ALG13-CDG with Infantile Spasms in a Male Patient Due to a De Novo *ALG13* Gene Mutation

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Abstract A boy presented at the age of 3.5 months with a developmental delay. He developed infantile spasms with hypersarrhythmia on EEG 1 month later. Additional symptoms were delayed visual development, asymmetrical hearing loss, hypotonia, and choreoathetoid movements. He also had some dysmorphic features and was vulnerable for infections. He was treated successively with vigabatrin, prednisolone, valproic acid, nitrazepam, and lamotrigine without a lasting clinical effect, but showed a treatment response to levetiracetam. Cerebral MRI showed hypoplasia of the corpus callosum and a mild delay in myelination. Further investigations including metabolic screening and glycosylation studies by transferrin isoelectric focusing were all considered to be normal. Whole-exome sequencing identified a de novo mutation in the *ALG13* gene (c.320A>G, p.(Asn107Ser)). Mutations in this gene, which is located on the X-chromosome, are associated with congenital disorders of glycosylation type I (CDG-I). Mass spectrometric analysis of transferrin showed minor glycosylation abnormalities. The c.320A>G mutation in *ALG13* has until now only been described in girls and was thought to be lethal for boys. All girls with this specific mutation presented with a similar phenotype of developmental delay

and severe early onset epilepsy. In two girls glycosylation studies were performed which showed a normal glycosylation pattern. This is the first boy presenting with an epileptic encephalopathy caused by the c.320A>G mutation in the *ALG13* gene. Since glycosylation studies are near-normal in patients with this mutation, the diagnosis of ALG13-CDG can be missed if genetic studies are not performed.

Introduction

Infantile spasms is the most common early-onset epileptic encephalopathy (EOEE). The outcome can be poor and includes developmental delay and chronic refractory epilepsy in 70% of cases. Prognosis depends on the underlying etiology, which is diverse and includes infections, perinatal events, and genetic disorders (Michaud et al. 2014).

The underlying etiology is unknown in about 40% of patients with infantile spasms, once called “idiopathic” cases. Nowadays, it is assumed that genetic factors play an important role in these patients (Osborne et al. 2010; Berg and Scheffer 2011). However, in only a small proportion a particular monogenetic defect has been identified (Dimassi et al. 2016; Møller et al. 2016; Michaud et al. 2014). A specific epilepsy syndrome can be caused by mutations in different genes, but at the same time the same gene and even same mutation can lead to broad phenotypic variations (Møller et al. 2016; Berg and Scheffer 2011). De novo mutations seem to play an important role in sporadic patients with infantile spasms. Genomic analyses, such as whole-exome sequencing (WES), can help to identify causative genes and significantly improve the diagnostic

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yield (Helbig et al. 2016; Allen et al. 2013; Michaud et al. 2014). In several studies a family-based WES approach is used to reveal the underlying genetic cause in patients with an unexplained intellectual disability or epileptic encephalopathy. After sequencing the exomes of the patient and unaffected parents, the exome of the patient can be analyzed for mutations in genes involved in autosomal recessive and X-linked inheritance, but also for de novo mutations by excluding inherited variants (de Ligt et al. 2012; Allen et al. 2013).

Previous reports implicated a role of *ALG13* mutations in the etiology of epileptic encephalopathies (Myers et al. 2016; Smith-Packard et al. 2015; Allen et al. 2013; Dimassi et al. 2016; Michaud et al. 2014; Hino-Fukuyo et al. 2015; Kobayashi et al. 2016). Particularly, the c.320A>G mutation in *ALG13* has been identified several times in girls with infantile spasms. *ALG13* gene mutations were also described as a cause of X-linked intellectual disability and congenital disorders of glycosylation type I (ALG13-CDG) (Bissar-Tadmouri et al. 2014; de Ligt et al. 2012; Timal et al. 2012). The *ALG13* gene is located on the X-chromosome and encodes a protein that heterodimerizes with ALG14 to form a complex in the endoplasmic reticulum that catalyzes the second step of protein N-glycosylation (Averbeck et al. 2008). This multistep process is identical for each N-glycosylated protein and is important for the structure and function of these glycoproteins. Genetic defects in this process cause CDG-I, with a highly variable multisystem phenotype. The diagnostic test for almost all N-linked CDG-I-subtypes is serum transferrin isoelectric focusing (IEF) (Sparks and Krasnewich 2005). However, there are CDG-I-subtypes with a normal transferrin profile, such as ALG13-CDG and ALG14-CDG, which expresses the need for additional glycoprotein biomarkers. With advanced mass spectrometry it is possible to analyze released glycans from serum proteins and perform intact glycoprotein analysis, which provides quantitative glycan structural information. Until now, advanced mass spectrometry has mostly been used in research settings. But since it is a quick test with high sensitivity and specificity the added value of mass spectrometry in the diagnostic process is increasingly recognized (Van Scherpenzeel et al. 2016). For most CDG-I-subtypes the defective enzyme is known, but enzymatic assays have not yet been developed or are scarcely available for most (Sparks and Krasnewich 2005). Also the clinical phenotypes of CDG-I-subtypes are not discriminative. Therefore, genetic testing can be helpful to define the specific CDG-I-subtype (Timal et al. 2012).

