Role for GLUT1 in diabetic glomerulosclerosis

Charles W. Heilig, Frank C. Brosius III and Carol Cunningham

Numerous studies have investigated specific pathways that link diabetes and high extracellular glucose exposure to glomerulosclerosis and mesangial cell extracellular matrix production. However, only in the past ten years has a role for glucose transporters in this process been addressed. Many different glucose transporters are expressed in glomeruli; of these, the GLUT1 facilitative glucose transporter is upregulated in the diabetic renal cortex and in response to glomerular hypertension, as well as in cultured mesangial cells exposed to high glucose. Transgenic mouse and cell models have recently been developed to test the role of GLUT1 in the pathogenesis of glomerulosclerosis with and without diabetes. Clinical studies of *GLUT1* alleles performed in humans have identified *GLUT1* susceptibility alleles for diabetic nephropathy. Studies are also currently under way to assess the potential role of GLUT1 in nondiabetic renal disorders.

A chronic effect of diabetes in many patients is the development of impaired kidney function, or diabetic nephropathy, in which the microvasculature (glomerulus) of the kidney is damaged. This process occurs in both type 1 and type 2 diabetic patients, where it is a major cause of morbidity and mortality (Refs 1, 2). Studies in humans have confirmed an important role for hyperglycaemia in the development of diabetic kidney disease (Refs 1, 2), and investigations in rodents have also described an important role for excess glucose metabolism in the stimulation of extracellular matrix (ECM) production. The excess ECM production is believed to arise mainly from the mesangial cells (MCs), which are located in the glomerular tufts of the kidney (Refs 3, 4).

Mesangial ECM provides a structural role in supporting the glomerular capillaries, and also serves to convey molecular information to MCs, for example by modulating responses to cytokines (Ref. 5). Furthermore, MCs express receptors for ECM proteins, which may stimulate MC replication

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1

Role for GLUT1 in diabetic glomerulosclerosis

and more matrix protein production (Ref. 5). Thus, changes in MC ECM can affect MC phenotype and behaviour. Although numerous studies have been performed to identify and characterise the roles of various signalling pathways in mediating diabetes-induced glomerular ECM accumulation in vivo and MC ECM production in vitro, it is only in the past ten years that a potential role for glucose transporters in diabetic glomerular disease has been addressed (Refs 6, 7, 8, 9, 10). The finding in the Diabetes Control and Complications Trial (DCCT) in 1993 (Ref. 2) that hyperglycaemia plays a major role in the development of diabetic kidney disease, and the fact that the glucose transport system of the glomerulus and MCs had not been defined, led to investigations in cultured cells and animals to explore this uncharted territory (Refs 6, 7, 11, 12). The discovery that the facilitative glucose transporter protein GLUT1 was a major glomerular and MC glucose transporter stimulated by diabetic levels of glucose was followed by a number of human studies of *GLUT1* single-nucleotide polymorphisms (SNPs), examining their potential relationship to diabetic nephropathy. This review therefore focuses on the roles of GLUT1 and glucose uptake in diabetic glomerulosclerosis, and the implications of *GLUT1* susceptibility alleles for diabetic nephropathy.

Biology of the facilitative glucose transporters

GLUT1, initially characterised as the erythrocyte glucose transporter (Ref. 13), is one of 12 GLUT isoforms, which exhibit tissue-specific expression and regulation (Ref. 14). GLUTs transport glucose across the plasma membrane into mammalian cells in a gradient-mediated manner, with uptake achieved with varying kinetics of transport depending upon the GLUT isoform(s) expressed (Refs 15, 16). GLUT1 has been intensively studied over the past 25 years. These investigations yielded valuable and detailed information on its primary and secondary structures, membrane topology and kinetics (Refs 17, 18). Multiple membrane-spanning α-helices of GLUT1 form a central aqueous channel in the plasma membrane where glucose is believed to pass (Ref. 19). GLUT1 is a high-affinity glucose transporter, with a K_m of approximately 3 mM, which is thought to be important in providing basal nutrition to many cell types (Refs 15, 16). More-recent investigations have implicated this glucose transporter in the pathogenesis of diabetic nephropathy (Refs 6, 7, 8,

9, 20, 21, 22, 23), and even nondiabetic nephropathy (Ref. 24).

Many of the known GLUT isoforms were discovered only recently. Their varying patterns of expression and kinetics suggest specific roles for the different isoforms (Refs 15, 16). They have been divided into three major classes based upon their sequences, and it is hoped that this classification might help us better understand their origins and contributions to glucose metabolism in the whole organism as more information becomes available (Ref. 25). Class 1 consists of GLUTs 1–4, Class 2 consists of GLUTs 6, 8, 10 and 12, and Class 3 consists of GLUTs 5, 7, 9 and 11 and HMIT (Ref. 16). HMIT is primarily a Na$^+$/myo-inositol transporter.

Glucose transporters of the glomerulus and implications for diabetic glomerular disease

Several studies have identified GLUT1 as a major glucose transporter of the glomerulus and the MC (Refs 6, 7, 8, 9, 11, 23). GLUT1 in the glomerulus is also detectable in podocytes and endothelial cells (Refs 11, 26, 27), indicating a broad intraglomerular distribution for this glucose transporter (Table 1). The many new GLUT isoforms discovered also allow for testing of their expression in the kidney and other tissues (Ref. 16). It is important for this discussion to note that expression of GLUTs 1, 3, 4, 5 and 8 has been reported in glomeruli (Refs 11, 28, 29, 30), whereas GLUTs 1 and 4 have been reported in cultured MCs (Refs 6, 7, 26, 29). GLUT9 has been detected in whole-kidney samples (Ref. 31); however, detection of GLUT9 in isolated glomeruli or MCs has not to our knowledge been reported. Future studies of the newly described GLUT isoforms might provide meaningful information concerning the pathogenesis of diabetic nephropathy, in addition to a better understanding of physiological glucose metabolism in the normal kidney.

