT1, GLUT2, GLUT3, and GLUT4, respectively. Use of 2-deoxy-D-glucose as a substrate indicated that GLUT5 was also a very low affinity glucose transport system in the brush border membrane. However, its primary substrate is fructose and it is responsible for much of the fructose uptake at the apical surface of the intestine.[23]

Initially, Waddell described GLUT7 in 1992 as an hepatic microsomal transporter protein that had 68% identity with GLUT2.[24] Subsequently, it was reported to be a cloning artifact, and its mRNA could not be found in either the human or rat liver.[25] In 2004, human GLUT7 was the last member of the GLUTs to be cloned using an intestinal cDNA and a polymerase chain reaction-based strategy.[26] Having 528 amino acids, GLUT7 shares 68% similarity and 53% identity with GLUT5 and is predominantly expressed at the apical membrane of the enterocytes in the small intestine. This GLUT can transport both glucose and fructose when expressed in *Xenopus* oocytes with high affinity, but with very low transport capacity, and it is not sensitive to cytochalasin B.[26,28] It is likely that its correct physiological substrate has yet to be identified (see GLUT9 below).

In 2000, Phay et al cloned human GLUT9 from cancer tissues using polymerase chain reaction-based methods.[27] GLUT9 has two splice variants, a 540 amino acid length transporter (*hSLC2A9a*) targeted to basolateral membranes in the liver and kidney and an N-terminal truncated form (*hSLC2A9b*) found in the renal apical membrane, apparently in the distal convoluted tubule.[28] It shares 38% and 44% identity with GLUT1 and GLUT5, respectively. Human GLUT9 was initially shown to be a high affinity/low capacity glucose and fructose transporter, but more recently as a consequence of a series of genome-wide association studies (GWAS) was identified as a high capacity urate transporter.[29–32]

Human GLUT11 was cloned from the human heart in 2001 and shares 35% and 41.9% identity, respectively, with GLUT1 and GLUT5.[33] To date, it has only been detected in heart and skeletal muscle and has three splice variants of unknown function. GLUT11 transports glucose and fructose when expressed in Cos-7 cells.

Class III GLUTs include GLUT6, GLUT8, GLUT10, GLUT12, and GLUT13 (HMIT). A non-functional pseudo gene called GLUT6 with high sequence identity to GLUT3 was initially identified in 1990.[22] Subsequently, the functional human GLUT6 was cloned in 2000 from leucocytes but was initially named GLUT9.[30] Later, the gene was designated as SLC2A6 (GLUT6) by the HUGO Gene Nomenclature committee.[31] Human GLUT6 is a 507 amino acid protein, and has high sequence identity with GLUT8 (44.8%) and has 28.5% identity with GLUT1. It appears to be exclusively expressed in the brain and lymphoid tissues and transports glucose when reconstituted into Cos-7 membrane vesicles. GLUT8, another glucose transporter, was cloned from the human testis, its primary expression site, and has 477 amino acids with 29.4% sequence identity to GLUT1.[30,34,35]

GLUT10 was cloned from human liver cDNA as a 541 amino acid protein and has 29.7% and 33.6% sequence identity with GLUT3 and GLUT8, respectively.[36] GLUT10 is expressed in most human tissues, with the highest level found in the liver and pancreas. GLUT10 displays very low transporter activity and shows high affinity for 2-deoxyglucose when expressed in *Xenopus* oocytes.[37] Human GLUT12 was identified using a malignant breast cancer cell line (MCF-7) as a 617 amino acid protein.[38] GLUT12 shares 40% and 29% sequence identity with GLUT10 and GLUT4, respectively. Immunocytochemistry assays indicated that GLUT12 is expressed in skeletal muscle, the small intestine, and adipose tissue. Other studies indicated that GLUT12 is also present in the heart, prostate, brain, placenta, and kidney tissues.[39–42] GLUT12 shows glucose transporter activity when expressed in *Xenopus* oocytes and this activity is inhibited by other hexoses, including fructose, galactose and 3-O-methyl-D-glucose (3-OMG), suggesting that they may also be substrates.[43]

Human GLUT13, is also known as the H^+-myo-inositol transporter, HMIT, for its proton-coupled activity. Both human and rat GLUT13 were cloned at the same time.[44] The rat GLUT13 cDNA encoding a 618 amino acid protein, and the human GLUT13 has 629 amino acids. GLUT13 is expressed mainly in the brain, in particular in ganglion

cells and some neurons.[44] GLUT13 is the only GLUT that has specific electrogenic transport activity for myo-inositol. To date, no glucose transport activity has been reported for GLUT13, although it does appear to mediate inositol-3-phosphate (IP3) movement.

Structural comparisons among Class I, II, and III GLUT transporters

Hydropathy analysis of the GLUTs reveals that they all have 12 predicted transmembrane domains (TMs) with both the C-terminus and N-terminus and a long loop connecting the TM6 and TM7 present on the cytosolic side of the membrane.[45–47] Protein sequence alignments of all GLUTs reveal that many amino acids are conserved among these proteins. Good examples are PMY in TM4, PESPRY/FLL in TM7, GRR in Loop8, GPGPIP/TW in TM10, and VPETKG in the C-terminus (Figure 1).[48] These motifs are considered to be the signature characteristics of glucose transporters. Other residues, like R92 in Loop2, E146 and R153 in Loop4, Y293 in Loop7, E329, R333, and R334 in Loop8, E393 and R400 in Loop10, and P385 in TM10, are also highly conserved in all the GLUT members. (Note that all amino acid residue numbers are assigned according to the corresponding residues in GLUT1). Mutations of these residues in GLUT1 reduced or abolished glucose transport activity with or without affecting cytochalasin B binding.[48–51]