Here, we present a boy with infantile spasms and near-normal glycosylation studies. WES identified a c.320A>G mutation in *ALG13*, which has previously only been described in girls.

Case Report

A 3.5 months old boy was referred with delayed motor development. Before, he had been admitted to the hospital twice for recurrent infections. Parents had noticed at the age of 6 weeks that he did not make eye contact or smile yet. Apart from a delay in motor development with axial hypotonia, a plagiocephaly, mild retrognathia, mild torticollis, and scoliosis with a hemivertebra L1–L2 were present. Furthermore, asymmetrical hearing loss of 60–70 dB with an underdeveloped right auricle was detected. At the age of 4.5 months he presented with infantile spasms and hypsarrhythmia on EEG.

Cerebral MRI showed hypoplasia of the corpus callosum and a mild delay in myelination. Genetic and metabolic screening at the age of 3.5 months, including glycosylation studies by transferrin isoelectric focusing, were considered to be normal. Mass spectrometric analysis of transferrin revealed a lack of one glycan of ~6%, while a repeat plasma sample at the age of 15 months revealed a lack of one glycan of ~8% (in controls <4%). A family-based WES in the proband and parents was performed, revealing a de novo c.320A>G mutation (p.(Asn107Ser)) in the *ALG13* gene, confirming the diagnosis ALG13-CDG.

Treatment with vigabatrin was started, with addition of nitrazepam. Because of a lack of treatment response after 2 weeks, the vigabatrin was withdrawn, and high dose prednisolone (10 mg four times daily) was started, leading to a cessation of seizures and disappearance of hypsarrhythmia on EEG within 2 weeks. Two weeks later prednisolone was decreased slowly and valproic acid was started. At that time, generalized choreatiform movements were noted. A few weeks later he presented with generalized tonic-clonic seizures, occurring during an upper respiratory tract infection. Lamotrigine was added, but did not result in a cessation of seizure activity and was withdrawn. Recently, levetiracetam was started which resulted in a reduction of seizure activity for several weeks up to now.

Discussion

The infantile spasms and developmental delay in our patient are due to ALG13-CDG caused by a de novo c.320A>G mutation in the *ALG13* gene, resulting in the substitution of serine for asparagine at position 107 in the ALG13 protein (p.(Asn107Ser)). This de novo mutation is the most frequently described *ALG13* mutation, leading to a developmental delay and epileptic encephalopathy. It has so far only been described in 12 girls in a heterozygous state, but never in a male patient (de Ligt et al. 2012; Allen et al. 2013; Michaud et al. 2014; Smith-Packard et al. 2015;

Dimassi et al. 2016; Kobayashi et al. 2016; Myers et al. 2016; Wong 2016). Previously, it was even assumed that this variant caused embryonic lethality in males because only female patients were reported (Smith-Packard et al. 2015).

It is unclear by which mechanism the c.320A>G variant in *ALG13* is acting. Haploinsufficiency, causing the loss of one functional *ALG13* copy, could have been the case in girls with this mutation. However, one would expect that it would cause a more severe phenotype or even lethality in boys because of the hemizygous state. Therefore, a dominant negative effect as mechanism of action seems more likely in this *ALG13* variant. In girls this would lead not only to the loss of one normal functioning *ALG13* copy, but also to a dysfunction of the remaining copy resulting in even less or no residual function. This could lead to a more or less similar phenotype in both male and female patients.

