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## **Biotin-thiamine responsive basal ganglia disease: Identification of a pyruvate peak on brain spectroscopy, novel mutation in *SLC19A3*, and calculation of prevalence based on allele frequencies from aggregated next-generation sequencing data**

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### **Abstract**

Biotin-thiamine responsive basal ganglia disease is an inborn error of metabolism caused by mutations in *SLC19A3*, encoding a transporter of thiamine across the plasma membrane. We report a novel mutation identified in the homozygous state in a patient with typical brain MRI changes. In addition, this patient had markedly elevated CSF pyruvate, a low lactate-to-pyruvate molar ratio, and an abnormal pyruvate peak at 2.4 ppm on brain magnetic resonance spectroscopy. Using aggregated exome sequencing data, we calculate the carrier frequency of mutations in *SLC19A3* as 1 in 232 individuals in the general population, for an estimated prevalence of the disease of approximately 1 in 215,000 individuals. The disease is thus more frequent than previously recognized, and the presence of a pyruvate peak on spectroscopy could serve as an important diagnostic clue.

### **Keywords**

biotin-thiamine responsive basal ganglia disease; magnetic resonance spectroscopy; pyruvate; *SLC19A3*; thiamine metabolism dysfunction syndrome 2; thiamine transporter-2 deficiency

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URLs: ExAC, <http://exac.broadinstitute.org/>; Mercury pipeline, <https://github.com/dsexton2/Mercury-Pipeline>; Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>

#### **CONFLICTS OF INTEREST**

The authors report no conflict of interest.

#### **SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of this article at the publisher's web-site.

## 1 | INTRODUCTION

Biotin-thiamine responsive basal ganglia disease (BTBGD), also known as thiamine transporter-2 deficiency, or thiamine metabolism dysfunction syndrome 2 (OMIM #607483) is an autosomal recessive condition caused by biallelic mutations in *SLC19A3*. The disease was initially named biotin-responsive basal ganglia disease, but once it was recognized that the culprit gene encodes a thiamine transporter and not a biotin transporter, its nomenclature was changed to BTBGD (Alfadhel et al., 2013). This inborn error of metabolism presents with neurologic dysfunction and associated bilateral basal ganglia lesions on brain imaging, both of which can worsen intermittently. The severity of the disease is quite variable, ranging from Leigh syndrome in infancy (Haack et al., 2014), to Wernicke's encephalopathy in later life (Kono et al., 2009). Since its initial description in 1998 (Ozand et al., 1998), there have been 116 affected individuals reported in the medical literature, and 21 mutations in *SLC19A3* have been described so far. Here, we report a new patient with typical neurologic and brain imaging findings of BTBGD, and an additional pyruvate peak on brain magnetic resonance spectroscopy (MRS) at 2.4 ppm—present during an acute crisis, but absent at baseline. The patient was found to have a previously unreported homozygous mutation in *SLC19A3*. Given the rarity of the condition, the prevalence of BTBGD remains unknown. We calculated its prevalence based on next generation sequencing data collected in the Exome Aggregation Consortium (ExAC), a cohort of 60,706 exomes (Lek et al., 2016) obtained from unrelated individuals, not ascertained for BTBGD. The calculated prevalence demonstrates that BTBGD remains likely underdiagnosed.

## 2 | PATIENT AND METHODS

### 2.1 | Clinical report

The patient was born at 41 weeks' gestation after an uneventful pregnancy and delivery. Over the course of the first 2 years of life, she was noted to be delayed, as she did not sit up unsupported until 10 months of age, and did not start walking until 2 years of age. A neurological exam obtained at 2 years and 5 months old revealed truncal hypotonia with appendicular hypertonia, with a wide-based stiff gait and hyperreflexia of lower extremities without clonus. Further recommended workup included a normal chromosome microarray, plasma amino acids, urine organic acids, and plasma acylcarnitine profile. Lactate concentration was 18.4 mg/dl (2.04 mmol/L, reference range: 9–16 mg/dl), while pyruvate was normal at 0.84 mg/dl (0.095 mmol/L, ref: 0.3–1.5 mg/dl), for a lactate-to-pyruvate molar ratio of 21 (normal: 10–20). Baseline brain magnetic resonance imaging (MRI) and MRS were performed at 2 years and 6 months (see Neuroimaging section below), and an electroencephalogram performed at 2 years and 8 months was normal.

At 3 years and 1 month the patient was admitted with a 1-week history of intermittent subjective fever and emesis 4–5 times a day, followed by a 1-day history of decreased alertness and gait instability. Her lactate and pyruvate levels were normal one day after hospital admission, with a lactate of 1.5mmol/L (ref: 1–2.4), a pyruvate of 0.87 mg/dl (0.099mmol/L), and a molar ratio of 15.2. A lumbar puncture on hospital day 4 was unrevealing; neurotransmitters were sent showing low CSF tetrahydrobiopterin at 10 nmol/L (ref: 18–50), but normal neopterin at 20 nmol/L (ref: 7–65) and normal