Class I GLUTs

Although the Class I GLUTs have a similar putative topology, only 38% of all the amino acids are conserved in GLUT1–4.[52] They all appear to have a single N-linked glycosylation site on Loop1, which connects TM1 and TM2.[11,13–15] A glutamine residue (part of the QL motif) in

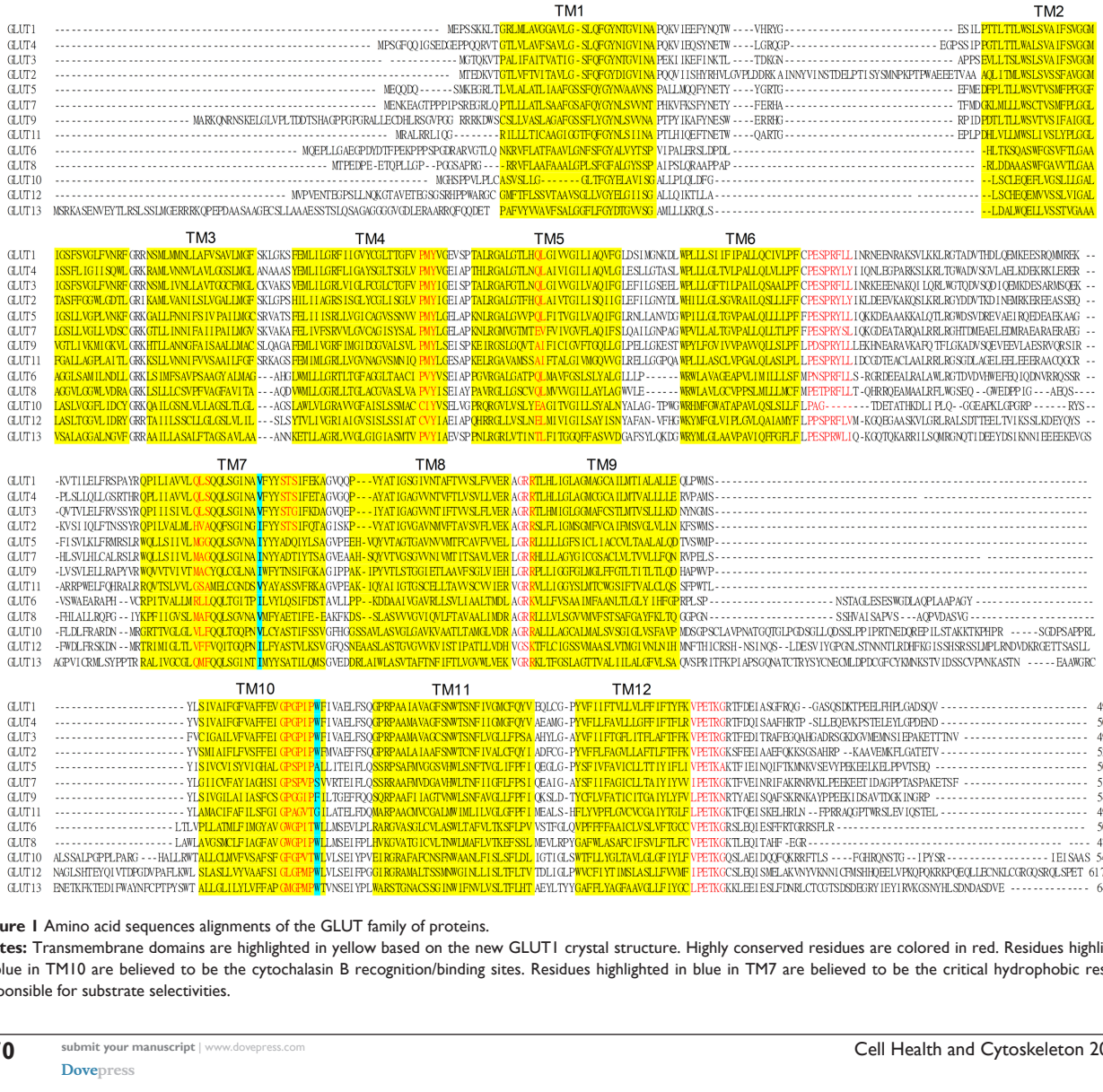

Figure 1 Amino acid sequences alignments of the GLUT family of proteins.

Notes: Transmembrane domains are highlighted in yellow based on the new GLUT1 crystal structure. Highly conserved residues are colored in red. Residues highlighted in blue in TM10 are believed to be the cytochalasin B recognition/binding sites. Residues highlighted in blue in TM7 are believed to be the critical hydrophobic residues responsible for substrate selectivities.

TM5 is shared among the Class I GLUTs, which suggests this motif is important for glucose recognition.[53] The serine-threonine-serine motif in extracellular Loop7 between TM7 and TM8 is also conserved in Class I GLUTs.[54] Mutation of these serine or threonine residues locks the protein conformation, suggesting this is a critical site for conformation change. Another highly conserved motif is GPXXXP in TM10, where a tryptophan also appears immediately after this sequence.[55,56] It has been proposed that this tryptophan is a crucial residue for binding the two inhibitors, cytochalasin B and forskolin, without directly affecting glucose transport. In addition, a QLS motif in TM7 is only present in transporters like GLUT1, GLUT3, and GLUT4 which carry glucose, but not in those that transport fructose, including GLUT2, suggesting that this motif is involved in glucose and fructose selectivity.[57]

