Mutations in the SLC2A10 gene cause arterial abnormalities in mice

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Aims Glucose transporter 10 (GLUT10), encoded by the SLC2A10 gene, is a member of the class III facilitative glucose transporter family. Mutations in the SLC2A10 gene cause arterial tortuosity syndrome (ATS) in humans. To further study the pathogenesis of the disease, we generated mice carrying GLUT10 mutations.

Methods and results Using a gene-driven N-ethyl-N-nitrosourea (ENU)-mutagenesis approach, we generated mice carrying GLUT10 mutations c.383G→A and c.449C→T, which resulted in missense mutations of glycine to glutamic acid (p.G128E) and serine to phenylalanine (p.S150F), respectively. Both mutant strains appeared normal at birth, gained weight appropriately and survived to adulthood (>18 months). Blood and urine glucose were normal. Echocardiogram and electrocardiogram were also normal and brain magnetic resonance angiography revealed normal cerebral arteries without tortuosity, stenosis/dilatation, or aneurysm. Histopathology revealed thickening and irregular vessel wall shape of large and medium size arteries characterized by markedly increased elastic fibres, both in number and size. There was also intima endothelial hypertrophy and deranged elastic fibres that resulted in disruption of internal elastic lamina in the aorta of older mice.

Conclusion Abnormal elastogenesis with early elastic fibre proliferation provides a clue to the pathogenesis of arterial tortuosity in human ATS. Availability of this mouse model will allow testing of the relationship between diabetes and its vascular complications, including diabetic retinopathy, nephropathy and peripheral vascular disease.

Keywords Glucose transporter 10; Arterial tortuosity syndrome; SLC2A10

1. Introduction

Glucose is an important substrate to maintain glucose homeostasis in most mammalian cells. The entry of glucose into cells must be mediated by specific transporter proteins present on the plasma membrane. These glucose transporters consist of two functionally and structurally distinct gene families, the sodium-dependent glucose transporters and the facilitative glucose transporters (GLUT).1 Based on sequence similarity, the GLUT family has been further divided into three subclasses: class I, class II, and class III. All GLUTs are predicted to contain 12 transmembrane domains (TM) with cytoplasmic N-terminal and C-terminal and glycosylation sites on the extracellular loop.2 To date, more than 13 members of the GLUT family have been identified in humans; however, the physiological functions of many of the GLUTs have not been fully elucidated.

Glucose transporter 10 (GLUT10), encoded by the SLC2A10 gene, is a member of the class III facilitative glucose transporter family. Like other GLUTs, the secondary structure of GLUT10 contains 12 putative TMs with an intracellular loop between TM domains 6 and 7 and an extracellular loop between TM domains 9 and 10. The human SLC2A10 gene is localized on chromosome 20q12-13.1, a region that has been linked to type 2 diabetes.3–7 Human GLUT10, a 541 amino acid protein, was demonstrated to be highly expressed in the liver and pancreas.3 Owing to the distribution and properties, the genetic polymorphisms of GLUT10 have been studied and shown not to be a major contributor to the type 2 diabetes susceptibility; however, a rare haplotype may have some protective effects.8,9 In comparison, the murine SLC2A10 gene, mapped to mouse chromosome 2, encodes a 537 amino acid protein with 12
putative TMs, as in humans. The amino acid sequence of murine GLUT10 shares 77% homology with human GLUT10. Genetic defects in GLUTs cause inborn errors of metabolism in humans. Heterozygous mutations in the SLC2A1 gene result in deficiency of cerebral glucose transport, whereas mutations in the SLC2A2 gene cause Fanconi-Bickel syndrome, which is characterized by hepatorenal glycogen accumulation, proximal renal tubular dysfunction, and impaired utilization of glucose and galactose. Recent studies have indicated that loss-of-function mutations in the SLC2A10 gene cause arterial tortuosity syndrome (ATS), an autosomal recessive disorder with typical features comprising tortuosity and elongation in the major arteries due to disorganization of elastic fibres in the arterial wall. The upregulation of the transforming growth factor-β (TGF-β) signalling pathway is involved in the elastogenesis and has been described in human ATS patients. Other clinical features of ATS include aneurysm, stenosis, and laxity in the skin and joints. Surprisingly, mutations in the human SLC2A10 gene do not contribute to type 2 diabetes.

Arterial vascular diseases associated with vessel tortuosity and aneurysm have been seen in patients with atherosclerosis, hypertension, diabetes, and obesity as well as in patients suffering from connective tissue disorders due to genetic defects in elastogenesis or collagen synthesis. Mouse models of some of these connective tissue disorders have provided a new insight into the pathogenesis of vascular diseases. However, at present time, there is no animal model of GLUT10 deficiency.

