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Genetic variations in the *GLUT3* gene associated with myelomeningocele

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Abstract

Objective—Our objectives are to examine the extent of described sequence variation in the glucose transporter 3 (*GLUT3*) gene in myelomeningocele (MM) children, identify novel variations in the *GLUT3* gene in these children and determine whether these variations may confer risk of MM.

Study Design—We sequenced the 10 exons of *GLUT3*, including exon-intron boundaries, on 96 children with MM. Sequencing was performed with Sanger methods and results analyzed with DNA analysis software. Frequencies of known single nucleotide polymorphisms (SNPs) were identified, those differing from the reference sequence (GRCh37/hg19 assembly) were considered variations.

Results—Six novel and nine previously described, genetic variations were identified in our population. The novel variations include a large, 83 base pair deletion involving the core promoter region and part of Exon 1 (1/96 children), and a two base pair deletion in the coding sequence of Exon 4 (1/96 children). The remaining novel variations are located in the introns in the proximity of the splice sites. Novel mutations in *GLUT3* were observed among 6.25% of our population. Additionally, the frequency of the rare allele for rs17847972 located in a splice donor site is higher ($P < 0.001$) in MM in our population than expected.

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Conclusion—We identified previously undescribed deletions and single nucleotide variations involving the *GLUT3* gene that may be associated with increased susceptibility to MM. Of particular interest, the two deletions involve both an important core promoter site and a coding region predicted to have deleterious effect. The functional significance of these findings is under investigation.

Keywords

GLUT3 gene; genetic variations; myelomeningocele; Sanger sequencing

Introduction

Neural tube defects (NTDs) constitute a heterogeneous category of fetal malformations that result from failure of neural tube closure by the 4th week of embryologic development.¹ NTDs are the most common structural central nervous system defect and occur at an incidence of 0.5–2/1000 live births worldwide.^{2,3} The majority of NTDs can be classified as anencephaly or spina bifida.⁴ The most common NTD associated with survival is myelomeningocele (MM), a severe form of spina bifida that occurs due to defective closure of the caudal neural tube with herniation of the spinal cord and meninges.⁴ Most infants born with MM survive and these individuals often have multiple disabilities and increased mortality rate.^{5,6}

The etiology of NTDs is not entirely understood, but involves both genetic and environmental in association with critical timing during embryogenesis.⁷ Clustering of NTDs within families, and associations with various genetic syndromes, underlines the importance of identifying the underlying hereditary basis of NTDs.^{7,8} Maternal folate status has been established as an important factor in the development of NTDs. The association between folate deficiency and NTDs led to mandated fortification of grain products in the U.S. in the late 1990s. This fortification has been associated with a 20–30% decrease in the NTD rate.^{9,10} The mechanism by which folate deficiency causes NTDs remains unclear despite an extensive number of investigative studies.¹¹ It appears that other genetic and environmental influences may contribute to a folate resistant phenotype of NTDs.¹² Teratogenic exposures to anti-epileptic medications are associated with increased risk of NTDs although these risks may be mitigated with appropriate folate supplementation based on evidence from animal studies.^{13,14}

Other environmental factors with genetic controls implicated in the development of NTDs include derangements in glucose metabolism and maternal obesity.^{15–20} Mexican American women are particularly interesting in that they have the highest rates of offspring with NTDs, maternal obesity and type 2 diabetes mellitus in the U.S.^{5,21} A recent analysis from the National Birth Defects Prevention Study showed the factors most associated with NTDs were Hispanic ethnicity, maternal obesity and low dietary folate intake.²²

Previous animal studies have shown increased glucose levels during embryogenesis can alter expression of proteins involved in glucose metabolism and homeostasis.^{23–25} Hyperglycemia during this critical period is associated with increased apoptosis, and increased production of reactive oxygen species generation that favor cell death.^{25–27} More

recent human studies have further exhibited an association between high maternal dietary glucose intake and the risk of NTDs in non-diabetic women.²⁸