Class II GLUTs

Class II GLUTs also have 12 TMs and a single N-linked glycosylation site on exofacial Loop1 between TM1 and TM2.[22,26,27,30] A major difference between Class I and II is that Class II lacks the tryptophan residue after the GPXXXP motif.[58] Another striking difference is that all Class II GLUTs have a single hydrophobic residue, isoleucine, in TM7 associated with fructose/glucose selectivity.[59] It was speculated that this residue was facing the aqueous pore at the exofacial vestibule of GLUT7 using a computer model of the GLUT7 protein based on the GlpT crystal structure.[60] However, analysis using a GLUT9 computer model based on the recent GLUT1 crystal structure indicates that this isoleucine is more likely to be facing away from the aqueous pore and to be acting as a structural regulator through hydrophobic interactions with adjacent TMs.[61] At the equivalent position in Class I GLUTs (except for GLUT2 which can also transport fructose) there is a valine residue.[7] This suggests that subtle interactions between TMs mediated by hydrophobic residues can affect substrate specificity within this family of transporters. Another important structural variation between the Class I and II GLUTs is that Class II do not contain the QLS motif.[57]

Class III GLUTs

Class III GLUTs have similar topology to classes I and II and many of the motifs found in the other classes are conserved, such as PESPR in TM6 (GLUT6 and GLUT8), GRR in Loop2 and Loop8, PETKGR in TM12, and arginine and glutamate residues in Loop4 and Loop10.[30,34,36,38,41] Class III GLUTs also have a tryptophan residue after the GPXXXP motif in TM10. However, one significant difference is that Class III GLUTs do not have an N-linked glycosylation site in Loop1 between TM1 and TM2. Instead, Loop1 is shorter, and the predicted glycosylation sites are in the long Loop9 between TM9 and TM10.[30,34,36,38] GLUT13 (HMIT) is predicted to have more than one N-linked glycosylation site within this loop.[44] In addition, GLUT6 has two arginine residues present in TM7 and TM8, which are absent in other GLUTs.[31] Again, Class III GLUTs do not contain the QLS motif, which is unique to the Class I proteins.[8]

Structure of human GLUT1

Despite the amino acid sequence differences among all the GLUTs, the overall structural protein arrangements are predicted to be very similar. Extensive studies have been carried out to elucidate the structure–function relationship, primarily using GLUT1.

The secondary structure of GLUT1 was first proposed by Mueckler et al[11] to have 12 transmembrane α-helical domains. They also noted that over half of the residues are hydrophobic and proposed that amphipathic helices TMs 3, 5, 7, 8, and 11 might form a central aqueous channel allowing glucose to be translocated through the membrane.[11] Later, this structure was confirmed by Fourier transform infrared spectroscopy, circular dichroism spectroscopy analysis, scanning glycosylation mutagenesis, and mass spectrometric analysis.[45,62,63] Cysteine scanning mutagenesis was subsequently employed to probe which residues lined this proposed central pore.[64–74] The resulting model indicated that TMs 2, 4, 5, 7, 8, 11, and possibly 1 and 10 formed a central aqueous transport channel for glucose, whereas TMs 3, 6, 9, and 12 formed a structural scaffold on the outside of the protein.[70] A possible substrate binding site was also proposed, involving Q161, Q282, and W412.[70]

Subsequently, a three-dimensional inwar facing computer model of GLUT1 was created based on the newly generated GlpT crystal structure.[75] Remarkably, this model agreed with most of the previous data for GLUT1, in which the 12 transmembrane helices an be viewed as two symmetrical six α-helical bundles connected by a long intracellular loop between TM6 and TM7.[70] This also supports the hypothesis that the 12-TM GLUT proteins arose from a gene duplication event from a protein with six TMs.[70,76,77] The new model also indicated that the central transport cavity was formed by TMs 2, 4, 5, 7, 8, and 10, and that most of the important residues previously described as crucial for substrate/inhibitor transport/binding functions faced the pore. However, docking studies for the binding site of the GLUT inhibitor, cytochalasin B, predicted that it

was on the intracellular side, a positioning that contradicts the experimental data.[75,78]

Other models for GLUT3, GLUT4, GLUT5, and GLUT9 were also constructed based on computer models of the mechanosensitive ion channels of the Mscl homolog from *Mycobacterium tuberculosis*, GlpT, and an *Escherichia coli* homolog of GLUTs 1–4 (XylE).[79–85] The GLUT4 model was very similar to that for GLUT1 (also based on the GlpT) structure with the same TM arrangement with conserved residues lining the transport cavity, and both ATP-binding and cytochalasin B-binding sites in the cytosolic side. The GLUT3 computer was based on a Mscl crystal structure. However, the authors need to manipulate the gaps of GLUT3 model due to the TMs differences between the two proteins.[79,83] The authors also suggested that glucose is transported by the key flexible TM segments and a network of polar and aromatic residues with two different glucose binding sites on each side of the transport pathway, rather than by an alternating access mechanism with one stable binding site, as proposed based on the GlypT crystal structure.[79]

In 2014, new GLUT1 and GLUT5 computer models were developed based on the XylE crystal structure, which is a bacterial D-xylose transporting homolog sharing a high degree of similarity and identity with GLUT1–4.[81] In parallel with other MFS protein transporters, the topology of these models fitted into the common structural fold with 12 TMs. More important, this study proposed that the 12 TM topology of MFS may have arisen by two gene duplication events through an initial triple-helix bundle into the six-helix bundle, then into two linked pseudo symmetrical six-helix bundles.[4] Thus, the alignment of GLUT1 and GLUT5 sequences to XylE is in the triple-helix array. This modeling also predicted that Asn411 in GLUT1 and His419 in GLUT5 are the corresponding substrate binding sites for glucose and fructose, respectively.

More recently, a GLUT9 model was also constructed based on XylE.[82] The resulting GLUT9 model topology is similar to the XylE structure, with 12 TMs arranged in a double six-helical bundle arrangement. In the same study, single monomer GLUT9 protein was purified from the GLUT9 overexpressing oocyte. Consequently, it allowed the construction of a low resolution crystal structure of GLUT9. The structure revealed a transport cavity, which contains the plausible urate binding sites. These included amino acids: H23, R31, L182, Q203, A206, Q328, L332, N333, F426, W459, and N462.

