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Case report

A case with congenital disorder of glycosylation with defective fucosylation 2 and new mutation in *FUK* gene

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Abstract

Introduction: Congenital disorders of glycosylation (CDG) is a group of rare, hereditary, multisystem disorders, predominantly affecting nervous system. There are *N*- and *O*- types of glycosylation. Fucosylation, a form of *N*-glycosylation, involves many enzymes. Until today, type 1 and type 2 fucosylation defects were identified, having pathogenic variants in genes encoding α-1,6-fucosyltransferase and fucokinase enzymes, respectively. In this article, a patient with type 2 fucosylation defect will be presented, with hypotonia, developmental delay and blindness and a pathogenic variant that was previously described in two patients.

Method: Whole exome sequencing (WES) was performed, since the patient had no time to implement diagnostic algorithm for hypotonia etiology.

Results: WES revealed a new pathogenic variant of homozygous *c.993_1011del (p.Glu335Hisfs*55)* frameshift variant of the *FUK* gene NM_145059 transcript. She had milder clinical manifestation than reported two patients.

Conclusion: Congenital Defect of Glycosylation should be considered when the clinical findings cannot be explained by other known diseases, particularly in patients with multisystemic, predominantly neurological involvement.

Keywords: Congenital defect of glycosylation; Type2 fucosylation defect; Hypotonia; Developmental delay

1. Introduction

Congenital disorders of glycosylation (CDG) is a group of rare, genetic diseases characterized by deficient glycosylation of proteins and lipids due to congenital glycan metabolism defect [1,2]. Glycosylation occurs in several processes in the cytoplasm, endoplasmic reticulum, and Golgi apparatus of the cell. It is termed as *N*- or *O*-glycosylation according to the amino acid or hydroxyl group that the glycan binds to, in the protein [1,3].

Fucosylation is a common form of *N*-glycosylation, incorporating L-Fucose (6-deoxy-L-galactose) into many glycans [3]. Fucosylation requires guanosine diphosphate L-fucose (GDP-Fucose) and a set of fucosyltransferase enzymes as substrate.

Two pathways supply GDP-Fucose in mammals, the de-novo pathway is predominant, involving a number of fucosyltransferases, and the salvage pathway, with fucokinase and other enzymes.

Pathogenic variants in the fucosyltransferase 8 (FUT8) gene encoding α-1,6-fucosyltransferase enzyme of the de-novo pathway are classified as type 1

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fucosylation defect, while pathogenic variants in the *FUK* gene encoding fucokinase enzyme, phosphorylating β-L-fucose and converting it to β-L-fucose-1-phosphate in the salvage pathway, as type 2 [3,4].

CDGs can present with multisystemic involvement, including neurological, hematological, gastrointestinal, renal, cardiovascular, ophthalmological, musculoskeletal, and skin and connective tissue involvement [1,5]. Neurological findings such as hypotonia, developmental delay, ataxia, cerebellar syndrome, peripheral neuropathy, strabismus, nystagmus, retinis pigmentosa, and stroke-like events were determined in more than 90% of the patients in the most common type of CDGs [6].

In this article, a patient with hypotonia and type 2 fucosylation defect will be presented with a view to contribute to the genotype and phenotype spectrum, due to being a novel pathogenic variant in the *FUK* gene and milder clinical findings with respect to those two patients described in the literature so far.

2. Patient's history

An 11-month-old Somalian girl was admitted due to hypotonia, development delay, and blindness. The family suspected an abnormality when the patient could not hold her head and follow the mother when she was three months old. There was no abnormality in the patient's history or family history. She was born at 38 weeks weighing 3100 g, by normal spontaneous vaginal delivery. She was born from an uneventful first pregnancy of a 21-year-old mother and a 39-year-old father, both Somalians. The family had no other pregnancy history and no children. It was not a consanguineous marriage, but they were from the same village.

Body weight and height were in the normal percentiles, system examinations were normal, and there was no dysmorphic finding at the first visit. There was a 1.5x1.0 cm café-au-lait macule on the lower outer edge of the left scapula. Neurological examination revealed central hypotonia, increased deep tendon reflexes, and normal values for joint range of motion. She had no spasticity or contracture. She failed in tracking objects or light and uttering a word but could turn towards the voice calling her name. She couldn't sit without support, maintaining head control was only briefly. The fundus was normal in the eye examination. Head control was determined during the next examination at 12 month, sitting unsupported for a few seconds at 15 month, and prolonged sitting without support at 18 month. The patient's other examination findings were still continuing. Brain MRI taken at 11 month revealed hyperintensities in T2 and fluid attenuated inversion recovery series of bilateral frontal, parietal and occipital periventricular white matter, cortical sulcal prominence and lateral ventricular enlargement. Encephalomalastic areas were detected in the bilateral parietal and right temporal lobes. Abnormal discharge or ground rhythm irregularity was not observed in electroencephalography.

