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CASE REPORT

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The trap of genetic tag: The importance of pathogenicity prediction tools in the correct interpretation of variants of uncertain significance in the era of high-throughput genome sequencing

Francesco Pellegrino

Department of Pediatrics, AOU Città della Salute e della Scienza di Torino, University of Torino, Turin, Italy

Correspondence
Francesco Pellegrino, Department of Pediatrics, University of Torino, Piazza Polonia 94, Torino 10126, Italy.
Email: f.pellegrino@unito.it

Abstract

Although recent advancements in DNA sequencing technologies and their widely used, the interpretation of variants of uncertain significance from these large datasets is not clear-cut. Here, we present the case of a family referred to our metabolic disease department, in which three males' individuals were affected by a suspected a genetic inherited disease, resulting from next-generation sequencing results. A correct assessment of the clinical significance of the genetic variant found in our cases, with a review of the literature, the evaluation of population database and the use of computational predictive program changed the initial suspect. Despite NGS technologies have increased diagnostic sensitivity, most of these variants remains of uncertain clinical significance. An efficient systematic approach is fundamental to determine the pathogenicity of a variant, avoiding incorrect interpretation in a clinical setting.

KEYWORDS

ALG13-CDG, variants of uncertain significance, variants prediction tools

1 | INTRODUCTION

Molecular genetic testing support medical decision-making in the diagnosis of symptomatic individuals and in the prediction of disease risk assessment for patients and their family members.

Recent advancements in low-cost, high-throughput DNA sequencing, as next-generation sequencing (NGS), have generated massive amounts of genetic data, resulting in dramatically increased numbers of DNA variants identified. However, despite NGS technologies have increased diagnostic sensitivity, most of these variants remains of uncertain clinical significance (VUS). This is a critical limitation for translating genetic data into clinical practice. Thus, there is a need for an efficient systematic approach in determining and scoring the pathogenicity of a VUS, based on scientific literature and on the prediction tools.

Here, we present the case of a family referred to our metabolic disease department, in which three males' individuals were affected by epileptic encephalopathy (EE), autistic spectrum disorder, intellectual disability (ID), and developmental delay (DD). From next-generation sequencing resulted the mutation of ALG13 gene, initially considered responsible for the clinical spectrum of our patients. However, the Computational (In Silico) Predictive

software reported this mutation as non-pathogenetic, surprisingly confuting our aetiologic hypothesis. These cases illustrate the importance of combining clinical, laboratory and prediction tools in evaluation of the variants of uncertain clinical significance (VUS).

2 | CASE DESCRIPTION

The index case is a 5-year male child affected by EE, DD, ID, and autistic spectrum symptoms. Neurological evaluation revealed mild language delay and motor stereotypies pattern. The brain magnetic resonance imaging (MRI) was normal. Advanced psychometric tests were performed, confirming motor and cognitive stereotype patterns (Autism Diagnostic Interview-Revised), IQ of 73 with attention deficit (Leiter International performance scale-revised), and evidence of autistic spectrum symptoms (Autism Diagnostic Observation Schedule-Second Edition).

During hospitalization in our metabolic department, we performed extensive metabolic work-up, including isoelectricfocusing serum transferrin, without any pathologic results. Before performing NGS, the mother of our patient informs us that she has another 16-year-old male child with EE, DD, ID and autistic spectrum disorder, followed by another hospital. She also reported that she has a 53-year-old brother (uncle of our patient) with the same symptoms.

We decided to perform NGS to the proband and the parents (so-called "trio" testing: mother, father, affected child), but we retain valuable to test other family members (affected brother and uncle). NGS identified a variant of unknown significance c.581C > T (p.Pr0194Leu) in ALG13 gene (NM_018466.4) confirmed in the index case, his brother, his mother, and his uncle.

ALG13 gene is located on Xq23 and encodes asparagine-linked glycosylation 13 protein, a domain of the UDP-N-acetylglucosamine (GlcNAc) transferase.[1]

Pathogenic mutation of ALG-13 results in a congenital disorder of glycosylation (CDG), known as X-linked CDG type 1 (ALG13-CDG), a defect of N-glycosylation, reported in 68 individuals.