5-methyltetrahydrofolate at 79 nmol/L (ref: 40–150). The CSF pyruvate was markedly elevated at >3 mg/dl (>0.34 mmol/L; ref: 0.54–1.7 mg/dl), lactate 2.8 mmol/L (ref: 1.11–2.44), and a molar ratio of <8.5, which was reported as compatible with pyruvate dehydrogenase deficiency. CSF amino acid concentrations were unremarkable, including a normal alanine level of 19.5 umol/L (ref: 12.6–34.7). On hospital day 5 the patient developed hypoxemia and impending respiratory failure requiring intubation. She also had moderately decreased left ventricular systolic function with severe septal hypokinesis, with a fraction of shortening of 17.3 % (ref: 28–40%) and an ejection fraction of 45.7 % (ref: 55–70%), requiring pressors. At this point, her lactate levels peaked at 13.78 mmol/L, and a new brain MRI with MRS was obtained that same day (see below).

She had a 7-week hospital stay, during which she developed other complications including seizures, responsive to levetiracetam. She eventually required a tracheostomy, and was discharged on minimal ventilator settings. Of note, a dual genome sequencing panel—including all 37 mitochondrial genes and over 100 nuclear genes encoding mitochondrial proteins—was obtained during this admission, and no variants were found.

When seen for follow-up at the age of 3 years and 5 months, the patient remained non-verbal. After the diagnosis of BTBGD was made via whole exome sequencing, she was placed on thiamine 750 mg/day (25 mg/kg/d) and biotin 30 mg/d (1 mg/kg/d), with no further metabolic decompensations by the age of 5 years and 1 month.

## 2.2 | Neuroimaging

Brain MRI and MRS were initially performed at 2 years and 6 months of life on a 3T magnet (General Electric, Milwaukee, WI). The following pulse sequences of the brain were acquired: sagittal spoiled gradient echo (SPGR) T1WI, axial T2WI, axial T2 FLAIR, axial susceptibility weighted angiography (SWAN), coronal fat-saturated T2WI, and axial diffusion tensor imaging (DTI) with seven non-collinear directions of encoding. Single voxel MRS was performed with a  $2 \times 2 \times 2$  cm voxel over the left cerebral deep gray nuclei (TR 1500; TE 35 and 288 msec).

A follow-up brain MRI was performed at age 3 years and 2 months (3T MRI; General Electric) with similar sequences. Axial arterial spin-labeling (ASL) perfusion images and coronal high resolution T2WI through the hippocampi were also acquired. Single voxel MRS was performed with a  $2 \times 2 \times 2$  cm voxel over the left cerebral deep gray nuclei (TR 1500; TE 144 msec) and left parietal white matter (TR 1500; TE 35 and 144 msec).

## 2.3 | Pyruvate dehydrogenase enzyme activity

Pyruvate dehydrogenase (PDH) complex activity was assayed at 3 years and 9 months in fresh isolated blood lymphocytes by measuring the decarboxylation of  $1\text{-}^{14}\text{C}$ -pyruvate in the presence of thiamine pyrophosphate,  $\text{NAD}^+$  and coenzyme A—both after activation with dichloroacetate and inactivation with fluoride—as previously described (Kerr et al., 1987; Sheu, Hu, & Utter, 1981).

## 2.4 | Whole exome sequencing (WES)

WES was obtained at a commercial lab at the age of 3 years and 9 months. Briefly, fragmented DNA was ligated to paired-end adapters. Target enrichment was achieved as previously described (Bainbridge et al., 2011), sequencing was performed on an Illumina HiSeq platform (Illumina Inc, San Diego, CA), and short reads were aligned using the Burrows–Wheeler transform (Li & Durbin, 2010). Data analysis and interpretation was performed by using the Mercury analysis pipeline.

## 2.5 | Calculation of prevalence

We queried all published *SLC19A3* mutations in the literature, as well as performed a gene search of the Human Gene Mutation Database (HGMD<sup>®</sup>) (Stenson et al., 2014). The pathogenicity of these variants was assessed by reviewing publications with clinical and/or functional data. To be considered pathogenic, the variant had to be reported in a patient with a classical phenotype who had biallelic variants in *SLC19A3*.

The Exome Aggregation Consortium (ExAC) was used to obtain the allele frequencies of the *SLC19A3* pathogenic variants, and it also allowed us to identify potentially pathogenic variants that may be predicted damaging to *SLC19A3* function, but that have not been described in the medical literature. ExAC is a database of 121,412 alleles from 60,706 unrelated individuals (Lek et al., 2016), including 33,370 non-Finnish European individuals, 8,256 South Asians, 5,789 individuals of Latino descent, 5,203 individuals of African or African-American descent, 4,327 East Asians, 3,307 persons of Finnish heritage, and 454 people of other ethnicity. In order to perform a bioinformatic analysis of variant potential pathogenicity, we queried ExAC for canonical splice site, missense, frameshift, nonsense (stop gain), and stop loss mutations. None of the cohorts or consortia in ExAC includes patients ascertained for the presence of BTBGD, so we considered them to be unbiased with respect to variation in the *SLC19A3* gene. Variants were excluded if: (1) they had a minor allele frequency of >1% in any population; (2) they were found in sites covered in fewer than 80% of individuals, as this may indicate a low-quality site; (3) they were only present in a non-canonical transcript; and (4) they were in an untranslated region. Variants that passed these filters were evaluated for their potential to alter protein function by using in silico prediction models: PolyPhen-2 (Adzhubei et al., 2010), SIFT (Kumar, Henikoff, & Ng, 2009), and the Combined Annotation-Dependent Depletion (CADD) score (Kircher et al., 2014). Unpublished variants predicted to be benign by one or more in silico models were not considered for the calculation of prevalence. For likely pathogenic variants, such as frameshift, stop-gain (non-sense), and canonical splice site mutations, only the CADD Phred-scaled score was provided.