Since the patient came from abroad and did not have time for a detailed examination, a whole exome sequencing (WES) was requested directly, without following the examination algorithms for the etiology of hypotonia. WES revealed a homozygous *c.993_1011del (p.Glu335Hisfs*55)* frameshift variant according to the NM_145059 transcript of the *FUK* gene located on the 16th chromosome.

3. Genetic analysis

Genomic DNA was extracted from peripheral blood cells according to the manufacturer's standard procedure (DNeasy Blood & Tissue Kits - QIAGEN). gDNA was broken into 150–500 bp fragments using a BGI enzymes kit (Segmentase, BGI), the fragments were collected using magnetic beads. Extracted DNA was amplified using a ligation-mediated polymerase chain reaction (LM-PCR). A mean exome coverage of more than 99% was obtained. The sequencing depth was greater than 100 × for capture regions. Lastly, the qualified products were sequenced with PE100 + 100 on MGISEQ-2000 (BGI, China).

Variants were filtered based on frequency, inheritance pattern, clinical phenotype and pathogenicity. After that, the *c.993_1011del (p.Glu335Hisfs*55)* variant was detected to be homozygous according to the NM_0145059 transcript in the *FUK* gene (Alternative gene title; FCSK gene), which was considered to explain the patient's clinical state. Sanger sequencing was performed to verify the variant at proband and parents. The variant submitted in ClinVar database (Submission ID: SUB9433789). The study was conducted in accordance with the Declaration of Helsinki and written informed consent was obtained from the participants. Ethics committee approval is not required as WES is performed for diagnostic purposes.

4. Discussion

Since the first case published in 1980, more than 140 types of CDG have been described to date, most of them with *N*-glycosylation defects [1,6,7]. We report a new *FUK* gene pathogenic variant in this article, leading to a milder clinical presentation than the previous two patients.

The *FUK* gene encoding the fucokinase enzyme, involved in the reuse of fucose degraded from oligosaccharides, was identified in 2002 [8]. Given that GDP-fucose is largely produced by the de-novo pathway in fucosylation, loss-of-function in the salvage pathway can be considered to cause no serious adverse effects on total cellular fucosylation. However, compound

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heterozygous *c.667 T > C (p.Ser223Pro)* and *c.2047C > T (p.Arg683Cys)* as well as homozygous *c.2980A > C (p.Lys994Gln)* mutations in the *FUK* gene were shown to cause loss-of-function in the salvage pathway and severe multisystemic disease in two patients in 2018 [4].

In our study, we detected the *c.993_1011del (p.Glu335Hisfs*55)* variant as homozygous in the *FUK* gene according to the NM_0145059 transcript. To our knowledge, this variant is novel. The children had inherited the variant from the unaffected mother and father, both in heterozygous state (Fig. 1). The frameshift variant *p.Glu335Hisfs*55* presumably leads to the loss-of-function of gene *FUK* (PVS1) and is extremely infrequent in GnomAD, ExAC and 1000 Genomes (PM2) [9] and our in-house database (n = 1978 Turkish individuals). At the same time, it is located in a highly conserved residue and GERP score showed the mutated region is conserved among the species. Bioinformatic Prediction analysis with in silico algorithms, such as Mutation Taster 2021 and CADD showed this alteration to be pathogenic and disease causing. A variant downstream of *p.Glu335Hisfs*55* have previously been reported as disease-causing, including *p.Arg741** (ClinVar ID: 1018138). *FUK* gene frameshift variants are considered to cause disruption in glycoprotein synthase and therefore cause disease.

To date, two patients with type 2 fucosylation defect were published, a six-year-old Hispanic boy and a seven-year-old Arabic girl, whose symptoms started at 3–4 years. A history of central hypotonia, developmental delay, epilepsy, feeding difficulties, cerebral atrophy, corpus callosum abnormality, delayed myelination, maculopathy, cortical blindness, optic nerve atrophy, skeletal contractures, respiratory difficulties, and frequent infections were reported in these patients [4].

Central hypotonia started almost in the neonatal period in our patient. She had no feeding difficulties. Height and weight percentiles were within normal limits. She gained new motor skills like sitting on her own at her 15-month examination while she could not sit unsupported at 11-month. She had cortical blindness despite normal macula and fundus examination. Gaining new motor skills in time and not having skeletal contractures, recurrent infections, respiratory difficulties, seizures or epilepsy can be interpreted as having a milder

A) Parts of the Sanger sequencing electropherograms are depicted and demonstrate

homozygosity for the variant *c.993_1011del* in patient, heterozygosity in father and mother.

Fig. 1. Sanger Sequencing Electropherograms A) Parts of the Sanger sequencing electropherograms are depicted and demonstrate homozygosity for the variant *c.993_1011del* in patient, heterozygosity in father and mother. B) Display of IGV (Integrative Genomics Viewer) showing a novel *FUK* gene *c.993_1011del* frameshift variant in homozygous state in patient. The gray letters represent the wild type nucleotides even so the black lines represent a 19 base-nucleotide deletion.

