Molecular Genetics and Metabolism Reports 26 (2021) 100717

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Case Report

SLC35A2-CDG: Novel variant and review

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ARTICLE INFO

Keywords:
CDG
Congenital disorder(s) of glycosylation
IGF1
Phenotype
SLC35A2
Variant
Sequence comparison

ABSTRACT

SLC35A2 encodes the X-linked transporter that carries uridine diphosphate (UDP)-galactose from the cytosol to the lumen of the Golgi apparatus and the endoplasmic reticulum. Pathogenic variants have been associated to a congenital disorder of glycosylation (CDG) with epileptic encephalopathy as a predominant feature. Among the sixty five patients described so far, a strong gender bias is observed as only seven patients are males. This work is a review and reports a SLC35A2-CDG in a male without epilepsy and with growth deficiency associated with decreased serum IGF1, minor neurological involvement, minor facial dysmorphism, and camptodactyly of fingers and toes. Sequence analysis revealed a hemizygosity for a novel *de novo* variant: c.233A > G (p.Lys78Arg) in SLC35A2. Further analysis of SLC35A2 sequence by comparing both orthologous and paralogous positions, revealed that not only the variant found in this study, but also most of the reported mutated positions are conserved in SLC35A2 orthologous, and many even in the paralogous SLC35A1 and SLC35A3. This is strong evidence that replacements at these positions will have a critical pathological effect and may also explain the gender bias observed among SLC35A2-CDG patients.

1. Introduction

The solute carrier family SLC35 comprises several members of an evolutionary conserved family of nucleotide sugar transporters (NSTs) such as the UDP-GlcNAc (NGT) and UDP-galactose transporters (UGT). The solute carrier family SLC35 of human NSTs is divided into 7 subfamilies (SLC35A-G), identified on the basis of sequence similarity. The SLC35A subfamily includes 5 ancient paralogous (SLC35A1–5) of which SLC35A1 and SLC35A3 share the highest identity with the CDG-associated SLC35A2 (Table 1).

SLC35A2-CDG is an X-linked congenital disorder of glycosylation (CDG), caused by the deficiency of the Golgi-localized UGT. It results in the reduction of galactosylation needed for N-glycan remodeling and O-glycan synthesis in the Golgi, and thus affects the synthesis of glycoproteins, glyco(sphingo)lipids and proteoglycans [1]. The *SLC35A2* cDNA which encodes for the human UGT was first cloned and characterized by Miura et al. in 1996 [2]. Interestingly, two splice variants of *SLC35A2* were identified encoding two proteins UGT1 and UGT2, which differ in 3 amino acids in the C-terminus [2,3]. UGT1 is localized only in the Golgi apparatus, whereas the UGT2 C-terminus contains a dilysine motif that is responsible for dual localization in the Golgi and endoplasmatic reticulum [4].

The overexpression of NGT (*SLC35A3*) in mutant cells defective in UGT (*SLC35A2*) has been found to restore galactosylation of N-glycans [5]. Although NGT overexpression restored UDP-Gal transport, it also resulted in the decrease of transport of its natural substrate UDP-GlcNAc

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https://doi.org/10.1016/j.ymgmr.2021.100717
Received 8 December 2020; Received in revised form 17 January 2021; Accepted 18 January 2021
Available online 30 January 2021

into the Golgi. This observation suggested that the biological function of both the NGT and UGT in galactosylation might be coupled. Maszczak-Seneczko et al. in 2012 suggested that NGT/UGT complexes either mediate transport of both substrates (UDP-Gal and UDP-GlcNAc) or alternatively just bring the NGT and UGT homodimers together [6]. Either way, the ability of NGT and UGT to interact with each other may be a regulatory mechanism of N-glycan biosynthesis in the Golgi by ensuring adequate supply of both natural substrates to their respective glycosyltransferases. It seems clear now that the NGT and the UGT function in glycosylation is achieved *via* their mutual interaction [6,7].

Compared to several other well-characterized transporters, the NGT shows limited amino acid sequence identity to other NGTs that transport the same substrate, in particular yeast and mammals [2]. An important exception to this is the UGT short N-terminal region comprising 35 amino acid residues that is crucial for N-glycan galactosylation [8].

