Human Glucose Transporters in Health and Diseases

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CONTENTS

PREFACE

Eukaryotes rely on the efficient distribution of energy and carbon skeletons between organs in the form of sugars. Glucose in animals and sucrose in plants serve as the dominant distribution forms of energy. Cellular uptake and release of sugar requires vesicular and/or plasma membrane transport proteins. Humans and plants use related proteins from three superfamilies for sugar translocation: the major facilitator superfamily (MFS), the sodium solute symporter family (SSF; only in the animal kingdom), and SWEETs.

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CHAPTER 1

CHARACTERISTICS OF HUMAN GLUCOSE TRANSPORTERS

The cell membrane is made up of a lipid bilayer, which is selectively permeable to the majority of molecules; it separates the inner environment from that of the outer environment. Molecules and ions move through the cell membrane spontaneously down their concentration gradient by the process of diffusion. They can also move against their concentration gradient, but this requires the expenditure of energy. The lipid bilayer of plasmalemma is impermeable to most essential molecules and ions. However, it is permeable to water molecules and a few other small uncharged molecules such as oxygen and carbon dioxide. These molecules diffuse freely in and out of the cell. The lipid bilayer is impermeable to cations (such as K^+, Na^+, and Ca^{2+}), anions (such as Cl^- and HCO_3^-), small hydrophilic molecules, and macromolecules (such as proteins and RNA). Glucose cannot penetrate the lipid bilayer because it is hydrophilic in nature; therefore, it requires specific carrier proteins to undergo diffusion through the bilayer. Carrier proteins or transporters of water-soluble molecules across tissue barriers have attracted greater attention since 1952, when Widdas proposed that the transport of glucose across the erythrocyte membrane required a carrier mechanism to facilitate diffusion [Widdas, 1952].

There are two general classes of membrane transporters: channels and carriers (Figure 1-1). Uniporters are integral membrane proteins that are involved in facilitated diffusion. They are classified into two groups: ion channels or carrier proteins. They mediate the transport of a single solute and transport it across its gradient (uniporters may not utilize energy). Glucose transporters (GLUTs) are examples of uniporters, which transport glucose across the cell membrane. Symporters are integral membrane proteins that are involved in the movement of two or more molecules or ions across the plasma membrane in the same direction and are therefore a type of cotransporter. Typically, ion(s) move down their electrochemical gradient, thereby allowing other molecule(s) to pass through the lipid

bilayer along with them. Sodium–glucose transporters (SGLTs) are examples of symporters, which transport sodium ions and glucose across the cell membrane. The other example of a symporter is the $Na^+/K^+/2Cl^-$ carrier protein found in the loop of Henle in the renal tubules of the kidneys; it transports four molecules of three different types. Antiporter is an integral membrane protein that is involved in the transport of two different types of ions or molecules, which are pumped in opposite directions across the membrane. During the transport *via* antiporters, one of the species is transported along its concentration gradient, which yields the energy to drive the transport of the other solute against its concentration gradient. First, a substrate binds and is transported across the membrane and then another substrate binds, which is transported in the opposite direction. In this case, only change is catalyzed and not the net transport, because carrier proteins cannot undergo conformational transition in the absence of a bound substrate. Adenine nucleotide translocase is an example of an antiporter, which catalyzes the exchange of ADP for ATP across the inner mitochondrial membrane. The sodium-calcium exchanger is another example of an antiporter, which allows three Na^+ ions into the cell in exchange for one Ca^{2+} ion out of the cell.

There are three types of gated ion channels depending on the method by which the opening of uniporter channels may be regulated.

1) Voltage-regulated or voltage-gated ion channels, which are present in "excitable" cells such as neurons and muscle cells. These channels open or close in response to the changes in the charge across the plasma membrane.
2) Stress-regulated or mechanically gated ion channels open or close depending on the physical pressure exerted on the transporter. This type of channel is expressed in the ear.
3) Ligand-regulated or ligand-gated ion channels are regulated by the binding of the ligand to the transporter; however, the ligand itself is not transported. The following are some examples of ligands: acetylcholine, gamma amino butyric acid (GABA), cyclic AMP (cAMP), ATP, and so on.

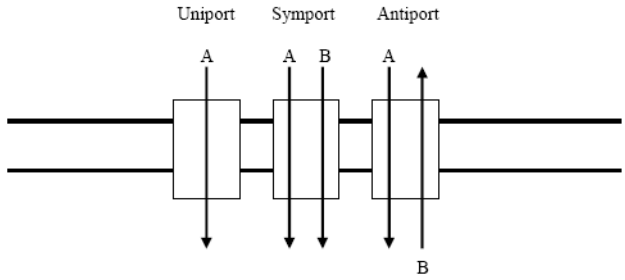

Figure 1-1. Classes of carrier proteins.

In the case of mammalian cells, glucose is an essential metabolic substrate. It is the major source of energy and is an important substrate for the biosynthesis of proteins and lipids in mammalian cells. D-glucose is the major form of glucose presented to the cell for energy production and many other anabolic requirements. It is obtained directly from the diet, following the hydrolysis of ingested disaccharides and polysaccharides and is also synthesized in organs such as the liver. Glucose derived from the hydrolysis of ingested saccharides is transported from the lumen of the small intestine into the blood circulation. Both dietary glucose and the glucose synthesized within the body must be quickly transported from blood circulation to the target cells. In the target cell, glucose and other monosaccharides are transported across the plasma membrane. Due to the hydrophilic nature of glucose, it requires specific transporters to cross cellular membranes. This type of transport is, as in the case of glucose, other monosaccharides, and other molecules, mediated by energy as well as *via* facilitative mechanisms.

Transporter proteins are classified based on phylogenetic and functional data. Within the major facilitator superfamily (MFS), the largest group is the sugar porter family. Sugar porters are found in bacteria, archaea, and eukaryote [Pao et al., 1998; Saier, 2000].

Glucose transporters belong to the MFS, which contains 74 families of membrane transporters, each of which transport a particular substrate; more than 10,000 members have been sequenced. Membrane proteins belonging to the MFS are ubiquitously expressed and are highly conserved from bacteria to humans; they import or export target molecules, such as amino acids, sugars, nucleotides, drugs, peptides, organic and inorganic anions, metabolites, neurotransmitters, polyols, and so on. Over the past few years, different isoforms of glucose transporters have been identified, which exhibit different substrate specificities, kinetic properties, and tissue expression profiles. The number of distinct gene products, together with

the presence of different transporters in certain tissues and cells, indicates that the transport of glucose into the cell is a process of considerable complexity.

There are three families of genes that encode for glucose transporters in humans: *SLC2A*, *SLC5A*, and *SLC50A* [Wright, 2013].