GLUT3 is a glycoprotein with 12 transmembrane domains that transports glucose across cell membranes and is a member of a superfamily of transport proteins comprised of 14 members.²⁹ This group of proteins is encoded by the *SLC2* family genes that is subclassified into 3 classes based on sequence similarity of which GLUT3 is in class 1.^{29,30} The *GLUT3* (*SLC2A3*) gene is located on chromosome 12p13.3 and is comprised of 10 exons.³⁰ GLUT3 is expressed predominantly in neuronal tissue functioning as a high affinity glucose transporter in conditions of low ambient glucose levels surrounding neurons.²⁹ Studies in animal models have shown that GLUT3 is critical in the both the developing pre and post-implantation murine embryo.^{31–33} More recently GLUT3 expression in human placenta tissue has been shown to be present throughout gestation with a predominance in the 1st trimester placenta.³⁴ Increasing DNA methylation of the *GLUT3* gene has been reported to be associated with decreased expression throughout gestation, thus GLUT3 as a placental transporter may be of greater significance in the 1st trimester during periods of embryogenesis.^{34,35}

In previous studies we have demonstrated associations between coding single nucleotide polymorphisms (SNPs) in three genes (*HK1*, *LEPR*, *GLUT1*) involved in glucose metabolism and NTDs.³⁶ Additionally, we have described novel and rare variations in the *GLUT1* gene associated with MM.³⁷ The objective of our study is to examine the relationship between the sequence of the GLUT3 gene and MM. We sought to study both previously identified polymorphisms as well as potentially identify new variations.

Materials and Methods

Children with MM and their parents were enrolled into the study from 1996–2006 from 3 primary sites (Houston, Texas; Los Angeles, California; Toronto, Canada). Study approval by the Institutional Review Board (IRB) of the University of Texas Health Science Center at Houston and informed consent was obtained. 864 subjects were enrolled in the study, of which 96 were selected to be included in this project. The characteristics of the study population are described in detail elsewhere.³⁸ The children were selected based on ethnicity (48 Hispanic; 48 Caucasian). As mentioned previously the Hispanic population was of particular interest due to increased rates of NTDs, maternal obesity and type 2 diabetes mellitus.^{5,21} Additionally, we included only MM affected children born after the 1998 federally mandated folate fortification to select those children most likely to have had adequate dietary folate intake.

Blood samples were obtained from children and parents, and genomic DNA was extracted from blood lymphocytes utilizing the Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN). When a parent was not available for blood sampling, a saliva DNA kit was sent to obtain a sample and the DNA (DNA Genotek; Kanata, Ontario, Canada) was subsequently extracted from the sample. DNA was amplified with whole genome amplification (Genomiphi; GE Healthcare, Piscataway, NJ) and working stocks of 10ng/μl

were prepared for PCR and stored at -80°C . DNA from the selected children has been used in previous studies with consistent genotyping results.^{36,38}

PCR and nested-sequencing primers were designed based on reference genomic sequences of *GLUT3* (NM_006931, GRCh37/hg19 Assembly) extracted from the University of Santa Cruz Genome Browser (UCSC Genome Browser; <http://genome.ucsc.edu/cgi-bin/hgGateway>). The primers were designed containing 50–100 bases in flanking regions of the exons to include splice sites. A total of 10 PCR primer pairs were designed (available upon request) and synthesized by Integrated DNA Technologies USA (Commercial Park, Coralville, IA). PCR amplification of exons with MyTaq Hot Start DNA Polymerase (Bioline USA Inc, Taunton, MA) was completed using the MJ Research PTC-100 Programmable Thermal Cycler (MJ Research, Waltham, MA). PCR product sizes were confirmed with 1.8% agarose gel electrophoresis. The amplified exon DNA was treated with T7 exonuclease 1 and Shrimp Alkaline Phosphatase (United States Biochemicals, Affymetrix, Cleveland, OH) to remove excess nucleotides and primers. Amplicons were sequenced using the BigDyeTerminator (Applied Biosystems, Foster City, CA) reagents with nested sequencing primers. The sequenced product was resolved on the ABI3100 Genetic Analyzer (Life Technologies Inc, Grand Island, NY).

Our sequence results were analyzed using the DNA Sequencing Analysis Software v5.1 from ABI. To identify variations in the sequences, we compared results manually to the reference sequences (NM_006931) from the UCSC Genome Browser. The Caucasian reference population (CEU) includes Caucasians from Utah included in the Centre d'Etude du Polymorphisme Humain (CEPH) Collection for mapping genetic markers and the National Heart, Lung and Blood Institute (NHLBI) Exome Sequencing Project. The Mexican American (MEX) reference population includes Mexican Americans recruited from Los Angeles, CA, used in the international HapMap project and data from the 1000 Genomes Project in the single nucleotide polymorphism database (dbSNP). Admixture analysis was performed to confirm genetic equivalency between our Caucasian and Hispanic children and the referenced populations.³⁸

Our sample size of 96 children was selected primarily due to the size of the polymerase chain reaction plate (96 wells) allowing for efficient utilization of DNA and reaction buffers. Additionally, the statistical convenience of utilizing 96 lies in the overall allele count of 192 (2 alleles per child $\times$ 96 children), allowing for detection of rare alleles, described as a frequency of 0.5% or roughly 1/192 alleles.

