Short Report

Multiple sequence variations in SLC5A1 gene are associated with glucose–galactose malabsorption in a large cohort of Old Order Amish


Glucose-galactose malabsorption (GGM) is an autosomal recessive disease with life-threatening newborn diarrhea caused by mutations in the Na+/glucose cotransporter gene SLC5A1. Because of its rarity, the clinical course of the disease has not been well studied. Here, we report 33 patients with GGM from a large Old Order Amish pedigree and the associated mutations in SLC5A1 gene. Clinically, all affected individuals presented with classic watery diarrhea and dehydration. The increased bowel sounds, distended abdomen, vigorous nursing regardless of their illness, and irritability and apathy were also noted as part of the initial presentation. Patients underwent a dramatic turnaround with an immediate cease of the diarrhea and a quick rehydration if they were correctly diagnosed and adequately managed, followed by a normal growth and development pattern afterwards; whereas a prolonged clinical course would follow if the disease was not recognized. Sequence analysis of the 15 protein-coding exons and the corresponding exon–intron boundaries of SLC5A1 gene revealed four homozygous missense mutations, c.152A>G (p.N51S), c.1231G>A (p.A411T), c.1673G>A (p.R558H), and c.1845C>G (p.H615Q), that co-segregate with the GGM phenotype in all of the affected individuals. These findings suggest that founder effect of the SLC5A1 mutations associated with the disease in Amish and a population specific genetic testing is in need to pursue an early diagnosis which is critical for a favorable outcome.

Glucose–galactose malabsorption (GGM) is a rare autosomal recessive disorder caused by a defect in glucose and galactose transport across the intestinal brush border (1, 2). Patients with GGM present with the neonatal onset of severe life-threatening watery diarrhea and dehydration (1, 3). Since its first report in 1962, about 200 individuals affected by GGM have been identified worldwide (3–5). Mutations in the Na+/glucose cotransporter gene SLC5A1 have been determined to be associated with congenital GGM (6, 7). To date, more than 40 mutations of SLC5A1 responsible for GGM have been described (7–13).

SLC5A1 is a member of a large gene family, the sodium:solute symporter family (14). Human SLC5A1 gene is located in chromosome 22q13.1, comprises 15 exons and encodes a 73-kda glycoprotein predicted to possess 14 transmembrane segments (15–17). After expression, the SLC5A1 protein is localized to the brush border membrane of the intestinal epithelium and actively imports luminal glucose or galactose into the enterocyte.
Variations in SLC5A1 gene are associated with glucose–galactose malabsorption

by coupling sugar transport with Na\(^+\) gradient across the membrane (17). Mutations in SLC5A1 have been shown to cause defect in sugar transport (6–10).

Because of the rarity of GGM, the clinical description of the disease has been based on the collection of individual case reports, with no systematic clinical study reported in the literature in the past. In this study, we describe a cohort of 33 individuals with GGM associated with multiple homozygous mutations in the SLC5A1 gene in an extended Amish pedigree. To our knowledge, this is the first clinical study of the disease in a sizable cohort.

Patients and methods

Patients

The study was approved by DDC Clinic for Special Needs Children (DDC Clinic) Institutional Review Board, and written informed consent was obtained from each participant or their legal guardian. A total of 33 affected individuals were included in this study. All of the patients were Old Order Amish, with 32 of them from the Geauga County settlement of Ohio and one from Pennsylvania. DDC Clinic provided primary medical services to 29 of them, and 7 from birth. As part of routine medical care, each patient’s detailed medical history and records were collected. The diagnosis of GGM was established based on family history, physical examination and characteristic clinical course of the disease, and later confirmed by DNA mutation analysis. Most parents and siblings of the patients also chose to participate in the study.

Mutation analysis

Total genomic DNA from whole blood was isolated using the PUREGENE DNA Isolation Kit (Gentra Systems, Minneapolis, MN) according to the manufacturer’s protocol. Polymerase chain reaction (PCR) primers were designed to amplify each of the 15 protein-coding exons and their flanking intronic sequences of SLC5A1. We designed primer sequences using Primer3 software (http://frodo.wi.mit.edu/primer3). PCR amplifications were performed using 50 ng of genomic DNA in each reaction. The PCR products were examined on 1% agarose gels and purified for sequencing using Qiagen spin columns (QIAGEN, Valencia, CA). Sequencing reactions were performed using the Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and the extension products were analyzed on an Applied Biosystems 310 Genetic Analyzer. The identified mutation was verified with repeated PCR amplification and sequencing in both orientations. Sample sequences were compared to the GenBank reference sequences using Mutation Surveyor software (SoftGenetics LLC, State College, PA) for the identification of sequence variants. The GenBank accession numbers of both genomic DNA and mRNA reference sequences used in this study are NT_011520 and NM_000343, respectively.