Glucose is transported across the cell membranes and tissue barriers by a specific saturable transport process involving members of three different classes of glucose transporters: sodium-independent glucose uniporters (facilitated transport, GLUT proteins, and *SLC2* genes), sodium-dependent glucose symporters (secondary active transport, SGLT proteins, and *SLC5* genes), and a new class of glucose uniporters, SWEET proteins (*SLC50* genes), each with different kinetic properties. Most cells express a variety of glucose transporters, and the pattern of expression in different tissues is related to the specific metabolic requirements. Even though these integral membrane proteins are called glucose transporters, they possess various substrate specificities and are involved in the transport of several different molecules, not just glucose.

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CHAPTER 2

EXPRESSION OF GLUCOSE TRANSPORTERS IN HEALTH

The human *SLC2* (GLUT) family of membrane proteins

In 1948, LeFevre postulated that a specific component within the cellular plasma membrane is necessary for the transport of glucose across the lipid bilayer [LeFevre, 1948]. In 1952, Widdas proposed a mobile carrier mechanism to explain the observed kinetics of glucose transport across sheep placenta [Widdas, 1952]. In 1977, the human GLUT1 protein was purified by Kasahara and Hinkle from human erythrocytes [Kasahara and Hinkle, 1977]. In 1985, the first GLUT to be isolated was cloned from HepG2 cell line. The complete genetic and protein sequence of GLUT1 was thus obtained [Mueckler et al., 1985].

In humans, 14 members of the family of GLUTs have been identified. They are encoded by the solute-linked carrier family 2, subfamily A gene family, *SLC2A* [Long and Cheeseman, 2015; Thorens and Mueckler, 2010]. Many of the genes belonging to this subfamily show varying amounts of substrate specificity, tissue distribution, and kinetic behavior [Pao et al., 1998; Saier et al., 1999] (Table 2-1).

All GLUTs are predicted to contain 12 hydrophobic membranes spanning, α-helical transmembrane (TM) domains. These domains are connected by the hydrophilic loop between TM6 and TM7 of the protein [Joost et al., 2002; Joost and Thorens, 2001; Uldry and Thorens, 2004]. GLUTs contain a short intracellular N-terminal segment, a large C-terminal segment, and a single site for glycosylation on the exofacial end, either in the large loop between TM1 and TM2 (first extracellular loop) or between TM9 and TM10 (fifth extracellular loop) [Mueckler and Thorens, 2013] (Figure 2-1). According to a computer-simulated model, based on GlpT crystal structure, GLUT1 shows two symmetrical six α-helical boundless connected by a long intracellular loop between TM6 and TM7 [Mueckler and Makepeace, 2009]. This also supports the hypothesis that the 12-TM GLUTs originated from a gene duplication event from a

protein with six TMs [Mueckler and Makepeace, 2009]. The TM domain contains a water-filled pathway through which the substrate moves [Lachaal et al., 1996; Zeng et al., 1996]. As was proposed for GLUT1, helices 1, 2, 4, 5, 7, 8, 10, and 11 form an inner bundle that is stabilized by the outer helices 3, 6, 9, and 12 [Mueckler and Makepeace, 2009] (Figure 2-2).

Sequence comparison of all 14 members belonging to the GLUT family shows that the sequences are better conserved in the putative TM regions and more divergent in the loops between the TM domains and the C- and N-termini. The most divergent regions are loops 1 and 9 and the two terminal regions. The sequences among members of the GLUT family are 14–63% identical and 30–79% conserved [Zhao and Keating, 2007]. Sequence alignments of all members revealed several highly conserved structures: PMY in TM4, QQLSGIN in TM7, GPGPIP/TW in TM10, and VPETKG in the C-terminal tail. PESPRY/FLL in loop 6 and GRR in loop 8 are also highly conserved. In addition, there are 18 conserved glycine residues; of them, 11 are adjacent to at least one other conserved amino acid [Zhao and Keating, 2007]. The conserved glycine residues in helices 1, 2, 4, 5, 7, 8, and 10 indicate a critical role in the structure of these helices. Helix 7 appears to be important for the binding of substrate from the exofacial site. The presence of a common motif suggests that this family originated from a common ancestral gene.

Based on the phylogenetic analysis of sequence similarity and characteristic elements, the GLUT family of sugar transporters is divided into three classes [Joost et al., 2002; Joost and Thorens, 2001; Manolescu et al., 2007b; Zhao and Keating, 2007]: an N-linked glycosylation site for family members of Classes I and II is positioned in the first exofacial loop between TM1 and TM2 and family members of Class III contain a shorter extracellular loop 1 and harbor the glycosylation site within the larger loop 9 [Joost and Thorens, 2001]. Wilson-O'Brien et al. [2010] proposed that Class I and II mammalian GLUTs form distinct, well-supported clades (respectively 100% and 99% bootstrap). Class III GLUTs do not form a distinct clade, but the members are dispersed in the evolutionary tree amongst non-mammalian sequences. However, Joost and Thorens [2001] suggest that Class III GLUTs are the "oldest" isotypes, from which Classes I and II have evolved. It might have been due to an adjustment to the additive requirements of glucose homeostasis in mammals. Class I GLUTs include GLUT1–GLUT4 and GLUT14, which are 48–63% identical in humans and have been extensively characterized. Residues that appear specific for the Class I GLUTs are the following: 1) glutamine in α-helical structure 5 (QL motif corresponding with Q161 in GLUT1), which

