Received: 15 November 2021 | Revised: 28 March 2022 | Accepted: 3 November 2022

DOI: 10.1002/mgg3.2099

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

Whole exome sequencing reveals several novel variants in congenital disorders of glycosylation and glycogen storage diseases in seven patients from Iran

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Abstract

Background: Congenital disorder of glycosylation (CDG) and Glycogen storage diseases (GSDs) are inborn metabolic disorders caused by defects in some metabolic pathways. These disorders are a heterogeneous group of diseases caused by impaired O- as well as N-glycosylation pathways. CDG patients show a broad spectrum of clinical presentations; many GSD types (PGM1-CDG) have muscle involvement and hypoglycemia.

Methods: We applied WES for all seven patients presenting GSD and CDG symptoms. Then we analyzed the data using various tools to predict pathogenic variants in genes related to the patients' diseases.

Results: In the present study, we identified pathogenic variants in Iranian patients suffering from GSD and CDG, which can be helpful for patient management, and family counseling. We detected seven pathogenic variants using whole exome sequencing (WES) in known *AGL* (c.1998A>G, c.3635T>C, c.3682C>T), *PGM1* (c.779G>A), *DPM1* (c.742T>C), *RFT1* (c.127A>G), and *GAA* (c.1314C>A) genes.

Conclusion: The suspected clinical diagnosis of CDG and GSD patients was confirmed by identifying missense and or nonsense mutations in *PGM1*, *DPM1*, *RFT1*, *GAA*, and *AGL* genes by WES of all 7 cases. This study helps us understand the scenario of the disorder causes and consider the variants for quick disease diagnosis.

KEYWORDS

CDG, GSD, Iran, Pathogenic genetic variants, WES

[Correction added on February 14, 2023 after first online publication. The co-authors Samira Negahdari and Mohammad Hamid affiliations are updated in this version.

1 | INTRODUCTION

Biochemical and biological role of glycoproteins and glycolipids are determined by the specific structure of its glycan parts (Varki, 2017). Glycosylation, the attachment of oligosaccharides to proteins and lipids, involving >250 gene products, plays a crucial role in many biological processes. Therefore, the deficiency of glycosylation enzymes or transporters can result in a variety of medical problems such as congenital disorder of glycosylation (CDG) (Supraha Goreta et al., 2012). Most of CDG disorders have an autosomal recessive inheritance pattern, but autosomal dominant and X-linked forms have also been identified (Chang et al., 2018). CDGs comprise a broad range of mild to severe genetic diseases because the defective genes are involved in a variety of diverse steps in metabolic pathways (Péanne et al., 2018).

Patients with CDG typically present with multisystemic signs, mainly growth retardation, developmental delay, hypotonia, and neurologic abnormalities. Other symptoms include skeletal dysplasia, dysmorphic features, cardiac, hepatic, hematological, and endocrinological abnormalities have been reported (Grünewald et al., 2002; Péanne et al., 2018).

Glycogen storage diseases (GSDs) comprise a group of inherited inborn errors of glycogen metabolism. GSDs are classified according to enzyme deficiency and the affected tissue into several types; GSDII (OMIM Entry # 23230) also known as Pompe disease and GSDIII (GSDIII; Cori-Forbes Disease; OMIM 232400) are two rare, autosomal recessive disease with a wide spectrum clinical severity range affecting the liver, heart, skeletal muscle, and accessory muscles of respiration (Dagli et al., 2016; Kanungo et al., 2018).

In this present study, we report genetic variants identified using WES and comprehensive bioinformatic analysis in some Iranian patients with clinically diagnosed CDG and GSD. We found putative pathogenic mutations in known related genes.

2 | MATERIALS AND METHODS

2.1 | Subjects and clinical assessment

Here, detailed clinical assessment included standard history, physical evaluations, neurological examination, and metabolic profiling were performed in seven individuals who had been primarily suspected to be affected by various types of CDG and GSD by specialists. This research was approved by ethical committee of Ahvaz Jundishapur University of Medical Sciences.

