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Novel Insights from Clinical Practice

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# A Novel Homozygous *ALG12* Mutation in a Patient with CDG Type Ig: New Report of a Case with a Mild Phenotype

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**Established Facts**

- Congenital disorders of glycosylation (CDG) are a group of rare genetic diseases caused by the deficiency of enzymes involved in the biosynthesis or remodeling of the glycan moieties of glycoconjugates.
- The clinical features of ALG12-CDG include dysmorphic features, developmental delay, hypotonia, progressive microcephaly, hypogammaglobulinemia, coagulopathies, and failure to thrive.

**Novel Insights**

- The present case report describes the assessment of the severity of the c.367G>A mutation.
- The patient described in this study contributes to understanding the milder ALG12-CDG cases and to further expanding the genotype-phenotype spectrum.

**Keywords**
*ALG12* · Congenital disorders of glycosylation · Developmental delay · Mild phenotype · Missense mutation

**Abstract**
Congenital disorders of glycosylation (CDG) are a group of rare genetic diseases caused by the deficiency of enzymes involved in the biosynthesis or remodeling of the glycan moieties of glycoconjugates. Most of CDG are autosomal recessive; however, few of them show autosomal dominant or X-linked inheritance. ALG12-CDG is an autosomal recessive inherited defect caused by a deficiency in the α-mannosyltransferase, dolichyl-P-mannose: Man7-GlcNAc-2-PP-dolichyl-alpha-6-mannosyltransferase (mannosyltransferase 8), which determines Man7GlcNAc2-PP-dolichol accumulation in tissues including fibroblasts. The clinical features of ALG12-CDG include dysmorphic features, developmental

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delay, hypotonia, progressive microcephaly, hypogammaglobulinemia, coagulopathies, and failure to thrive. Herein, we describe the case of a Sicilian patient with a milder phenotype bearing an *ALG12* homozygous mutation. To date, including this patient, only 16 cases have been described with this form of CDG. Furthermore, our study contributes to understanding the milder ALG12-CDG cases and to further expanding the genotype-phenotype spectrum.



## Introduction

Congenital disorders of glycosylation (CDG) are a group of rare genetic diseases caused by the deficiency of enzymes involved in the biosynthesis or remodeling of the glycan moieties of glycoconjugates [Jaeken, 2013; Monticelli et al., 2016]. To date, CDG are classified in 2 groups including CDG type I and CDG type II. CDG type I is caused by defects of the glycan chain assembly and its attachment to the nascent glycoprotein in the cytosol or the endoplasmic reticulum. CDG type II is caused by abnormal remodeling or processing of the glycan moieties in the Golgi network, generally resulting in truncated or structurally deficient carbohydrate chains [Grubenmann et al., 2002; Jaeken, 2013; Monticelli et al., 2016]. The clinical phenotype of these defects can range from milder phenotypes with mono-organ involvement to multisystemic manifestations and early lethal outcomes. Commonly, CDG presents with neurological signs and symptoms such as psychomotor and speech delay, intellectual disability, ataxia, seizures, strabismus and abnormal eye movements, macro-/microcephaly, and neuroimaging abnormalities [Francisco et al., 2019]. Most of CDG are autosomal recessive; however, few of them show autosomal dominant or X-linked inheritance. Isoelectrofocusing (IEF) of serum transferrin is a validated screening test for CDG due to defects in the N-glycosylation pathway, and it allows to discriminate type I from type II patterns [Aebi et al., 1999].

However, serum transferrin IEF can be normal in various CDG or may even normalize during adulthood [Tahata et al., 2019]. Genetic investigations and the broader application of NGS have allowed an increase in the rate of diagnosis.