Finally, almost four decades after the first purified GLUT, a high resolution (3.2 Å) crystal structure was reported in 2014. The purified protein was generated using a baculovirus transfection system with High Five insect cells.[84] This GLUT1 model reveals a partially open inward facing conformation that is locked due to a single missense mutation, E329Q, and confirms a 12-TM transporter protein formed of two six-helical domains, with cytoplasmic N-domains and C-domains. One additional feature was the presence of a helical bundle, an intracellualr coiled heical, ICH, domain, within the long intracellular loop. This ICH domain has also been observed in other sugar transporter crystals, such as XylP and GlcP,[85,86] and is believed to be unique to the sugar porter family and not in other MFS. This ICH domain may function as a latch to ensure the closing of an intracellular gate in the inward facing conformation of GLUT1. In addition, only a single sugar binding site was identified, located toward the C-domain of the transporter, which substrate could access from either side of the protein. The residues forming this proposed binding site are Q282/Q283/N288 from TM7, N317 from TM8, and N415 from TM11. The N-domain of the structure is believed to play a role in regulation of the conformational change during transport. Another notable feature of this GLUT1 crystal is the extracellular gate formed by TM1 and TM7. N34 is an essential residue in TM1 that serves as the central coordinator for hydrogen bonding between S294, T295 in TM7, and T310 in TM8. Note added in proof: a new GLUT3 crystal structure was published with a D-glucose bound to the outward-occluded conformation and with maltose bound to outward-open and outward-occluded conformations.[87]

Importance of transmembrane domain 7 in GLUTs

The new GLUT1 crystal structure indicated that TM7 is critical for both structural regulation and substrate binding. TM7 forms an extracellular gate in the inward facing conformation of GLUT1,[84] and three of the potential binding residues were observed within the substrate-bound crystal structure.[84] Interestingly, Q282, Q283, and N288 match the molecular data observed in Mueckler and Makepeace's original cysteine scanning mutagenesis studies.[70] Substitution of these residues into cysteines reduced the glucose transport substantially. However, only Q282 and Q283 were shown to be facing the aqueous environment, but not N288.

Based on the GLUT1 crystal structure, a revised model of GLUT9 and a new GLUT5 model have also been generated.[61] These models indicate that this hydrophobic residue (I335 in GLUT9 and I296 in GLUT5) is not projecting toward the aqueous transport pathway as previously proposed; instead, it appears to interact with residues on the adjacent TMs via an complex hydrophobic network (Figure 2). Additional functional studies confirmed that replacement of I335 in GLUT9 with valine resulted in a loss of trans-stimulation

