



ALG3-CDG: lethal phenotype and novel variants in Chinese siblings

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Received: 13 February 2020 / Revised: 17 June 2020 / Accepted: 28 June 2020 / Published online: 12 July 2020
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Abstract

Congenital disorders of glycosylation (CDG) are a group of genetic, mostly multisystem disorders, which often involve the central nervous system. ALG3-CDG is one of the some 130 known CDG. Here we report two siblings with a severe phenotype and intrauterine death. Whole-exome sequencing revealed two novel variants in *ALG3*: NM_005787.6:c.512G>T (p.Arg171Leu) inherited from the mother and NM_005787.6:c.511C>T (p.Arg171Trp) inherited from the father.

Introduction

Congenital disorders of glycosylation (CDG) are a group of inherited disorders characterized by deficient glycosylation [1]. They are mostly multisystem disorders often involving the central nervous system [2–5]. ALG3-CDG is a rare disorder, with only 23 patients reported so far [2–13]. ALG3 adds the 6th mannose to the growing dolichol-linked oligosaccharide in the ER [2, 14]. Here we report two siblings with a severe malformation syndrome and intrauterine death.

Materials and methods

Genetic analysis

Genomic DNA was extracted from the muscle tissue of the fetuses and from peripheral blood of their parents. Whole-exome sequencing (WES) and Sanger sequencing were performed according to standard procedures.

Western blotting

Muscle tissues from the patients and a control were washed with phosphate-buffered saline and western blot analysis was performed according to standard procedures.

Results

Clinical report

We report two siblings with severe multisystem dysmorphism who were born to healthy parents. The mother stated that she was never exposed to alcohol, warfarin, or other drugs. The present study was approved by the hospital's Institutional Review Board, and written informed consent was obtained from the parents. The control tissue was obtained from a healthy fetus who was born with induced labor. The tissue sample was voluntarily donated to our hospital, which is in accordance with the ethical guidelines.

Patient 1

Patient 1 was a male fetus. Multiple congenital abnormalities were detected on prenatal ultrasound scan (USS) at 26 weeks

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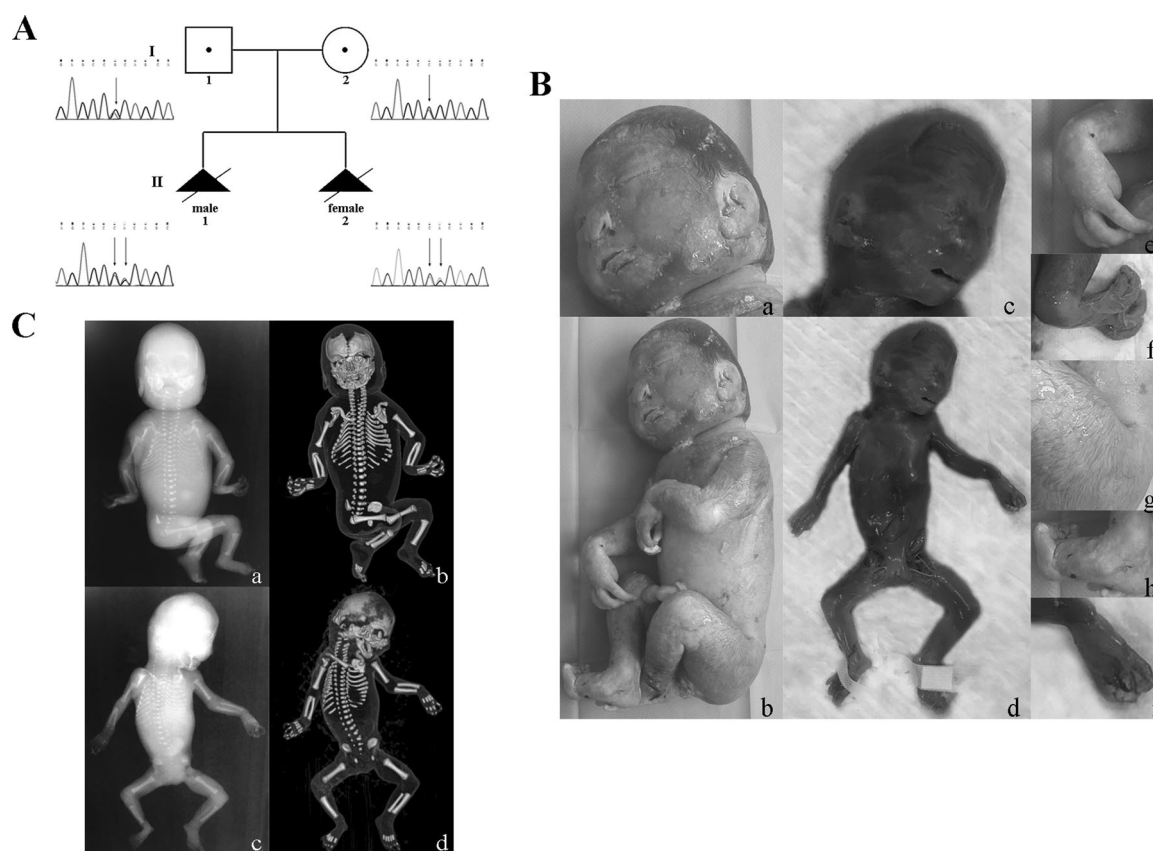