The carrier frequency was calculated as the number of individuals carrying an *SLC19A3* variant known or predicted to alter protein function, divided by the total number of individuals ascertained. The prevalence of the disease was calculated based on the carrier frequency and/or the allele frequency using Hardy–Weinberg equilibrium. The Hardy–Weinberg principle establishes that  $p^2 + 2pq + q^2 = 1$ , with  $p$  being the reference allele frequency,  $q$  being the minor allele frequency,  $2pq$  the carrier frequency, and  $q^2$  the frequency of the disease.

## 3 | RESULTS

### 3.1 | Neuroimaging findings

Brain MRI and MRS at 2 years and 6 months (Figure 1a–d): small hyperintense lesions were present in the putamen and right caudate head that demonstrated T1 and T2 prolongation, partial T2 FLAIR signal suppression, and facilitated diffusion without mass effect, consistent with chronic areas of encephalomalacia, and necrosis. Trace susceptibility was present in associated putaminal lesions, likely representing hemosiderin from prior hemorrhage. Brain parenchyma was otherwise normal in signal intensity for age. Bilateral hippocampal dysgenesis was also noted; the hippocampi were globular and dysmorphic in appearance. MRS of the left basal ganglia demonstrated normal metabolic ratios for age. No abnormal pyruvate or lactate was detected.

Brain MRI and MRS at 3 years and 2 months (Figure 1e–h): multiple new brain lesions intervally developed in the striatum, thalami, hypothalami, midbrain, pons, and medulla oblongata. The new lesions in the striatum and thalami demonstrated hyperintense signal on T2WI, mass effect, and mixed restricted and facilitated diffusion representing a combination of cytotoxic and vasogenic edema. The remainder of the brain was normal in signal. MRS of the left basal ganglia revealed moderately decreased NAA to creatine ratio, consistent with neuronal loss, and a moderate lactate peak. MRS of the left parietal white matter demonstrated an elevated peak at 2.4 ppm, consistent with pyruvate. Although protons associated with glutamine and glutamate (Glx) co-resonate in this location, adjacent peaks associated with these protons were not elevated and there was no elevation Glx alpha proton at 3.8 ppm to support glutamine and/or glutamate elevation.

### 3.2 | PDH enzyme testing

The presence of a pyruvate peak on brain MRS had previously been reported in a case of PDH deficiency (Zand et al., 2003). This, in addition to the elevated CSF pyruvate—above the limit of quantitation—with a decreased lactate-to-pyruvate ratio raised suspicion for PDH deficiency. Pyruvate dehydrogenase complex activity in lymphocytes was thus obtained, but it revealed an activity of 3.28 nmol/min/mg protein (ref: 0.98–2.72), relatively high in reference to concurrent and prior controls, corresponding to 201% of the control mean. The assay showed good activation and inactivation after pre-incubation with dichloroacetate and fluoride, respectively. The activity of dihydrolipoamide dehydrogenase (E3) was within the reference range, and the ratio of PDC/E3 was 3.6 (ref: 1.41–3.55), also above the reference range at 156%. Thus, the activity was relatively high both relative to total cell protein and to the activity of the internal mitochondrial reference enzyme, E3.

### 3.3 | Whole exome sequencing

A homozygous variant of unknown significance was detected in the *SLC19A3* gene (NM\_025243.3:c.416T>A; p.Val139Glu). Both parents were noted to be heterozygous for this change. This variant is exceedingly rare, as it was not found in ExAC. It is also well conserved through evolution (see Supplementary Figure S1), and prediction models suggest this change is probably damaging (Polyphen-2) or deleterious (SIFT), with a Phred-scaled CADD score of 27.1. In addition, a novel de novo heterozygous frameshift variant

was detected in the *DDX3X* gene (NM\_001356.3:c.14\_17delCAGT; p.Ala5Glyfs\*14), associated with X-linked dominant intellectual disability (OMIM#300958) (Snijders Blok et al., 2015).

### 3.4 | Calculation of prevalence

The allele count for known pathogenic variants was 19 (see Table 1), while the allele count for predicted pathogenic variants was 243 (see Table 2), for a total of 262 variants found in 121,412 alleles. The allele frequency is thus 0.216%, or 1 in 463, for a carrier frequency of 1 in 232 individuals in the general population. The estimated prevalence of the disease is thus calculated as 1 in 214,744 individuals.