In two girls with the c.320A>G mutation in the *ALG13* gene, serum transferrin isoelectric focusing was performed and showed a normal glycosylation pattern. In one girl mass spectrometric analysis was also performed, which was normal as well (Smith-Packard et al. 2015). It is unclear if mass spectrometry was performed in the second girl (Dimassi et al. 2016). In our patient analysis of glycosylation by transferrin isoelectric focusing was normal, but a slightly reduced glycosylation with a lack of one glycan was demonstrated by mass spectrometry. In a patient with a different *ALG13* mutation (c.280A>G; p.(Lys94Glu)), transferrin isoelectric focusing revealed clearly increased asialo- and disialotransferrin fractions, in agreement with a CDG-I (Timal et al. 2012). Given that positions 1–125 of the *ALG13* gene are related to glycosyltransferase activity, one would expect that both mutations would lead to a defect in glycosylation and thus a clearly abnormal glycosylation pattern (Uniprot 2017). Since glycosylation studies are near-normal in patients with the c.320A>G mutation in *ALG13*, the diagnosis of ALG-13 CDG can be missed if genetic studies are not performed.

Because most *ALG13* mutations were identified with WES in patient cohorts with either epileptic encephalopathies or intellectual disability, there is only limited data available on the clinical spectrum caused by *ALG13* mutations and outcome of these patients.

Nearly all girls described with the c.320A>G mutation were normal at birth, but developed early onset seizures and a severely delayed development or even developmental regression after a few months. One case showed a developmental delay since birth (de Ligt et al. 2012). Most patients presented initially with infantile spasms. Later on they developed more polymorphic seizures, such as myoclonic-tonic spasms, focal seizures, or generalized epilepsy. Vigabatrin did not improve infantile spasms in one patient (Michaud et al. 2014), and even worsened the

additional movement disorder in another (Myers et al. 2016). In about half of the patients, the infantile spasms initially responded to ACTH treatment (Allen et al. 2013; Michaud et al. 2014; Smith-Packard et al. 2015; Hino-Fukuyo et al. 2015). However, during follow-up the majority regained epileptic seizures, which sometimes responded to anti-epileptic drugs (such as topiramate) or a ketogenic diet, and were refractory in others (Allen et al. 2013; Michaud et al. 2014; Smith-Packard et al. 2015; Kobayashi et al. 2016; Myers et al. 2016).

As in our case, some patients with a mutation in *ALG13* had an extrapyramidal movement disorder such as dyskinesias or choreoathetoid movements (Myers et al. 2016; Kobayashi et al. 2016; Timal et al. 2012), whereas also dysmorphic features were reported (de Ligt et al. 2012; Dimassi et al. 2016). Furthermore, visual development may be affected by a defect of the *ALG13* gene, since it has been described in almost half of the cases (our report, Timal et al. 2012; de Ligt et al. 2012; Allen et al. 2013; Smith-Packard et al. 2015; Dimassi et al. 2016; Hino-Fukuyo et al. 2015).

Other *ALG13* variants have been described previously in males (Table 1). In most of these cases, the mutation was maternally inherited. Timal et al. described the first male patient with ALG13-CDG, due to a de novo c.280A>G mutation in the *ALG13* gene, who died at the age of 1 year. He presented with refractory epilepsy with polymorphic seizures, multiple congenital anomalies, bilateral optic nerve atrophy, recurrent infections, increased bleeding tendency, as well as extrapyramidal and pyramidal signs. His glycosylation pattern suggested a CDG-I. No structural abnormalities were found by assaying lipid-linked oligosaccharides (LLO) synthesis, neither was a clear deficiency shown by indirect analysis of the GlcNAc transferase activities. Direct assaying of ALG13/ALG14 enzyme activity was necessary to detect a functional deficit in glycosylation and confirm the diagnosis of ALG13-CDG. Unfortunately this assay is not available anymore at this moment. Since this entire analysis is a lengthy biochemical process and the clinical phenotypes of CDG-I-subtypes are not discriminative, the authors recommend the use of next generation sequencing techniques to diagnose CDG-I-subtypes like ALG13-CDG (Timal et al. 2012).