It has recently been proposed that a gene-driven approach to mouse mutagenesis can be applied to study mammalian gene function. Treatment with N-ethyl-N-nitrosourea (ENU) efficiently induced point mutations in the mouse genome. An allelic series of genes of interest can be screened by sequence-based analysis. Using this approach, we describe in this report the generation and phenotypical characterization of mice carrying GLUT10 mutations.

2. Methods

2.1 Mice

GLUT10 mutant mice (G2 generation) in a C3HeB/FeJ background, generated by Ingenium Pharmaceuticals, Germany, were used. ENU mutagenesis and screening of mice carrying the mutations were done by Ingenium Pharmaceuticals. Briefly, C3HeB/FeJ male mice were treated with three intraperitoneal injections of ENU (90 mg/kg × 3) to generate G0 mice. G0 male mice were then mated with wild-type C3HeB/FeJ female mice to produce G1 heterozygous offspring. Genomic DNA from G1 male mice was archived for mutation screening, and the corresponding sperm samples were frozen for recovery of mutant mice. The mutations of interest were screened through the collected DNA archives by sequencing data were analysed by the DNA Sequencing manufacturer's instructions on a 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing data were analysed by v1.1/3.1 (Applied Biosystems, Foster City, CA, USA) following the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) for sequence analysis. The sequencing data were then compared with the murine GLUT10 sequence.
by flushing with 1× phosphate-buffered saline (PBS) at a speed of 6 mL/min for 1 min, then 4% paraformaldehyde (Merck, Darmstadt, Germany) in 0.1 M PBS, pH 7.4 at a speed of 4 mL/min for 20 min. After perfusion fixation, the ascending aorta and descending thoracic aorta were removed and dissected into segments to cut cross and longitudinal sections, respectively. Other tissues from lung, kidney, heart, brain, skin, spleen, pancreas, skeletal muscles, adipose tissues, and joints were then removed, stored in the same fixative at room temperature until use, and embedded in paraffin. Paraffin sections (5 µm) were cut and stained with haematoxylin–eosin (H&E) for the general pathological examinations. Other serial sections were also processed for Verhoeff-van Gieson’s stain to detect elastic and collagen fibres distributed in aorta, artery, and arteriole in other tissues.

2.6 Statistical analysis
Data were expressed as mean ± standard deviation. Differences between groups were analysed by one-way analysis of variance (ANOVA). The P-value < 0.05 was considered significantly different.

3. Results
3.1 Generation of mice carrying GLUT10 mutations
The ENU-mutagenized mouse DNA archives were screened for mutations in exon 2 of the murine GLUT10 (SLC2A10) gene. We chose exon 2 because it contains 80% of the amino acid coding sequence. We identified seven missense mutations with non-synonymous amino acid changes within the 1272 bases of exon 2 (c.367G>A, c.383G>A, c.449C>T, c.527T>C, c.674A>T, c.763T>C, and c.796G>T), which resulted in p.V123M, p.G128E, p.S150F, p.L176P, p.Q225L, p.S255P, and p.G266C, respectively (Figure 1). Six of the seven missense mutations were of conserved residues in the mouse, rat, and human SLC2A10 genes (V123M, G128E, S150F, L176P, S255P, and G266C). Using Garnier analysis, we predicted that mutations G128E, S150F, and L176P severely interfere with the coil, turn, and helix structure of TM domains 4, 5, and 6, respectively, which would give rise to a change in the protein structure with a strong probability of phenotypic effects. Therefore, mice carrying these three mutations were revitalized by in vitro fertilization. However, mice with a mutation causing L176P could not be recovered.

3.2 Phenotypic characterization of mice carrying GLUT10 mutations
The progeny of the mice carrying heterozygous c.383G>A (p.G128E) or c.449C>T (p.S150F) were bred to generate mice homozygous (−/−), heterozygous (+/−), and wild-type (+/+) for the introduced mutations (Figure 2A and B). After four generations of breeding, littermates of all three genotypes were studied (n=4 for each group).

Figure 1  Predicted secondary structure for GLUT10. The 12 transmembrane domains predicted by TMHMM v2.0 are numbered 1 through 12. Missense mutations with amino acid changes and their respective locations are shown.