Fig. 1 Pedigree, photographs, and radiographic examination results of the two patients. **a** II 1: patient 1 (the first pregnancy of the family); II 2: patient 2 (the second pregnancy of the family). **b** a, b, e, g, h

Pictures of patient 1. **c**, **d**, **f**, **i** Pictures of patient 2. **c**, **a**, **b** Radiographic examination result of patient 1. **c**, **d** Radiographic examination result of patient 2

of gestation. The parents decided to terminate the pregnancy at 28 weeks of gestation. The following multiple anomalies were detected: intrauterine growth restriction with short limbs, micrognathia, separation of bilateral renal collecting system, hydramnios, the hands postural fixation, increased cardiothoracic proportion, and fetal edema especially of the scalp tissue. Facial dysmorphism included eyelid ptosis, hypertelorism, short nose, flat nasal bridge, anteverted nares, thin lips, low-set, large and thick ears with malformed pinnae and micrognathia (Fig. 1b (a)). In addition, the patient had a short neck, short limbs, inverted widely spaced nipples; joint contractures, long fingers and toes, ulnar deviation of fingers, adducted thumbs, toes valgus, abnormal subcutaneous fat distribution at neck and buttock, and hirsutism (Fig. 1b (b, e–h)). Radiographic examination showed short long bones with wide metaphyses, short vertebral bodies, short ribs, a narrow thorax, paddle-shaped iliac wings, and bilateral small scapulae (Fig. 1c (a, b)). Fetal karyotype was normal.

Patient 2

This patient was the sister of patient 1 and the mother's second pregnancy. USS at 18 weeks of gestation showed

scalp tissue edema and mildly short limbs. After genetic counseling, the parents decided to terminate the pregnancy at 22 weeks of gestation. Dysmorphism was similar to that in patient 1. Contractures of wrists, knees, and left elbow were milder. Hirsutism and short neck were not observed (Fig. 1b (c, d)). Radiographic examination revealed short limbs, short ribs, a narrow thorax, vertebral hypoplasia, and scoliosis (Fig. 1c (c, d)).

Whole-exome sequencing and Sanger sequencing results

Whole-exome sequencing showed two novel variants in the *ALG3* gene: NM_005787.6:c.512G>T (p.Arg171Leu) inherited from the mother and NM_005787.6:c.511C>T (p.Arg171Trp) inherited from the father. The second patient showed the same variants. (Fig. 1a). The effect of NM_005787.6:c.512G>T (p.Arg171Leu) was predicted using the computational tools Sorting Intolerant from Tolerant (SIFT), PolyPhen-2, and MutationTaster, M-CAP, and the results indicated that the protein variant is “damaging,” “damaging,” “disease causing,” and “damaging,” respectively. The CADD_phred score of the variant is 35. SIFT,