ALG12-CDG is an autosomal recessive defect caused by a deficiency in the α-mannosyltransferase, dolichyl-P-mannose: Man7-GlcNAc-2-PP-dolichyl-alpha-6-mannosyltransferase (mannosyltransferase 8), which determines Man7GlcNAc2-PP-dolichol accumulation in tissues including fibroblasts [Chantret et al., 2002]. The clinical spectrum is characterized by dysmorphic features, developmental delay, hypotonia, progressive microcephaly, failure to thrive, and male genital hypoplasia. Cardiac and musculoskeletal malformations are described in some patients. Routine laboratory analyses reveal low levels of antithrombin and some coagulation pa-

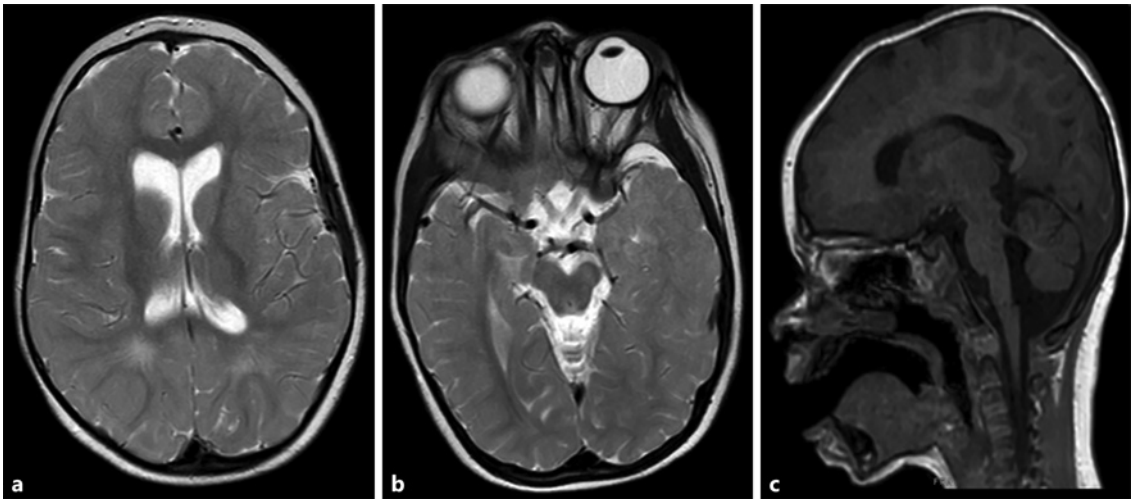


**Fig. 1.** Axial (**a**, **b**) and sagittal (**c**) MRI view of our patient. The brain MRI shows cerebellar hypoplasia of cortical gyri, inferior cerebellar vermis hypoplasia with an enlarged fourth ventricle communicating with the cisterna magna (suggesting a Dandy-Walker variant), focal reduction in size of the corpus callosum (part of the isthmus and splenium), lateral ventricle enlargements with square-shaped anterior horns, and subarachnoideal space enlargements in the temporo polar area.

rameters, low levels of Immunoglobulins (mainly IgG) and B cell dysfunction.

Herein, we describe the case of a Sicilian patient with a milder phenotype bearing an *ALG12* homozygous mutation. A compound heterozygous mutation has been seen in another Sicilian patient.

## Case Report

The patient is a 29-month-old girl of Caucasian origin. She is the second child of unrelated healthy parents. She was born full-term after a normal pregnancy with a birth weight of 3,180 g (25th–50th centile), length 50 cm (50th–75th centile), head circumference 32 cm (3rd–5th centile). At the age of 10 months, she was hospitalized for recurrent episodes of vomiting and gastroesophageal reflux. She was diagnosed with failure to thrive, worsening after weaning and persisting during the first year of life; she also had had 2 episodes of infective gastroenteritis. Global developmental delay was evident (sat alone at 9 months, stood alone at 11, walked without support at 18, and she could babble at 11 months). She also showed microcephaly, bilateral epicanthal folds, cup-shaped ears, pear nose, and joint laxity. At 29 months of age, her physical parameters were within normal limits, but head circumference was 44 cm (below the 3rd centile); she had axial hypotonia and walked unsupported with a wide-based ataxic gait. Hyperactivity and hand stereotypes were also present. Language was limited to babbles and sounds. She also showed fine hypertrichosis, palpebral edema, left eye convergent squint, and low-set ears. Lab-