2.2 | DNA extraction

Approximately 10 ml of peripheral blood from all participants and available family members were collected. Genomic DNA was extracted using the routine salting out protocol from blood leukocytes. DNA quantity and quality were evaluated by gel electrophoresis and nano-drop.

2.3 | Exome sequencing

In an effort to identify rare alleles associated with *AGL*, we applied WES for the enrolled patients. For the library preparation, SureSelect Human All Exon V6, followed by sequencing using the Illumina HiSeq 2000 genome analyzer platform was used by Macrogen Company.

2.4 | PCR and sanger sequencing

The PCR primers were designed using OLIGO 7. The PCR was performed using 100 ng genomic DNA, 12.5 µl Master Mix (Amplicon Co.), 10 pmol of each primer and the total volume of the PCR was 25 µl. The amplification condition was 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s with a final extension at 72°C for 5 min. The results of Sanger sequencing were aligned with the reference genomic sequence and analyzed with Chromas LITE 2.1.1. Also, the presence of the detected mutation was confirmed by the parent analysis and bi-directional Sanger sequencing. Purified DNA fragments were directly sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems 3500 DNA Analyzer) and analyzed by Chromas and DNA Baser v4 software.

2.5 | In silico pathogenicity assessing of genetic variants

Synonymous variants were excluded from our considerations. Further filtering was carried out based on allele frequency in the ExAC (Exome Aggregation Consortium), 1000 g ESP, Kaviar databases, and removal of common variants (i.e., minor allele frequency >0. 1). Subsequently, homozygous variants were analyzed and reported variants associated with diseases were considered. Next, the variants selected in the previous step were evaluated with several in silico prediction programs.

3 | RESULTS

3.1 | Clinical findings

In this study, the seven patients, three infant girls and four boys, mostly were born with consanguineous parents with different ethnicities comprising, Persian, Lur, and Arab, were included.

All patients showed a relatively hypotonia with neck muscle weakness. In P4, P5, P6, and P7 liver shows mild prominent size; in addition, P6 has an increase in size of the spleen. Blood sepsis and dyspnea were also observed in P6. Cardiac problems were seen in P1 and P4 (Table 1). Microcephaly was diagnosed in P3 case. Polydactyly was seen in P7. Hearing loss was observed in one patient (P2), while others had no auditory problems. P1 and P2 had vision problems; Retinitis pigmentosa (RP) and cataracts was seen in P2, while P1 was completely blind. Skeletal problem-skyphosis-was seen in P1.

Consanguineous relationships have been mentioned for all these families (Table 1), origin from southwestern of Iran.

3.2 | Genetic findings

Using WES, we detected seven putative pathogenic or likely pathogenic variants (according to ACMG criteria by step to step filtering variants as described above), in known *AGL*, *PGM1*, *DPM1*, *RFT1*, and *GAA* genes. We identified homozygous mutations (six novel and one previously reported) in seven affected individuals belonging to unrelated families initially diagnosed as CDG and GSD patients (Table 2).

The variants were verified by PCR amplification of the corresponding, coding, and flanking intronic sites following standard protocols. The filtering strategy allowed us to identify two novel homozygous nonsense variants in the *AGL* gene (RefSeq NM_000028.2): c.3682C>T; p.(Arg1228*) (exon27) in case 7 and *GAA* gene (RefSeq NM_000152.5); p.(Thy438*) (exon9) in case 4.

Similar screening of the remaining patients resulted in the identification of five causative mutations in *AGL*, *PGM1*, *DPM1*, and *RFT1* genes has been described as follows.