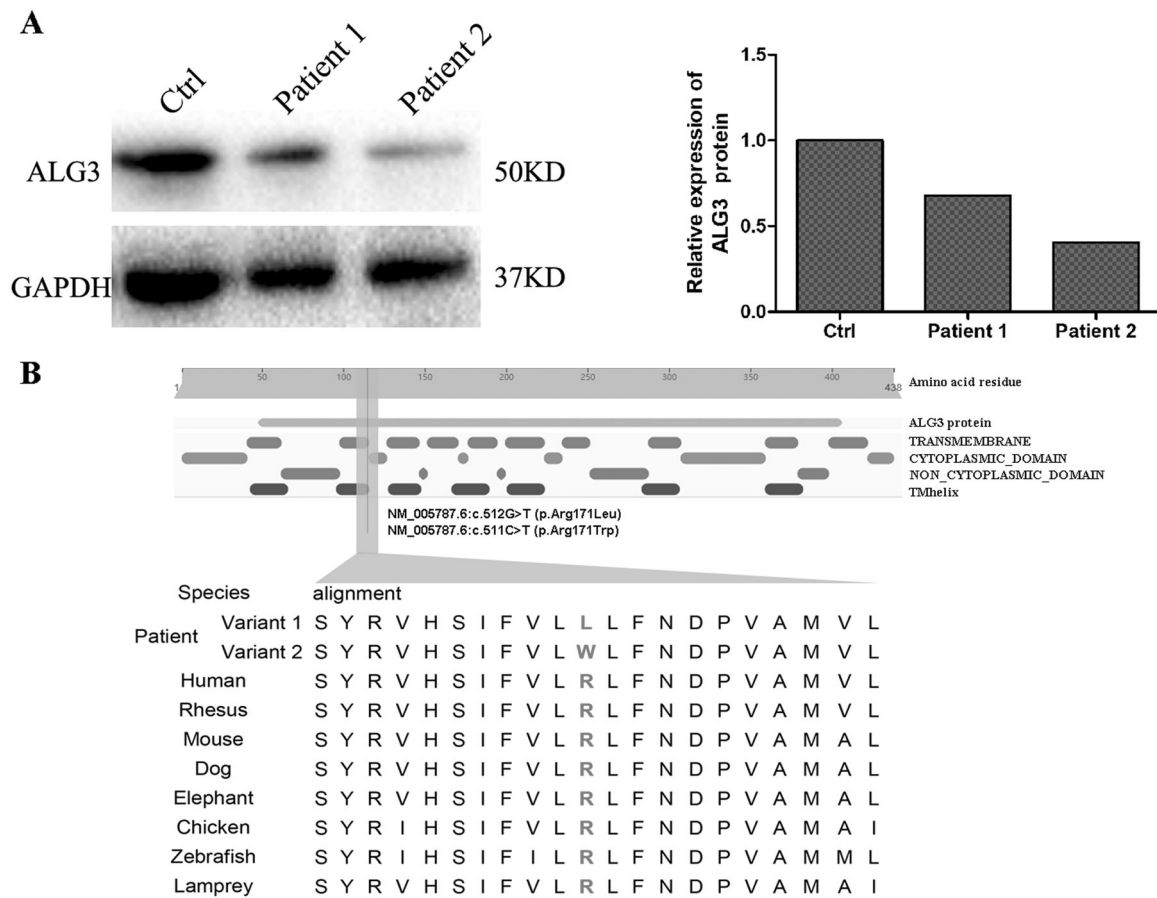


Fig. 2 The effects of the variants on ALG3 protein. **a** Relative protein expression of ALG3 in the two patients. GAPDH was used as an internal reference protein. **b** The location of the variants and

conservation of ALG3 residues. The variants were located in ALG3 protein domain, and the amino acid residue was highly conserved

PolyPhen-2, MutationTaster and M-CAP predicted NM_005787.6:c.511C>T (p.Arg171Trp) to be a “damaging,” “damaging,” “disease causing,” and “damaging” variant, respectively. The CADD_phred score of this variant is 33. According to the ACMG criteria, both variants were classified as uncertain significance (PM2, PM5 and PP3).

ALG3 protein expression in the two patients

Western blot analysis of ALG3 in muscle tissues showed a clearly decreased expression in the two patients compared to that in the control (Fig. 2a).

Discussion

Twenty-three ALG3-CDG patients have been reported so far. These patients showed a broad phenotypical spectrum that mainly includes dysmorphism and neurological, cardiac, skeletal, gastrointestinal, and urogenital abnormalities (summary of reported patients in Table 1). The phenotypes

of our patients were already extremely severe in utero and resembled those of the patients reported by Lepais et al. [3] and Sun et al. [10]. The patient described by Sun et al. died at 19 days and showed multiple dysmorphisms with prominent cerebral, cerebellar, skeletal, cardiac, pulmonary, urogenital, hepatic, and pancreatic involvement. Lepais et al. [3] reported similarly affected siblings. In one, the abnormalities were detected at 23 weeks of gestation, which was terminated at 25 weeks. In the other, intrauterine growth retardation and short long bones were noted at 13 weeks. The parents refused to terminate the pregnancy, and the child died at 12 days due to heart failure. This severe fetal phenotype resembles that of a patient reported with ALG9-CDG (OMIM: 608776) and patients with Schneckenbecken dysplasia, also known as SLC35D1-CDG (OMIM: 269250).