## 4 | DISCUSSION

In the present paper we describe a novel mutation in the *SLC19A3* gene, which we deem pathogenic given the frequency, conservation and in silico prediction data, but more importantly because it is consistent with the patient's clinical phenotype and characteristic brain MRI findings. BTBGD is a treatable genetic condition characterized by episodic encephalopathy, often triggered by febrile illness. It is caused by mutations in the *SLC19A3* gene, encoding the human thiamine transporter 2 (hTHTR-2), a high-affinity thiamine transporter (Rajgopal, Edmondson, Goldman, & Zhao, 2001), but not a biotin transporter (Subramanian, Marchant, & Said, 2006). It is thus intriguing that the disease was initially described as being responsive to biotin (Ozand et al., 1998), but this is likely related to the fact that biotin increases the expression of *SLC19A3*, and individuals with biotin deficiency will only show 33% gene expression as compared to controls, ranging from 16 to 70% (Vlasova, Stratton, Wells, Mock, & Mock, 2005). However, a recent open-label, prospective, comparative study showed that the combination of thiamine plus biotin was not superior to thiamine alone in terms of number of recurrences, neurologic sequelae or brain MRI findings, but it was associated with decreased duration of the acute crises (Tabarki et al., 2015). Indeed, it is known that normal cells exhibit upregulation of *SLC19A3* expression under situations of stress, but this adaptive stress-induced upregulation of gene expression is lost in patients with BTBGD (Schänzer et al., 2014). Since about 48% of the intestinal transport of thiamine is mediated by hTHTR-2 (Said, Balamurugan, Subramanian, & Marchant, 2004), and because *SLC19A3* is also expressed at the blood–brain barrier (Geier et al., 2013), large doses of thiamine should be administered in order to overcome the transport block. In addition, drugs that are known to inhibit hTHTR-2—such as metformin, famotidine, chloroquine, and verapamil—carry the theoretical risk of worsening the condition, and thus should be avoided in these patients (Liang et al., 2015).

The prevalence of the disease was previously unknown, but it is reportedly very rare based on the fact that there have been only 116 case reports published in the literature to date. We sought to generate an accurate estimate of disease prevalence based on the carrier frequencies of pathogenic and potentially pathogenic variants in a large cohort that was not selected for the presence of BTBGD, and should thus be free of ascertainment bias. We found a surprisingly high birth prevalence of about 1 in 215,000 individuals. It should be noted that this estimated prevalence corresponds to all forms of the disease, regardless of

its severity—ranging from early infantile lethal Leigh syndrome to late-onset Wernicke-like encephalopathy. Based on this calculated prevalence, there should be about 1,500 individuals with this disease in the United States alone; the fact that the number of patients is much lower indicates that the disease remains largely underdiagnosed.

Since the disease is treatable but underdiagnosed, new diagnostic clues for BTBGD would be welcome. We describe the presence of a pyruvate peak on brain MRS during an episode of acute encephalopathy. It should be noted that pyruvate dehydrogenase is one of the three main mitochondrial enzymes that require thiamine as a cofactor. The presence of a high CSF pyruvate, low CSF lactate-to-pyruvate molar ratio, and high intracerebral pyruvate as assessed by brain MRS all point toward a deficiency of PDH *in vivo*. The measured activity of PDH *in vitro* was likely not decreased because it is measured in the presence of added thiamine pyrophosphate (TPP); in fact, the PDH enzyme activity in our patient was elevated. It should be noted that pyruvate and succinate both peak around 2.4 ppm on brain MRS (Chawla, Kumar, & Gupta, 2004), and thus a diagnosis of succinate dehydrogenase (SDH) deficiency should be entertained when such a peak is found (Helman et al., 2016). However, when a tall 2.4 ppm peak is found, SDH deficiency can be readily excluded by a normal urine organic acid analysis, while PDH deficiency can be excluded by normal—or even increased—enzyme activity in cells in the presence of TPP.

It is also interesting to note that our patient was found to have two separate conditions, BTBGD and X-linked dominant intellectual disability due to *DDX3X* mutations. Such blended phenotypes resulting from two unrelated genetic conditions coexisting in the same individual were found in 4.6% of positive cases in a study of 2,000 patients in whom clinical exome sequencing was performed (Yang et al., 2014).

In summary, we identified a novel mutation in *SLC19A3*, causative of biotin-thiamine responsive basal ganglia disease. Based on the calculated prevalence of the disease, it is very likely that the condition remains underdiagnosed. A pyruvate peak identified by brain MRS during episodes of acute encephalopathy might aid in the identification and earlier diagnosis of this treatable inborn error of metabolism.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENTS

