Girls with Seizures Due to the c.320A>G Variant in *ALG13* Do Not Show Abnormal Glycosylation Pattern on Standard Testing

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Received: 30 October 2014 / Revised: 05 January 2015 / Accepted: 28 January 2015 / Published online: 03 March 2015
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Abstract A girl with early onset severe epilepsy, developmental delay, intellectual disability, visual maturation delays, and feeding problems was without a diagnosis despite an extensive genetic and metabolic evaluation. She initially manifested infantile spasms which responded to high-dose ACTH. Seizures seemed to resolve, but then at age 5, she developed complex partial seizures resistant to antiepileptics that responded to a ketogenic diet. Additional features included visual impairment, hypotonia, reflux, and severe feeding problems requiring a G-tube. She was referred to the Geisinger Health System whole-genome sequencing clinical research program. A variant in the X-linked gene *ALG13* (c.320A>G p. 107 N->S) was identified. Four additional girls from three published exome sequencing studies were found to have the identical c.320A>G variant in *ALG13*. All presented with early onset severe epilepsy and intellectual disability. Three of the five exhibited visual impairment and possible developmental regression. A boy with a variant in *ALG13* presented with a severe congenital disorder of glycosylation type Is. Glycosylation studies in the case reported here were normal; none of the other girls reported in the literature have had glycosylation studies. X-inactivation studies have not been done. The N107 residue and the surrounding

region – MNNHQ – are highly conserved across species and are found in a presumed functional domain of this glycotransferase superfamily. The consistent clinical presentation of a severe phenotype in girls coupled with identical variants in an X-linked gene strongly suggests a critical position effect. Negative glycosylation studies in one individual suggest the possibility of a new mechanism requiring investigation.

Introduction

Over 100 congenital disorders of glycosylation (CDG) have been described (Freeze et al. 2014). These are generally grouped into disorders of *N*-glycosylation, disorders of *O*-glycosylation, and disorders of the Glycosylphosphatidylinositol-Anchor Pathway (Freeze et al. 2014). Timal et al. (2012) reported a boy with refractory epilepsy with polymorphic seizures, hepatomegaly, horizontal nystagmus, optic nerve atrophy, susceptibility to infections, a bleeding diathesis, swelling of the eyelids and distal extremities, and extrapyramidal and pyramidal signs. He died at 1 year of age. Biochemical analysis identified a CDG with a metabolic signature consistent with a defect in *N*-glycosylation type Is (aka CDG-ALG13). Exome sequencing identified a 280A>G transition in the *ALG13* gene, resulting in a lys 94-to-glu (K94E) substitution at a highly conserved residue in the C-terminal glycosyltransferase domain thought to be causal for the CDG. Cell studies demonstrated only 17% residual enzyme activity of UDP-GlcNAc transferase. The variant was inherited from his unaffected mother. *ALG13* is located on the X-chromosome and encodes the protein ALG13 which forms the UDP-GlcNAc transferase with ALG14 and catalyzes a key step in endoplasmic reticulum N-linked glycosylation (Averbeck

Communicated by: Robert Steiner

Competing interests: None declared

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et al. 2007). To date, this is the only CDG reported due to a variant in *ALG13*. Bissar-Tadmouri et al. (2014) identified a novel missense variant, 3221A>G in *ALG13* (p.Y1074C), that segregated with a non-syndromic X-linked intellectual disability disorder family with four affected boys. No additional clinical information was provided in the report. The mother was heterozygous for the variant and was normal. X-inactivation studies in the mother were normal. Glycosylation studies were not performed for these boys.

We report a case of a girl with a severe epileptic encephalopathy identified with a variant in *ALG13* and review the literature relative to this variant.

Case Report and Literature Review

A 7-year-old girl was referred to the Geisinger Health System IRB-approved whole-genome sequencing clinical research project focused on children with undiagnosed intellectual disability. Parents underwent genetic counseling and were consented for participation including return of primary and secondary findings and reporting of deleterious variants.

This child was the product of a pregnancy complicated by cholecystectomy at 13 weeks gestation, several urinary tract infections, and dehydration. During the pregnancy, her mother took multiple medications at various times (albuterol, budesonide, fluticasone propionate + salmeterol, fexofenadine [after first trimester], promethazine HCl, oxycodone HCl, acetaminophen, propofol, fentanyl, midazolam, labetalol ondansetron HCl, atropine, neostigmine, meperidine, trimethoprim and sulfamethoxazole, melatonin, cephalexin, misoprostol). Newborn screening was normal. She was hospitalized at 2 weeks of age with RSV infection, gastroesophageal reflux, and apnea. At 5 months of age, she developed repetitive eye rolling and was initially diagnosed with delayed visual maturation. On multiple occasions, eyes were normal on examination, and she was ultimately diagnosed with cortical visual impairment. At 6 months, the parents were concerned about regression (lost cooing, responsive smile, and laugh).