In conclusion, our cases expand the gene variant database of ALG3-CDG. In addition, we provide a new prenatal genotype-phenotype correlation of ALG3-CDG, which may be beneficial for the future prenatal diagnosis of other CDG cases.

Table 1 *ALG3* genetic defects and clinical features of present and reported patients

	Current study		Körner et al. / Stibler et al.	Denecke et al.	Scholten et al.	Sun et al.	Kranz et al.	Rimel-la-Le-Huor et al. / Riess et al.	Lepais et al.	Fumara et al. / Alsubhi et al. / Himmelreich et al.									
	1	2	1	1	1	1	1	1	1	1	1	1	2	3	4				
Gene mutation	c.368G>T/ c.367C>T	c.368G>T/ c.367C>T	c.353G>A	c.160_1-96del	c.796C>T	c.512G>A	c.470T>A	c.116C>T/ c.512G>A	p.P39L / p.R171Q	p.P39L / p.R171Q	p.P39L / p.R171Q	p.P39L / p.R171Q	p.P39L / p.R171Q	c.512G>A	c.160_1-96del	c.126S>A	c.380G>C/ c.286G>A	c.296+4-A>G (c.197_2-96del)/ c.1037-A>G	
Protein change	p.R123L/ p.R123W	p.R123L/ p.R123W	p.G118D	p.V54Fs-X66*	p.R266C	p.R171Q	M157K	p.P39L / p.R171Q	p.P39L / p.R171Q	p.P39L / p.R171Q	p.P39L / p.R171Q	p.P39L / p.R171Q	p.P39L / p.R171Q	p.R117P/ p.G96R	p.W421*	p.A65Fs-X42*/ p.N346S			
Gender	M	F	M	M	F	F	M	M	M	M	M	M	M	F	F	F	M	M	
Pregnancy	Abnormal	Abnormal	Normal	Normal	Normal	Abnormal	n.d.	Normal	Normal	Abnormal	Normal	Normal	Abnormal	Normal	Normal	Normal	Normal	Normal	
Consanguinity	-	-	-	n.d.	n.d.	-	n.d.	-	-	-	-	-	-	-	-	-	-	-	-
Amniotic fluid	Hydramnios	n.d.	n.d.	n.d.	Oligohydramnios	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Intrauterine growth restriction (IUGR)	+	+	-	n.d.	-	+	n.d.	-	+	+	-	-	-	-	-	-	-	-	-
Birth weight	n.d.	n.d.	4150 g	4700 g (>97th centile)	3440 g (85th centile)	3250 g (25th-50th centile)	n.d.	3790 g (>90th centile)	2760 g	755 g (-0.5 SD)	2870 g (50th centile)	2550 g (50th centile)	2550 g (50th centile)	n.d.	>97th centile	n.d.	n.d.	n.d.	n.d.
Birth length	n.d.	n.d.	54 cm	48 cm (75th centile)	52 cm (90th centile)	50 cm (50th centile)	n.d.	50 cm (50th-90th centile)	47 cm (10th centile)	n.d.	n.d.	n.d.	n.d.	n.d.	>97th centile	n.d.	n.d.	n.d.	n.d.
Hc	n.d.	n.d.	36 cm	36 cm (75th centile)	31.5 cm (5th centile)	31 cm (<3rd centile)	n.d.	34 cm (50th centile)	33 cm (10th centile)	n.d.	n.d.	n.d.	n.d.	n.d.	Normal	Normal	Normal	Normal	Normal
Microcephaly	n.d.	n.d.	Present at 4 months	<3rd centile by 5 years	<3rd centile by 5 years	<3rd centile by 4 years	Present at 4 months	<3rd centile by 5 years	<3rd centile by 10 months	+	+	+	+	+	+	+	+	+	+
Micrognathia	+	+	n.d.	n.d.	+	+	n.d.	+	n.d.	+	+	+	+	+	+	+	+	+	+
Ocular ptosis	+	+	n.d.	n.d.	+	n.d.	n.d.	+	+	+	+	+	+	+	+	+	+	+	+
Hypertelorism	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	-	+	+	+	+	+	+	+	+	+	+	+
Short nose	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Flat nose bridge	+	+	n.d.	n.d.	n.d.	+	n.d.	+	+	-	-	-	-	-	-	-	-	-	-
Anevverted nares	+	+	n.d.	n.d.	n.d.	+	n.d.	-	-	+	+	+	+	+	+	+	+	+	+
Thin lip	+	+	n.d.	n.d.	+	+	n.d.	+	+	+	+	+	+	+	+	+	+	+	+
Abnormality of the ear	+	+	+	n.d.	n.d.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Short neck	+	-	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Short limbs	+	+	-	n.d.	n.d.	+	-	-	+	+	+	+	+	+	+	+	+	+	+
Inverted wide-spaced nipples	+	+	-	n.d.	n.d.	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Flexed joint	+	+	n.d.	n.d.	n.d.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Long fingers and toes	+	+	n.d.	n.d.	n.d.	+	n.d.	+	+	+	+	+	+	+	+	+	+	+	+
Ulnar deviation of fingers	+	+	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Adducted thumbs	+	+	+	n.d.	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lateral deviation of toes	+	+	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	+	+	+	+	+	+	+	+	+	+
Abnormal subcutaneous fatty distribution	+	+	-	n.d.	n.d.	+	n.d.	-	+	+	+	+	+	+	+	+	+	+	+
Hirsutism	+	-	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-	-	-	-	-	-	-
Status	Induced labor	Induced labor	Alive at 12 months	Alive at 6 years	Alive at 4 years	Died at 19 days of sepsis	Alive at 9 years	Alive at 5 years	Alive at 21 years	Alive at 15 years	Alive at 15 years	Died at age 22 months	Neonatal death	Alive at 2 months	Alive at 7 months	Alive at 14 months	Died at age 3.5 years	Alive at 6 years	Alive at 6 years