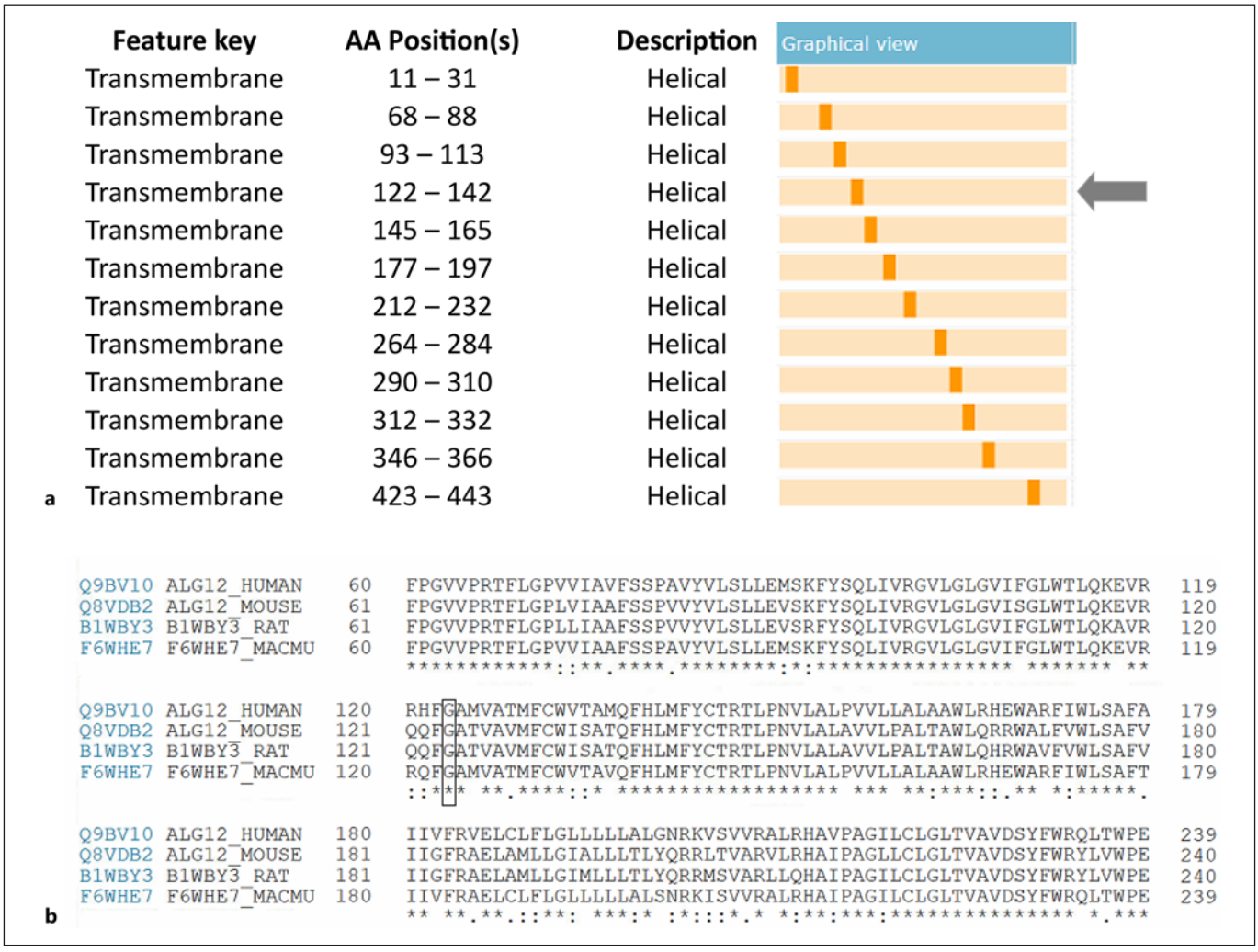


**Fig. 2.** Position of the mutation in endoplasmic reticulum domain and sequence alignment of ALG12 among different species and the patient. **a** The location of the p.Gly123Arg (black arrow) is comprised within the endoplasmic reticulum transmembrane domain of the ALG12 protein (adapted from https://www.uniprot.org). **b** Sequence alignment among vertebrate species showing (rectangle) that the "Glycine" at position 123 is highly conserved. ALG12_HUMAN, *Homo sapiens* (human); ALG12_MOUSE, *Mus musculus* (mouse); B1WBY3_RAT, *Rattus norvegicus* (rat); F6WHE7_MACMU, *Macaca mulatta* (Rhesus macaque).