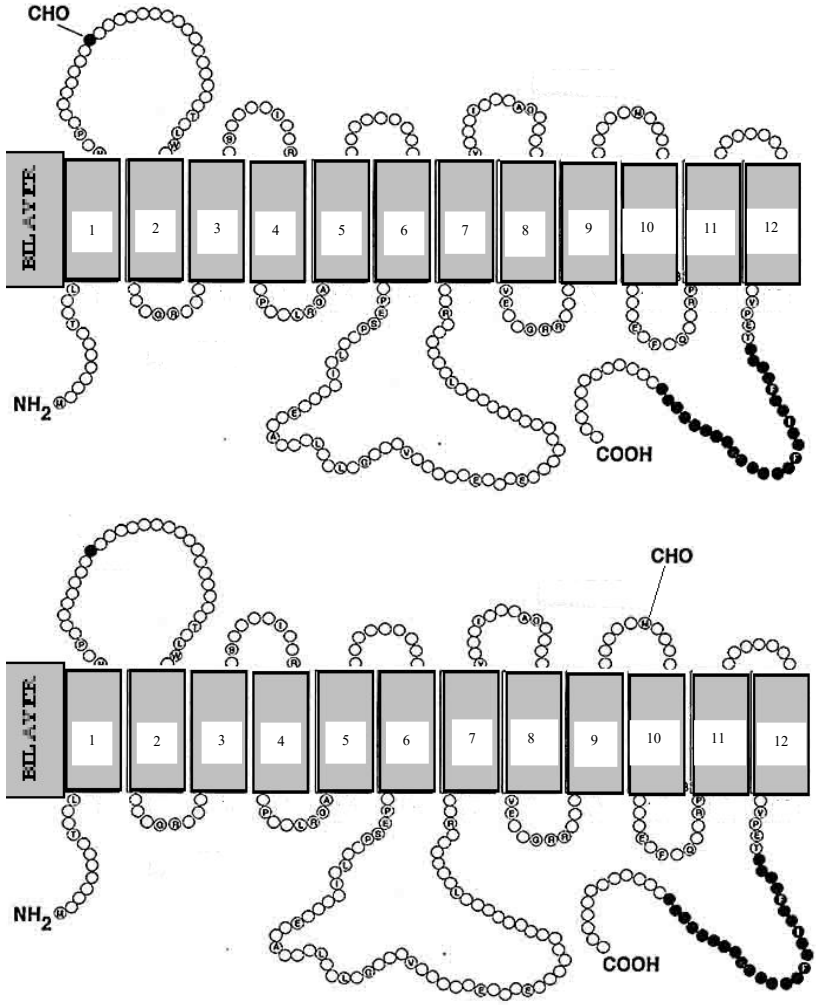

Figure 2-1. The N-linked glycosylation sites in Class I and II of glucose transporters (GLUTs) (upper panel) and in Class III (lower panel). 1–12 – α-helical transmembrane domains [Mueckler and Thorens, 2013].

suggests that this motif is important in the identification of glucose [Mueckler et al., 1994]; 2) the STS (serine–threonine–serine) motif in the extracellular loop 7, which is a critical site for the conformational change during the transport process [Doege et al., 1998]; 3) highly conserved motif GPXXXP in TM10, where tryptophan also appears immediately after this sequence, which is crucial for the binding the two inhibitors: cytochalasin B and forskolin, without directly affecting the transport of glucose [Garcia et al., 1992]; and 4) a QLS motif in TM7 is only present in GLUTs that transport glucose but not fructose, suggesting that this motif is involved in the selectivity of glucose and fructose [Seater et al., 1998].

Class II GLUTs comprise GLUT5, GLUT7, GLUT9, and GLUT11. These transporters are 36–40% identical. The striking sequence characteristic of Class II GLUTs is the lack of tryptophan residue following the conserved GPXXXP motif in helix 10, corresponding with tryptophan 388 in GLUT1 and the absence of the QLS motif [Seater et al., 1998].

Class III GLUTs include GLUT6, GLUT8, GLUT10, GLUT12, and GLUT13 (HMIT). Class III GLUTs are only 19–41% identical. Many of the motifs found in the other classes are conserved, such as PESPR in TM6, GRR in loops 2 and 8, PETKGR in TM12, and arginine and glutamate residues in loops 4 and 10. Class III GLUTs also have a tryptophan residue after the GPXXXP motif in TM10 [Long and Cheeseman, 2015]. Class III GLUTs do not contain the QLS motif, which is unique to the Class I GLUTs [Thorens and Mueckler, 2010].

One or more GLUTs are expressed in every type of cell in the human body. The human GLUTs possess various substrate specificities and are involved in the transportation of several hexoses in addition to *myo*-inositol, urate, glucosamine, and ascorbate [Mueckler and Thorens, 2013]. All the members of the GLUT family are facilitative transporters except for GLUT13 (HMIT), which is an H^+/*myo*-inositol symporter [Uldry et al., 2001]. It is highly likely that the major substrates for several GLUTs have not been definitively identified [Thorens and Mueckler, 2010].

Class I family members

GLUT1

As mentioned earlier, GLUT1 is one of the first membrane transport proteins to be purified [Kasahara and Hinkle, 1977] and cloned [Mueckler et al., 1985]. It has been detected in the human erythrocytes and brain as well as in HepG2 cells; it comprises 3–5% of the total erythrocyte protein.

The gene encoding GLUT1 (*SLC2A1*) maps to chromosome 1p35-p31.3. It comprises 10 exons and is about 33 kb in length and the size of mRNA is about 2.8 kb [Longo and Elsas, 1998]. GLUT1 is a highly hydrophobic heterogeneously glycosylated protein. The deduced sequence of GLUT1 comprises 492 amino acids with a molecular weight of approximately 54.2 kDa [Hruz and Mueckler, 2001]. An extracellular loop of 33 amino acids located between TM1 and TM2 is the location of a single site for ASN-linked oligosaccharide addition. On SDS PAGE, GLUT1 runs as a band between 50 and 60 kDa, due to the N-linked glycosylation at ASN-45.

The molecular weight of GLUT1 in the brain ranges between 45 and 55 kDa [Maher et al., 1991; Maher et al., 1994; Olson and Pessin, 1996]. The differences in molecular weight are due to the differences in the N-linked glycosylation. The functional effect of the different types of glycosylation is not yet clear. It is suggested that they are involved in GLUT1 trafficking [McMahon et al., 2000] and substrate affinity [Onetti et al., 1997]. The large molecular weight of GLUT1 is present in the cerebral cortex and in cerebral microvessels [Maher et al., 1994]. The low molecular weight isoform is readily detectable in the microvessel-depleted membranes of the brain (neuronal/glial membranes) and synaptosomes [Maher et al., 1994; Pardridge et al., 1990]. An intermediate form of GLUT1 is present in the choroid plexus [Kumagai et al., 1994]; however, according to the results obtained by Maher et al. [1994], the choroid plexus contains a low molecular weight isoform of GLUT1. Although absent in hepatocytes, GLUT1 appears to be the most ubiquitously distributed GLUT. It is expressed in many fetal (throughout early mammalian embryo development from the oocyte to the blastocyst) and adult mammalian tissues and cell types. GLUT1 transports glucose to various cells comprising of a barrier between body tissue and blood supply. Thus, the endothelial or epithelial cell-like barrier cells of the brain, eye, peripheral nerve, and placenta express a relatively high level of GLUT1 [Takata et al., 1990]. It is also present in the blood cells (e.g., erythrocytes and granulocytes), adipocytes, muscle cells, kidney cells, the colon, and in fetal tissues [Gould and Bell, 1990; Gould and Holman, 1993; Korgun et al., 2002; Longo and Elsas, 1998]. In the adipocytes and muscle cells, GLUT1 is present constitutively in the plasma membrane, where it presumably provides the low level of glucose required for basal cellular activity [Marette et al., 1992; Zorzano et al., 1989].