Table 1 (continued)

Current study	Körner et al. Sibler et al.	Denecke et al.	Schollen et al.	Sun et al.	Kranz et al.	Rimel-le-Huu et al.	Riess et al.	Lepais et al.	Fumara et al.	Alsubhi et al.	Himmelfreuch et al.
Other phenotypic features					Limb normal	Limb normal	Limb normal	Limb normal	Limb abnormalities	Limb abnormalities	Limb abnormalities
Glycosylation pattern	Accumulation of the LLO intermediate; abnormal of N-glycosylation	Accumulation of the LLO intermediate; hypoglycosylated glycoproteins		Accumulation of the truncated LLO species	Accumulate glycans the size of Man ₅ GlcNAc ₂	Accumulation of truncated Man ₅ GlcNAc ₂ oligosaccharide in addition to the mature Glc ₂ Man ₆ GlcNAc ₂ oligosaccharide	Serum transferrin IEF showed a type 1 pattern	Serum transferrin IEF showed a type 1 pattern	Accumulation of the truncated Man ₅ GlcNAc ₂ oligosaccharide	Serum Tf IEF showed a type 1 pattern	IEF of Patient Tf revealed a type 1 pattern. Accumulation of Man ₅ GlcNAc ₂ -PP-dolichol
separation of bilateral renal collecting system							Pregnancy history was unremarkable	Pregnancy history was unremarkable			
Skeletal dysplasia											

F Female, M Male, n.d. Non-detected, LLO lipid-linked oligosaccharide, Tf serum transferrin, IEF isoelectric focusing.

Acknowledgements We are grateful to the patients and their family for providing samples and clinical histories.

Funding This research was supported in part by the 345 Talent Project to YL and National Natural Science Foundation of China (81701462 to YL) and National Key R&D Program of China (2018YFC1002900 to YL).

Author contributions YB and SGZ performed the research and analyzed and interpreted the data. YB and HQ drafted the manuscript. HL, ZTZ, and YSW helped in sample collection. CXL performed phenotyping of patients. YL and CQ helped in analysis and interpretation of ES data. HKJ provided technical support. YB, HL, ZTZ, YSW, YL, JLL, and CQ were involved in scientific discussion and offered suggestions. CQ and YL conceived and designed the study, revised the manuscript, and provided final approval of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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