Case1: In this case, a novel homozygous variant was identified in *PGM1* (RefSeq NM_001172818.1): c.779G>A; p.(Gly260Asp) (exon5) and confirmed by Sanger sequencing, as well as formal segregation analysis the probands' parents were performed. The same variant filtering strategy was applied in case 2 as the previous cases. The detected mutation was previously reported in *DPM1* gene (c.742T>C), p.(Ser248Pro). Case 3: a novel disease-causing

TABLE 1 A brief description of four affected individuals in seven families

Description	Affected individuals						
	P1	P2	P3	P4	P5	P6	P7
Age of onset, years, month	At birth	At birth	At two months	At four months	At year one	At two months	At birth
Hypotonia	+	+	+	+			
Dysphagia			+				
Developmental delay	+	+	+		+	+	
Skeletal problem	+						+
Neurologic abnormalities		+					
Cardiac, hepatic & splenic abnormalities	+	+		+	+	+	+
Polyneuropathy			+				
Vision problems	+	+					
Ethnicity/Gender	L/M	P/M	L/F	A/F	A/M	L/F	L/F
Parental consanguinity degree	Third relative	No-relativity	No-relativity	Third degree relative	Third degree relative	Forth degree relative	Forth degree relative

Abbreviations: A, Arab; F, female; L, Lur; M, male; P, Persian.

TABLE 2 All the genes and their variants in seven participated patients in this study

Patient	Gene	Coding sequence location	Amino acid changes	Exon number	Reference	Disease	Inheritance
P1	PGM1	c.779G>A	p.Gly260Asp	5	—	DPM1-CDG	AR
P2	DPM1	c.742T>C	p.Ser248Pro	9	rs587777114	DPM1-CDG	AR
P3	RFT1	c.127A>G	p.Ile43Val	2	rs1299936893	RFT1-CDG	AR
P4	GAA	c.1314C>A	p.Thy438*	9	rs1001018149	GSDII	AR
P5	AGL	c.1998A>G	p.Glu407Gly	9	—	GSDIII	AR
P6	AGL	c.3635T>C	p.Met1212Thr	27	rs779439947	GSDIII	AR
P7	AGL	c.3682C>T	p.Arg1228*	27	rs113994131	GSDIII	AR

variant in the *RFT1* gene (RefSeq NM_052859.4) c.127A>G; p.(Ile43Val) (exon2) was found in the homozygous state in the proband and both sequenced, heterozygous carrier siblings. We also identified three novel distinct homozygous missense variants, c.6686T>G, p.(Glu407Gly) (exon 9), c.3635T>C, p.(Met1212Thr) (exon 27), and c.3682C>T, (Arg1228X) (exon 27), in *AGL* gene (RefSeq NM_000028.2), in case 5, 6, and 7respectively.

3.3 | In silico analyses

Prediction of pathogenic effects of missense mutations on the function of the proteins was done using various in silico prediction tools (Table 3).

4 | DISCUSSION

Here we performed clinical analysis and mutation diagnostics using WES in seven distinct families in southwest of Iran with primary overlapping clinical manifestations of a CDG and GSD conditions. The suspected clinical diagnosis of CDG and GSD patients confirmed by identifying missense and or nonsense mutations in *PGM1*, *DPM1*, *RFT1*, *GAA*, and *AGL* genes by WES of all seven cases.

In the case of GSDIII condition, after clinical and molecular analysis of three cases (P5, P6, and P7) from unrelated consanguineous families, we were able to identify three novel variants (c.3682C>T, p.Arg1228X), (c.6686T>G, p.Glu407Gly), and (c.3635T>C, p.Met1212Thr) in *AGL* gene. The novel mutation c.3635T>C, p.(Met1212Thr), and c.3682C>T, p.Arg1228* was located in the glucosidase domain of in on the C-terminal of the glycogen debranching enzyme in exon 27. Deficiency of GDE domains results in impaired degradation of glycogen and increased abnormal glycogen accumulation in affected tissues (Kanungo et al., 2018). Also, c.3635T>C mutation, causes methionine-threonine substitution that can physically affect the content and protein yield. In the case of the nonsense mutation c.3682C>T, p.Arg1228* our findings show it may results in a shortened protein whose function could be altered or even be stopped.

We detected a novel nonsense mutation (c.1314C>A, p.Thy438*) that causes a defect in acid α-glucosidase (GAA) that leads to GSDII in case 4 (GSDII-GAA). Deficiency or absence of this enzyme results in the accumulation of glycogen in the lysosomes in multiple tissues in GSDII condition (Dagli et al., 2016; Kishnani et al., 2006).