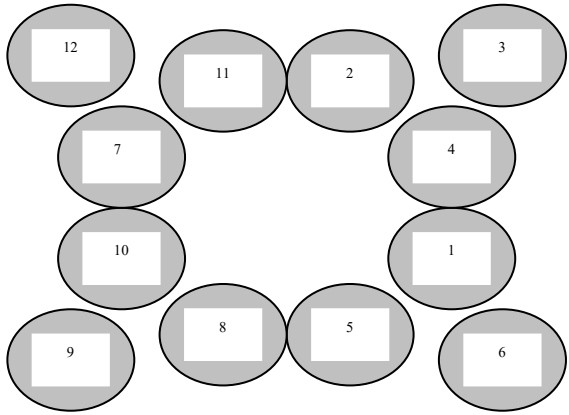

Figure 2-2. Model of the substrate-binding site of GLUT1. Helices 1, 2, 4, 5, 7, 8, 10 and 11 form an inner bundle that is stabilized by the outer helices 3, 6, 9 and 12 [Mueckler and Makepeace, 2009].

GLUT1 is present not only in the cell membrane but also in the mitochondria. *In vitro* mitochondrial import of GLUT1, immunoblot analysis of mitochondrial proteins, and cellular immunolocalization studies have indicated that GLUT1, a 54 kDa protein, localizes in the mitochondria of the human kidney cells (293 T) and has been identified in the murine fibroblasts (NIH/3T3) as well. The oxidized form of vitamin C, dehydroascorbic acid, enters mitochondria *via* facilitative GLUT1 and accumulates in the mitochondria as ascorbic acid. It protects mitochondria from oxidative injury [Sagun et al., 2005].

GLUT1 also performs other functions. Studies conducted on GLUT1 using adherent cell lines have shown that it can function as a receptor for human T cell leukemia virus type-1 (HTLV). Infection of CD4+ T lymphocytes by the HTLV is mediated by GLUT1. GLUT1 plays an essential role in CD4+ T cell activation [Deng and Yan, 2016; Jin et al., 2006; Jones et al., 2006; Manel et al., 2004].

GLUT1 has affinity for glucose (Km = 3–7 mM) [Zhao and Keating, 2007]. The other transport substrates of GLUT1 include galactose, mannose, and glucosamine [Mueckler and Thorens, 2013; Wieczorke et al., 2003].

GLUT2

In 1998, human GLUT2 was cloned from rat and human liver cDNA libraries [James et al., 1988; Kayano et al., 1988]. It is encoded by the *SLC2A2* gene located on the human chromosome 3q26.1-q26.2 and comprises 11 exons [Joost and Thorens, 2001; Zhao and Keating 2007]. The gene is about 31 kb in length [Zhao and Keating, 2007]. GLUT2 comprises 524 amino acids with a molecular weight of 58 kDa [Longo and Elsas, 1998]. Human GLUT2 has an 80% homology and 55% identity with GLUT1. The amino acid sequence of GLUT2 displays 81% identity between the human, mouse, and rat origin [Zhao and Keating, 2007]. GLUT2 lacks the QLS motif at helix 7, which is thought to confer substrate specificity on the transporter; this explains the high affinity for glucosamine toward GLUT2 [Uldry et al., 2002]. Compared to other GLUTs, GLUT2 exhibits several distinctive characteristics. It is the only GLUT that catalyzes the bidirectional transportation of glucose depending on fed or fasting state [Deng and Yan, 2016].

GLUT2 is primarily expressed in the intestinal absorptive epithelial cells, hepatocytes, pancreatic β-cells, and in the kidney cells [Fukumoto et al., 1988; Thorens et al., 1988]. In the epithelial cells, GLUT2 is expressed exclusively in the basolateral membrane, where it works in conjunction with the SGLT co-transporter of the apical membrane in order to facilitate the absorption and reabsorption of glucose from the intestinal lumen or for forming urine from the blood [Thorens et al., 1990a; Thorens et al., 1990b]. In the hepatocytes, GLUT2 is involved in the uptake of glucose during the fed state and release of glucose, synthesized by gluconeogenesis, into the blood during the fasted states [Zhao and Keating, 2007]. In pancreatic β-cells, an increase in the concentration of blood glucose triggers insulin secretion. GLUT2 is also required as a glucose sensor in the area of hepatoportal vein and in the central nervous system. Low levels of GLUT2 mRNAs are present in the nuclei of the brain, including the nucleus tractus solitatius, the motor nucleus of the vagus, the paraventricular hypothalamic nucleus, the lateral hypothalamic area, the arcuate nucleus, and the olfactory bulbs [Leloup et al., 1994; Piroli et al., 2007]. These sensors appear to control glucagon secretion, feeding behavior, insulin secretion, and peripheral tissue glucose uptake [Marty et al., 2007]. Because GLUT2 as well as glucokinase are present in the nuclei of the brain that are involved in the control of energy homeostasis, GLUT2 may be associated with glucose sensing mechanisms that control feeding, energy expenditure, and counter regulation.

GLUT2 is a low-affinity transporter of glucose (Km ~17 mM), galactose (Km ~92 mM), mannose (Km ~125 mM), and fructose (Km ~76 kM). It has high affinity for glucosamine (Km ~0.8 mM) [Conville et al., 1993; Johnson et al., 1990; Uldry et al., 2002].

GLUT3

The information regarding GLUT3 is scarce, especially in humans [Mueckler, 1994]. It was cloned from a human fetal muscle cDNA library [Kayano et al., 1988]. A single copy gene *SLC2A3*, is located on the human chromosome 12p13.3 [Joost et al., 2002; Joost and Thorens, 2001; Longo and Elsas, 1998]. This gene comprises 10 exons (spanning 17 kb) [Zhao and Keating, 2007]. The open reading frame codes for a 496-amino-acid membrane protein with a predicted weight of 54 kDa [Longo and Elsas, 1998]. It shares about 64% identity with *SLC2A1*. GLUT3 is highly expressed in the brain [Nagamatsu et al., 1992; Piroli et al., 2007], especially in the neurons [Maher et al., 1991; Maher et al., 1994] and therefore it is also referred to as a "neuronal glucose transporter" [Nagamatsu et al., 1992]. It is also expressed in tissues with high demand for glucose such as spermatozoa [Burant and Davidson, 1994], placenta [Illsley, 2000], preimplantation embryos [Pantaleon et al., 1997], fibroblasts [Longo and Elsas, 1998], human platelets [Heijnen et al., 1997], and retinal endothelial cells [Knott et al., 1996]. It is abundantly expressed in human white blood cells, where in the resting cell, it is largely confined to the intracellular storage vesicles which get translocated to the plasma membrane in response to various proliferative stimuli [Simpson et al., 2008]. GLUT3 mRNA has been detected only in the differentiated neurons in rat brains, and gradually increases its levels from embryonic day 14, attaining adult levels in most regions by postnatal day 20 [Bondy et al., 1992]. The highest levels of GLUT3 have been found in large neurons of the olfactory system, hippocampal formation, neocortex, deep cerebellar, pontine, and brain stem nuclei.