Results

Clinical phenotype

GGM was diagnosed in 33 patients (15 males, 18 females), from age 0 to 26 years. All affected individuals were Old Order Amish from 11 families showing multiple lines of common descent among the parents of affected children (Fig. 1).

Fig. 1. Partial pedigree of the Old Order Amish family with congenital GGM. Affected individuals are indicated by the filled symbols and unaffected individuals by unfilled symbols. Circles and squares denote females and males, respectively. Arrows indicate the probands used for the full-length SLC5A1 gene sequence analysis. The pedigree was developed based on genealogical information taken from the Swiss Anabaptist Genealogical Association (SAGA) group, James C. Hostetler database (website: http://www.omii.org).
Xin and Wang

The typical signs and symptoms were not found in the parents and 61 unaffected sibs. All children affected with GGM were born with normal birth weight (3584 ± 457 g) and length (51.5 ± 2.7 cm) after an uneventful full-term pregnancy. There were no dysmorphic features noticed in those newborns at birth and afterwards. Routine hematologic and metabolic assays at birth were generally within normal ranges.

The initial presentation of the disease was watery diarrhea, usually appearing on day 2 or 3 after birth, soon after the breast feeding or formula was initiated. The diarrhea was often described by parents as ‘nonstopping’, ‘keep running’, ‘in each diaper change no matter how often you change the diaper’. The infants became irritable, gradually developed moderate to severe dehydration, even though their nursing remained strong. More than 20% of body weight might have been lost when the patients presented in a medical setting at the age of 1–2 weeks (Fig. 2). The patients generally were extremely irritable at the presentation, but might become apathetic if the disease had further progressed. They usually had distended abdomen and increased bowel sounds at the examination. The stool was acidic with pH near 5 or lower, positive for reducing substances. Other laboratory findings included significant metabolic acidosis, hypernatremic and hyperosmolar dehydration. At this point, the patients might follow one of three typical clinical courses as described in Table 1.

Patients would have a dramatic turnaround if they were correctly diagnosed and appropriately managed as illustrated in Fig. 2, whereas a prolonged clinical course could follow if the disease was not recognized. Nonetheless, as soon as the correct diagnosis was made, and the carbohydrate-free formula, either supplemented or not with fructose was adequately administrated, and the children would easily rehydrate and follow a normal growth and developmental pattern afterwards (Table 1 and Fig. 2).

The infants might be weaned from carbohydrate-free diet to low-carbohydrate diet before 1-year old while more frequent bowel movements (2–5 times/day) with loose stool were found after weaning in all affected children. Mild abdomen distention and increased bowel sounds were also noted in these patients. The routine urine analysis performed in random urine samples collected from 15 patients revealed various amount of ketones in three patients, but ketonuria was not found in other 12 patients tested. No glycosuria was documented in any of these patients.

The tolerance to carbohydrate-containing diet improved gradually over time in all individuals in this cohort, although the time and degree of improvement varied among them. Most individuals were able to reasonably tolerate regular carbohydrate-containing diets when they reached to their teens, before their twenties at latest.

\[\text{Fig. 2. Body weight changes before and after dietary intervention in two infants. The birth weights are 4281 and 3515 g for case 1 and 2, respectively, and the body weight changes are expressed as percentage to the birth weight. Arrows indicate the time of diagnosis and the initiation of dietary intervention (the body weights were 75\% and 79\% of their birth weights for case 1 and 2, respectively).}\]
Variations in SLC5A1 gene are associated with glucose–galactose malabsorption

Table 1. Typical clinical courses of glucose–galactose malabsorption after initial presentation