Figure 2  Sequence analysis of the c.383G>A and c.449C>T mutations in the exon 2 of murine SLC2A10 gene. (A) Genomic sequence of murine SLC2A10 gene showing the point mutation homozygous for A (−/−) at position c.383; heterozygous was G/A (+/−), and wt G/G (+/+ ) at the same position. (B) Nucleotide sequence mutation homozygous for T (−/−), heterozygous for C/T (+/−), and wt C/C (+/+ ) at position c.449.
3.3 General appearance

Homozgyous and heterozygous mutant mice of both strains appeared normal; specifically there were no obvious facial dysmorphisms, joint contractures, or skeletal abnormalities. Skins were not loose. The weight gains were normal and the lifespan of −/− mice was similar to +/+ and +/- mice (>18 months).

Table 1  Blood chemistry of G128E male mice at 10 months of age

<table>
<thead>
<tr>
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<th>+/+ (n = 5)</th>
<th>+/- (n = 5)</th>
<th>−/− (n = 5)</th>
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</thead>
<tbody>
<tr>
<td>Glucose-AC (mg/dL)</td>
<td>90.8 ± 20.41</td>
<td>96.2 ± 11.12</td>
<td>81.0 ± 9.14</td>
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<tr>
<td>Insulin (IU/mL)b</td>
<td>16.4 ± 1.66</td>
<td>25.3 ± 7.06</td>
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<td>TG (mg/dL)</td>
<td>129.8 ± 28.72</td>
<td>121.6 ± 22.13</td>
<td>108.4 ± 23.45</td>
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<tr>
<td>T-Cholesterol (mg/dL)</td>
<td>144.8 ± 23.75</td>
<td>146.6 ± 12.60</td>
<td>130.0 ± 14.78</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>5.8 ± 0.11</td>
<td>5.7 ± 0.19</td>
<td>5.7 ± 0.13</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.7 ± 0.26</td>
<td>3.6 ± 0.27</td>
<td>3.7 ± 0.18</td>
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<tr>
<td>Globulin (g/dL)</td>
<td>1.9 ± 0.16</td>
<td>2.0 ± 0.11</td>
<td>2.0 ± 0.23</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>18.0 ± 2.99</td>
<td>16.5 ± 1.35</td>
<td>18.4 ± 2.06</td>
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<tr>
<td>Creatinine (mg/dL)</td>
<td>&lt;0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>GOT (U/L)</td>
<td>68.1 ± 13.35</td>
<td>66.3 ± 5.42</td>
<td>69.6 ± 9.30</td>
</tr>
<tr>
<td>GPT (U/L)</td>
<td>33.4 ± 5.82</td>
<td>31.5 ± 2.08</td>
<td>32.3 ± 3.02</td>
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</tbody>
</table>

Table 2  Urine chemistry of G128E male mice at 10 months of age

<table>
<thead>
<tr>
<th></th>
<th>+/+ (n = 5)</th>
<th>+/- (n = 5)</th>
<th>−/− (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-AC (mg/dL)</td>
<td>21.4 ± 4.50</td>
<td>24.1 ± 2.85</td>
<td>22.5 ± 3.92</td>
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<tr>
<td>Creatinine (mg/dL)</td>
<td>82.2 ± 11.32</td>
<td>97.3 ± 7.43</td>
<td>91.3 ± 13.25</td>
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<tr>
<td>T-Bilirubin (mg/dL)</td>
<td>0.6 ± 0.08</td>
<td>0.7 ± 0.06</td>
<td>0.7 ± 0.08</td>
</tr>
<tr>
<td>Urine-N (mg/day)</td>
<td>3690 ± 249.4</td>
<td>3810 ± 162.4</td>
<td>3861 ± 146.1</td>
</tr>
<tr>
<td>Microalbumin (mg/L)</td>
<td>31.8 ± 3.11</td>
<td>33.6 ± 6.61</td>
<td>31.6 ± 6.39</td>
</tr>
</tbody>
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*Values are expressed as mean ± SD. Differences between groups were analysed by one-way analysis of variance (ANOVA), none achieved statistical significance (all P-values > 0.05).

3.4 Laboratory findings

Fasting blood glucose showed no hyperglycaemia in −/− mice when compared with the +/+ mice (81.0 ± 9.14 mg/dL for −/− and 90.8 ± 20.41 mg/dL for +/+ mice, measured at 10 months of age). Other blood chemistry including insulin, triglyceride, total cholesterol, total protein, albumin, BUN, and creatinine were all within normal limits (Table 1). Urinalysis showed no glycosuria and no microalbuminuria (Table 2). Echocardiogram (M mode and Doppler study) and EKG revealed regular heart rhythm and normal heart structure and ventricular function (data not shown). Brain MRA demonstrated that the cerebral vessel pattern in −/− mice was similar to that in +/+ mice, and there was no specific arterial tortuosity, stenosis, dilatation, or aneurysm (Figure 3).