**Table 1.** In silico prediction analysis of the detected mutation in *ALG12* (NM_024105.3) c.367G>A (p.Gly123Arg)

| In silico predictive tool | Prediction/Score | PHRED-scaled |
|---|---|---|
| CADD_phred | 33 | >30 highly pathogenic; >20 pathogenic |
| DANN_score | 0.999 | Range from 0 to 1* |
| FATHMM_pred | DAMAGING | |
| GERP++_RS | 4.7 | Range from −12.3 to 6.17* |
| LRT_pred | Deleterious | |
| MetaLR_pred | DAMAGING | |
| MutationTaster_pred | DAMAGING | |
| MutationAssessor_pred | Medium | Neutral, low, medium, high |
| Polyphen2_HDIV_pred | Probably damaging | |
| PROVEAN_pred | DAMAGING | |
| SIFT_pred | DAMAGING | |
| SiPhy_29way_logOdds | 17.700 | Range from 0 to 37.9718* |
| UMD prediction | Pathogenic | |
| VEST3_score | 0.917 | Range from 0 to 1* |
| CLINSIG | Likely_pathogenic | |
| PhyloP20way_mammalian | 0.932 | Range from −12.51 to 1.2* |

* A larger number indicates a higher probability to be damaging.

oratory analysis revealed hypogammaglobulinemia (0.52 g/dL; normal range 0.7–1.6 g/dL). Moreover, IgG levels were slightly lower (646 mg/dL; normal range 700–1600 mg/dL), and low antithrombin III levels (58 and 62% on different occasions; normal range 80–120%) were also reported. A brain MRI showed cerebellar hypoplasia of cortical gyri, inferior cerebellar vermis hypoplasia with an enlarged fourth ventricle communicating with the cisterna magna (suggesting a Dandy-Walker variant), focal reduction in size of the corpus callosum (part of the isthmus and splenium), lateral ventricle enlargements with square-shaped anterior horns, and subarachnoideal space enlargements in the temporo polar area (Fig. 1). Electroencephalogram, electrocardiogram, echocardiogram, abdomen ultrasound, and fundus oculi showed normal findings. According to reported methods, organic acids were assayed in urine and turned out to be normal [Di Rosa et al., 2006]. Finally, serum transferrin IEF showed a decrease in tetrasialo- (76%; normal range 78–99%) and an increase in disialotransferrin (13.2%; normal range 0.5–1.6%), indicating a CDG type I pattern. Array-CGH analyses yielded normal results. The Clinical Exome Solution by Sophia Genetics was performed following the protocol in the ThermoFisher Scientific NGS machine, covering 4,493 genes related to the most common inherited diseases. This approach achieved an 80x average coverage over the sequenced, with 95% regions covered at least 20x. We filtered the identified variants according to recessive/de novo pattern of inheritance, gene features, and minor allele frequency <1% using 1000 Genomes, ESP6500, ExAC, and gnomAD as references. NGS detected c.367G>A (p.Gly123Arg) homozygous missense mutations in *ALG12* (NM_024105) (rs1555930118), inherited from the parents, and confirmed by Sanger sequencing. The variant located in the transmembrane helical domain is highly conserved across species (Fig. 2) and classified as pathogenic or possibly damaging by in silico predictive tools (Table 1); it is also classified in ClinVar as uncertain significance/likely pathogenic. This variant was not detected in GnomAD_exome, GnomAD, ExAC, 1000 Genomes, or in ALFA project. Moreover, screening of this variant was not detected in a population of 50 healthy controls (100 alleles) of the same geographic area, ruling out the possibility of a population polymorphism.

## Discussion

In the present patient, we identified a homozygous missense mutation c.367G>A (p.Gly123Arg) in the *ALG12* gene (NM_024105) inherited from both parents, who are unrelated but come from the same area in Sicily. A compound heterozygous Sicilian patient harboured the same mutation [Sturiale et al., 2019], suggesting that this rare genetic variant might have spread in our region. However, the present case report contributes to the assessment of the severity of the c.367G>A mutation; in fact, in homozygotes, the severity of mutations is directly correlated with the phenotype [Romano et al., 1996].