GLUT1, GLUT3, GLUT4 and GLUT8 are all high-affinity, low-capacity glucose transporters, with the potential for saturation at or near physiological glucose concentrations (Refs 16, 30, 32). In mice and rats, GLUT1 protein is now known to increase in whole glomeruli (Refs 33, 34) and in MCs (Refs 7, 8) in response to diabetes or high glucose exposure, respectively. This increase allows excess glucose entry into the MCs as previously reported (Refs 6, 7, 9), providing fuel for metabolism, signalling pathway activation and

2

Role for GLUT1 in diabetic glomerulosclerosis

Table 1. Glucose transporter expression in whole glomeruli and in specific glomerular cell types

	Glomerulus	Effect of diabetes	Mesangial cell	Podocyte	Endothelial cell
GLUT1	+	Increase	+	+	+
GLUT2	NA	NA	NA	+	NA
GLUT3	+	NA	NA	NA	NA
GLUT4	+	Decrease/Increase[a]	+	+	NA
GLUT5	+	Increase	+	NA	NA
GLUT8	+	Increase	NA	+	NA
SGLT	NA	NA	+	NA	NA

[a] In diabetes, GLUT4 is decreased in mesangial cells and increased in podocytes.
Abbreviations: GLUT, glucose transporter; NA, information not available; SGLT, sodium–glucose cotransporter.

ultimately increased ECM production (Refs 6, 9, 35). The increased MC GLUT1 expression appears to be sustained in response to a persistent elevation of the extracellular glucose concentration (Ref. 7), which could contribute to accumulation of excessive ECM in the glomerulus over time. By contrast, glomerular GLUT4 appears to be suppressed with diabetes (Ref. 29). A major difference in the distributions of GLUTs 1 and 4 in glomerular cells appears to be that GLUT1 is expressed largely in the plasma membrane, whereas GLUT4 is detected in a perinuclear, cytoplasmic location (Ref. 11). Therefore, increases in glomerular GLUT1 have the potential to translate into increased plasma membrane GLUT1 and glucose uptake. Such a scenario has been observed in cultured MCs designed to overexpress GLUT1, where the glucose uptake rate markedly increases in the absence of elevated extracellular glucose (Ref. 6). By contrast, suppression of GLUT1 in MCs provides a protective effect against high glucose exposure, preventing increased MC GLUT1 expression and ECM production in response to high glucose concentrations (Ref. 8). The GLUT5 isoform has also been identified in glomerular MCs, and increases in response to sterptozotocin diabetes (Ref. 28). This isoform is believed to function predominantly as a fructose transporter, and therefore its increased expression could influence formation of advanced glycation end products in the MC (Ref. 28). The GLUT3 isoform is a high-affinity glucose transporter

detected in whole glomeruli (Ref. 11), although its precise cellular localisation there remains to be defined. Each of these glomerular glucose transporters, GLUTs 1, 3, 4 and 5, has also been detected in renal tubule segments. However, the renal tubular GLUTs were recently reviewed, and are therefore not discussed further in this review of the glomerulus.

Several recent studies have characterised the potential for cytokines implicated in diabetic nephropathy to regulate GLUT1 and glucose transport in cultured MCs (Refs 7, 8, 23, 36, 37): insulin-like growth factor 1 (IGF-1) increased glucose transport into MCs, particularly under conditions where GLUT1 expression was increased (Ref. 7); transforming growth factor β1 (TGF-β1) increased MC GLUT1 expression and glucose uptake (Refs 23, 38); and angiotensin II (ANG II) stimulated MC glucose uptake (Refs 37, 39). All of these cytokines are believed to play a role in diabetic nephropathy, and also have in common the property of stimulating glucose uptake into MCs. Since this effect on MCs has the potential to enhance ECM protein production (Ref. 6), these cytokines might play an important role in facilitating glomerulosclerosis via GLUT1. For example, TGF-β increases in diabetic glomeruli (Ref. 40), in high-glucose-exposed MCs (Ref. 41), in animals with glomerular hyperfiltration (Ref. 24), and in MCs mechanically stretched in vitro (Ref. 24). The increased TGF-β might then stimulate expression of GLUT1 (Ref. 23), leading

3

to increased MC glucose uptake, metabolism and stimulation of ECM production. The positive feedback loop of glucose–TGF-β–GLUT1–glucose in MCs is not unique: a glucose–GLUT1–glucose positive feedback loop has been described (Ref. 7) in which PKC may increase MC GLUT1 expression (Refs 9, 36), thereby causing increased MC glucose uptake. Both of these positive feedback loops enhance glucose uptake into the MC where it can be metabolised and serve to stimulate excessive ECM production.

Data on GLUTs in the human glomerulus and in human MCs (HMCs) are limited, but GLUT1 has been detected in both whole glomeruli (Ref. 42) and in cultured HMCs (Refs 38, 43). In HMCs cultured from nondiabetic and diabetic kidneys, cells from diabetics had increased GLUT1 expression and an increased glucose-uptake rate (Ref. 43), consistent with the data described above in the high-glucose-exposed rat MCs (Refs 7, 8). In addition, TGF-β1 stimulated HMC GLUT1 expression and glucose uptake. Similar effects of high glucose and TGF-β1 have been observed in cultured rat MCs (Refs 7, 23, 38).

Glucose uptake in other glomerular cells
Podocytes

The glomerular filtration barrier consists of the capillary endothelial cells, the glomerular basement membrane, and the foot processes of the visceral epithelial cells (podocytes) (Refs 44, 45, 46). Podocytes are important for regulating turnover of the basement membrane and for maintaining the filtration function of glomeruli (Refs 44, 45, 46). Recent work in glomerular podocytes has identified GLUT1, GLUT2, GLUT4 and GLUT8 as major GLUTs in these cells (Refs 27, 47, 48). Mechanical stretch, insulin and increased extracellular glucose concentration each result in enhanced glucose uptake in these cells (Refs 48, 49). Insulin induces both GLUT1 and GLUT4 translocation in cultured human podocytes (Ref. 48). High glucose exposure has been found to potentiate the induction of glucose uptake by mechanical stretch, despite decreased GLUT2 and GLUT4 levels (Ref. 24). Since GLUT1 accounts for a substantial amount of basal and insulin-stimulated glucose uptake in podocytes (Ref. 48), these data suggest that high glucose exposure and mechanical stretch could induce GLUT1 expression or translocation. It appears that diabetes in humans induces GLUT4 expression (Ref. 27), and GLUT8 is increased in podocytes

with type 2 diabetes mellitus in mice (Ref. 30). It is not yet known whether GLUT1 levels are altered in diabetic podocytes. The increase in podocyte glucose uptake that occurs with diabetes and/or mechanical stretch might lead to adverse effects related to excess glucose metabolism, such as increased ECM production, and possibly apoptosis (Ref. 30).