3 | ALG13-CONGENITAL DISORDER OF GLYCOSYLATION

The glycosylation processes, set in the endoplasmic reticulum and Golgi apparatus, are the most common post-translational modification of protein and lipids. More than 150 different congenital disorder disorders of glycosylation (CDG) are currently described.[2] These disorders may be classified into four categories: N-linked glycosylation (also CDG-I), O-linked glycosylation, combined N- and O-linked/multiple glycosylation, and lipid and glycosylphosphatidylinositol (GPI) anchor biosynthesis defects. The broad phenotypic spectrum includes developmental delay (DD), intellectual disability (ID), seizures, endocrinological and coagulation abnormalities.[3] Although the majority of CDG are inherited in an autosomal recessive pattern, ALG13-CDG is a rare X-linked congenital disorder of N-glycosylation, and a total of 67 individuals (50 females and 17 males) affected have been reported in literature.[4]

Most of the affected individuals are females presenting with early onset epileptic encephalopathy (EE) (MIM 300884) with DD and mild-to-severe ID. Only 17 affected males have been reported, suggesting that ALG13 variants may be life-threatening conditions in males. It is enigmatic that an X-linked disorder affects primarily female patients. Also, from a biochemical point of view, although ALG13-CDG is a disorder of glycosylation most of the individuals affected have normal IEF of serum transferrin, which is the routinely used biochemical screening test for CDGs. In particular, a type 1 IEF pattern (observed in CDG-I) is characterized by a decrease of tetrasialotransferrin and an increase of di- and asialotransferrin bands, whereas a type 2 pattern (observed in CDG-II) shows in addition an increase of tri- and monosialotransferrin.[4,5]

Possible explanations of normal transferrin glycosilation include unbalanced X-inactivation, that might explain the normal glycosylation tests in females, and the eventual differential expression of the disease in liver (a mosaic pattern), where some genetically intact cells compensate for the glycosylation impairment with higher residual enzyme activity. Considering the high prevalence in females, it has been speculated that ALG13 pathogenic mutations do not lead to loss of function, but rather to gain of function.[6] Therefore, glycan abnormalities might be different from a glycan impairment, and we are not using the right biochemical method to identify the glycosylation abnormalities and alternate testing methods need to be utilized. Since glycosylation tests are near-normal in most of the patients, and due to highly variable phenotype, the diagnosis of ALG13-CDG can be missed if genetic studies are not performed. Prenatal diagnosis has become reliable for CDG-Ia (7) and in all other types of CDG for which the molecular defect is known. Early attempts of prenatal diagnosis based on transferrin IEF in fetal blood have failed, such as this method is not reliable also in postnatal life.

The most representative de novo variant is c.320A > g, resulting in the substitution of serine for asparagine at position 107 in the ALG13 protein (p. Asn107Ser).

This variant was found de novo in a total of 45 patients, 42 unrelated heterozygous symptomatic females, but only in three affected males. All these individuals

TABLE 1 Clinical, biochemical, and genetic data of male individuals affected by ALG13-CDG reported in literature.

	Timal 2012 [15]	Bissar-Tadmouri 2013 (4 pts) [16]	Hino- Fukuyo 2015 [17]	Moller 2016 [18]	Galama 2017 [19]	Gadomski 2017 [20]	Ng 2020 (2pts) [5]		Sobering 2021 (3 pts) [10]			Montano 2021 [21]	Cai 2022 [22]	
DNA var	c.280A > G	c.3221A > G	c.880C > T	c.1641A > T	c.320A > G	c.1388A > g	c.320A > G	c.2915G > T	c.3013C > T	c.2458-15_2486 del	c.2272G > T	c.482C > T	c.23T > C	c.862C > G
Protein var	P. Lys94Asp	p. Tyr1074Cys	p.Pro294Ser	p.Gln547His	p. Asn107Ser	P.Glu463Gly	p.Asn107Ser	p.Gly972Val	p.Pro1005Ser		p.val758Phe	p.Pro143Leu	p.Val8Ala	p.Leu288Val
Inheritance	De novo	Maternal	Maternal	Maternal	De novo	Maternal	De novo	De novo	Maternal	Maternal	Materanl	Maternal	Maternal	Maternal
Intellectual disability	Yes	Yes	Yes	Yes	Yes	ND	ND	ND	Yes	Yes	No	Yes	Yes	Yes
Seizures	Yes	ND	Yes	Yes	Yes	Yes	Yes	ND	ND	ND	ND	No	Yes	No
Hypotonia	ND	ND	ND	ND	Yes	Yes	ND	ND	ND	ND	ND	Yes	No	No
Skeletal findings	Yes	ND	ND	ND	Yes	Yes	ND	ND	ND	Polydactyly	ND	Microcephaly	No	No
Other Symptoms	Extrapiramidal symptoms, hepatomegaly, recurrent infections, prolonged APTT, delayed visual maturation	No	Delayed visual maturation	ND	Chorea, delayed visual maturation	ND	ND	ND	ND	ND	ND	Cardiopathy	No	Binocular strabysmus
EEG	ND	ND	ND	Lennox-Gasteaut pattern	ND	ND	Hypsiaritmia	ND	ND	ND	ND	ND	Abnormal slow-wave activities in bilateral brain	Normal
Brain anomalies	ND	Normal	Anomaly corpus callosum	ND	Corpus callosum hypoplasia, mild delay in myelination	Non -specific white matter changes	Cerebral atrophy		ND	ND	ND	Normal	Slightly wider bilateral frontotemporal extracerebral space	Delayed myelination and widening of bilateral frontotemporal extracerebral space
CDG Testing	TIEF: abnormal; LLO: normal; GlcNAc transferase activity: low	ND	ND	ND	Normal;	Abnormal cellular glycosylation	ND	ND	Normal	Normal	ND	ND	ND	ND
Survival	Died, 1 year	Alive, 6–15years	Alive as adult	ND	Alive, 15 months	Alive, 3 years	ND	ND	Alive, 6 years	Alive, 8 years	Alive, 13 years	Alive, 11 years	Alive, 2 years	Alive, 3 years