3.5 Histopathological analyses

Most notable lesions in both mutant mouse strains were found in the arterial vascular system, especially in vessels close to the heart, such as the aorta, pulmonary trunk and the larger arteries that originate from them. In general, mutant mice carrying the p.G128E mutation had more severe arterial abnormalities than the p.S150F mutant mice and older mice, examined at 10 months, had more severe phenotypes than 6-month-old mice. The vascular phenotypes were characterized by the thickening and irregular shape of the vessel wall (Figure 4B) due to striking increases in the number and size of elastic fibres (Figure 4D and F). In cross-sections of the ascending aorta, the endothelial and smooth muscle cells formed a small plaque in the lamina interna and media where the elastic fibres were more disorganized and fragmented with disruption of the internal elastic lamina membrane (Figure 4D, arrow). The latter findings were only seen in the 10-month-old mice. The endothelial cells along the lamina interna of the descending aorta from wild-type mice were flat in shape (Figure 4G), whereas those in mutant mice showed
thickened intima with hypertrophy and faint nuclear staining of the endothelial cells (Figure 4H, arrows).

The elastic fibres along the arterial walls and alveolar walls of the lungs of the mutant mice were also increased, resulting in what appeared to be a more darkly stained lung tissue in the mutant mice when compared with wild-type (Figure 5A and B). There was also an increase of collagen fibres in the adventitia of the artery (Figure 5B). The vascular morphology in the mutant mouse spleen was also abnormal. There was thickening of the vessel walls in the central arteries in the white pulp of the mutant mouse spleen, which were due to accumulation of elastic fibres in the internal elastic lamina and a marked increase of collagen fibres in the tunica adventitia (Figure 5F). These findings also resulted in widening of the trabecula where arteries, veins, nerves, and lymphatics travel in parallel (Figure 5H). The increasing elastic fibres and zigzag-shaped internal elastic lamina were also detected in the arterial vessel walls of the kidneys (Figure 5J) in the mutant mice. There was no evidence of glomerulosclerosis.

Figure 4  Histopathological analyses of aorta from wt (+/+) and p.G128E mutant (∼/−) mice at 10 months of age. Cross-sections from ascending aorta (A–D) and longitudinal sections from descending thoracic aorta (E–H): Normal ascending aorta was round in shape (A), whereas mutant mouse aorta appeared irregular with uneven thickness. Elastic lamina in mutant mouse ascending aorta (D) was thicker than in wild-type aorta (C). There was a small plaque in the lamina interna and media where the endothelial and smooth muscle cells disoriented, and the internal elastic lamina was discontinuous in this part (arrow in D). The lamina adventitia harboured a leukocyte infiltrate. Longitudinal section of wild-type (E) and mutant (F) descending thoracic aorta: Elastic fibres in the lamina in mutant mouse aorta (F) were coarse and more densely stained than in wild-type aorta (E). In wild-type aorta (G), the endothelial cells faced towards the vessel lumen were thin and flat and were not always well-defined in sections, whereas those in mutant mouse descending aorta showed hypertrophy with faint nuclei (arrows in H). A, B, G, and H were stained with haematoxylin and eosin; C, D, E, and F stained with Verhoeff-van Gieson for elastin. Bars = 100 μm.
Figure 5  Histopathological analyses of lung, spleen, and kidney of wild-type (+/+) mouse and p.G128E mutant mouse (−/−) at 10 months of age. Lung (A and B): Compared with the thin vessel wall in wild-type artery (A), the elastic lamina in mutant mouse lung were thicker, uneven, and protruded into the lumen (arrows in B) and collagen fibres (pink colour) of the adventitia were increased. Note that elastic fibres around alveolar walls in mutant mouse lung were also more pronounced than those in the wild-type lung. Spleen (C–H): Thickened vessel walls of the central arteries (arrows in D and F) in white pulp of mutant mouse spleen (D and F) when compared with the wild-type (C and E). Note that elastic fibres were more abundant and thicker in the internal lamina and there were increased collagen fibres in the adventitia of the mutant mouse artery (F). In comparison to wild type (G), there was widening of trabecula with marked increase of elastic fibres in the mutant mouse spleen (H). Kidney (I and J): The artery walls in wild-type mice were thin and smooth (I), while the elastic fibres were thicker and zigzag-shaped around the vessel lumen in mutant mice (J). A, B, and E through J were stained with Verhoeff-van Gieson for elastin; C and D were stained with haematoxylin and eosin. Bars = 100 μm.
arterial abnormalities, there were no pathological changes in the cellular components of the liver, pancreas, heart, skeletal muscle, brain and skin that were examined.