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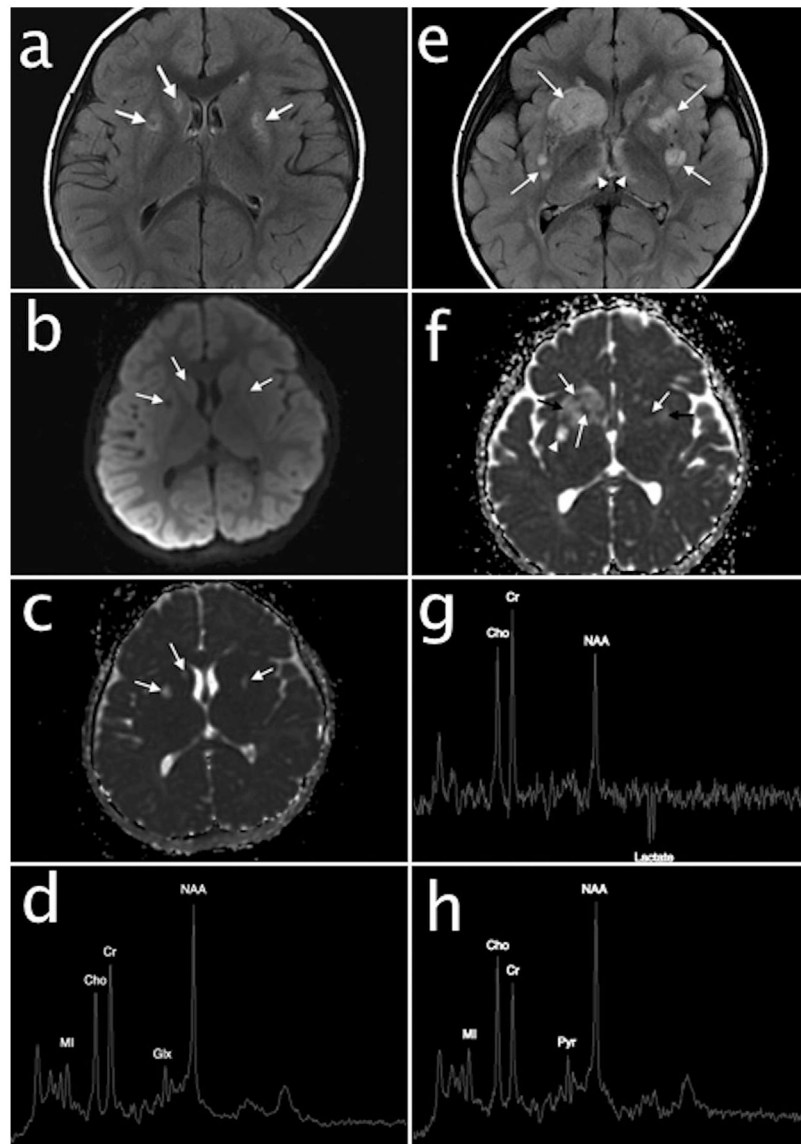
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**FIGURE 1.**

Baseline brain MRI and MRS performed at 2 years and 6 months. Axial T2 FLAIR image (a) and diffusion weighted images (b and c) demonstrates hyperintense lesions in the putamen and right caudate head with partial FLAIR signal suppression (arrows, a) and facilitated diffusion (arrows, b and c) consistent with areas of chronic encephalomalacia and necrosis. Short echo single voxel MRS (d) over the basal ganglia shows normal metabolic ratios; no abnormal pyruvate or lactate are present. Follow-up brain MRI and MRS at 3 years and 2 months. Axial T2 FLAIR (e) demonstrates acute on chronic cerebral deep gray nuclear lesions with new hyperintense lesions in the striatum (arrows) and medial thalami (arrowheads). Mixed diffusion abnormalities in the lesions (arrows, f) represent a combination of cytotoxic edema (restricted diffusion, white arrows), vasogenic edema (facilitated diffusion with mass effect, black arrows), and encephalomalacia/necrosis (facilitated diffusion without mass effect, arrowhead). Intermediate echo single voxel MRS

(g) over the basal ganglia reveals an inverted lactate doublet at 1.3 ppm consistent with anaerobic metabolism and a decreased NAA to creatine ratio consistent with neuronal loss. Short echo MRS (h) over the left parietal white matter demonstrates an unusually prominent peak at 2.4 ppm consistent with pyruvate (Pyr). NAA, N-acetylaspartate; Cho, choline; Cr, creatine; MI, myoinositol