Infantile spasms were diagnosed at 8 months by EEG (hypsarrhythmia was present). She was treated with ACTH with a good response. She had no clinically apparent seizures until 5 years of age although EEGs were not normal (findings included excessive, superimposed, theta frequency background activity; rare, irregular bursts of higher amplitude and somewhat sharply contoured activity seen asymmetrically over the left hemisphere; and diffuse excessive slowing) and all visual evoked response tests showed greater than the expected latency. At 5 years old, she developed complex partial seizures resistant to anti-epileptics. EEG was abnormal with irregular generalized

polyspike and wave activity, as well as left and right paroxysmal fast wave activity over the temporal regions suggesting generalized, multifocal seizures arising from right and left hemispheres. She responded well to a ketogenic diet and achieved better seizure control. She continues to experience severe reflux and significant feeding problems which required placement of a G-tube. She exhibits pica. She has severe cognitive impairment with limited expressive language; IQ is estimated to be in the range of 20–25.

She has undergone an extensive diagnostic evaluation including high-resolution chromosomes, chromosomal microarray, extensive metabolic evaluation (plasma and urine amino acids, urine organic acids, lysosomal studies, lactate, pyruvate, ammonia, very long-chain fatty acids, creatine kinase, carnitines), CSF protein, multiple MRIs, thyroid studies, hearing evaluations, and *FOXG1* sequencing, all of which were normal. A PET scan showed changes consistent with a seizure focus. CDG was not suspected clinically and no diagnostic studies were performed.

Whole-genome sequencing was performed using an Illumina platform. The sequence was analyzed using the SimulConsult Genome-Phenome Analyzer (Segal et al. 2014). Multiple variants were identified and assessed. A de novo variant in *ALG13* (c.320A->G) was initially given a low priority given that this is an X-linked gene that would not be expected to cause disease in a female heterozygote. However, a review of the literature identified four additional girls that were part of exome sequencing studies with the identical de novo c.320A->G *ALG13* variant.

The first girl was reported by de Ligt et al. (2012) from a large cohort of subjects undergoing diagnostic exome sequencing for severe intellectual disability, although the authors did not assign causality based on the available information. Subsequently, three additional girls were identified, two from the Epi4k (2013) epileptic encephalopathy exome sequencing project and one from a genetic study of infantile spasms (Michaud et al. 2014). All four girls from the three studies presented with severe early onset seizures and severely delayed psychomotor development. Hypotonia and feeding problems were noted in all but the Michaud patient (limited clinical information presented). Three of the girls (Epi4k et al. 2013; Michaud et al. 2014) had hypsarrhythmia on EEG. Both of the girls from the Epi4k study were reported to have normal or mildly delayed development prior to the onset of the seizures, while the de Ligt et al. patient was noted to be delayed from birth. One of the Epi4k patients responded well to high-dose ACTH but seizures returned after tapering. This girl was also reported to have no visual tracking. The de Ligt et al. patient was noted to have no visual fixation until 18 months of age. None of the four previously reported cases had CDG studies performed,

although personal communication regarding the case reported by de Ligt et al. indicated the clinical presentation "...would fit in the N107S 'epilepsy phenotype' as reported by Michaud and in the Epi4K paper" [personal communication]. Since our patient has been evaluated at multiple institutions, we confirmed that she had not been included in any of the referenced sequencing projects.

Including our patient, all five girls share features of severe early onset seizures, severe psychomotor delay and intellectual disability, and feeding problems and demonstrated hypsarrhythmia on EEG. Four of the five had possible developmental regression and abnormalities of visual development that seem to be central in nature. All five have the identical variant in *ALG13*. The authors of the Epi4k project estimated that the probability of their two patients having the same variant by chance was $p = 7.8 \times 10^{-12}$, a number that is large compared to the chance of this occurring in five unrelated patients with strikingly similar presentations.

Since CDG studies had not been performed in the reported patients, we sent a sample for transferrin isoform analysis and isoelectric focusing with affinity chromatography and mass spectrometry to a laboratory with extensive experience in diagnostic testing for CDG (tests included the mono-oligosaccharide/di-oligosaccharide transferrin ratio, the a-oligosaccharide/di-oligosaccharide transferrin ratio, the tri-sialo/di-oligosaccharide transferrin ratio, the apolipoprotein CIII-1/apolipoprotein CIII-2 ratio, and the apolipoprotein CIII-0/apolipoprotein CIII-2 ratio). These studies were normal. Specifically, no abnormalities were seen that were suggestive of a disorder of *N*-glycosylation (Table 1).