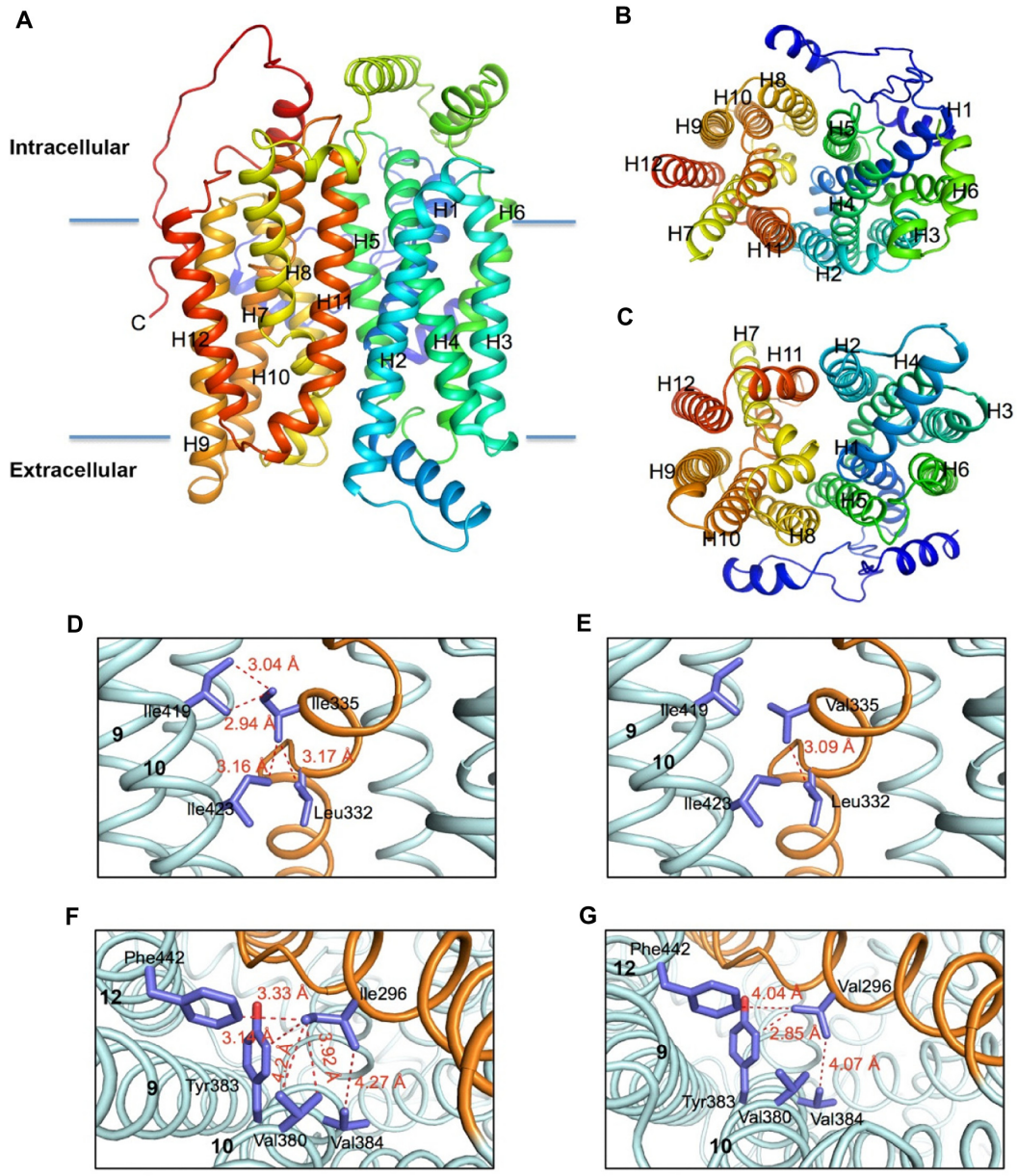

Figure 2 Molecular models of the human hGLUT9 and hGLUT5 transporters comparing possible hydrophobic interactions.
Notes: (**A**) Computer model of hGLUT9a based on the human hGLUT1 crystal structure. (**B** and **C**) Views from the intracellular face and extracellular face. (**D**) Potential interactions of I335 indicate a hydrophobic network with residues within TM10. (**E**) Structural model of the mutant SLC2A9 I335V was generated, demonstrating that the intricate linkage to helix 10 is disrupted when I335 is converted to Val. (**F**) In SLC2A5, Ile296, the equivalent of Ile335 in SLC2A9, forms a more extensive hydrophobic cluster with neighboring residues in TM10 and TM12. (**G**) Structural model of the mutant SLC2A5 I296V, highlighting loss of the hydrophobic network, which subsequently leads to alteration in substrate specificity. Adapted from Long W, Panwar P, Witkowska K, et al. Critical roles of two hydrophobic residues within human glucose transporter 9 (hSLC2A9) in substrate selectivity and urate transport. *J Biol Chem*. 2015;290: 15292–15303.[61]

between fructose and urate. It has been hypothesized that I335 in GLUT9 affects the rigid body movement of one of the two six-helix bundles, and subsequently the orientation of helix 7 (TM7) in the translocation pore of the transporter.

Mechanisms of substrate transport by GLUTs

The mechanism of how glucose is transported has mainly been investigated using GLUT1. Initial explanations of how glucose is transported across the cell membrane were based on mathematical analysis of the transport activities of hexose utilizing erythrocyte preparations. The fluxes were best fit with the Michaelis–Menten enzyme kinetics model[89–90] which predicts that the rate of hexose absorption depends on both the initial hexose concentrations and the binding affinity of the protein for the substrate and that there is a maximum rate of transfer (rate of transfer is saturable).[91] The earliest mechanistic theory, ie, the "simple carrier model", was proposed by

Widdas and assumes four transport stages: an empty carrier that opens to one side of the membrane (cis side), to which glucose can bind; the substrate binding carrier has to translocate to the other side of the membrane (trans side); the carrier releases the glucose on the trans side; and the empty carrier switches back to the cis side. Moreover, Widdas postulated that both phosphorylation and metabolism of glucose were the two possible mechanisms providing the necessary energy for glucose transfer. However, due to the complexities of the erythrocyte system, differences between experimental methods and variations in kinetic data, many other models have been proposed over the same time period.[92] These models include the following: the Regen–Tarpley asymmetrical carrier model,[93] the Eilam model,[94] the lattice-pore model,[95] the tetramer model,[96] and the introversion model.[97] Despite the differences between these models, they all tried to explain two principal scenarios that occur during glucose transfer: the asymmetry of the transport affinities (K_M, substrate concentration at half the maximal transport rate) of zero trans between hexose influx and efflux; and the trans-acceleration that occurs when hexose is present on the trans side.[92,98]

Purification and cloning of GLUT1 subsequently permitted a better picture of how glucose is transported to be proposed. Today there are two popular models still under consideration. One is the two-site/fixed sites transporter, in which both substrate binding sites are simultaneously available from either side of the membrane.[99–101] After binding, hexoses can then exchange between these sites and accelerate the binding process. In another words, this model suggests that GLUT1 can work as an antiporter. However, human GLUTs (all except GLUT13) were thought to be uniporters,[100] in which they transport one hexose molecule at a time in a unidirectional approach, whereas an antiporter (or exchanger) will simultaneously transport two molecules in opposite directions. It is argued that this two-site model can explain the complex asymmetry and multiphasic transport kinetics, while the simple carrier model cannot sufficiently account for these incidences without violating the energy conservation law.[98,99] However, asymmetric glucose transport activities are not seen in hepatocytes or adipocytes, which are mediated by GLUT2 and GLUT4, respectively.[16,102,103] Several recent investigations also indicate that GLUT1 can form oligomers, such as dimers or tetramers, which could account for the three phases and asymmetric kinetics.[104–107] Cloherty et al[106] and De Zutter et al[107] proposed that the glucose transporters in mammalian cells present as cooperative dimers or tetramers of GLUT1, and they hypothesized that one (or two in a tetramer) exofacial and one (or two in a tetramer) endofacial hexose binding site(s) present simultaneously. In both dimers and tetramers of GLUT1, cis-allosteric hexose transport was observed, ie, hexose binding to one oligomer subunit induces the transport by other subunit(s). Therefore, the GLUT1 dimers are able to transport cis-hexose in exchange with the trans side substrate, which is similar to the two-state model assumption; the tetramer GLUT1 cooperative transporter could also further explain the observed multiphasic transport.