GLUT4

In 1989, various research groups cloned GLUT4 from human [Fukumoto et al., 1989], rat [Birnbaum, 1989; Charron et al., 1989], and mouse tissues [Kaestner et al., 1989]. In addition to GLUT1, GLUT4 is one of the most extensively studied GLUTs, which is attributed to its important physiological role as the rate-limiting step in insulin-stimulated glucose uptake in the skeletal and cardiac muscle cells and brown and

white adipose tissue [Huang and Czech, 2007]. GLUT4, classically referred to as an "insulin-responsive" transporter has been widely studied due to its role as the primary insulin-sensitive member of this family and thus for its role in diabetes. GLUT4 is encoded by *SLC2A4* located on the human chromosome 17p13 [Zhao and Keating, 2007]. It has 13 exons with a gene size of approximately 6 kb [Zhao and Keating, 2007]. GLUT4 is a 509-amino acid protein with a molecular weight of approximately 55 kDa. It is highly conserved with 91–96% sequence identity among human, bovine, rat, and mouse origin [Thorens and Mueckler, 2010; Zhao and Keating, 2007].

GLUT4 is a facilitative GLUT characterized by preferential expression in the skeletal muscle, cardiomyocytes, and adipose tissue, where it is responsible for insulin-stimulated glucose uptake and for the entry of glucose into the skeletal muscle cells during contraction/exercise. The expression of GLUT4 in the brain [Piroli et al., 2007] has been located in the hypothalamus [Leloup et al., 1996], cerebellum [Vanucci et al., 1998], cortex, and hippocampus [McEwen and Reagan, 2004; Vanucci et al., 1998]. The insulin receptor and GLUT4 exhibit overlapping distributions in the brain, including the cerebellum, hypothalamus, and hippocampus [Doré et al., 1997; Marks et al., 1991], suggesting that insulin elicits the translocation of GLUT4 from internal pools to the plasma membrane in the central nervous system as it does in the periphery [Piroli et al., 2007]. GLUT4 is unique among other members of the GLUT family in its dynamic cycling within the muscle cells and adipocytes. Unlike other human GLUT proteins which constitutively reside in the cell membrane, more than 95% of the total amount of GLUT4 is stored intracellularly [Medina and Owen, 2002; Pascoe et al., 1996].

GLUT4 is constitutively active during hexose transport, and glucose uptake is regulated by insulin controlling the amount of GLUT4 in the plasma membrane [Huang and Czech, 2007]. In unstimulated cells, GLUT4 is rapidly removed from the plasma membrane, which gets recycled slowly, leading to a steady-state accumulation in intracellular organelles. All the intracellular GLUT4 of L6 muscle cells recycle to the plasma membrane within 6 h [Foster et al., 2001], whereas 3T3 L1 adipocytes do not show similar behavior [Govers et al., 2004; Korylowski et al., 2003]. In muscle cells, GLUT4 molecules from the plasma membrane reach the endosomal recycling compartment within 20 min and rapidly exit within 20 min [Foster et al., 2001]. Western blot experiments showed that GLUT4 is present in low-density microsomes and that insulin elicits the trafficking of GLUT4 from the microsomal fraction to the plasma membrane [Birnbaum, 1989]. In the presence of insulin or other

stimuli, such as exercise, the equilibrium of the recycling process is altered to favor the process of translocation (regulated movement) of GLUT4 from the intracellular storage vesicles to the plasma membrane, and in the case of muscle, the translocation takes place to the transverse tubules as well. The net effect is an elevation in the maximal velocity of glucose transported into the cell [Gould and Holman, 1993; Kandror and Pilch, 1996]. Activation of the intrinsic activity of GLUTs may also contribute toward the increased uptake of glucose [Somwar et al., 2001]. Within 10 min, the amount of surface GLUT4 increases 2- to 3-fold in the skeletal muscle cells and in human adipocytes [Sweeney et al., 2004], but the increase is >10-fold in rodent adipocytes [Thong et al., 2005]. Insulin signaling results in changes in GLUT4 trafficking parameters, for example, GLUT4 exocytosis is accelerated, whereas GLUT4 endocytosis is inhibited. In mature adipocytes and muscle cells, GLUT4 is a longlived protein ($t_{1/2}$ of about 40 h); therefore, each polypeptide chain is likely to cycle to the plasma membrane many times during its lifetime. GLUT4 trafficking in adipocytes differs greatly from that of the other recycling proteins in some cells, indicating that GLUT4 contains specific sequences that control its trafficking. The $F^{5}QQI$ sequence located in the N-terminal region mediates the internalization process of GLUT4 through a clathrin–adaptin protein complex-2 (AP2)-dependent pathway in insulin-stimulated adipocytes. The LL^{490} motif is dispensable for both basal and insulin-stimulated endocytosis. The $TE^{499}LE^{501}$ sequence located in the C-terminus is involved in basal retention [Blot and Mc Graw, 2008]. N-glycosylation of GLUT4 also plays a role in its translocation [Ing et al., 1996]. Phosphatidic acid, a lipid second messenger, is produced by phospholipase D in a variety of intracellular signaling pathways. It transduces the signal by altering the localization and/or the activity of its target proteins. Several proteins are known to be regulated by phosphatidic acid. Insulin appears to be capable of stimulating phospholipase D activity. The putative phosphatidic acid-binding motif, SQWL, is located in the first intracellular loop of GLUT4, proximal to the third TM and suitably placed to mediate interactions with lipid molecules of the membrane. The other members of the GLUT family, which are not regulated by phospholipase D, do not contain the SQWL motif. Mutation in the sequence of SQWL in 3T3-L1 adipocytes reduces the exposure of GLUT4 in response to insulin by affecting the fusion of the GLUT4 storage vesicles (GSVs) with the plasma membrane [Heyward et al., 2008].

It is unclear whether in the absence of insulin, GSVs represent a separate, distinct class of vesicles. Several, so-called nonspecialized proteins have been found to co-localize with GLUT4 in the adipocytes.