Rare allele frequencies of all known SNPs identified in our population were tallied and compared to ethnically matched frequencies reported in the dbSNP using Fisher's test. A two-tailed $P < 0.05$ was considered significant. We corrected for multiple testing using Bonferroni methods. Variations identified in our population, and not reported in dbSNP were considered novel. These novel findings were then reverse-sequenced with a reverse sequencing primer for confirmatory testing. Variations present on forward and reverse sequencing were considered valid. The parents of children found to have novel variations were sequenced to assess for inheritance of the mutation.

Novel variations of particular interest were selected for expanded study in our entire cohort population of 864. In addition to our cohort we added 92 Mexican American controls, recruited from the Houston area, and 93 Caucasian controls from the Human Variation Panel-Caucasian Panel of 100 (HD100CAU; Coriell Institute for Medical Research, Camden, NJ). Custom TaqMan SNP genotyping assays with fluorescently labeled probes were utilized for the interrogation. Genotyping results were read utilizing the 7900HT Fast Real-Time PCR System (Life Technologies) and analyzed using the 7900 HT Sequence Detection System Software.

Results

After sequencing the 10 exons of the *GLUT3* gene we found six previously undescribed variations including two deletions (table 1). A large 83bp deletion (Exon1:g:8088945_8088862del83bp) was found spanning the core promoter and 5' untranslated region (UTR) of exon 1 in a single Hispanic child. We performed DNA sequencing of both parents of the child, neither of which had the deletion thus confirming the variation as *de novo*.

A second deletion, in a separate Hispanic child, (Exon 4: c.470_471delTC) was identified in the coding sequence of Exon 4 (Figure 1). The deletion resulted in a frameshift mutation in the coding sequence. DNA sequencing of both parents found the deletion was paternally inherited. Genotyping of 864 additional children using fluorescently labeled probes designed by custom genotyping Taqman assays (Life Technologies) to determine whether the deletion we identified in exon 4 (Exon 4: c.470_471delTC) was present in our larger cohort was completed. As expected, the deletion mutation was not identified in any additional subjects, likely confirming the variation as an isolated, inherited mutation.

Four additional novel variations were identified, all occurring in different intronic regions of the gene. Of note, one of the variations (Intron 3: g:8085543C>G) is located in a cryptic splice acceptor site and was identified in a single Caucasian child with MM (Figure 2). Aligning sequences of this variant in humans with 24 known vertebrate sequences available through the UCSC Genome Browser (available at <http://genome.ucsc.edu/index.html>) showed the reference allele to be highly conserved amongst vertebrates (Figure 3). The 3 remaining variations (Intron 5: g:8083043T>C, Intron 6: g:8082222C>A, Intron 9: g:8074240C>A) were also aligned and compared with vertebrate sequences and were noted to be highly conserved amongst primates, and less highly conserved when compared to other vertebrates (information available on request).

Nine previously described SNPs within or flanking the exons of *GLUT3* were evaluated. Eight of the nine SNPs evaluated had population frequencies reported both for the Caucasian reference population (CEU) and the Mexican American reference population (MEX). Heterozygosity was demonstrated in all nine of the SNPs in Caucasian children, and in all but one SNP (rs151144610) in the Mexican American children (Table 2). Hardy-Weinberg equilibrium testing on all SNPs utilizing Haploview 4.2 (Broad Institute, Cambridge, MA) was performed and all SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$) with the exception of rs17847972 ($P < 0.001$).³⁹

Table 2 describes the rare allele frequencies in our cohort and shows the comparisons to ethnically matched reference population frequencies. There was a statistically significant higher rare allele frequency for rs17847972 (C/T) in the splice donor site of exon 8, in both our Caucasian and Mexican American children compared to population frequencies ($P = <0.001$) that remained significant after correction for multiple testing. Additionally, a significantly higher rare allele frequency was observed for rs17847967 (T/C) in Caucasian children compared to the reference population. When comparing the allele frequencies between our Caucasian and Mexican American children we found no significant differences.