Another proposed mechanism is the alternating access model, and this appears to be supported by the available MFS crystal structures.[77,84–86,108,109] Jardetzky explained the assumptions of the mechanism as early as 1966.[110] They are the following three premises: the transporter has a cavity to admit a small substrate; the transporter contains a substrate binding site; and the transporter has two different configuration openings to one side of the cell membrane or the other.[110] This alternating access mechanism supports the presumption that GLUT1 is an uniporter, and has only one binding site that substrate can alternately access from either side of the membrane in the course of conformational changes. The newly crystallized GLUT1 also fits with the alternating access mechanism,[84] which predicts four conformational states during a complete hexose transport cycle: an empty outward-open transporter state, a ligand-bound and occluded transporter state, an inward-open state, and a ligand-free and occluded state. These four states appear as a slightly more detailed version of the original simple carrier theory that Widdas proposed in 1952.[88] Additionally, the crystal structure of GLUT1 suggests that the transporter has a favorable conformation when it is substrate-free. Note added in proof: The recently reported GLUT3 crystal structure shows strong evidence to support the alternating access mechanism with a bound D-glucose in an outward-occluded conformation.[87]

Mechanism of trans-acceleration

Another often observed property of GLUT-dependent glucose transport is that of trans-acceleration. This was first observed in erythrocytes as uphill hexose counterflow (other terms include countertransport acceleration and trans-stimulation transport).[111,112] In these experiments, hexose was detected flowing apparently against its concentration gradient from the *cis* side into the *trans* side of the membrane where hexose is also present, and vice versa. Rosenberg and Wilbrandt argued that the phenomenon was due to two different transport systems present in the red cell, which Naftalin and Holman referred to as a two binding site carrier.[92,112]

Furthermore, it was believed that hexoses could exchange freely between these two sites within the carrier cavity, a process that Naftalin termed a geminate exchange.[92,99,100] Alternatively, others argued that the carrier contains only one binding site, and the carrier can move from outward facing to inward facing with and without substrate binding. It is the empty carrier return from the trans side to the cis side of the membrane that limits the rate of transport. Therefore, trans-acceleration occurs when hexose is presented on the trans side, which allows the carrier to return faster to the cis side with a bound substrate.[84,100]

Currently, hexose/hexose trans-acceleration has only been shown in GLUT1, GLUT3, and GLUT9,[29,100,113] and few studies have probed the mechanism at the molecular level. One study indicated that the ATP-binding site of GLUT1 contains residues that are necessary for glucose trans-acceleration to occur[114] and proposed that mutations in the ATP-binding sites would alter the tertiary structure of GLUT1, and thus restrict the flexibility of the transporter for sensing substrates on both sides of the membrane. A second study employed chimeras constructed from GLUT1 and GLUT4 expressed in human embryo kidney (HEK-293) cells.[100] It found that the TM6 of GLUT1 contains residues necessary for trans-acceleration with glucose-glucose exchange and is responsible for constraining the relaxation of GLUT1, but not direct binding or translocation of substrate during transport. Hence, this study was not able to distinguish between the simple carrier model or the two-site model to explain trans-acceleration. GLUT2 and GLUT4 do not show trans-acceleration of hexose transport; instead, they show symmetrical transport in both oocyte and mammalian cell systems.[103,115]

Human GLUT9 is the only Class II GLUT reported to show not only hexose/hexose trans-acceleration activity but also hexose/urate trans-acceleration when using the *Xenopus* oocyte expression system.[29] GLUT5, GLUT7, GLUT11, and Class III GLUTs have not been subjected to detailed molecular examination of their transport mechanisms or substrate-binding pockets.

Subcellular trafficking of GLUT transporters

In the 1990s it was discovered that while GLUT1 is primarily present in the plasma membrane, GLUT4 is mostly localized to intracellular compartments.[116–121] The C-terminus is primarily responsible for the differential targeting of GLUTs[122–126] to the plasma membrane or intracellular vesicles that can translocate these proteins to other sites.[127–131] GLUT1 and GLUT4 are believed to be recycled by exocytosis and endocytosis between the plasma membrane and cytosol, but to varying degrees. Insulin promotes their surface expression, but the regulatory mechanisms are thought to be different for the two proteins.[123,127,128,130]

GLUT1

The cytoskeleton and microtubules within cells are important in regulating the trafficking of GLUT1. For example, GLUT1 carrying intracellular vesicles are upregulated by protein kinase C and casein kinase substrate 3 (PACSIN 3) in adipocytes and downregulated by Syntaxin 1C (STX1C) in a lung epithelial cell line.[132,133] STX1C is a soluble syntaxin suppressing the stability of microtubules and vesicle transport mobility, whereas PACSIN 3 is an adaptor protein involved in regulation of cellular cytoskeletal elements and the clathrin-coated pit pathway. Trafficking of GLUT1 from an intracellular pool to the plasma membrane is also increased by AMP kinase in murine brain microvasculature endothelium bEnd.3 cells.[134] In the human megakaryocytic leukemia M07e cell line, GLUT1 trafficking is also influenced by cytokine stem cell factor and cholesterol depletion induced by methyl-β-cyclodextrin.[135]

The most recent studies of subcellular trafficking of GLUT1 have focused on the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway.[129,136] Increased PI3K/Akt activity regulates GLUT1 trafficking and glucose uptake in T-cells/B-cells.[137] Factors that activate the Akt pathway include the growth factors interleukin-7 and interleukin-3, granulocyte/macrophage colony-stimulating factor, the inhibitor of NF-kB-kinase b, CD28, and Kaposi sarcoma-associated herpes virus infection.[137–139]