Endothelial cells

Although GLUT1 has been reported in vascular endothelial cells outside the kidney (Ref. 50), and has also been detected in glomerular endothelial cells (C. Heilig, unpublished), there is a lack of published information addressing the mechanism of glucose uptake and metabolism in the latter. It is notable in the literature that GLUT1 in endothelial cells from different parts of the body might respond differently to a given stimulus (Ref. 51), such as diabetes. For example, examination of GLUT1 in endothelial cells from retina and brain has shown that the retina does not reduce GLUT1 in the face of hyperglycaemia (Ref. 50), whereas brain endothelium does, suggesting retinal endothelial cells are susceptible to excessive glucose uptake and resulting adverse effects. Although glomerular endothelial cells have typically been difficult to maintain in primary culture, multiple passages are feasible (Ref. 52). In vitro studies should allow for detailed characterisation of the glucose transport system and downstream pathways to ECM production, with and without high glucose exposure. It is known that glomerular endothelial cells produce ECM proteins and profibrogenic cytokines, which could contribute to the development of glomerulosclerosis (Ref. 53). Some of the glomerular endothelium is exposed to the mesangium without an intervening basement membrane, and these are sites where endothelial production of ECM potentially could affect the mesangium.

Vascular endothelial growth factor (VEGF) is increased in diabetic glomeruli (Ref. 54), and is known to upregulate TGF-β1 in glomerular endothelial cells (Ref. 53). Furthermore, TGF-β1 is a known stimulator of GLUT1 expression and glucose uptake in MCs. Therefore, VEGF potentially could modulate glomerular endothelial cell GLUT1 expression, glucose transport and the activity of glucose-dependent pathways in these cells, such as those leading to excess matrix production. Clearly, studies are needed to determine the potential contributions of glomerular endothelial

4

cell glucose transporters and glucose uptake to endothelial cell function and the development of diabetic glomerulosclerosis. This is currently a neglected area of research.

Models of altered glucose transport into glomerular MCs

As mentioned above, GLUT1 and the glucose uptake rate increase in rat MCs in response to high glucose exposure (Refs 7, 8). The excess glucose available to the cell can then be metabolised, which is an important step in the stimulation of MC ECM production (Refs 4, 6, 35). The recent production of transgenic mice and MCs with overexpression and underexpression of GLUT1 compared with the normal mouse and with normal cultured MCs allows for investigation of the role played by GLUT1 in the pathogenesis of glomerulosclerosis with and without diabetes. Important features of the MC models are described below.

MCLacZ, MCGT1 and MCGT1AS cells

MCLacZ cells have been used as control transduced MCs. These cells were derived from the spontaneously immortalised $16KC_2$ rat MC line, which maintains the normal adult MC phenotype in culture (Refs 6, 55). The $16KC_2$ MCs were stably transduced with a retroviral (MoMuLV) LacZ-expression vector to persistently overexpress the gene product β-galactosidase, which has no known effect on glucose transport (Ref. 6). The cells turn blue, as expected, when exposed to the β-galactosidase substrate X-Gal, and maintain a stable phenotype in culture (Refs 6, 8). GLUT1 protein expression in the control MCLacZ cells is detectable at a low level, providing sharp contrast for the overexpression of GLUT1 in MCGT1 cells.

MCGT1 cells were produced by stably transducing the $16KC_2$ rat MC line with a retroviral (MoMuLV) sense-*GLUT1* expression construct, leading to tenfold overexpression of GLUT1 protein (Ref. 6). The glucose uptake rate is persistently elevated in these cells at fivefold control, despite growth in standard 8 mM glucose medium (i.e. 140 mg/dl glucose) (Ref. 6). Over time, these cells respond to the increased glucose metabolism in multiple ways, eventually becoming rapid growers, outpacing the control MCLacZ cells (Ref. 8). Glucose does not equilibrate across the plasma membrane of the MCGT1 cells, even after 3 months in culture, and the cells

continue to take up glucose at an excessive rate. The appetite of the MCs for glucose is apparently very large, as glucose utilisation in the GLUT1-overexpressing MCGT1 cells is 43-fold control (Ref. 6), while free intracellular glucose measured by [^{13}C]glucose NMR spectroscopy is reduced compared with controls (C. Heilig, unpublished). The MCGT1 cell line has been of particular value for studying the mechanisms by which intracellular glucose (as opposed to extracellular glucose) stimulates ECM production (Refs 6, 56).

MCGT1AS cells were produced by stably transducing the $16KC_2$ rat MC line with a retroviral (MoMuLV) antisense-*GLUT1* expression construct (Ref. 6). Their GLUT1 protein content is suppressed approximately 50% (Ref. 8), and the glucose uptake rate in these cells is reduced approximately 33–40%. Baseline GLUT1 transcription and the fibronectin protein level are also reduced. High glucose in the diabetic range does not stimulate GLUT1 expression or fibronectin expression in these cells, and so they appear to be protected (Ref. 8). Although the MCs with an approximate 50% reduction of GLUT1 protein grow more slowly than control MCs (Ref. 7), they are quite viable and continue to grow easily when passed in culture (Refs 8, 56).

Therefore, in both the MCGT1AS and MCGT1 cells, the GLUT1 expression level regulates the MC growth rate and matrix production. Additional data described below suggest that GLUT1 plays a major role in the induction of select MC genes by glucose, and that manipulation of GLUT1 expression might have potential therapeutic value.