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Clinical course</th>
<th>Outcomes</th>
<th>Other clinical notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>10</td>
<td>Admitted to hospital, often to PICU. Extensive work-ups being normal, including intestinal biopsy, multiple formula attempts and prolonged inpatient stays (up to 6 months)</td>
<td>Good only after the diagnosis was made</td>
<td>Diarrhea stopped almost immediately after a carbohydrate-free formula was started, and weight gain and discharge followed shortly</td>
</tr>
<tr>
<td>Group 2</td>
<td>20</td>
<td>The disease was recognized at the initial presentation by experienced clinicians, mostly by their parents for the repeated cases in the same family</td>
<td>Excellent</td>
<td>Patients were rehydrated with carbohydrate-free formula at outpatient setting, and then followed the normal growth curve</td>
</tr>
<tr>
<td>Group 3</td>
<td>3</td>
<td>Patients seemed less severely affected. Remained undiagnosed for years (up to 8 years)</td>
<td>Good after the diagnosis was made</td>
<td>Chronic diarrhea until the diagnosis was reached</td>
</tr>
</tbody>
</table>

Mutational analysis

The entire coding region and adjacent splice sites of SLC5A1 gene were screened by direct sequencing in four probands of the pedigree, one from group 1, one from group 2 and two from group 3, as indicated in Fig. 1. A total of seven homozygous alterations were identified in the open reading frame of SLC5A1 in all four probands (Fig. 3A). Of these sequence variants, three were synonymous changes with no alteration of the predicted amino acid, other four led to substitution of amino acid residues (N51S, A411T, R558H, and H615Q). The four missense mutations occurred in four different exons (exon 2, 11, 14, and 15, respectively). Targeted sequencing of SLC5A1 exon 2, 11, 14, and 15 in parents and unaffected siblings of the four probands revealed that all the parents were obligate carriers, and their unaffected siblings were either homozygous normal or heterozygous for each of the four missense mutations.

We next tested all other affected individuals from the pedigree (n = 29) by targeted sequencing of the four exons bearing the missense mutations. Sequence analysis showed all of them were homozygous for these mutations. We further screened 100 healthy Amish control individuals (n = 200 chromosomes) for the four mutated loci and found that none were homozygous for any of these sequence variants, but five of them were heterozygous carriers with an estimated carrier frequency of 5.0%.

Discussion

In this study, we describe a cohort of 33 individuals with GGM, a rare genetic disorder, in a large consanguineous Old Order Amish pedigree. All patients presented with typical profuse watery diarrhea with neonatal onset and severe dehydration. Many other signs and symptoms, such as increased bowel sounds, distended abdomen, vigorous nursing regardless of their illness, and irritability or apathy have been observed at the initial presentation and reported here for the first time, although they may not be specific for this condition and might be manifestations of dehydration. The unique position as a primary care facility has given us the opportunity to carefully and continually observe these patients, while we provide medical services, and to reveal some previously unnoticed clinical signs and symptoms. The most dramatic clinical feature is the patients’ response to carbohydrate-free formula, which leads to an almost immediate cease of their diarrhea, and a quick rehydration. The enteric carbohydrate-free formula with or without fructose addition seems a very effective and safe measures for the rehydration. If the patients are not diagnosed and treated properly in a timely manner, the disease is usually lethal. It is noted that no patient in this cohort is older than 26 years old, the age of the first patient clinically diagnosed within this area.

Because of its rarity, the diagnosis of GGM for a clinician remains challenging. However, the reward for an early diagnosis is substantial as all patients who were correctly diagnosed and adequately managed accordingly have followed a very favorable clinical course as group 2 in Table 1. It is noted that the majority of the patients in this cohort were recognized easily by local primary care physicians, more often by their experienced parents, which once again highlights the importance of knowledge of the disease and recognition of clinical signs and symptoms. The extremely high incidence of this disease in the community has been intriguing both the community members and health professionals and as a consequence, it
has greatly improved their knowledge toward the condition. Therefore, we expect that the increasing knowledge of this disease in neonatologists, pediatricians and pediatric gastroenterologists will improve the disease diagnosis. The additional signs and symptoms at the initial presentation described in this report will help the early recognition of the disease as well.