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TABLE 1

Known pathogenic variants in *SLC19A3*

Genomic coordinate (GRCh37/hg19)	cDNA (NM_025243.3)	Protein (NP_079519.1)	dbSNP ID	Type of mutation	In silico prediction					Reference	
					Polyphen-2	SIFT	CADD Phred	Allele number	Allele count		Allele frequency
chr2:228,568,440–228,613,489	–	–	–	Promoter deletion	–	–	–	–	–	–	Fjølves et al. (2016)
chr2:228567015	c.20C>A	p.Ser7*	–	Stop-gain	–	–	35	–	–	–	Gerards et al. (2013)
chr2:228566967	c.68G>T	p.Gly23Val	rs121917882	Missense	Probably damaging	Deleterious	28.1	121372	1	0.0008239%	Kevelam et al. (2013); Pérez-Dueñas et al. (2013); Zeng et al. (2005)
chr2:228566960	c.74dupT	p.Ser26Leufs*19	–	Frameshift	–	–	35	–	–	–	Debs et al. (2010); Sremba, Chang, Elbalalesy, Cambray-Forker, & Abdenur, (2014)
chr2:228566953–228566954	c.81_82dupGA	p.Met28Argfs*2	–	Frameshift	–	–	29.6	121374	2	0.001648%	Sremba et al. (2014)
chr2:228566905	c.130A>G	p.Lys44Glu	rs137852957	Missense	Possibly damaging	Deleterious	25.5	–	–	–	Kono et al. (2009); Miyajima and Kono (2010)
chr2:228564094	C.337T>C	p.Tyr113His	rs145999922	Missense	Probably damaging	Deleterious	25.5	121402	7	0.005766%	Fjølves et al. (2016)
chr2:228563967	c.464C>T	p.Ser155Leu	–	Missense	Probably damaging	Deleterious	27.3	–	–	–	Kohrogi et al. (2015)
chr2:228563924	c.507C>G	p.Tyr169*	–	Stop-gain	–	–	35	–	–	–	Kevelam et al. (2013)
chr2:228563914	c.517A>G	p.Asn173Asp	–	Missense	Probably damaging	Tolerated	18.23	–	–	–	Fassone et al. (2013)
chr2:228563904	c.527C>A	p.Ser176Tyr	–	Missense	Probably damaging	Deleterious	22.3	121400	1	0.00082%	Kevelam et al. (2013)
chr2:228563890	C.541T>C	p.Ser181Pro	–	Missense	Probably damaging	Tolerated	22.1	121404	11	0.00906%	Fjølves et al. (2016); Kevelam et al. (2013)
chr2:228563506–228563536	c.895_925del31	p.Val299Profs*11	–	Frameshift	–	–	35	–	–	–	Kevelam et al. (2013)

Genomic coordinate (GRCh37/hg19)	cDNA (NM_025243.3)	Protein (NP_079519.1)	dbSNP ID	Type of mutation	In silico prediction					Reference	
					Polyphen-2	SIFT	CADD Phred	Allele number	Allele count		Allele frequency
chr2:228563473	c.958G>C	p.Glu320Gln	-	Missense	Probably damaging	Deleterious	29.7	-	-	-	Kono et al. (2009); Miyajima and Kono (2010); Yamada et al. (2010)
chr2:228560811	c.980-14A>G	-	rs200542114	Exon 4 skipping	-	-	0.223	120582	3	0.002488%	Debs et al. (2010)
chr2:228560795	c.982delG	p.Ala328Leufs*10	-	Frameshift	-	-	35	-	-	-	Haack et al. (2014)
chr2:228560623	c.1154T>G	p.Leu385Arg	rs563607795	Missense	Probably damaging	Deleterious	33	121338	1	0.0008241%	Kevelam et al. (2013)
chr2:228552882-228553023	r.1173_1314del	p.Gln393*	-	Exon 5 deletion	-	-	-	-	-	-	Kevelam et al. (2013)
chr2:228553000	c.1196A>T	p.Asn399Ile	-	Missense	Benign	Deleterious	24	-	-	-	Kohrogi et al. (2015)
chr2:228552932	c.1264A>G	p.Thr422Ala	rs121917884	Missense	Benign	Deleterious	23.5	-	-	-	Alfadhel et al. (2013); Distelmaier et al. (2014); Zeng et al. (2005)
chr2:228552272	c.1332C>G	p.Ser444Arg	-	Missense	Possibly damaging	Deleterious	26.3	-	-	-	Kevelam et al. (2013)

TABLE 2

## Predicted pathogenic variants in SLC19A3

Genomic coordinate (GRCh37/hg19)	cDNA (NM_025243.3)	Protein (NP_079519.1)	dbSNP ID	Type of mutation	Polyphe-2	SIFT	CADD Phred	Allele number	Allele count	Allele frequency (%)
chr2:228566999	c.36G>C	p.Trp12Cys	-	Missense	Probably damaging	Deleterious	32	121308	1	0.0008243
chr2:228566979	C.56T>C	p.Leu19Pro	rs142553867	Missense	Probably damaging	Deleterious	27.9	121356	2	0.001648
chr2:228566967	c.68G>A	p.Gly23Asp	rs121917882	Missense	Probably damaging	Deleterious	26.9	121372	1	0.0008239
chr2:228566935	c.100T>G	p.Phe34Val	rs201603239	Missense	Probably damaging	Deleterious	29.8	121370	3	0.002472
chr2:228566923	c.112T>C	p.Tyr38His	rs368305641	Missense	Possibly damaging	Deleterious	25.9	121356	2	0.001648
chr2:228566922	c.113A>G	p.Tyr38Cys	-	Missense	Probably damaging	Deleterious	25	121358	1	0.000824
chr2:228566883	c.150 + 2T>C	-	-	Canonical splice	-	-	23.3	121304	1	0.0008244
chr2:228564282	c.151-2A>T	-	-	Canonical splice	-	-	23.2	120730	1	0.0008283
chr2:228564259	c.172G>T	p.Val58Phe	rs200412423	Missense	Probably damaging	Deleterious	23.8	121114	1	0.0008257
chr2:228564244	c.187T>C	p.Tyr63His	rs144817990	Missense	Probably damaging	Deleterious	24.3	121286	14	0.01154
chr2:228564225	c.206C>T	p.Pro69Leu	-	Missense	Probably damaging	Deleterious	25.6	121356	1	0.000824
chr2:228564223	c.208G>C	p.Val70Leu	-	Missense	Probably damaging	Deleterious	25.7	121356	2	0.001648
chr2:228564216	c.215T>C	p.Val72Ala	-	Missense	Possibly damaging	Deleterious	25.4	121382	1	0.0008238
chr2:228564214	c.217C>T	p.Leu73Phe	rs373943165	Missense	Possibly damaging	Deleterious	25.2	121384	2	0.001648
chr2:228564189	c.242C>T	p.Pro81Leu	-	Missense	Probably damaging	Deleterious	27.5	121402	2	0.001647
chr2:228564177	C.254T>C	p.Leu85Ser	-	Missense	Possibly damaging	Deleterious	22.7	121404	1	0.0008237
chr2:228564126	c.305G>A	p.Gly102Glu	-	Missense	Probably damaging	Deleterious	31	121412	1	0.0008236