Here, we also present three cases (P1, P2, and P3) with CDG conditions that have been described below.

A novel missense mutation (c.779G>A, p.Gly260Asp) in the *PGM1* (phosphoglucomutase-1) gene in P1 was identified. The phosphoglucomutase 1 deficiency causes an autosomal recessive type of DPM1-CDG. The phosphoglucomutase-1 is a well-known enzyme for its key role in glucose homeostasis by catalyzing the inter-conversion of glucose 1-phosphate and glucose 6-phosphate and participating in both glycogenolysis and glyconeogenesis (Tegtmeyer et al., 2014).

We identified c742T>C (S248P) mutation in exon 9 of the *DPM1* gene in P2 case. Deficiency of DPM1, impairs the assembly of N-glycans and causes DPM1-CDG (Garcia-Silva et al., 2004; Yang et al., 2013).

Here, we also report a female patient (P3) with a novel homozygous mutation in *RFT1* (NM_052859.4, c.127A>G, p.Ile43Val) leads to the accumulation of dolichol-PP-GlcNAc2Man5 in impaired glycosylation. Rft1 protein proposed to involve in the translocation of the dolichol-linked oligosaccharide GlcNAc2Man5 across the endoplasmic reticulum membrane. Mutations in the *RFT1* gene lead to the RFT1-CDG (Haeuptle et al., 2008; Haeuptle & Hennet, 2009).

In the present study, the purpose was the identification of pathogenic variations using WES in some Iranian patients in order for patient management, and family counseling. WES is a comprehensive, cost-efficient, and effective method to identify mutations in genetic diseases, including inherited metabolic disorders, especially in complex cases.

TABLE 3 Pathogenicity assessment of the variants identified by WES

Case	Gene	Amino acid changes	PredictSNP	MT	Polyphen2	SIFT	PANTHER	gnomAD
P1	PGM1	p.Gly260Asp	D,61%	D	D Score:0.997	AFFECT PROTEIN FUNCTION Score: 0.01	Probably damaging Preservation time: 4200	HOM = 0 HET = 0 ƒ = 0
P2	DPM1	p.Ser248Pro	N,63%	D	B Score:0.261	TOLERATED Score: 0.29	Probably damaging Preservation time: 797	HOM = 0 HET = 0 ƒ = 0
P3	RFT1	p.Ile43Val	N,83%	D	B Score:0.036	TOLERATED Score: 0.88	Probably damaging Preservation time: 1215	HOM = 0 HET = 0 ƒ = 0.0000318
P4	GAA	p.Thy438*	—	—	Stop Codon	—	—	HOM = 0 HET = 1 ƒ = 0.00000466
P5	AGL	p.Glu407Gly	D,61%	D	D Score:0.999	TOLERATED Score: 0.33	Probably damaging Preservation time: 1237	HOM = 0 HET = 3 ƒ = 0.0000119
P6	AGL	p.Met1212Thr	N,65%	D	B Score:0.001	TOLERATED Score: 0.53		HOM = 0 HET = 4 ƒ = 0.000016
P7	AGL	p.Arg1228*		—	Stop Codon	—	—	HOM = 0 HET = 2 ƒ = 0.00000799

Abbreviations: F, Allele frequency; MT, Mutation Taster.

AUTHOR CONTRIBUTIONS

None.

ACKNOWLEDGMENTS

We grateful to the staff of Narges Genetic Laboratory and all family members of the patients and their relatives for their cooperation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

None.

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How to cite this article: Papi, A., Zamani, M., Shariati, G., Sedaghat, A., Seifi, T., Negahdari, S., Sedighzadeh, S. S., Zeighami, J., Saberi, A., Hamid, M., & Galehdari, H. (2023). Whole exome sequencing reveals several novel variants in congenital disorders of glycosylation and glycogen storage diseases in seven patients from Iran. *Molecular Genetics & Genomic Medicine*, *11*, e2099. https://doi.org/10.1002/mgg3.2099