GLUT4

The targeting, trafficking, and recycling of GLUT4 between the intracellular compartments and the plasma membrane has been reviewed extensively elsewhere.[6,98,131,140–142] Consequently, we will simply outline some of the key features of the GLUT4 trafficking process. Cushman and Wardzala[143] proposed the first schematic mechanism of how insulin stimulates GLUT translocation to and from the membrane in the rat adipose cell in 1980. This study hypothesized that vesicles containing glucose transporters were stimulated to bind and fuse with the plasma membrane by undefined "secondary messengers" upon association of insulin with its receptor, and that when insulin disassociates from the receptor, the process reversed. Subsequently, numerous potential pathways involved in the insulin-dependent pathway have been identified.[140,142] They include insulin receptor substrate, PI3K

and its regulatory subunit, P85 and catalytic P110, protein kinase C, ADP-ribosylation factors, phosphoinositide-dependent protein kinase 1, Golgi-localized, g-ear-containing ADP-ribosylation factor-binding proteins and protein kinase B (or Akt) and its substrate protein of 160 kDa (AS160).[144–154]

GLUT4 trafficking and recycling are also regulated independently from the insulin signal, which is referred to as a general (insulin-independent) pathway and that the movement of GLUT4 is also controlled by muscle contraction. Muscle contraction activates several signaling messengers, including calcium, nitric oxide, and reactive oxygen species within the muscle cells themselves, that regulate glucose homeostasis endogenously.[142,155–157]

Subcellular trafficking of other GLUTs

GLUT2 and GLUT3

There is good evidence that GLUT2 can be trafficked to the small intestinal enterocyte apical membrane under the influence of protein kinase C bII, which is activated by phorbol 12-myristate 13-acetate PMA, extracellular signal-regulated kinase p38, and glucagon-like-peptide 2 in the rat intestine.[158–163] In contrast, stress, a high-fat diet, and glucocorticoids inhibit GLUT2 trafficking to the brush border membrane.[164–166] Most recently, subcellular localization studies using live cell imaging demonstrated that GLUT2 is endocytosed through a caveolae-dependent mechanism, which is partially recovered in Rab11A-positive recycling endosomes.[99] However, what is still not entirely clear is the role that this transporter plays while it is transiently expressed at the cell surface. It has been proposed that this transporter provides a high capacity entry pathway for glucose and fructose at the start of a meal when the luminal concentrations are high, and is then withdrawn as the meal progresses, leaving SGLT1 to bring in the remaining hexose upward into the cells. Another theory is that apical GLUT2 provides a shunt, moving glucose back out into the lumen and providing an osmotic control during the absorptive process.[167]

Trafficking of GLUT3 is mediated by intracellular vesicles with a SNARE complex in the neuronal PC12 cell line, and is possibly regulated by Rab11 in neurons from mice with Huntington's disease.[168,169] However, only a few studies have been conducted on the actual processes for these trafficking pathways.

GLUT8

Trafficking of GLUT8 is different from that of the other GLUTs. GLUT8 expression levels at the plasma membrane are controlled by glucose and insulin, and a t-SNARE protein, syntaxin 4, which has also been found in blastocyts to be necessary for the fusion of GLUT8 carrying vesicles with the plasma membrane.[170–172] Other targeting/trafficking studies showed that GLUT8 distributes more to the intracellular compartment in neurons and blastocysts than at the cell surface.[172–174] Piroli et al pointed out that, in rat hippocampal neurons, GLUT8 rapidly translocated to the rough endoplasmic reticulum following peripheral glucose administration.[175] Therefore, they hypothesized that GLUT8 transports glucose out of the rough endoplasmic reticulum into the cytosol to maintain cellular glucose homeostasis in neurons.[172,173] On the other hand, it has been observed that a highly conserved dileucine-containing motif (DEXXXLLI) was critical for GLUT8 sorting to the late endosomal/lysosomal compartments in mouse blastocytes.[172–176]

GLUT12

Similar to GLUT8, GLUT12 has the conserved DEXXXLLI in its sequence; however, this motif affects the cell surface expression level instead of directing GLUT12 to the intracellular compartment as seen for GLUT8 in mouse blastocytes.[172,173] In the case of MCF-7 cells, GLUT12 was localized perinuclearly when insulin was absent.[38] However, the acute change in subcellular distribution of GLUT12 was not detected after insulin treatments. Another study indicated that GLUT12 trafficking is influenced by the mTOR-raptor signaling pathway.[39]

Pathophysiologies associated with GLUT proteins

Given the essential roles played by this family of proteins in cellular metabolism, it is not surprising that there are really no common diseases associated with genetic mutations in the GLUTs. In most situations, such mutations would be lethal for the embryo and development could not occur. However, it is now becoming appreciated that there are a number of disease states in which the expression patterns of hexose transporters are altered, providing an opportunity for diagnosis or even treatment. Therefore, this section covers the few documented GLUT-specific genetic diseases and then looks at recent advances in diagnosis and treatment.

GLUT1: deficiency syndrome

A limited number of patients (~250) have been described since 1991 with autosomal dominant haplo-insufficiency mutations in their *hSLC2A1* gene leading to reduced the concentrations of glucose in cerebrospinal fluid.[177] This

mutation in turn results in seizures, delayed development, and small brain size. The types of mutation are numerous and could affect functional activity of the protein directly, its ability to form dimers or tetramers, or its trafficking to the plasma membrane.[177–180]

GLUT2: Fanconi–Bickel syndrome

This is an extremely rare glycogen storage disease, for which a little over 100 affected patients have been reported worldwide, the first described in 1949.[181] Three mutations in hSLC2A2 responsible for this autosomal recessive disease were identified in 1997, and they appeared to result in a truncated non-functional protein.[182] This would fit with a number of the clinical symptoms associated with the syndrome, including glucosuria, presumably as a consequence of significantly impaired glucose reabsorption across the renal proximal convoluted tubular basolateral membrane where GLUT2 is expressed.[182] The diarrhea could be explained by poor hexose absorption in the small intestine, again resulting from lack of GLUT2 in the epithelial basolateral membrane. Patients also suffer from poor regulation of blood glucose and galactose levels, which after a meal, rise rapidly and are maintained for some time. This condition could result from both the inability of the liver to take up hexoses and store them as glycogen and also probably from impaired insulin release by pancreatic beta cells as GLUT2 forms part of the normal blood glucose-sensing mechanism.[183,184] Similarly, after a meal, the liver would be unable to release glucose back into the circulation, which could be responsible for the severe accumulation of glycogen in this organ seen in many patients. The lack of renal reabsorption of glucose would further exacerbate the situation, leading to hypoglycemia. Patients with a variety of mutations in GLUT2 continue to be reported, some with a full-length but non-functional transporter, while in others the defective protein fails to be targeted to the plasma membrane.[184,185]