However, many questions regarding the localization and trafficking pathways of GLUT4 remain unanswered. The majority of intracellular GLUT4 is found in small 50-nm vesicles referred to as GSVs. The formation of GSVs is catalyzed by a budding stage where coat proteins are recruited to the donor membrane *via* adaptor proteins [Chavier and Goud, 1999; Kirchhausen, 1999]. They may form either from endosomes or from parts of the trans-Golgi network or both. The existence of a specialized GLUT4 compartment that is exclusively insulin sensitive has been postulated, but such an organelle has not been fully characterized. In the absence of insulin, GLUT4 is segregated from the compartment containing the recycling receptors. Studies related to GSVs reveal the presence of synaptobrevin, vesicle-associated membrane protein 2 (VAMP2), cellubrevin (VAMP3), secretory carrier membrane proteins (SCAMP), phosphatidylinositol 4-kinase, GTP-binding protein Rab4, and zinc-dependent aminopeptidase (165 – for vesicle protein of molecular weight 165 kDa or gp160 – glycoprotein of molecular weight 160 kDa) [Hanpeter and James, 1995; Malide et al., 1997]. These observations suggest the existence of GLUT4 in a specialized compartment that is regulated by insulin, which is characterized by the presence and functional requirement of VAMP for final fusion with the plasma membrane [Ishiki and Klip, 2005].

In both *Xenopus* oocytes and Chinese hamster ovaries [Asano et al., 1992; Nishimura et al., 1993], GLUT4 has been found to be a high affinity transporter of glucose with a Km of 1–4 mM, whereas in *Saccharomyces cerevisiae*, it is 5–6 mM [Kasahara and Kasahara, 1997]. It can also transport dehydroascorbic acid and glucosamine (Km ~3.9 mM) [Kasahara and Kasahara, 1997].

GLUT14

Wu and Freeze identified and cloned GLUT14 as a duplication of GLUT3 with 94.5% identity [Wu and Freeze, 2002]. In humans, the gene encoding GLUT14 (*SLC2A14*) is located on chromosome 12p13.3, about 10 Mb upstream of GLUT3. GLUT14 contains 11 exons and exhibits a genomic organization similar to that of GLUT3. It has two alternatively spliced forms. The short form of GLUT14 (GLUT14-S) contains 10 exons and produces a 497-amino acid protein. It shares 94.5% identity with GLUT3. The long form of GLUT14 (GLUT14-L) has an additional exon and codes for protein with 520-amino acids that differs from GLUT14-S only at the N-terminus. Both isoforms of GLUT14 are specifically

expressed in the human testis. The ortholog of GLUT14 is not found in mice [Wu and Freeze, 2002].

Class II family members

GLUT5

Kayano et al. [1990] initially cloned *SLC2A5* from the human small intestine. *SLC2A5* encodes GLUT5 and is located on human chromosome 1p36.2 [Joost et al., 2002; Joost and Thorens, 2001]. It comprises 12 exons (spanning 33 kb) [Zhao and Keating, 2007] and produces a 501-amino acid protein with a molecular weight of approximately 43 kDa [Drozdowski and Thomas, 2006; Longo and Elsas, 1998]. GLUT5 exhibits the weakest inter-isoform homology of any of the members of the GLUT family [Kayano et al., 1990]. Buchs et al. [1998] have found two domains of GLUT5 to be responsible in the uptake of fructose (amino-terminus to the first intracellular loop, and the sequence between the 5th and 11th TM stretches), whereas at least parts of the carboxyl-terminal of GLUT5 seem to be required for transport function [Oka et al., 1990].

GLUT5 is expressed at high levels in the apical membrane of enterocytes [Davidson et al., 1992]. GLUT5 mRNA levels increase with age and the highest levels are found in the small intestine of adults [Cheeseman, 2008a]. In the intestine, a proximal–distal gradient has been observed, with GLUT5 mRNA levels being higher in the proximal small intestine when compared to the distal small intestine [Drozdowski and Thomas, 2006]. It is also expressed at high levels in the plasma membrane of mature spermatozoa [Burant et al., 1992]. Moderate to significant levels of GLUT5 mRNA and/or protein have now been detected in the kidney, adipose tissue, skeletal muscle, and in the brain [Kayano et al., 1990; Longo and Elsas, 1998; Maher et al., 1994; Piroli et al., 2007; Stuart et al., 2006]. The expression of GLUT5 was discovered in the microglial cells of the brain [Payene et al., 1997], and its expression is present only in the microglia among cells of the mononuclear phagocyte system and is absent in the monocyte-derived cells that infiltrate lesions in the brain infarcts [Sasaki et al., 2003]. The physiological role of GLUT5 in the microglia remains unclear, because fructose levels in the brain are low and GLUT5 transporter activity for glucose is much lower than that of fructose [Leloup et al., 1994]. In adipocytes and muscle cells, GLUT5 is not subjected to acute insulin regulation as in the case of GLUT1 and GLUT4 [Mueckler, 1994].

Among the seven members of the GLUT family that are able to transport fructose, GLUT5 is the sole transporter that is specific for fructose, with no ability to transport glucose or galactose. It mediates the transport of fructose with a Km of about 6 mM [Burant et al., 1992; Douard and Ferrais, 2008] and one of its primary functions is to mediate the uptake of dietary fructose across the apical membrane of the small intestine [Douard and Ferrais, 2008]. Fructose is then released into the bloodstream *via* GLUT2 in the intestinal basolateral membrane [Mueckler and Thorens, 2013].

GLUT5 is an interesting topic of research because total fructose consumption has dramatically increased, for example, in the United States, there has been an increase in the consumption from about 20 to 80 g/day in the last 20–30 years [Bray, 2007; Schürmann, 2008]. Fructose is a major sweetener in Western diets, and for a long time, it was used in the treatment of diabetes as it did not cause acute hyperglycemia. Increased consumption of fructose, particularly in the form of carbonated beverages, has been attributed to participation in the increased prevalence of obesity and type 2 diabetes mellitus (DM).

GLUT7

Human GLUT7 has been cloned from the human intestinal cDNA library by using a PCR-based strategy [Li et al., 2004]. Initially, in 1992, Waddell described GLUT7 as a hepatic microsomal transporter protein that had 68% identity with GLUT2 [Waddell et al., 1992]. Subsequently, it was reported to be a cloning artifact, and its mRNA could not be found, either in human or rat liver [Burchell, 1998]. *SLC2A7* is adjacent to *SLC5A5* (gene encoding GLUT5) on the chromosome 1p36.22 [Joost and Thorens, 2001; Zhao and Keating, 2007]. The protein encoded by *SLC2A7* comprises 524-amino acid residues and shares 68% similarity and 53% identity with GLUT5; it is the most closely related isoform [Li et al., 2004]. Sequence alignments between fructose and non-fructose transporting GLUT isoforms identified a motif in GLUT7 that potentially confers its ability to transport fructose. GLUT7 is primarily expressed in the apical membrane of the small intestine (distal region) and colon, although GLUT7 mRNA has been detected in the testis and prostate as well [Cheeseman, 2008b; Schürmann, 2008].