Comment

To our knowledge, this is the first large-scale sequencing study of the *GLUT3* gene in children with MM. DNA sequencing was used to identify six novel variations, all of which occurred in separate children with MM, yielding an overall novel mutation rate of 6% in our population. This includes two potentially important deletions, both of which occur early in the gene sequence, and are predicted to have deleterious consequences with regards to function. The first, an 83bp deletion spans the core promoter region and the 5' UTR region of exon 1. Due to the size and location of the deletion it overlaps many transcription factors binding sites demonstrated by Transcription Factor ChIP-Seq from ENCODE (UCSC Genome Browser) and is likely to impact transcription of the DNA sequence. This deletion is also predicted to alter 17 of 19 transcription factor target sites (TFSEARCH: Searching Transcription Factor Binding Sites, available at <http://www.rwcp.or.jp/papia/>). This would likely cause loss of regulatory function of the involved transcription factors, thus altering *GLUT3* transcription (information available on request). The 5'-UTR is an important region for ribosomes to bind to the messenger ribonucleic acid (mRNA) for initiation of translation and this deletion is expected to alter the translation initiation process of *GLUT3*. In addition, there are noted to be 9 potential microribonucleic acids (miRNA) that recognize the deleted 5'-UTR region of the gene thus potentially altering the mRNA stability and expression of *GLUT3* (miRBase, available at <http://mirbase.org/>. Accessed 11/07/2013; available on request).

The second deletion, a 2bp deletion in exon 4, results in a frame shift mutation. There are 502 amino acids in the *GLUT3* protein and the frame shift mutation results in a conversion of 156Leu to 156Gln and a stop codon 22 amino acids downstream from the conversion with a subsequent loss of 324 amino acids. Based on structural analysis of class I GLUT proteins (GLUT 1–4, 14) the frame shift mutation leads to termination of the protein structure in the region of transmembrane domain number four.⁴⁰ *GLUT3* has 12 transmembrane domains, thus a termination at domain number 4 would result in a loss of the remaining 8 transmembrane domains, including sites for both extracellular and cytoplasmic substrate binding regions.^{40,41}

Four previously unknown variations were identified in intronic regions of the gene. Of particular interest, one variant (Intron 3: g:8085543C>G) is predicted to resemble mRNA splicing enhancer and silencer target motifs (Human Splicing Finder, available at <http://www.umd.be/HSF/>;) with alterations in binding predicted in the variant. It is important to note that this is a prediction tool and actual loss or gain of splicing function would need to

be experimentally confirmed. Additionally, this particular region is predicted to be target for two miRNAs, hsa-miR-7156-5p; hsa-miR-3667-5p, with alteration noted in the in hsa-miR-7156-5p noted in the variant. There appears to be loss of site recognition in the variant for hsa-miR-3667-5p. The previously undescribed variant, Intron 9: g:8074240C>A, is also predicted to be within the recognition sites of nine miRNAs. Of note, the variant allele A at the location is associated with loss of recognition by all nine of the miRNAs, as well as being recognized as a new target site by three separate miRNA (information available on request). Variations to miRNA recognition have the potential to alter mRNA stability and thus affect protein expression.

We reported the rare allele frequencies of nine previously described SNPs identified in our cohort. The rare allele for the SNP, rs17847972, was noted to be present at a much higher frequency ($P < 0.001$) in our cohort as compared to the reported rare allele frequencies in the 1000Genomes project. The location of the SNP is in the splice donor motif of exon 8. The significance of this finding is unclear but the higher association of this variation in our cohort compared to reference databases may indicate functional significance.

Our study has several strengths. Sequencing of all 10 exons of the *GLUT3* gene, including the 50–100 bases in the intron-exon boundaries of each exon, was performed with the Sanger technique. This technique is the gold-standard in sequencing but is costly and labor intensive. The Sanger technique does provide benefit over newer whole exome sequencing techniques that may miss important exon flanking regions. Several variations were identified in these exon-intron boundaries and are predicted to be important binding sites for miRNA and splicing proteins. Another strength of our study is that our cohort includes the two ethnicities (Mexican American; Caucasian) that have the highest rates of NTDs in North America, and we only included those born after the 1998 federally mandated folic acid fortification. Because our gene of interest is involved in glucose metabolism this inclusion criteria allowed us to further enrich our study population for folate-resistant cases.