### Signalling pathways in MCGT1 GLUT1-overexpressing cells
PKC pathway

The protein kinase C isoforms PKCα and PKCβ1 are persistently increased and activated in MCGT1 cells when compared with the MCLacZ control cells (Refs 9, 56). The increase in PKC expression appears to be due to an increase in mRNA levels (Ref. 9). Other PKC isoforms, including PKCδ, PKCε and PKCζ, were not increased or activated in the MCGT1 cells (Ref. 9). The increased PKCα and PKCβ1 expression might have particular importance as both of these isoforms have previously been implicated in diabetic nephropathy (Refs 57, 58, 59). Furthermore, the data from MCGT1 cells grown in normal glucose medium (140 mg glucose/dl is normal for

the rat) indicate an important role for intracellular glucose in the PKC activation (Refs 9, 56). Subsequent investigations linked the increase in PKC activity in MCGT1 cells to increased fibronectin expression, via the AP1 transcription factor complex (Ref. 56). AP-1 can bind to TPA-responsive elements (TREs) in regulatory regions of the fibronectin (*FN*) and *GLUT1* genes to stimulate their expression (Ref. 56) (Fig. 1). The PKC pathway was the main signalling pathway activated in MCGT1 cells, and the examination of other signalling pathways in these cells is described below.

ERK1/2 MAPK pathway

The extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway was found not to be activated in the chronic GLUT1-overexpressing MCGT1 cells,

when tested by immunoblotting of cell proteins with phosphospecific antibodies (Ref. 56). This is in contrast to the acute response described in cultured MCs exposed to high extracellular glucose concentrations, and in glomeruli isolated from streptozotocin diabetic rats, where ERK1/2 has been shown to be activated (Ref. 60). It is possible that acute (early) activation of ERK1/2 in MCGT1 cells was missed, since this line is chronically maintained for study. It is also possible that extracellular glucose is more important than intracellular glucose in activating the ERK1/2 pathway. Regardless, it is evident that the chronic high production of ECM proteins typical of MCGT1 cells does not depend on persistent activation of ERK1/2. Comparisons of ERK1/2 activation between control MCs chronically exposed to high extracellular glucose versus MCGT1 cells chronically maintained in normal
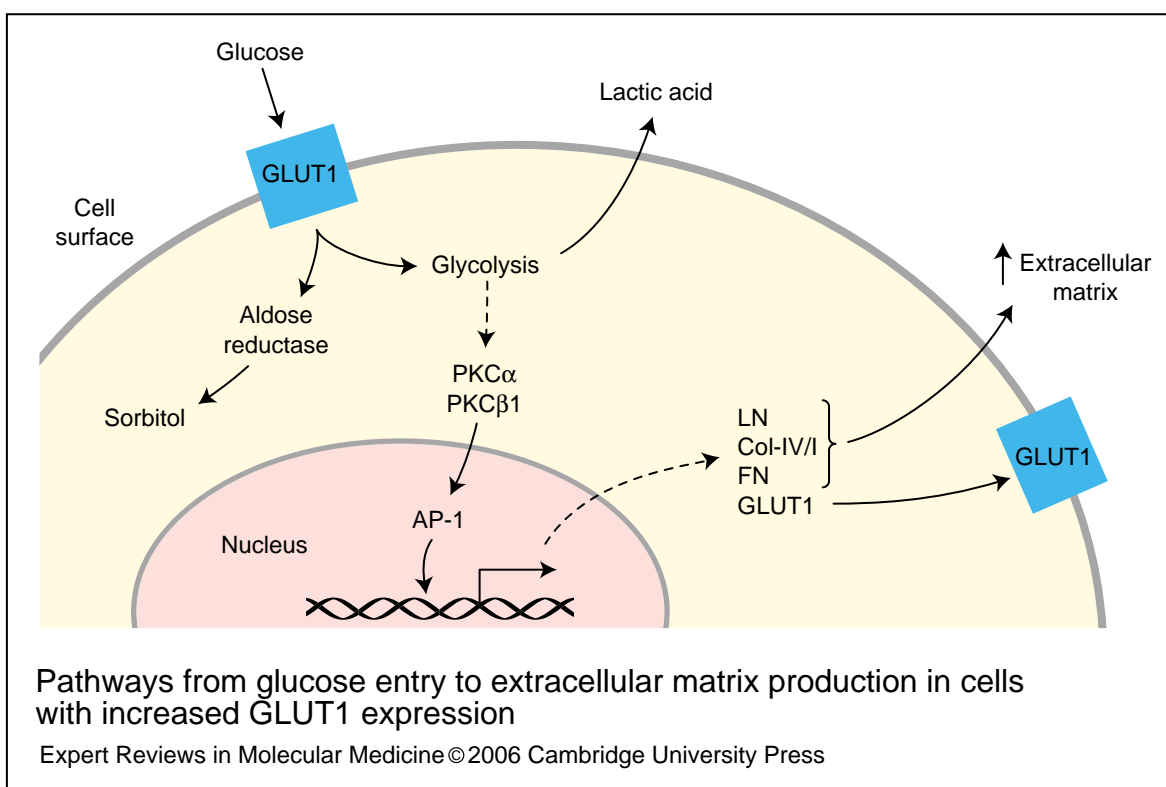
Pathways from glucose entry to extracellular matrix production in cells with increased GLUT1 expression

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Figure 1. **Pathways from glucose entry to extracellular matrix production in cells with increased GLUT1 expression.** The figure shows known (solid lines) and proposed (dashed lines) pathways from glucose entry to extracellular matrix (ECM) production in mesangial cells with increased GLUT1 expression. Mesangial cells with overexpression of GLUT1 have been shown to have increased flux of glucose through the aldose reductase and glycolytic pathways. As a result, they demonstrate persistently activated PKCα and PKCβ1, resulting in increased AP-1. The AP-1 transcriptional activator then leads to increased ECM production. Sorbitol and lactic acid are produced in excess. Abbreviations: Col-I, collagen type 1; Col-IV, collagen type IV; FN, fibronectin; GLUT1, facilitative glucose transporter 1; LN, laminin; PKCα, protein kinase C alpha; PKCβ1, protein kinase C beta-1.

6

glucose medium will help differentiate the chronic extracellular and intracellular effects of glucose on this signalling pathway.