showed a similar phenotype with DD, infantile spasms, and epilepsy.[4]

The molecular mechanisms by which ALG13-CDG causes epilepsy and impairs neuronal development are still unknown.

Several theories have been proposed to explain the role of ALG13 in the inhibitory synaptic transmission. Some suggest that ALG13 affected mRNA expression of Gabrα2 gene, compromising total and surface expression of GABA-A receptor α2.[8] Another recent study explored the pathogenic role of ALG-13 in epilepsy by showing hyperactive mechanistic target of rapamycin (mTOR) signaling pathways in the cortex and hippocampus of ALG 13 knock-out mice. However, there were no glycosylation defects detected in this mouse model.[9] The other 23 presented ALG13 pathogenetic variants different from c.320A>g, (p. Asn107Ser), with milder and more variegated phenotype.

An efficient treatment is still not available for the CDG-Ia patients and mannose or fucose supplementations did not improve the clinical or biochemical features. On the contrary, in CDG-Ib patients, treatment with oral mannose has shown significant improvement.[7]

4 | ASSESSMENT OF THE CLINICAL SIGNIFICANCE OF THE GENETIC VARIANT

As suggested by the guidelines of American College of Medical Genetics and Genomics (ACMG)[10] we review all previously reported males' individuals with ALG-13 variants, to search whether the variant has been reported previously in the literature and to summarize the phenotype of the previously reported males affected by ALG13 mutation. We consulted several population databases, but we did not find the detected mutation c.581C>T (p.Pr0194Leu). It was also absent in the gnomAD database (https://gnomad.broadinstitute.org/). Summary of the clinical, biochemical, and genetic data of all the reported male subject with ALG13- CDG is presented in Table 1.

Thus, considering the phenotypical similarities with previous reported affected males and the family segregation of our subject, we initially hypothesized that this VUS may be a novel pathogenetic variant with male segregation and that it may justify the clinical course. However, population databases did not contain extensive information regarding the functional effect of these variants or any possible associated phenotypes. If the evidence for disease association from existing data is not strong or the mechanism of gene function is unclear, as in our case, several bioinformatics tools may be used to predict the possible impact of the variant on the gene or protein, so we decided to use Computational (In Silico) Predictive Programs. Computational predictions are generally based on the type of change, the domain structure, sequence conservation, and biochemical properties of the affected amino acid residues. In our case, the prediction software CADD (http://cadd.gs.washington.edu) reported this mutation as CADD 0 and was supported by MutationTaster (http://www.mutationtaster.org), surprisingly suggesting that the mutation we found in our patients family members is not responsible for their clinical course.

5 | CONCLUSION

This case underline as, although molecular genetic testing has a unique place in the diagnosis, management, and prevention of genetic disorders, the risk to find a VUS and to label a patient as affected by a genetic disease is more common than we think. This field is still compromised by the absence of a standard, comprehensive, and efficient variant assessment protocol approved and shared by the community. It is fundamental to systematically evaluates multiple parameters for each variant observed in patients with suspected inherited disorders. Optimal results are realized when the referring healthcare provider and the clinical laboratory work collaboratively in the testing process. Finding of a rare or novel variant in a genomic sequencing sample, as in our case, must not be assumed as relevant to a patient just because it is rare, novel or de novo. The clinicians and the laboratory must evaluate together the variant and the gene in the clinical course of the patient's and family's history, using prediction tools to distinguish between variants that cause the patient's disorder and those that are incidental findings.

AUTHOR CONTRIBUTIONS

FP is responsible for study conception and design, reviewed the literature and wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

The author declares that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

Data are available on request due to privacy/ethical restrictions.

ETHICAL APPROVAL

All methods were performed in accordance with the ethical standards as laid down in the Declaration of Helsinki and its later amendments or comparable ethical standards. Ethics approval was obtained by Hospital Scientific Direction. Written informed consent was obtained from a parent and/or legal guardian.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

ORCID

Francesco Pellegrino https://orcid.org/0000-0002-4353-8324

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