GLUT7 has a high affinity for both glucose (Km = 0.3 mM) and fructose. It does not transport galactose, 2-deoxy-D-glucose, and xylose.

The observation that the distribution of GLUT7 is limited to the distal region of the small intestine, the ileum, which does not contain high

concentrations of glucose and fructose, suggests that GLUT7 does not play a major role in taking up glucose and fructose from the diet. It may be important toward the end of a meal when luminal concentrations of hexoses in the ileum are low [Li et al., 2004; Schürmann, 2008].

The unusual substrate specificity and close sequence identity with GLUT5 suggest that GLUT7 represents an intermediate between Class II GLUTs and the Class I member GLUT2. Comparison between these proteins may provide key information regarding the structural determinants for the recognition of fructose as a substrate [Li et al., 2004]. Cheeseman [2008b] introduced the hypothesis that GLUT proteins may have a filter for substrate selectivity at the exofacial opening of their translocation pore.

GLUT9

GLUT9 (earlier designated as GLUTX) was identified independently by two groups of researchers; they designated it as GLUTX [Tartiglia and Weng, 1999] and GLUT9 [Phay et al., 2000]. Human GLUT9 cDNA was isolated by PCR amplification from a human kidney cDNA library based on sequence information from ESTs and from genomic sequence [Phay et al., 2000]. The human *SLC2A9* is located on the chromosome 4p15.3-p16 and is approximately 214 bp in length [Zhao and Keating, 2007]. The gene encodes two isoforms through the use of alternative promoters [Augustin et al., 2004; Keembiyehetty et al., 2006; Phay et al., 2000]. It consists of 12 exons coding for 540-amino acid proteins (the major isoform i.e. GLUT9 or GLUT9a). An alternative splice variant of GLUT9 mRNA consists of 13 exons and codes for a putative protein of 512-amino acids (GLUT9ΔN or GLUT9b isoform). The predicted proteins differ only in their N-terminal end, suggesting a different subcellular localization and a physiological role [Augustin et al., 2004; Zhao and Keating, 2007]. The next relative to GLUT9 is the fructose transporter GLUT5 [Joost and Thorens, 2001]. GLUT9 contains a dileucine motif in the N-terminus, but the motif does not sequester GLUT9 to intracellular pools, unlike most other GLUT family members [Augustin et al., 2004]. GLUT9b is expressed only in the liver and the kidneys, whereas GLUT9 is expressed in the liver, kidneys, intestine, leukocytes, and chondrocytes [Mobasheri et al., 2005; Mueckler and Thorens, 2013]. The different amino-terminal tails are important for the differential targeting of GLUT9 to opposite poles of the epithelial cells [Mueckler and Thorens, 2013]. GLUT9a is expressed in the basolateral membrane, whereas GLUT9b is targeted to the apical pole [Augustin et al., 2004]. GLUT9 was initially considered a glucose or fructose transporter, but it is now established that it is a urate transporter

[Caraynnopoulos et al., 2004; Manolescu et al., 2007a]. GLUT9a and GLUT9b transport urate with the same kinetics (Km ~0.6 mM) [Bibert et al., 2009]. It was found that urate is transported by GLUT9 (two splice variants of the glucose transporter) at a rate of 45- to 60-fold faster than that of glucose and to a lesser extent, fructose [Caulfield et al., 2008]. The relationship between GLUT9 and uric acid is highly clinically significant. Elevated levels of uric acid have been associated with metabolic syndrome, obesity, diabetes, hypertension, and chronic renal failure. Uric acid may play a role in the pathogenesis of this disease. GLUT9 is expressed in the articular cartilage and is a uric acid transporter; thus, it is possible that GLUT9 plays a role in gout [Doblado and Moley, 2009].

GLUT11

GLUT11 (formerly designated GLUT10) was cloned from the human heart in 2001 by Doege et al. [2001]. The gene encoding human GLUT11 (*SLC2A11*) is 28 bp in length, contains 10–13 exons, and is located on chromosome 22q11.2 [Doege et al., 2001; Zhao and Keating, 2007]. In humans, three isoforms of GLUT11 have been cloned, because the separate exons (exons 1A, 1B, and 1C) of *SLC2A11* generated mRNA of three variants of GLUT11 (GLUT11-A, GLUT11-B and GLUT11-C), which differ only at their N-terminal sequence [Scheepers et al., 2005]. GLUT11 comprises 496 amino acids [Mueckler and Thorens, 2013]. Human GLUT11 exhibits similarity with the members of GLUT family, and the closest relative of GLUT11 is the fructose transporter GLUT5 (sharing 41.9% amino acid identity with GLUT11). It also shares 35% of its identity with GLUT1 [Doege et al., 2001].

The GLUT11 isoforms are expressed in a tissue-specific manner but do not appear to differ in their functional characteristics. GLUT11-A is expressed in the heart, skeletal muscle, and the kidneys; GLUT11-B is expressed in the kidneys, adipose tissue, and the placenta; and GLUT11-C is expressed in the adipose tissue, heart, skeletal muscle, and pancreas [Scheepers et al., 2005].

GLUT11 has an affinity for fructose comparable to, possibly even higher than, that of glucose (Km ~0.2 mM), but not for galactose [Doege et al., 2001].

GLUT11 has no rodent ortholog [Scheepers et al., 2005].

Class III family members

GLUT6

GLUT6 (formerly designated GLUT9). It is noteworthy that the symbol GLUT6 has previously been used for a pseudogene derived from GLUT3 (*SLC2A3P*) [Kayano et al., 1990]. Human GLUT6 was cloned from leukocytes by PCR and RACEPCR amplification based on the sequence information obtained from murine-expressed sequence tags and a human genomic sequence [Doege et al., 2000a]. *SLC2A6*, encoding GLUT6 protein is located on the human chromosome 9q34 [Joost et al., 2002; Joost and Thorens, 2001; Zhao and Keating, 2007]. It comprises 10 exons (spanning 8 kb) [Zhao and Keating, 2007]. GLUT6 is composed of 507 amino acid residues. The cDNA of human GLUT6 expresses a 46-kDa membrane protein [Doege et al., 2000a]. GLUT6 contains the N-terminal dileucine motif, which is responsible for the trafficking of GLUT6 to the intracellular pools. Mutation of the N-terminal dileucine motif to alanine results in the expression of the protein in the plasma membrane. In addition, cell stimulation by several factors such as insulin, phorbol esters, and osmotic shock does not recruit GLUT6 to the plasma membrane [Lisinski et al., 2001]. Human GLUT6 is predominantly expressed in the brain, spleen, and the peripheral leukocytes as well as in germinal cells of the testis [Godoy et al., 2006].