The phenotype of the reported SLC35A2-CDG patients reported so far is mainly characterized by epileptic encephalopathy and variable dysmorphism [9–24]. We here widen the phenotype and the genotype by the identification and characterization of a mildly affected male without epilepsy and carrying a novel variant in a highly conserved position of the protein.

2. Methods

2.1. Biochemical analysis

Serum transferrin (Tf IEF) and apolipoprotein C-III isoelectrofocusing were carried out as described previously [25,26]. Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry of immunopurified serum transferrin was performed according to Sturiale et al. [27].

2.2. Genetic analysis

Pathogenic variant identification was achieved by whole exome sequencing. Genomic DNA was sheared by sonication, platform-specific adaptors were ligated, and the resulting fragments were size selected. The library was captured using the SeqCap® EZ Human Exome Library v3.0 (Roche NimbleGen), and paired end (2 × 101 bp) sequenced on a HiSeq2000 (Illumina). Reads were aligned to the human reference genome (hg19) using BWA (v0.6.2), and duplicate reads were removed using Picard MarkDuplicates (v1.78). Local realignment around insertions and deletions, base quality score recalibration, and variant calling were performed using GATK (v2.4.9) RealignerTargetCreator, IndelRealigner, BaseRecalibrator, and UnifiedGenotyper. Variants were annotated using Annovar (v11-02-2013). Synonymous variants were excluded, whereas variants with a frequency < 5% in the 1000 Genomes project, ESP, GoNL, and our in-house database were further considered. Subsequent prioritization was then applied based on recessive inheritance, conservation, and pathogenicity prediction scores.

2.3. Sequence collection and alignment

Human SLC35A2 (NP 005651.1) protein sequence was used as reference to search NCBI database for orthologous sequences through blastp. SLCA35A2 orthologous were collected for all major vertebrate groups (mammals, reptile, birds, amphibian's, teleost fish and sharks) and for the model organism invertebrates namely: *Branchiostoma floridea*, *Ciona intestinalis*, *Oikopleura dioica*, *Drosophila melanogaster*, and *Caenorhabditis elegans*. The collected sequences were uploaded into Geneious R7.1.9 (https://www.geneious.com) and aligned using the MAFFT align v7.017 [28] plugin tool. SLC35A2 paralogues were identified through the Ensembl genome database (Release 99-January 2020) paralogue pipeline, corresponding Human SLC35A2 paralogue sequences were next collected and uploaded into Geneious R7.1.9 (https://www.geneious.com) and aligned as previously described. Accession number of all sequences collected are available in Fig. 2.

3. Results

3.1. Patient report

This boy is born in 1998 after a normal 40 weeks' pregnancy as the first child of non-consanguineous healthy parents with a normal stature (both parents 164 cm). Apgar score was 9/10, birth weight 3310 g (SDS: 1.0), length 47 cm (SDS: −1.0) and head circumference 34 cm (SDS: 0.0).

Initial psychomotor development was normal: sitting without support at 6 months, walking alone at 1 year, and speaking first words around 14 months. Biochemical investigation showed a normal blood count and normal levels of serum albumin, cholesterol, transaminases, creatine kinase, lactate dehydrogenase, thyroid stimulating hormone, amino acids and of blood coagulation factors IX, XI and antithrombin. Leukocyte karyotype was normal. Brain magnetic resonance imaging, echocardiography, ophthalmology and skeletal radiography were normal. Growth velocity started to decline at about four years. Evaluation at nine years showed a normal serum insulin-like growth factor 1 (IGF1), a normal growth hormone (GH) response to clonidine stimulation, and a bone age of eight years. At 13 years, a normal value of IGFBP3 was found (3 mg/L, normal: 1.3–6.6 according to Tanner stage). Height was 126 cm (SDS: −3.07), with a growth velocity of 3.89 cm/year (SDS: 1.39), and a target height of 170.5 cm (SDS: 0.63). There was mild facial dysmorphism with triangular shape of the face, prominent and broad base of the nose, and mild prognathism of the upper jaw.. Bone age was 11.5 years. Serum IGF1 was decreased (90 ng/mL; reference range: 202–957) and IGF binding protein 3 was normal. GH stimulation tests with clonidine and glucagon showed a normal GH release. An IGF1 generation test with GH elicited a minimal response, consistent

Table 1
Characterization of *SLC35A* paralogues and corresponding CDG linked information.