Limitations of our study include the small sample size. This inhibits our ability to identify rare variations with frequency less than 1% in our ethnic subgroups. Additionally, the power of our study to identify significant associations between SNPs with low rare allele frequency and our cohort affected with MM is limited. Comparisons between the rare allele frequency in our population and reported population frequencies should be made with some caution as well due to our overall sample size. Another limitation of our study is that we have yet to define the functional effect of these variations on GLUT3 protein function, and the functional impact of these novel variations need to be confirmed in biologic assays. Gene sequencing of our entire cohort of 864 would be somewhat cost-prohibitive at this time, but may represent an area of future research with whole exome sequencing technology.

In summary, our study identified six novel genetic variations of the *GLUT3* gene in 96 children with MM. Due to the low allele frequencies of these variations it is unlikely they play a major role in the development of MM. However, our findings do contribute to the larger evidence supporting genetic associations of genes involved in the regulation of numerous metabolic pathways and the susceptibility to NTDs. It is important that we pursue additional studies assessing the potential impact these genetic variations have on the

physiologic function of the GLUT3 transporter in our cohort and compare these to findings to unaffected controls. An improved understanding of the contribution that both genetic and environmental factors play in the development of NTDs is needed.

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Figure 1.

Sanger sequencing results of variant Exon 4: c.470_471delTC

A, Represents the Sanger sequence of the normal reference sequence at the allele location c. 470_471 (*arrows*). **B**, Represents Sanger sequence of the heterozygote sequences for the myelomeningocele child with c.470_471delTC (*arrows*). The normal allele sequence of the child is present on top of the allele sequence with deletion (*labeled*) with the subsequent sequence listed to depict the effect of the deletion.

Figure 2.

Sanger sequencing results of variant Intron 3: g:8085543C>G.

A, Sanger sequence result of the reference allele Intron 3: g:8085543G (*arrow*). **B**, Sanger sequence result of the myelomeningocele child with the variant allele Intron3: g:8085543C>G (*arrow*).

MM Subject	ttgtcctcaatgtggctggtagagcccactt
Human	cac--ttgtcctcaatgtggctggtagagcccactt
Chimp	cac--ttgtcctcaatgtggctggtagagcccactt
Gorilla	cac--ttgtcctcaatgtggctggtagagcccactt
Orangutan	cac--ttgtcctcagtgcggctggtagagcccactt
Rhesus	cac--ttgtcctcagtgcagct-gtagagcccactt
Baboon	cac--ttgtcctcagtgcagct-gtagagcccactt
Marmoset	tgc--ttgtcctcagtgcagctggtagagcccactt
Tree shrew	ca-----ggatcagcaatagctgctt
Mouse	tg---ctgtctttcgggtaaccagtgcaggcagact
Rat	tg---ctgtgtcccacgtaaccagtac-----att
Guinea pig	tgt--ttgtcctcagcgcaccagtactgtccactt
Squirrel	tgc--ttatcctcaaagcaacctgtatagcccactt
Rabbit	cac--ttgtcctcaaggaaactggtagaacctgctc
Megabat	tac--ttgtcttcactgcaaccagaaacagcccc--t
Dog	tac--ctgttcccaatgcaaccagtatagccccctt
Cat	tac--ctattctcaatgcaaccggcatagcctcctt
Horse	tac--cttttctcagcataaccagtaaaaccctctt
Dolphin	ttc--ttgtcctcaatgcaaccagtatagaccctt
Cow	tgc--ttgtcttcaatgcaaccagtacagccccctt
Alpaca	tac--cggttctcagtgcaccagtgtagccccctt
Tenrec	taa--ctgtgctcactggaaccagaaacagcacaatt
Rock hyrax	tac--gtgttctcaatgcagccagcata---actg
Elephant	tac--ctgtcttcagtacaaccagtataaccactt
X. tropicalis	ccaagaggcccctctgaagctggtag-gcccatgt

Figure 3.

Sequence of 24 vertebrate species at the splice site location involved by variant Intron 3: g:8085543C>G

The figure depicts the sequence alignment of the allele location Intron 3: g:8085543G in 24 vertebrate species with the variant sequence identified in our MM subject. The sequence is conserved in all species with the exception of the marmoset and tree shrew (*bold*).