4. Discussion

In this study we described the first animal model of ATS due to mutations in the SLC2A10 gene. The pathological changes in our mice were most notable in the large and medium-size arteries. The vessel walls were thickened with markedly increased elastic fibres, both in number and size. The increased elastic fibres were not only restricted to the lamina interna, but also extended to the lamina media and adventitia. There was also intima endothelial hypertrophy, and deranged elastic fibres resulted in disruption of the internal elastic lamina in the aorta of older mice (10 months). Human ATS is also characterized by the alteration of the vascular elastic fibres, however, the affected elastic fibres are often described as less abundant, fragmented, and disorganized. Although fragmented elastic fibres were observed in some areas of the aortic walls, the predominant feature in our mutant mice was elastic fibre proliferation. It is possible that elastic fibre proliferation was an early sign of the disease and when the disease progressed, fragmentation and disruption of the elastic fibres followed. Along this line was the observation that only 10-month-old mutant mice showed disruption of elastic lamina and more severe pathological lesions and more fragmented elastic fibres than 6-month-old mutant mice, suggesting that the elastic fibre pathology in arterial vessel walls progressed in an age-dependent manner.

Also differing from human ATS, we did not observe arterial tortuosity, stenosis/dilatation, or aneurysm and our mice appeared to have normal lifespan and no other connective tissue manifestations. The milder clinical and pathological features of our mice could be due to milder missense mutations vs. the nonsense mutations often seen in severe ATS patients.

It is known that the endothelial cells are crucial in angiogenesis and vasculogenesis, and are a key player in the regulation of vasomotor tone and in the development of atherosclerotic vascular diseases. More importantly, they exert their effects mainly by secreting several different mediators either laminally or towards the surrounding lamina media and adventitia in the vessel wall. It is also known that elastin matrix is a critical component of the arterial wall, with both structural and biological signalling functions. It is essential during vascular morphogenesis and functions to maintain the homeostasis of the mature vessel wall. The disruption of elastin–vascular smooth muscle cell (VSMC) interactions might contribute to the pathogenesis of a spectrum of vascular diseases. Our mouse model with its endothelial hypertrophy and abnormal elastogenesis will be useful to further study the role of endothelial cells and elastin in the development of vascular diseases. It is also reasonable to speculate that the polymorphisms in the SLC2A10 gene might contribute to the pathogenesis of a spectrum of vascular diseases, including atherosclerosis, hypertension, and diabetes arteriopathy.

In human ATS, it was found that the downstream effectors of the TGF-β signalling pathway including Smad2 and its downstream target the connective tissue growth factor (CTGF) are increased. The link between GLUT10 and increased TGF-β signalling is unknown. A possible association involves a TGF-β inhibitor called decorin, a proteoglycan that is regulated by a glucose-response element in its gene promoter. In patients with ATS, decorin expression was severely reduced in vascular smooth muscle cells. It has been proposed that glucose is transported into the smooth muscle cell nucleus by GLUT10, activating decorin transcription via CREB. Decorin then binds to and inactivates TGF-β ligand extracellularly, attenuating signalling via the TGF-β/Smad pathway. Loss of GLUT10 may reduce nuclear glucose levels, releasing the decorin restraint on TGF-β signalling. Smad-responsive genes are activated, such as those encoding CTGF and versican, both of which have been shown to disrupt the elastic fibre network of blood vessels, resulting in aortic weakness. However, the proposed pathway does not explain the dramatic increase of elastic fibres in the early stage of the disease.

Hyperinsulinaemia, secondary to insulin resistance, is a major factor in the induction of vasculopathy and atherosclerosis in type 2 diabetes. In our mutant mice, increased elastic fibres were seen in the vessel walls of many organs, including kidney. Although GLUT10 mutant mice exhibited no hyperglycaemia, no hyperinsulinaemia, and no evidence of glomerulosclerosis, it is possible that these vessel changes may contribute to the development of arteriopathy in the setting of diabetes, especially the arterial tortuosity, which is seen in diabetes in tissues undergoing postnatal angiogenesis, including retinal and coronary microcirculations. Availability of this mouse model will allow testing of the relationship between diabetes and its vascular complications, including diabetic retinopathy, nephropathy, and peripheral vascular disease.

Taken together, although the phenotypes of the GLUT10 mutant mice were not as severe as in patients with ATS, abnormal elastogenesis with early elastic fibre proliferation may provide a clue to the pathogenesis of arterial tortuosity in human ATS and provides a mouse model to study the aetiology and mechanism of other vascular diseases, including diabetic angiopathy.

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Conflict of interest statement: None.

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