Through mutational analysis in four probands, we have identified four homozygous missense mutations, N51S, A411T, R558H, and H615Q, in SLC5A1 gene that are associated with the disease. All these sequence variants co-segregate consistently with the disease phenotype, with all affected individuals being homozygous, their parents being heterozygous, and none of the unaffected siblings tested or the 100 normal controls being homozygous for the alterations. These findings suggest that either one or a combination of those sequence transversions contributes to the disease phenotype. By checking the public database, we found that only R558H variant in exon 14 is a novel mutation, while the N51S, A411T, and H615Q are three documented SNPs according to NCBI dbSNP (SNP ID# rs17683011, rs17683430, and rs33954001, respectively). It is still unclear how each individual mutation reported here affects the function of the SLC5A1 protein and consequently causes defect in sugar transporting. Given the fact that both N51S and R558H affected amino acid residues that are highly conserved across the SLC5A1 orthologs (Fig. 3B), we speculate that these two mutations might be responsible for the impaired sugar transport, whereas A411T and H615Q are probably benign polymorphisms. In support of the pathogenic effect of R558H, we noted a similar mutation, R558C, previously reported at the same position being association with GGM (18). Further functional study is underway to determine the role of each mutation and their combinations in causing sugar transport defect. Because SLC5A1 is also expressed at a low level in the proximal tubule of the kidney, most GGM patients reportedly have renal glycosuria (1). However, in contrast to previous reports, no glycosuria was found in this group of patients, suggesting that the function of renal proximal tubule to reabsorb glucose seems intact in these patients.

Over 40 mutations of SLC5A1 responsible for GGM have been identified so far, and most of these mutations result in either truncated protein or mistrafficking of the transporter in the cell (3, 6). In the current study, we for the first time report multiple mutations shared by all the patients in a

---

**Fig. 3.** Mutation analysis of SLC5A1. (A) Schematic representation of the SLC5A1 transcript, with the position of identified sequence variants. Shaded area represents the protein-coding region and the numbers indicate the specific exons. An asterisk (*) denotes polymorphisms reported in the SNP database; a number sign (#) denotes synonymous changes. (B) A partial amino acid sequence alignment of human SLC5A1 with seven other vertebrate orthologs. Identical residues are indicated by asterisks. Residues with substitutions that are found in the patients are highlighted. NCBI accession numbers used are as follows in parentheses: human (NP_000334), chimpanzee (XP_515093), mouse (NP_062784), rat (NP_037165), dog (NP_001007142), cow (NP_777031), chicken (XP_415247), and zebrafish (NP_956975).
large cohort, suggesting a common founder effect of the four sequence variants. These variations formed a particular haplotype associated with the disease in an Old Order Amish population where the disease was identified. Because an early low-carbohydrate diet intervention is so critical for the patients and the disease prevalence is so high in the Amish population, it would be very useful to develop a simplified population specific genetic testing for early diagnosis and carrier test based on the information obtained from this study. In fact, newborn screening with the targeted DNA mutation analysis through umbilical cord blood collected from the infants of high-risk families has become a routine practice in the community through our facility; thus an early diagnosis can be made before severe symptoms develop. We believe that this form of population specific genomic medicine is not only possible but should become standard of care for the medical centers that serve the Amish population. Although such practice may not be applicable in general population at present because of lack of DNA 'hot spots' for screening in non-Amish population, this approach suggests some future directions for disease management.

It is noted that the tolerance to carbohydrate-containing diet improves gradually over the time in all patients in this cohort and most individuals are able to tolerate regular carbohydrate-containing diets before their twenties. Although the mechanism of such adoption remains unclear, it is also noted that parents-initiated probiotics supplements, *Lactobacillus acidophilus* in most cases, seem to accelerate this process, as reported by multiple families. Thus, the further work to study the consequence of these mutations on the *SLC5A1* capacity of sugar transportation and detailed pathogenesis of the disease will not only be valuable in solving some fundamental physiological issues, but also help in developing more effective treatment strategies for the affected individuals.

**Acknowledgements**

We thank the families for their patience and support. We appreciate the physicians from Rainbow Babies and Children’s Hospital, Cleveland Clinic Children’s Hospital in providing outstanding and compassionate care to the children affected by the disease, and Ms Alicia Bright in helping with data collection. The study was supported in part by The Elisabeth Severance Prentiss Foundation, The Reinberger Foundation, and the Leonard Krieger Fund of the Cleveland Foundation (L2009-0078).

**Conflict of interest**

Nothing to declare.

**References**