GLUT6 exhibits glucose transport activity; however, it appears to be a low-affinity facilitator of glucose (Km = 5 mM); furthermore, the transport activity was found in the presence of 5 mM but not in 1 mM glucose [Augustin and Mayoux, 2014; Joost and Thorens, 2001]. The function of GLUT6 may be to transport hexose molecules or related compounds across the intracellular organellar membranes. The biochemistry, cell biology, and physiology of GLUT6 are currently not well understood.

GLUT8

Sequences for human, rat, and mouse GLUT8 (earlier designated as GLUTX1) were first identified by database mining, cloning, and functional characterization [Doege et al., 2000 b; Ibberson et al., 2000].

SLC2A8, the gene encoding GLUT8, is located on chromosome 9q33.3, and is 11 kb in length and has 10 exons [Joost et al., 2002; Zhao and Keating, 2007]; it encodes a protein with 477 amino acid residues. *In vitro*-translated GLUT8 migrates as a 35-kDa protein; however, COS-7 cells transfected with GLUT8 cDNA expressed a 42-kDa protein [Doege et al., 2000b]. GLUT8 exhibits significant sequence similarity with the

members of the GLUT family (29.4% of the amino acids are identical with GLUT1) [Doege et al., 2000b]. It differs from other GLUTs at the C-terminal cytoplasmic tail, which is quite short with 20 amino acids compared to 42–45 amino acids for GLUT1–GLUT5 [Zhao and Keating, 2007]. This may be important to mediate the conformational changes that accompany glucose transport [Zhao and Keating, 2007]. GLUT8 contains a dileucine motif in the N-terminus, which might target the protein to the intracellular compartment. It has been found to be associated with endosomes, lysosomes, and membranes of the endoplasmic reticulum [Schmidt et al., 2009]. The distribution of GLUT8 is not affected by insulin treatment [Lisinski et al., 2001]. *SLC2A8* mRNA is expressed at high levels in the testis and at lower levels in the cerebellum, adrenal glands, liver, spleen, and brown adipose tissue [Mueckler and Thorens, 2013]. In humans, GLUT8 is expressed in differentiating spermatocytes at stage 1 [Doege et al., 2001] and has also been reported in the mature spermatozoa [Joost et al., 2002]. Therefore, it has been hypothesized that GLUT8 plays a major role in the provision of glucose to mature spermatozoa [Zhao and Keating, 2007]. In the brain, GLUT8 is found in the hippocampus, as well as the dente gyrus, amygdala and primary olfactory cortex, hypothalamic nuclei, and in the tractus solitaries [Ibberson et al., 2002; Piroli et al., 2007]. It has been detected in blood in the retinal barrier [Henry et al., 2002] and in the endometrium and endometrial adenocarcinoma [Goldman et al., 2006]. It is also present in blastocysts, where it is supposed to play a crucial role in glucose metabolism [Pinto et al., 2002]. The GLUT8 mRNA and protein are differentially regulated in the liver, depending on the prenatal and postnatal stage of mice [Gorovits et al., 2003].

GLUT8 is a high affinity transporter of glucose (Km ~2 mM) [Schmidt et al., 2009]. It appears to catalyze the transport of sugars or sugar derivatives through the intracellular membranes [Schmidt et al., 2009].

The function of GLUT8 requires further investigation.

GLUT10

GLUT10 has been cloned from the human liver cDNA library by using 3′ and 5′ RACE PCR based on an EST sequence that was identified *via* a homology search with known sequences of GLUT proteins [Mc Vie-Wylie et al., 2001]. The human GLUT10 gene (*SLC2A10*) is located on the chromosome 20q13.1; it contains 5 exons and is approximately 27 bp in length [Joost and Thorens, 2001; Zhao and Keating, 2007]. It is composed of 541 amino acids. It shares only 18–22% of its identity with

human GLUT1–GLUT11 but shares 35% of its identity with GLUT12 [Zhao and Keating, 2007]. GLUT10 does not have the PESPR motif in loop 6, which is present in all other GLUTs, which may contribute to its high affinity toward glucose [Zhao and Keating, 2007]. Immunocytochemical studies have indicated a rather intracellular localization for GLUT10 under steady-state conditions [Augustin, 2010]. The presence of the potential internalization motif YSRI at the C-terminus of the transporter supports these findings. Although GLUT10 does not contain a dileucine motif, it is reported that it is expressed in the insulin-sensitive tissues of the skeletal muscle and heart [Mc Vie-Wylie et al., 2001; Woods and Trayhurn, 2003]. GLUT10 mRNA is expressed at the highest levels in the liver and pancreas. Low levels of GLUT10 mRNA are detected in the human placenta and the kidneys [Woods and Trayhurn, 2003]. It is expressed in the human adipose tissue (omental and subcutaneous) and is also detected in the human preadipocyte cell strains (SGBS) and in 3T3-L1 adipocytes [Woods and Trayhurn, 2003].

GLUT10 shows a very high affinity for both deoxy-D-glucose (Km ~0.3 mM) [Dawson et al., 2001] and D-galactose but does not have an affinity for fructose [Dawson et al., 2001; Manolescu et al., 2007b].

The association of GLUT10 with a known type 2 DM susceptibility locus on chromosome 20 [Dawson et al., 2001; Mc Vie-Wylie et al., 2001] makes it an interesting target of research. Polymorphisms of GLUT10, however, are not associated with type 2 DM in the investigated human populations [Mueckler and Thorens, 2013]. Mutations in the gene encoding GLUT10 have been found to be associated with human arterial tortuosity syndrome [Coucke et al., 2006].

GLUT12

The cDNA of GLUT12 (formerly designated GLUT8) has been detected in the breast cancer cell line, MCF-7; it was cloned from a human embryonic cDNA library [Rogers et al., 1998]. The human GLUT12 gene, *SLC2A12*, is located on chromosome 6q23.2. It is 65 bp in length and contains 5 exons [Joost et al., 2002; Zhao and Keating, 2007]. GLUT12 cDNA encodes a protein containing 617 amino acids [Rogers et al., 1998] which shares 40% and 29% of its sequence identity with GLUT1 and GLUT4, respectively [Long and Cheeseman, 2015]. Dileucine motifs are present in the N-terminal end. GLUT12 contains potential C- and N-terminal targeting motifs, in similar positions to the LL and FQQI targeting motifs of GLUT4 [Rogers et al., 2002]. When MCF-7 cells were grown in the absence of insulin, GLUT12 was found to be located in the