GFAT and the hexosamine biosynthetic pathway

The enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT) is the rate-limiting enzyme in the hexosamine biosynthetic pathway (Refs 61, 62). This pathway has been been shown to stimulate TGF-β1 and fibronectin expression in MCs, and has been implicated in the development of diabetic nephropathy (Ref. 52). However, this pathway was also not activated in GLUT1-overexpressing rat MCs (Ref. 6). This finding contrasts with the report of Kolm-Litty et al. in 1998 (Ref. 63), where high extracellular glucose exposure caused an increase in MC hexosamine pathway activity leading to activation of TGF-β and fibronectin expression. Again, the data suggest that the chronic effects of glucose are different from the acute effects, and / or that extracellular glucose is a more effective stimulus to the hexosamine pathway than intracellular glucose. Future experiments in which hexosamine pathway activity is examined both acutely and chronically after induction of isolated GLUT1 overexpression in MCs could help determine whether there is an acute stimulation of the pathway that later abates.

Reactive oxygen species

Reactive oxygen species (ROS) have been implicated in the development of diabetic nephropathy (Refs 64, 65, 66). Glucose auto-oxidation, metabolism and formation of advanced glycosylation end products are believed to be key mechanisms involved in the generation of ROS inside cells, and have been causally linked to activation of multiple pathogenic pathways in the diabetic condition (Ref. 55). Specifically, ROS might activate aldose reductase, induce diacylglycerol, activate PKC, induce formation of advanced glycation end products, and activate the transcription factor nuclear factor kappa B (NF-κB) (Ref. 65). A single unifying mechanism of induction – increased production of ROS – has been proposed to explain the effects of glucose on each of four major pathways involved in diabetic tissue damage: the polyol pathway, the hexosamine pathway, the PKC pathway, and the formation of advanced glycation end products (Refs 65, 66).

However, in the chronic GLUT1-overexpressing MCGT1 cells, ROS were not increased, despite persistent elevation of ECM production (Ref. 6). These provocative data indicated that increased ROS were not necessary for excess ECM production, at least in the chronic phase of GLUT1 overexpression. This report by Weigert et al. contrasts with the model of Ha et al. where acute, high extracellular glucose exposure of MCs led to production of ROS, followed by stimulation of ECM production (Ref. 64). The latter authors implicated ROS in the excessive MC ECM production, although they did not report chronic studies. It is possible that the acute stimulation of ROS expression by glucose might not persist chronically in MCs, or again that extracellular glucose might be a more effective stimulus than intracellular glucose. In either case, further research will be needed to sort this out.

In view of the above findings in MCs, it is important to consider that both the duration of excess glucose uptake and potentially different effects of extracellular versus intracellular glucose may affect the pattern of expression for downstream effectors of ECM production.

Glomerular GLUT1 expression in diabetic mice

Recent preliminary work identified increased GLUT1 protein and increased glucose uptake in glomeruli isolated from type 2 diabetic mice (Ref. 33). These changes could lead to increased glucose-induced ECM production and glomerular scarring. An increase in glomerular GLUT1 has also been observed in preliminary studies in a nondiabetic model of glomerular hyperfiltration, where severe glomerulosclerosis rapidly develops (Ref. 67). Both the high glucose and hyperfiltration characteristics of diabetes mellitus may therefore play roles in the GLUT1 contribution to diabetic glomerulosclerosis. Further work will help clarify the role of glomerular GLUT1 in the pathogenesis of diabetic kidney disease. A recent report in diabetic rats also demonstrated increased GLUT1 in the proximal tubules, indicating that the glomerular GLUT1 response to diabetes is not unique (Ref. 68).

GLUT1-deficient mice

Mice deficient in GLUT1 have been produced as a model for protection against development of diabetic glomerulosclerosis (Ref. 33). The antisense-*GLUT1* transgene in these mice with a

Role for GLUT1 in diabetic glomerulosclerosis

7

C57BL/6 background is driven by a modified human β-actin promoter (Ref. 69). Inside the kidney this promoter expresses predominantly in the glomeruli and small vessels, but not in the tubules (Refs 34, 70). These transgenic mice were crossed into the db (diabetic) mouse line (C57BL/6 background) for study, and a preliminary report on db/dbASGLUT1 transgenic mice has documented that they are diabetic, yet they demonstrate protection against the development of glomerulosclerosis (Ref. 33). Primary culture MCs from these mice exhibit reduced GLUT1 protein and a reduced rate of glucose uptake, which might account for the lack of accumulation of ECM proteins in the diabetic glomeruli in vivo. Studies are in progress to determine the degree of protection against the albuminuria and to fully characterise the renal phenotype of these transgenic mice, with and without diabetes mellitus. The concept that GLUT1 suppression in glomeruli is protective against diabetic glomerulosclerosis has potentially important implications for the design of new therapies to prevent this complication. However, preferential inhibition of renal GLUT1 would be valuable, as this glucose transporter is expressed in many other tissues, not the least of which is the blood–brain barrier where its deficiency leads to the human GLUT1-deficiency syndrome (Refs 71, 72). This syndrome includes hypoglycorrhachia, developmental delay, acquired microcephaly, recurrent seizures, and spastic ataxia.