Discussion

The c.320A>G variant in *ALG13* results in a substitution of serine for asparagine at position 107 in the ALG13 protein (N107S). The N107 residue and the surrounding region – MNNHQ – are highly conserved across species (from mammals to bird to yeast) and are found in a presumed functional domain of this glycotransferase superfamily. There are numerous human splice variants of ALG13: all but one splice variant included the N107 residue at a variable distance from the C-terminus (up to 100 bp from the C-terminus).

The evidence presented strongly suggests that this variant causes the severe phenotype seen in these girls; however, the mechanism by which the variant is acting is not apparent. Potential explanations could include skewed X-inactivation or a dominant negative variant resulting in reduced ALG13 activity. However, one would expect to see evidence of the *N*-glycosylation defect as observed in the

one affected boy that underwent testing unless the skewed X-inactivation was limited to the central nervous system. Haplo-insufficiency by itself does not seem to be sufficient to explain the severe phenotype as the two heterozygous mothers reported in the literature were said to be normal although only one had been tested. However, differential expression or enzymatic requirements in different tissues could result in a tissue-specific phenotype, such as an encephalopathy as seen in these girls. Enzyme activity studies have not been performed at this time. It is possible that the variant causes a form of CDG that escapes detection by the usual laboratory evaluation. It is also possible that this variant may act through an as yet unknown mechanism.

The variant appears to be on a conserved loop deep inside the protein. This suggests it may be important for the catalytic activity of the protein, although, as previously noted, ALG13 does not appear to have any activity unless combined with ALG14. If this variant does affect the catalytic domain, it could create novel catalytic activity leading to the severe neurocognitive phenotype. Finally, there is only one splice variant that does not include this residue, so it is possible that an overabundance or imbalance of the protein product that does not include this residue could have a deleterious effect on brain development.

It is of interest that three of the five girls were reported to have normal or mildly delayed development in the first months of life prior to onset of clinical seizures, suggesting that the impact of the variant does not manifest until after birth. The defect also seems to have a significant impact on visual development without evidence of retinal or optic nerve abnormalities. Given the severity of the phenotype in the girls reported thus far and that it has not been identified in any males in published exome projects, one might presume that this variant causes embryonic lethality in males. Additional studies including CSF protein glycosylation studies, enzyme activity, neuronal cell culture, and model organism knockouts or knockdowns will be needed to elucidate the mechanism of this variant.

In conclusion, while the mechanism of action is unknown, the c.320A>G variant in *ALG13* should be sought in girls presenting with severe early onset epileptic encephalopathy and possible developmental regression, particularly if associated with delayed visual development.

Summary Sentence

Girls with the c.320A>G variant in *ALG13* do not have laboratory evidence of congenital disorder of glycosylation type Is on standard testing suggesting a different mechanism of action which causes severe epileptic encephalopathy

Table 1 Summary of patients with *ALG13* variants

Patient(s)	Sex	<i>ALG13</i> variant	Protein change	Glycosylation studies	Clinical findings
This report	F	c.320A>G	N107S	Normal	ID, DR IS, HA, SZ, DVM, FP
de Ligt et al. (2012)	F	c.320A>G	N107S	Not reported	ID, SZ, HYP, DVM, FP
Epi4k #1 (2013)	F	c.320A>G	N107S	Not reported	ID, DR, SZ, HA, DVM
Epi4k #2 (2013)	F	c.320A>G	N107S	Not reported	ID, DR, SZ, HA,
Michaud et al. (2014)	F	c.320A>G	N107S	Not reported	ID, IS, SZ, HA
Timal et al. (2012)	M	c.280A>G	K94E	<i>N</i> -glycosylation defect type Is	SZ, MC, HM, died first year of life
Timal et al. (2012)	F mother	c.280A>G	K94E	Normal	Normal
Bissar-Tadmouri et al. (2014)	M (<i>n</i> = 4) brothers	c.3221A>G	Y1074C	Not tested	ID
Bissar-Tadmouri et al. (2014)	F mother	c.3221A>G (heterozygous)	Y1074C	Not tested	Normal

Key: *ID* intellectual disability, *DR* developmental regression, *IS* infantile spasms, *HA* hypsarrhythmia, *SZ* seizures other than IS, *DVM* delayed visual maturation, *FP* feeding problems, *HYP* hypotonia, *MC* microcephaly, *HM* hepatomegaly

with visual impairment and possible developmental regression.

Scott M. Myers: Provided data, critical revision, contribution of intellectual content

Compliance with Ethics Guidelines

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Conflict of Interest

Bethanny Smith-Packard declares no conflict of interest.

Scott M. Myers declares no conflict of interest.

Marc S. Williams declares no conflict of interest.

Author Contributions

Marc S. Williams: Conception and design, drafting article, coordination of revisions, guarantor

Bethanny Smith-Packard: Provided data, critical revision, contribution of intellectual content

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