Including our patient, only 16 patients with ALG12-CDG have been described so far (Table 2). Most of them had developmental delay (13/16; 81.25%) and dysmorphic features (14/16; 87.5%) (Table 2). The clinical spectrum also included hypotonia (11/16; 68.75%) and abnormal brain imaging (10/11; 90%). Although neuroimaging findings were not reported in 5 patients and were unremarkable in 1 patient, in the remaining 11 cases cerebral/

**Table 2.** Clinical features of ALG12-CDG compared to our patient

| | ALG12-CDG patients ($n$ = 15) | Our patient |
|---|---|---|
| Dysmorphic features | 13/15 | + |
| Developmental delay | 12/15 (2 NR) | + |
| Seizures | 2/15 (8 NR) | – |
| Ocular abnormalities | 4/15 (5 NR) | + |
| Sensorineural deafness | 2/15 (8 NR) | – |
| Hypotonia | 10/15 (4 NR) | + |
| Failure to thrive | 8/15 (4 NR) | + |
| Cardiac abnormalities | 3/15 (10 NR) | – |
| Genitourinary abnormalities | 5/15 (7 NR) | – |
| Recurrent infection | 8/15 (5 NR) | – |
| Hypogammaglobulinemia | 8/15 (3 NR) | + |
| Coagulation abnormalities | 10/15 (5 NR) | + |
| Abnormal CDT analysis | 12/15 (3 NR) | + |
| Microcephaly | 7/15 (4 NR) | + |
| Skeletal abnormalities | 4/15 (8 NR) | – |
| Abnormal brain imaging | 9/15 (5 NR) | + |

NR, not reported. Clinical features of ALG12-CDG: Esfandiari et al., 2019; Sturiale et al., 2019; Tahata et al., 2019; de la Morena-Barrio et al., 2020.

cerebellar atrophy features (i.e., widening of subarachnoidal spaces, ventricular enlargement, cerebellar hypoplasia, etc.) were the most frequent findings (9/11); in particular, in one of them, hypoplasia pontocerebellar was revealed. Also, 2 patients showed hypoplasia of the corpus callosum, a single patient had pachygyria and delayed myelination, and another single patient had prominent cavum septum pellucidum [Chantret et al., 2002; Sturiale et al., 2019; Tahata et al., 2019; de la Morena-Barrio et al., 2020].

Coagulation abnormalities (11/16; 68.75%), hypogammaglobulinemia (9/16; 56.25%) and recurrent infections (8/16; 50%) were also frequently reported in ALG12-CDG patients.

Compared to most patients with ALG12-CDG, and to the one with the same mutation in compound heterozygous, our 29-month-old girl seems to have a milder clinical phenotype since she had slight dysmorphic features and mild developmental delay and walked independently at 2 years of age. She showed a mild hypogammaglobulinemia without a history of recurrent infections. Recently, 3 patients with ALG12-CDG were diagnosed in adulthood with normal or mildly impaired cognitive levels and coagulopathy, thus, defining the very mild end of the spectrum [Tahata et al., 2019; de la Morena-Barrio et al., 2020]. Although further studies are needed to confirm these results, our study contributes to understanding the milder ALG12-CDG cases and to further expanding the genotype-phenotype spectrum.

It also is interesting to observe that in our case, the diagnosis was reached thanks to the use of NGS, meaning that the clinical picture can be caused by many different metabolic conditions and that, despite broad metabolic investigation, the diagnosis can be missed even by doctors with experience in the field of rare diseases.

On the contrary, early diagnosis is essential for offering genetic counseling to family members to assess the carrier status and establish the genetic risk. Regarding that this is a rare form of CDG, relatively mild cases may escape the final diagnosis; thus, a wider use of coagulation factors, protein assays, and transferrin IEF can help pinpoint the condition, especially among unexplained cases.

## Statement of Ethics

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Written informed consent was obtained from the patient's parents for conducting the evaluations and the molecular analyses, and for publishing this case report and all the accompanying tables and images in scientific journals.

## Conflict of Interest Statement

The authors have no conflicts of interest to disclose.


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## Author Contributions

F. Calì and A.G. Nicotera conceived the study. A.G. Nicotera wrote the manuscript with support from G. Spoto, A. Fiumara, and R. Barone. In addition, G. Spoto and G. Romeo collected and analyzed the data. F. Cali, M. Vinci, and A. Musumeci were involved in genetic analysis. G. Di Rosa and S.A. Musumeci supervised the project.

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