GLUT1-overexpressing mice

Transgenic mice have been produced that exhibit a 3.5-fold increase in glomerular GLUT1 protein, with a comparable increase in the glucose uptake rate (Ref. 34). These mice were produced on a C57BL6 mouse background, and the transgene is driven by a modified human β-actin promoter (Ref. 70) (as used in the antisense-*GLUT1* transgene described in the GLUT1- deficient mice above). Glomerular overexpression of GLUT1 in this model is detected predominantly in the MCs (Ref. 34), which have previously been implicated in diabetic glomerulosclerosis (Refs 6, 35). The transgenic GLUT1-overexpressing mice are not diabetic, yet in a preliminary report they have been found to have excessive albuminuria and to develop glomerulosclerosis with features similar to those seen in the glomerulosclerosis of diabetic mice (Ref. 34). Studies in progress will quantitate the degrees of proteinuria, glomerulosclerosis and
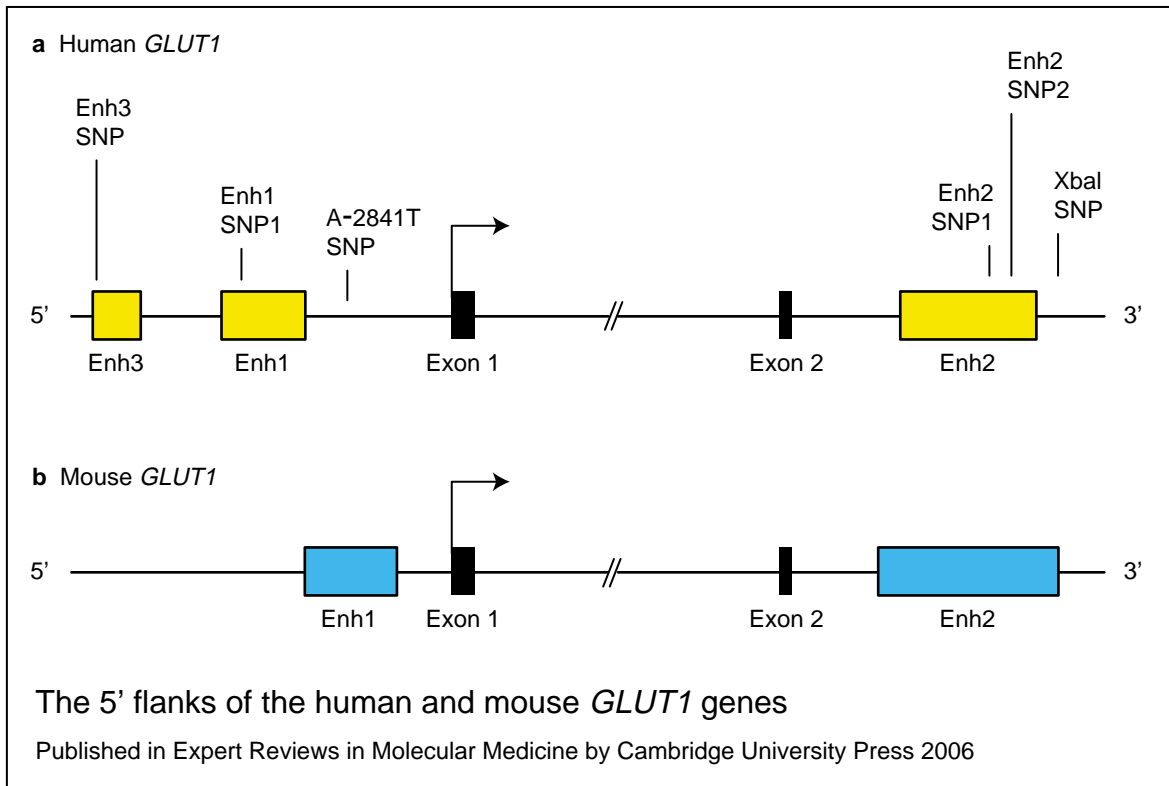
renal failure that develop in this model. The similarities to and differences from the diabetic kidney disease in db/db mice will also be assessed, to determine how an isolated increase in intracellular glucose availability differs from high extracellular glucose (diabetes) exposure with regard to the renal pathology produced. Although GLUT1 is increased in the diabetic kidney (Refs 20, 33), it also increases in response to glomerular hypertension and hyperfiltration in the absence of diabetes (Ref. 24), suggesting the GLUT1-overexpressing transgenic mouse might also have value as a model for the study of glomerulosclerosis in nondiabetic disorders.

Clinical studies of *GLUT1* alleles and their relationship to human diabetic nephropathy

Regarding genetic alterations of *GLUT1* in the setting of diabetic nephropathy, considerable attention has been given to the question of whether SNPs in this gene (Fig. 2) are associated with susceptibility to the nephropathy in types 1 and 2 diabetic patients. Clinical investigations of the XbaI polymorphic alleles in intron 2 of the human *GLUT1* gene, with and without diabetes, have provided conflicting results concerning their association with nephropathy (Refs 73, 74, 75, 76, 77). Specific details of these studies follow.

In an early study of type 2 diabetic Caucasian subjects (Ref. 78), Gutierrez et al. found no significant association between the *GLUT1* XbaI restriction length polymorphisms (RFLPs) and diabetic microvascular complications (Table 2). By contrast, a subsequent report in Asian subjects with type 2 diabetes mellitus determined that the *GLUT1* XbaI(−) allele was associated with a 1.9-fold increased risk for nephropathy (Ref. 74). Furthermore, another report found that the *GLUT1* XbaI(+) allele was associated with increased risk for nephropathy in Caucasian type 2 diabetics in Poland (Ref. 75), consistent with the concept that the XbaI polymorphic site may not be a disease allele itself, but rather is closely associated with a disease allele. Subsequent investigation in the UK of Caucasian type I diabetic patients homozygous for the XbaI(−) allele, found that these patients had an increased risk for nephropathy (Ref. 73), and that the diabetic patients with nephropathy tended to lack the Z+2 5'ALR2 protective allele for diabetic nephropathy (a polymorphism in the 5' flank of aldose reductase). Diabetic patients who were

a Human *GLUT1*

Enh3
SNP

Enh1
SNP1

A-2841T
SNP

Enh2
SNP2

Enh2
SNP1

Xbal
SNP

5'   3'

Enh3   Enh1   Exon 1   Exon 2   Enh2

b Mouse *GLUT1*

5'   3'