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clinical phenotype with respect to two previously reported patients. The comparison of the clinical features of our patient with the previous two patients is summarized in Table 1.

Clinical findings such as short stature, coarse facies or facial dysmorphic features, autistic features, microcephaly and Bombay blood group can also be seen in pathogenic variants of genes other than *FUK* participating in core fucosylation, which are not present in our patient or the previous two patients [10]. Eye anomalies such as visual impairment, optic disc hypoplasia, and nystagmus have been reported frequently, especially in *SRD5A3-CDG* patients [11]. In approximately 30 patients reported to date due to pathogenic variants in the *FUT8, FUK, POFUT1, SLC35C1, GFUS* genes involved in core fucosylation, especially common eye anomalies have not been reported. Congenital glaucoma was previously reported in one of the *FUT8* patients [10]. It is a remarkable finding that both the previous two patients and our patient had cortical blindness with a pathogenic variant in the *FUK* gene.

Various reasons can cause this different clinical phenotype. There are hundreds of incorrectly glycosylated products that impair cell signaling and migration or intercellular interaction in patients with CDG. It was suggested that each cell glycosylates differently, resulting in variable clinical phenotypes [12]. Analyzes of fibroblast cells in one of the previous two patients revealed decreased *FUK* activity and protein level. Again, some findings suggest that loss-of-function in the salvage pathway is critical for some cells whereas some can function without it [4]. The reason for the mild clinical phenotype in our patient may be attributed to the different glycosylation of each cell, the fact that fucosylation is not of the same critical importance for each cell, as well as the severity of the loss-of-function caused by the pathogenic variant. Other reasons for the mild clinical condition of our patient may be his young age, the fact

Table 1
Comparison of the clinical findings of our patient and the previous two patients [4].

	Patient 1	Patient 2	Our Patient
Sex	Male	Female	Fermale
Ancestry	Hispanic	Middle Eastern (Qatari)	Somalian
Current age	6 years	7 years	18 months
Age at onset of symptoms	3 years	4 years	Almost newborn term
Pregnancy complications	No	Born premature at 25 weeks (810 g)	No
Facial features or dysmorphism	No	No	No
Developmental delay	Yes	Yes	Yes
Stature	Normal for age	Normal for age	Normal for age
Intellectual disability	Yes, severe	Yes, severe	Yes, mild
Seizures/ epilepsy	Yes, epileptic encephalopathy	Yes, epileptic encephalopathy	No
Ataxia or gait problems	Nonambulatory	Nonambulatory	Nonambulatory
Hypotonia	Central hypotonia	Central hypotonia	Central hypotonia
Brain anomalies	Yes, dysplastic corpus callosum, delayed myelination in deep white matter	Yes, cerebellar atrophy, agenesis of corpus callosum, severe periventricular leukomalacia	Yes, hyperintensities of periventricular white matter, ventricular enlargement, encephalomalastic areas
Ocular	Symmetric maculopathy with severe visual impairment	Strabismus, nystagmus, cortical blindness, optic nerve atrophy	Normal macula and optic nevre, cortical blindness
Skeletal	Contractures	Contractures	Normal
Cardiac	None	None	None
Respiratory	Recurrent respiratory infections	Respiratory difficulties	Normal
Hepatopathy	Yes, elevated gamma glutamyl transferase (GGT)	No	No
Feeding problems/ Gastrointestinal	Dysphagia, GI dysmotility, reflux, poor gastric emptying, G-J tube feeding	Aspiration with oral feeds, G-tube feeding, chronic malabsorption, diarrhea, history of neonatal necrotizing enterocolitis with bowel perforation	Normal
Immunological	Recurrent infections, enlarged platelets	No	No

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that neurodegeneration will increase with age, or pathogenic variants that we do not know yet in other genes involved in glycolysis and fucolysis in patients with more severe clinical manifestations [12].

Inverted nipples, abnormal fat tissue distribution, dysmorphic facial features, brachydactyly, hypoplastic fingernails, developmental delay, cerebellar hypoplasia, major congenital anomalies especially in the central nervous system, high alkaline phosphatase and liver enzymes, coagulation disorders, and detection of transferrin isoforms in isoelectric focusing of serum transferrin. Although they suggest some types of CDG, these are not specific definitive diagnostic findings [6]. CDG diagnosis is difficult due to lack of specific diagnostic biological or biochemical markers for CDG and clinical findings look like other metabolic diseases. However, diagnosis frequency of CDG, the diversity and variability of the phenotype genotype spectrum are increasing day by day, thanks to the developments in genetics and increasing awareness. We could diagnose the patient with the new pathogenic variant and milder clinical findings by means of WES. In conclusion, especially in patients with multisystemic involvement with predominant neurological findings, CDG should be considered if the clinical findings cannot be explained by other known diseases.

Author contributions

Nezir Özgün, data collect, planning, writing. Yavuz Şahin, data collect, writing and genetic analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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