Another boy initially had a normal motor development until the age of 4 months. Then he developed bilateral optic atrophy with nystagmus and lack of visual fixation. Subsequently, infantile spasms appeared with hypersarrhythmia on EEG. A missense mutation of *ALG13* (c.880C>T) was detected. He inherited this variant from his mother who was an asymptomatic carrier. No glycosylation studies were reported (Hino-Fukuyo et al. 2015).

In a third boy with Lennox-Gastaut syndrome, a c.1641A>T mutation in *ALG13* was identified. Both his mother and grandmother were asymptomatic carriers. No

Table 1 Summary of male patients with *ALG13* mutation

	Age onset epilepsy	Sex	<i>ALG13</i> variant	Inheritance	Protein change	Clinical findings	MRI	Glycosylation studies	Response to treatment
This report	4.5 months	M	c.320 A>G	De novo	p.(Asn107Ser)	Developmental delay, infantile spasms/polymorphic seizures, hypotonia, dysmorphic features, delayed visual maturation, choreatiform movements	Hypoplasia corpus callosum and mild delay myelination	Transferrin IEF normal. Mass spectrometry lack of one glycan (~6–8% ref: <4%)	Spasms initially responded to prednisolone. No effect of vigabatrin, nitrazepam, and valproic acid. Response to levetiracetam
Timal et al. (2012)	Not reported	M	c.280A>G Hemizygous	De novo	p.(Lys94Glu)	Seizures, microcephaly, delayed visual maturation, extrapyramidal/hepatomegaly, bleeding tendency, swelling hand/feet/eyelids	Not reported	N-glycosylation defect type I (ALG13-CDG)	Refractory polymorphic epilepsy, died at age of 1 year
Bissar-Tadmouri et al. (2014)	Not applicable	M (<i>n</i> = 4)	c.3221A>G Hemizygous	Maternal inheritance	p.(Tyr1074Cys)	Intellectual disability	Normal	Not tested	Not applicable
Hino-Fukuyo et al. (2015)	5 months	M	c.880C>T Hemizygous	Maternal inheritance	p.(Pro294Ser)	Developmental delay, infantile spasms/seizures, delayed visual maturation	Anomaly corpus callosum	Not reported	Spasms responded to pyridoxine and ACTH, followed by tonic/myoclonic seizures responding to AED
Møller et al. (2016)	Not reported	M	c.1641A>T	Maternal inheritance	p.(Gln547His)	Lennox-Gastaut syndrome	Not reported	Not reported	Not reported

further information about this case was provided (Møller et al. 2016).

Finally, an *ALG13* missense mutation (c.3221A>G) was discovered in four male siblings with X-linked intellectual disability. Their mother was an asymptomatic carrier of the mutation as well. No epileptic seizures or other neurological symptoms were described in these boys, and glycosylation studies were not mentioned (Bissar-Tadmouri et al. 2014).

In conclusion, the c.320A>G mutation in the *ALG13* gene may not only cause ALG13-CDG with a severe early onset epileptic encephalopathy and developmental delay in girls, but can also be the cause of this phenotype in boys. Glycosylation studies in these patients are near-normal, which means that the diagnosis of ALG13-CDG can be missed if genetic studies are not performed (Myers et al. 2016; Smith-Packard et al. 2015; Allen et al. 2013; Dimassi et al. 2016; Michaud et al. 2014; Kobayashi et al. 2016).

Synopsis

The c.320A>G mutation in *ALG13*, which until now has only been described in girls, can also be a cause of ALG13-CDG with near-normal glycosylation studies in boys.

Author Contributions

Wienke H. Galama: Conception and design, literature review, drafted article, coordination of revisions, guarantor.

Sandra L. J. Verhaagen – van den Akker: Provided clinical data and drafted case report.

Dirk J. Lefeber: Provided glycosylation data, contribution of intellectual content and critical revision.

Ilse Feenstra: Provided genetic data, contribution of intellectual content and critical revision.

Aad Verrips: Conception and design, provided clinical data, contribution of intellectual content and critical revision.

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Compliance with Ethics Guidelines

Conflict of Interest

Wienke Galama declares that she has no conflict of interest.

Sandra Verhaagen – van den Akker declares that she has no conflict of interest.

Dirk Lefeber declares that he has no conflict of interest.

Ilse Feenstra declares that she has no conflict of interest.

Aad Verrips declares that he has no conflict of interest.

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. Additional informed consent was obtained from all patients for which identifying information is included in this article.

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