Enh1   Exon 1   Exon 2   Enh2

The 5' flanks of the human and mouse *GLUT1* genes

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Figure 2. The 5' flanks of the human and mouse *GLUT1* genes. (a) Human *GLUT1* gene. Single nucleotide polymorphism (SNP) sites in the 5' end of the human *GLUT1* gene that have been analysed for an association with diabetic nephropathy are shown. The AA genotype at the enhancer 2, SNP1 (Enh2SNP1) site (in intron 2) has been reported to be associated with increased risk for diabetic nephropathy in type 1 diabetics (Ref. 76). This SNP is reported to be located in a putative E-box motif (CCCATG), a potentially glucose-responsive site where USF transcription factors (upstream stimulating factors) may bind. A more recent, preliminary report has demonstrated that the AA *GLUT1* genotype is also associated with increased risk for nephropathy in type 2 diabetics, and even in nondiabetics (Ref. 77). The T allele at the A–2841T SNP in the human *GLUT1* promoter was recently shown to be tightly associated with increased risk for the nephropathy in type 1 diabetic subjects (Ref. 79). The polymorphic Xba1 diabetic nephropathy susceptibility site is shown in intron 2 and is described in the text. The human *GLUT1* map (not drawn to scale) was modified from Ref. 76 (Copyright © 2002 American Diabetes Association), with permission from the American Diabetes Association and from Andrzej Krolewski. (b) Mouse *GLUT1* gene. The mouse *GLUT1* gene is known to be responsive to glucose. The enhancer 1 and 2 sites each contain two putative E-boxes, plus either two nonconsensus TPA-responsive elements (TREs) (enhancer 1), or two consensus TREs (enhancer 2). All of these sites are potential glucose-response sites in the gene. By contrast, the mouse GLUT1 promoter has two putative E-boxes, but no typical TREs. The mouse map (not drawn to scale) was modified from Ref. 97 (Copyright © 1992 The American Society for Biochemistry and Molecular Biology, Inc.), with permission from The American Society for Biochemistry and Molecular Biology and from Yousuke Ebina.

homozygous for the XbaI(–) allele and who also carried the aldose reductase susceptibility allele (Z-2 5'ALR2) exhibited a greater than ninefold increased risk for diabetic nephropathy, suggesting this is a particularly high-risk genetic combination (Ref. 73). However, in contrast to this UK report, a study of Danish Caucasian type I diabetic patients in 2001 did not find a relationship between *GLUT1* XbaI alleles and

diabetic nephropathy (Ref. 79). Subsequent research in Caucasian type 1 diabetic subjects in the USA shed some light on these discrepancies. It provided evidence for a new *GLUT1* susceptibility allele [*GLUT1* enhancer 2, SNP1, A-allele, AA genotype (Enh2SNP1)], which might be involved in the pathogenesis of diabetic nephropathy (Refs 76, 77), and this site is in tight linkage disequilibrium with the nearby XbaI polymorphic

Role for GLUT1 in diabetic glomerulosclerosis

Table 2. *GLUT1* susceptibility alleles for diabetic nephropathy[a]

Study	Diabetes	Race, country	Patient numbers: controls versus diabetics	*GLUT1* SNP[b]	Association of SNP with nephropathy
Gutierrez et al. (Ref. 78)	Type 2	Caucasian, Spain	100 vs 20	XbaI	No
Liu et al. (Ref. 74)	Type 2	Asian, China	45 vs 64	XbaI	Yes
Grzeszczak et al. (Ref. 75)	Type 2	Caucasian, Poland	162 vs 132	XbaI	Yes
Hodgkinson et al. (Ref. 73)	Type 1	Caucasian, UK	44 vs 70	XbaI	Yes
Tarnow et al. (Ref. 79)	Type 1	Caucasian, Denmark	192 vs 175	XbaI	No
Ng et al. (Ref. 76)	Type 1	Caucasian, USA	207 vs 249	XbaI Enh2SNP1	Yes Yes
Hodgkinson et al. (Ref. 22)	Type 1	Caucasian, UK	101 vs 288	A–2841T	Yes

[a] Diabetic nephropathy was defined in these studies as persistent albuminuria or chronic renal failure.
[b] XbaI and Enh2SNP1 polymorphisms are located in intron 2; the A–2841T polymorphism is located in the promoter.

site (Ref. 76). Therefore, it has been suggested that the Enh2SNP1 site might be the location of a disease allele causing increased GLUT1 expression and diabetic nephropathy (Ref. 76), while its linkage disequilibrium with the nearby XbaI site may explain why the latter site has been implicated in some studies as a susceptibility allele (Ref. 76). Patients homozygous for the Enh2SNP1 allele (i.e AA) were also found to be homozygous for the XbaI(–) allele.

Investigations are under way to determine whether the *GLUT1* Enh2SNP1 causes increased GLUT1 expression in MCs, the cells most directly implicated in the development of diabetic glomerulosclerosis. The Enh2SNP1 site in intron 2 is also a putative binding site for USF (upstream stimulating factor) transcription factors. USFs are glucose- and insulin-responsive transcription factors that may stimulate gene expression in response to these stimuli (Refs 80, 81). Their role in regulating the transcription of glucose-inducible genes has been well described in liver cells (Refs 80, 81, 82, 83). More-recent preliminary studies have identified USF2 as a glucose-inducible transcription factor in MCs (Refs 84, 85),

where USF2 was shown to regulate GLUT1 transcription (Ref. 85). Site-directed mutagenesis of an E-box (putative USF-binding site) in the mouse *GLUT1* promoter, which has the same sequence as the E-box at the human Enh2SNP1 site, causes the *GLUT1* gene to become USF2-inducible (Ref. 84). Therefore, it is conceivable that the Enh2SNP1 site in the human *GLUT1* gene is USF2-responsive and that the AA genotype at the locus alters *GLUT1* gene expression to contribute to diabetic kidney disease.

A preliminary report from Hsu et al. in 2004 investigated approximately 3700 patients from the Atherosclerosis Risk in Communities (ARIC) study (Ref. 77). They found that both nondiabetic and type 2 diabetic patients had a significant twofold increased risk for nephropathy when they were homozygous for the Enh2SNP1 A allele (AA genotype). To our knowledge, this is the largest study to date of *GLUT1* alleles and nephropathy, and it is the first to identify increased risk for nephropathy in nondiabetic patients with the *GLUT1* AA genotype. This preliminary study is currently being extended to a larger population. The finding of an association between the *GLUT1*

Enh2SNP1 susceptibility allele and nephropathy in nondiabetic patients of course has potentially very important implications for patients with other renal diseases. The role of GLUT1 in nondiabetic glomerulsoclerosis is already being examined in experimental animals as noted above (Ref. 67), and further studies in humans might be important to pursue.