GLUT9: association with urate metabolism disorders

While GLUT9 can transport glucose and fructose, its primary physiological substrate appears to be the organic anion, urate. This observation is supported by reports of patients, primarily from Japan, who have mutations in the *hSLC2A9* gene.[28] Unlike the majority of mammals, humans maintain high plasma levels of urate, as a result of their modified metabolic pathway that is missing the enzyme uricase. Consequently, this metabolite is retained in the body as opposed to being broken down to allantoin and lost in the urine. Plasma levels of urate are regulated within narrow limits (250–300 μM) by secretion from the liver mediated by GLUT9 and by reabsorption in the proximal convoluted tubule of the kidney. Transport across the renal epithelium is achieved by the organic anion exchanger URAT1 in the apical membrane and by GLUT9a in the basolateral membrane. Loss of function of GLUT9 results in hypouricemia, presumably as a consequence of both a reduced release of urate from the liver and poor reabsorption from the urine in the kidney.[186–192]

Elevated levels of plasma urate have also been associated with hypertension, gout, and metabolic disease. A number of genome-wide association studies have identified polymorphisms in the *hSLC2A9* gene.[29] Consequently, it has been proposed that GLUT9 in the kidney and liver may well play a role in these disease processes. However, to date, few, if any, single nucleotide polymorphisms have been demonstrated to have direct effects on the function of the protein when it is expressed in vitro.[187–192] It is more likely that such mutations have subtle effects on levels of expression or interactions with other proteins in the cell.

GLUT expression in relation to disease

The most common and well described change of GLUT expression is in cancer cells that switch their metabolism primarily to glycolysis, which is far less energy-efficient and requires far more substrate. This rapid metabolic change is often referred to as the Warburg effect.[193,194] To supply the greatly elevated need for glucose, tumor cells increase their expression of some GLUT proteins, particularly GLUT1. This observation has been used to develop imaging tools, such as positron emission tomography, to detect some cancers.[195,196] Patients are injected with fluorodeoxy-D-glucose, a radioactively labeled glucose analog, which is rapidly taken up by the tumor cells and then metabolically trapped after phosphorylation.[197–199] Research efforts are also focusing on breast cancer cells, wherein some subtypes of the disease, the cells overexpress GLUT5 rather than GLUT1, suggesting that fructose-based probes may be of value.[199,200] However, this positron emission tomography technique is expensive and the probes are very short-lived. This has led to efforts to develop fluorescently labeled hexose analogs that would be preferentially taken up by tumor cells. There are no reports as yet of clinical trials for such compounds, but a number of reports have shown promising results obtained in vitro.[201,202]

Other studies observed that the GLUT1 gene rather than that for GLUT4 was predominantly expressed in the failing human heart, resembling the fetal stage heart.[40] Several

studies have also reported a similar phenomenon in the rodent heart.[203–205] However, the actual cause of the heart failure in humans has not been directly determined. Some recent studies indicate that upregulated GLUT1 expression is related to HIV infection in T-cells.[206–208] It was observed that increased expression of the GLUT1 gene in interleukin-7-induced T-cells rendered CD4 T-cells and thymocytes susceptible to HIV-1 infection.[206,207] These reports suggest that the GLUT1-mediated metabolic pathway is the major regulator in HIV-infected cells.[207] Thus, GLUT1 could be a potential marker of a diseased heart and inflammation in HIV-infected subjects.[40,202,207]

Moreover, other GLUTs, such as GLUT4, have been reported to be expressed on the myeloma cell membrane, where it is responsible for cell glucose consumption.[209] It was also found that GLUT4 expression levels were increased and GLUT2 expression levels were decreased in senescent hepatic cells and chronically diseased human liver tissues, which suggests that GLUT4 may play an important role in liver cirrhosis.[210] In many studies, GLUT4 was also found to be associated with type 2 diabetes.[142] While the expression level of GLUT4 did not change in type 2 diabetic rodents, trafficking of GLUT4 was affected in these animals.[210,211]

It has also been reported that the pattern of GLUT expression in cartilage changes in the presence of joint disease, offering a possible tool for diagnosis and ultimately treatment. Proinflammatory cytokines have been shown to alter the pattern of GLUT expression in primary cultured articular chondrocytes, and the patterns are very different under the anaerobic conditions that are normal for these cells.[212]

Summary

The *SLC2A* family of transporter proteins are essential for the handling of hexoses and a variety of other substrates. They are expressed in numerous cell types, some almost ubiquitously, while others have a very specialized cell localization and work with other protein families to perform a wide variety of functions. The recently reported first crystal structure for GLUT1 will undoubtedly lead to a better understanding of just how these proteins bind their substrates and move them across the cell membrane. Major genetic mutations are uncommon, underlining the critical roles played by GLUTs in metabolism; however, their patterns of expression can change significantly in disease. There is now a focus developing on profiling those changes for use as a diagnostic tool or even to develop treatments for a variety of conditions. Thus, it may prove possible in the future to develop inhibitors or activity modulators for GLUTs based on a detailed knowledge of their binding sites and how transport is mediated. However, the future development of therapies employing GLUT proteins will need to take account of their multi-tissue expression patterns which could easily result in unforeseen side effects.

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Disclosure

The authors report no conflicts of interest in this work.

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