Genomic coordinate (GRCh37/hg19)	cDNA (NM_025243.3)	Protein (NP_079519.1)	dbSNP ID	Type of mutation	Polyphen-2	SIFT	CADD Phred	Allele number	Allele count	Allele frequency (%)
chr2:228564112	c.319C>A	p.Gln107Lys	-	Missense	Probably damaging	Deleterious	27.7	121410	1	0.0008237
chr2:228564098	c.333C>A	p.Phe111Leu	-	Missense	Probably damaging	Deleterious	26.5	121398	1	0.0008237
chr2:228564087	C.344T>C	p.Met115Thr	-	Missense	Possibly damaging	Deleterious	23.5	121394	1	0.0008238
chr2:228564085	c.346G>A	p.Val116Ile	-	Missense	Possibly damaging	Deleterious	25.2	121388	1	0.0008238
chr2:228564082	c.349A>G	p.Thr117Ala	-	Missense	Possibly damaging	Deleterious	23.2	121370	1	0.0008239
chr2:228564058	c.373G>C	p.Ala125Pro	-	Missense	Probably damaging	Deleterious	29.8	121342	5	0.004121
chr2:228564054	c.377A>C	p.Tyr126Ser	-	Missense	Probably damaging	Deleterious	28.5	121334	1	0.0008242
chr2:228564050	c.381A>G	p.Ile127Met	-	Missense	Probably damaging	Deleterious	20.1	121330	1	0.0008242
chr2:228564048	c.383A>G	p.Tyr128Cys	-	Missense	Probably damaging	Deleterious	26	121330	15	0.01236
chr2:228564021	c.410A>C	p.Gln137Pro	-	Missense	Probably damaging	Deleterious	25.9	121288	2	0.001649
chr2:228564021	c.410dup	p.Arg138GlufsTer87	-	Frameshift	-	-	33	121272	2	0.001649
chr2:228564021	c.410A>G	p.Gln137Arg	-	Missense	Possibly damaging	Tolerated	23	121288	1	0.0008246
chr2:228564019	c.412A>G	p.Arg138Gly	rs201318094	Missense	Possibly damaging	Deleterious	24.9	121268	1	0.0008246
chr2:228564006	c.425A>G	p.Tyr142Cys	-	Missense	Possibly damaging	Deleterious	25.1	121226	1	0.0008249
chr2:228563965	c.466G>A	p.Val156Met	-	Missense	Probably damaging	Deleterious	25.3	121336	1	0.0008242
chr2:228563949	C.482T>C	p.Leu161Ser	-	Missense	Probably damaging	Deleterious	24.4	121352	1	0.000824
chr2:228563928	c.503C>G	p.Ser168Trp	rs139611208	Missense	Probably damaging	Deleterious	24.2	121382	1	0.0008238
chr2:228563911	c.520G>T	p.Val174Phe	rs59736804	Missense	Possibly damaging	Deleterious	26.4	121396	1	0.0008238
chr2:228563889	c.542C>T	p.Ser181Phe	-	Missense	Possibly damaging	Deleterious	21.8	121404	1	0.0008237