The most recent reports of *GLUT1* susceptibility alleles include a meta-analysis investigating the relationship between the *GLUT1* XbaI site alleles and diabetic nephropathy (Ref. 21), as well as a report describing a new *GLUT1* susceptibility allele (SNP) in the human *GLUT1* promoter (Ref. 22). The meta-analysis of multiple studies concluded that there is indeed a significant association between the *GLUT1* XbaI polymorphic site and diabetic nephropathy, but that additional and larger studies are needed (Ref. 21). The report on a new susceptibility allele in the *GLUT1* promoter identified the A–2841T polymorphic site as being strongly associated with diabetic nephropathy in patients with type 1 diabetes mellitus (Ref. 22). Specifically, the association of the TT genotype at this site (position –2841 of the *GLUT1* gene) with diabetic nephropathy was highly significant ($P < 0.000001$). The frequency of the TT genotype in the nephropaths was 54.9% versus 2.7% in patients without nephropathy after 20 years of type 1 diabetes ('uncomplicated diabetes'). The T allele at this same site was significantly increased in nephropaths versus patients with uncomplicated diabetes (70.9% versus 13.1%; $P < 0.000001$). In addition, the investigators noted a marked increase in the 'T –2841/T +22999' haplotype in the nephropaths versus patients with uncomplicated diabetes (32.9% versus 3.8%; $P = 0.0000007$). The T +22999 genotype refers to a thymidine at the XbaI polymorphic site in intron 2 of the *GLUT1* gene. Hodgkinson et al. have proposed that the T –2841/T +22999 *GLUT1* haplotype might be associated with an enhanced glucose uptake rate via GLUT1 into insulin-insensitive tissues such as MCs (Ref. 22). The increased glucose uptake could then stimulate ECM production by these cells. If the expression and/or activity of GLUT1 vary among diabetic patients, this might explain in part why only a fraction of them develop the kidney disease (Refs 6, 22).

The importance of *GLUT1* susceptibility alleles in the development of diabetic nephropathy can be put in perspective by recalling the results of other studies linking susceptibility alleles in other relevant genes to the diabetic renal disease (Refs 49, 73, 86, 87, 88). The increased risk for diabetic nephropathy associated with the individual *GLUT1* susceptibility alleles is approximately twofold. This is roughly in the same range of risk as observed for the TGF-β T869C (Leu10Pro) CC and CT genotypes (3.8-fold), the aldose reductase homozygous Z-2 genotype in the 5′ flank of this gene (3.3-fold), the transcription factor TSC-22 –396A/G homozygous AA genotype (twofold), and the angiotensin-converting enzyme (ACE) insertion/deletion II genotype (1.24-fold). However, the risk of diabetic nephropathy is increased ninefold when the *GLUT1* XbaI(−/−) genotype is combined with absence of the Z+2 5′ALR2 allele. Further investigations are indicated to fully delineate the contributions of the different *GLUT1* genotypes to the development of diabetic nephropathy, and the potential roles of other glomerular glucose transporters in this disorder such as GLUT5 and GLUT8, which also increase with diabetes mellitus (Table 1).

Clinical implications/applications

Diabetic patients with the *GLUT1* Enh2SNP1 homozygous AA genotype, or the TT genotype at A–2841T, have increased risk for diabetic nephropathy. In addition, even patients with the homozygous AA genotype at Enh2SNP1 who are not diabetic might have increased risk for kidney disease. Although multiple factors have been identified that contribute to the development of diabetic kidney disease, genetic factors have been recognised as important components in this process. Thus, the potential use of the Enh2SNP1 AA genotype or the TT genotype at A–2841T in a screening protocol that might include other risk factors [e.g. combination of a *GLUT1* susceptibility allele and an aldose reductase susceptibility allele that markedly increases the risk for nephropathy in type 2 diabetic patients (Ref. 73)], could assist in identifying patients at particularly high risk of developing the nephropathy. Such detection could then assist in targeting preventive measures to this population. Future studies should also clarify the relative importance of *GLUT1* susceptibility alleles versus other types of susceptibility alleles (e.g. aldose reductase, TGF-β) in the development of diabetic nephropathy.

Inhibitors of GLUT1 might have beneficial effects in preventing glucose-induced MC ECM production. One example is Rhein, an anthraquinone previously used as a Chinese

11

medicinal, which inhibits cellular glucose uptake (Refs 89, 90). Rhein was shown to inhibit the GLUT1-stimulatory effects of TGF-β1 (Ref. 38), and therefore holds some promise for the future prevention and treatment of diabetic kidney disease. Other known inhibitors of GLUT1 include isoflavones, flavanoids (e.g. quercetin) and methylxanthines (e.g. caffeine, theophylline) as described in recent reports (Refs 91, 92, 93). Furthermore, one recent study in type 1 diabetic rats reported prevention of diabetic nephropathy by treatment with the flavanoid quercetin (Ref. 94). Pentobarbital and several other anaesthetic drugs have also been shown to inhibit GLUT1 (Refs 95, 96). Therefore, numerous different GLUT1 inhibitors have been identified that could be tested for their potential to inhibit glomerular and MC glucose uptake.

Concluding remarks

Research to date indicates that altered MC GLUT1 expression leads to parallel changes in ECM production, which in vivo could contribute to the development of diabetic glomerulosclerosis. The mechanisms that regulate MC GLUT1 expression are the subjects of current investigations. Multiple different cytokines and glomerular hyperfiltration, which have been implicated in diabetic nephropathy, might alter MC GLUT1 expression and activity. Studies of the *GLUT1* gene in diabetic humans have implicated specific loci in the pathogenesis of diabetic nephropathy. It is now clear that additional investigation is needed to determine the relative contributions of the different loci to diabetic nephropathy and the mechanisms by which these loci may influence GLUT1 expression.

Acknowledgements and funding

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Role for GLUT1 in diabetic glomerulosclerosis

Further reading, resources and contacts

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The effects of increased mesangial cell GLUT1 expression in mimicking the diabetic phenotype are described in this work.

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Features associated with this article

Figures
Figure 1. Pathways from glucose entry to extracellular matrix production in cells with increased GLUT1 expression.
Figure 2. The 5' flanks of the human and mouse *GLUT1* genes.

Tables
Table 1. Glucose transporter expression in whole glomeruli and in specific glomerular cell types.
Table 2. *GLUT1* susceptibility alleles for diabetic nephropathy.

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