Generated from the University of California, Santa Cruz Genome Browser (Available at <http://genome.ucsc.edu/index.html>).

Table 1

Novel genetic variations in the GLUT3 gene of MM children with reported frequencies and summary of potential functional implications

Variations	Functional Site	A1/A2	All MM N=96	CEU N=48	MEX N=48	Vertebrate Conservation ^d	miRNA ^b	TF ^c
Exon 1: g:8088945_80 88862del83bp	Promoter/5'-UTR	83bp/-	0.5%	0%	1%	NA	Y	Y
Intron 3: g:8085543C>G	Intron	A/G	0.5%	1%	0%	92% (22/24)	Y	N
Exon 4: c:470_471delTC	Coding - Frameshift	TC/-	0.5%	0%	1%	100% (34/34)	Y	N
Intron 5: g:8083043T>C	Intron	T/C	0.5%	0%	1%	33% (8/24) ^d	N	N
Intron 6: g:8082222C>A	Intron	C/A	0.5%	0%	1%	53% (9/17) ^d	Y	N
Intron 9: g:8074240C>A	Intron	C/A	0.5%	0%	1%	31% (9/29) ^d	Y	N

Variations are presented with reference to the genomic location of the nucleotide change on chromosome 12 in the GRCh37/hg19 assembly. A1, reference allele; A2, variant allele; MM, myelomeningocele; CEU, Centre d'Etude du Polymorphisme; MEX, Mexican-American; miRNA, microribonucleic acid; TF, transcription factor binding site; UTR, untranslated region; NA, not applicable; Y, presence; N, absence.

^a Available vertebrate sequence data for the involved site of genetic variation, information listed as percentage of vertebrate species with conservation of the sequence (UCSC Genome Browser Multiz Alignments of 46 vertebrates).

^b Predicted miRNA binding location with alteration or loss of binding with change to variant allele (miRBase analyses, <http://www.mirbase.org/search.shtml>).

^c Predicted transcription factor binding site with loss of binding with change to variant allele (TF Search - <http://www.cbrc.jp/research/db/TFSEARCH.html>; and UCSC Genome Browser Transcription Factor ChIP-Seq from ENCODE).

^d Conserved in all primates sequences.

Table 2

Comparison of known genetic variations in the GLUT3 gene with rare allele frequencies reported between MM children and reference populations

Variable			Caucasian (CEU)		Hispanic (MEX)		CEU/MEX
SNP	A/A2	All MM	MM	Reference	MM	Reference	P value
rs2541279	T/C	27% (52/192)	30% (28/96)	29% (2383/8600) ^a	25% (24/96)	30% (40/132) ^b	.8/.4
rs17847967	T/C	20% (39/192)	23% (22/96)	14% (107/758) ^b	18% (17/96)	20% (26/132) ^b	.02/.7
rs74059377	G/A	1% (2/192)	1% (1/96)	0% (0/758) ^b	1% (1/96)	0.7% (1/132) ^b	.1/.1
rs17847972	C/T	47% (91/192)	49% (47/96)	0.1% (1/758) ^a	50% (48/96)	6% (8/132) ^b	<.001/<.001
rs41438344	C/G	1.5% (3/192)	1% (1/96)	0.2% (16/8600) ^a	2% (2/96)	8% (11/132) ^b	.2/.08
rs741361	A/G	41% (78/192)	43% (41/96)	37% (280/758) ^b	39% (37/96)	36% (48/132) ^b (42/98) ^c	.3/.7
rs151144610	C/T	0.5% (1/192)	1% (1/96)	0.6% (5/758) ^a	0% (0/96)	0% (0/132) ^b	.7/--
rs25684	G/A	40% (77/192)	43% (41/96)	48% (4119/8600) ^a	37.5% (36/96)	44% (58/132) ^b	.3/.3
rs112948014	A/G/--	2% (4/192)	1% (1/96)	ND	3% (3/96)	ND	--/--

All variations described with list reference sequence (rs) format

A1, reference allele; A2, variant allele; CEU, Centre d'Etude du Polymorphisme; MEX, Mexican-American; ND, no data available for reference population frequency; P two-tailed Fisher exact test; GLUT3, Glucose Transporter 3

^aNorth American Caucasian population from National Heart, Lung and Blood Institute (NHLBI) Exome Sequencing Project;

^b1000 Genomes Project;

^cMexican ancestry population from Los Angeles, CA (International HapMap Project).