Genomic coordinate (GRCh37/hg19)	cDNA (NM_025243.3)	Protein (NP_079519.1)	dbSNP ID	Type of mutation	Polyphen-2	SIFT	CADD Phred	Allele number	Allele count	Allele frequency (%)
chr2:228563839	c.592T>C	p.Phe198Leu	rs370725962	Missense	Probably damaging	Deleterious	29.2	121332	1	0.0008242
chr2:228563838	c.593T>C	p.Phe198Ser	-	Missense	Probably damaging	Deleterious	28.6	121334	1	0.0008242
chr2:228563832	c.599A>G	p.His200Arg	-	Missense	Probably damaging	Deleterious	24.5	121316	1	0.0008243
chr2:228563646	c.785T>C	p.Phe262Ser	-	Missense	Possibly damaging	Deleterious	25	121402	4	0.003295
chr2:228563614	c.817T>G	p.Tyr273Asp	-	Missense	Probably damaging	Deleterious	29.3	121404	1	0.0008237
chr2:228563577	c.854G>A	p.Trp285Ter	rs373198092	Stop-gain	-	-	40	121404	1	0.0008237
chr2:228563538	c.893A>G	p.Tyr298Cys	rs370104837	Missense	Possibly damaging	Deleterious	25.7	121326	2	0.001648
chr2:228563515	c.916A>T	p.Lys306Ter	-	Stop-gain	-	-	37	121028	1	0.0008263
chr2:228563476	c.955G>A	p.Val319Ile	rs372511010	Missense	Probably damaging	Deleterious	34	119876	2	0.001668
chr2:228560774	c.1003G>C	p.Gly335Arg	-	Missense	Probably damaging	Deleterious	34	121348	1	0.0008241
chr2:228560744	c.1033G>A	p.Gly345Arg	-	Missense	Probably damaging	Deleterious	35	121392	2	0.001648
chr2:228560725	c.1052T>A	p.Val351Asp	-	Missense	Possibly damaging	Deleterious	24.4	121402	1	0.0008237
chr2:228560723	c.1054T>G	p.Phe352Val	-	Missense	Possibly damaging	Deleterious	25	121402	1	0.0008237
chr2:228560707	c.1070C>T	p-Ala357Val	-	Missense	Possibly damaging	Deleterious	25.8	121398	7	0.005766
chr2:228560689	c.1088T>C	p.Met363Thr	-	Missense	Probably damaging	Deleterious	26.4	121402	2	0.001647
chr2:228560662	c.1115G>A	p.Cys372Tyr	-	Missense	Probably damaging	Deleterious	32	121390	3	0.002471
chr2:228560623	c.1154T>C	p.Leu385Pro	rs563607795	Missense	Probably damaging	Deleterious	33	121338	1	0.0008241
chr2:228560615	c.1162A>T	p.Thr388Ser	-	Missense	Probably damaging	Deleterious	29	121322	1	0.0008243
chr2:228560611	c.1166T>C	p.Ile389Thr	rs149282475	Missense	Possibly damaging	Deleterious	29.6	121308	1	0.0008243
chr2:228553024	c.1173-1G>A	-	rs531876613	Canonical splice	-	-	26.3	119782	1	0.0008248



Genomic coordinate (GRCh37/hg19)	cDNA (NM_025243.3)	Protein (NP_079519.1)	dbSNP ID	Type of mutation	Polyphen-2	SIFT	CADD Phred	Allele number	Allele count	Allele frequency (%)
chr2:228553013	c.1183G>T	p.-Ala395Ser	-	Missense	Probably damaging	Deleterious	34	120494	1	0.0008299
chr2:228553012	c.1184C>T	p.-Ala395Val	-	Missense	Possibly damaging	Deleterious	34	120506	1	0.0008298
chr2:228552992	c.1204C>T	p.Arg402Cys	rs150523975	Missense	Possibly damaging	Deleterious	31	120836	11	0.009103
chr2:228552992	c.1204C>G	p.Arg402Gly	rs150523975	Missense	Probably damaging	Deleterious	34	120836	1	0.0008276
chr2:228552991	c.1205G>A	p.Arg402His	-	Missense	Possibly damaging	Deleterious	34	120852	3	0.002482
chr2:228552981	c.1215G>T	p.Leu405Phe	-	Missense	Possibly damaging	Deleterious	27.6	120918	1	0.000827
chr2:228552961	c.1235T>C	p.Phe412Ser	-	Missense	Probably damaging	Deleterious	31	121022	1	0.0008263
chr2:228552944	c.1252C>G	p.Gln418Glu	rs368364131	Missense	Probably damaging	Deleterious	27.8	121048	83	0.06857
chr2:228552934	c.1262T>G	p.Met421Arg	-	Missense	Possibly damaging	Deleterious	24.8	121042	1	0.0008262
chr2:228552931	c.1265C>T	p.Thr422Ile	-	Missense	Probably damaging	Deleterious	28.5	121046	1	0.0008261
chr2:228552920	c.1276G>A	p.Val426Ile	-	Missense	Possibly damaging	Deleterious	27.4	120990	3	0.00248
chr2:228552905	c.1291C>T	p.Leu431Phe	-	Missense	Probably damaging	Deleterious	28.6	120926	1	0.000827
chr2:228552881	c.1314 + 1G>A	-	rs141957107	Canonical splice	-	-	25.8	120800	1	0.0008278
chr2:228552279	c.1325A>T	p.Tyr442Phe	-	Missense	Probably damaging	Deleterious	32	120246	3	0.002495
chr2:228552255	c.1349C>T	p.-Ala450Val	-	Missense	Possibly damaging	Deleterious	33	121132	1	0.0008255
chr2:228552255	c.1349C>G	p.-Ala450Gly	-	Missense	Possibly damaging	Deleterious	29.5	121132	3	0.002477
chr2:228552249	c.1355T>C	p.Ile452Thr	rs140706520	Missense	Possibly damaging	Deleterious	26.8	121202	4	0.0033
chr2:228552248	c.1356delA	p.Ile452PhefsTer3	rs140706520	Frameshift	-	-	34	121202	1	0.0008251
chr2:228552233	c.1371C>A	p.Ser457Arg	rs200054205	Missense	Possibly damaging	Deleterious	24.4	121328	3	0.002473
chr2:228552129	c.1475dup	p.Met492.AsnfsTer27	rs192876801	Frameshift	-	-	23.8	121118	1	0.0008256