SLC 35A paralogues	Protein name and aliases	Substrate (s)	Subcellular localization	Amino acid number	UniProtKB	Linked disease
SLC35A1	CMP-Sia transporter (CST)	CMP-Sia	Golgi	337	P78382	SLC35A1-CDG (OMIM #603585)
SLC35A2 *UGALT* *UGT* *UGTL*	UDP-Gal transporter (UGT) UDP-Gal	UDP-Gal; UDP-GlcNAc	Golgi and/or ER	396	P78381	SLC35A2-CDG (OMIM #300896)
SLC35A3	UDP-GlcNAc transporter (NGT)	UDP-GlcNAc	Predominantly Golgi	325	Q9Y2D2	CDG-SLC35A3 (OMIM #615553)
SLC35A4	Probable UDP-sugar transporter; MGC2541	Putative UDP-Gal		324	Q96G79	
SLC35A5 *(ORF) UNQ164* *(OFR) PRO190*	Probable UDP-sugar transporter	Putative UDP-sugar		424	Q9BS91	

with GH resistance. At 15 years, height was 136 cm (SDS -3.64) and treatment was started with recombinant IGF1 followed by growth acceleration (Fig. 1A).

At 9 years, he underwent a bilateral calcaneus-stop procedure for symptomatic flexible flatfeet. At 13 years, he was evaluated for scoliosis and progressive hand and feet deformities (clinodactyly, camptodactyly of the four ulnar fingers and toes, and ulnar deviation of the two distal phalanges of the fourth and fifth fingers). Hand deformities are shown in Fig. 1B and C.

Brain MRI, repeated at 18 years, was normal. Currently at 20 years, he has a normal gait, tendon hyperreflexia (especially in the lower limbs), no motor deficits, no Babinski, and no signs of cerebellar dysfunction nor epilepsy. There is a minor intellectual disability. He is autonomous in most daily activities, rides a bicycle, runs small errands and completed 12 years of school education with an individualized educational program. He currently works as a theatre box-office clerk.

Isoelectrofocusing (IEF) of serum transferrin (Tf) at six years revealed a type 2 pattern (asialo Tf: 0.5%, normal: 0–1.0; monosialo Tf: 7.5%, normal: 0–2.7; disialoTf: 17.1%, normal: 0.0–10.5; trisialo Tf: 23.4%, normal:6.5–18.5; tetrasialo Tf: 27.7%, normal: 35.3–63.5; pentasialo Tf: 18.9, normal: 17.1–31.1; hexasialo Tf: 4.8, normal: 0.0–13.4). Serum apolipoprotein C-III isoelectrofocusing (IEF) was normal.

MALDI-TOF analysis of serum Tf revealed hypogalactosylation (not shown).

Whole exome sequencing of leukocyte DNA showed a novel variant in *SLC35A2*: c.233A > G (p.Lys78Arg) in transcript NM_001042498.2 (ClinVar File Submission: SCV000840552), confirmed by Sanger sequencing also in patient's fibroblasts. This variant was absent in the mother (*de novo)* and was not found at gnomAD browser V3 [29]. Pathogenicity prediction using CADD revealed a score of 26.6, a strong indication of its functional impact. To further investigate the relevance of position 78 we performed a sequence alignment of 20 SLC35A2 orthologous collected from vertebrate and invertebrate species showing full conservation of lysine 78 (Fig. 2A). In addition, as paralogue annotation has previously proven to be efficient in distinguishing between disease causing and benign variants [30–32], we investigated position 78 of SLC35A2 in the corresponding human paralogues SLC35A1 and SLC35A3, SLC35A4 and SLC35A5 mentioned previously (Fig. 2B). Lysine 78, located in the N-terminal of the protein, was shown

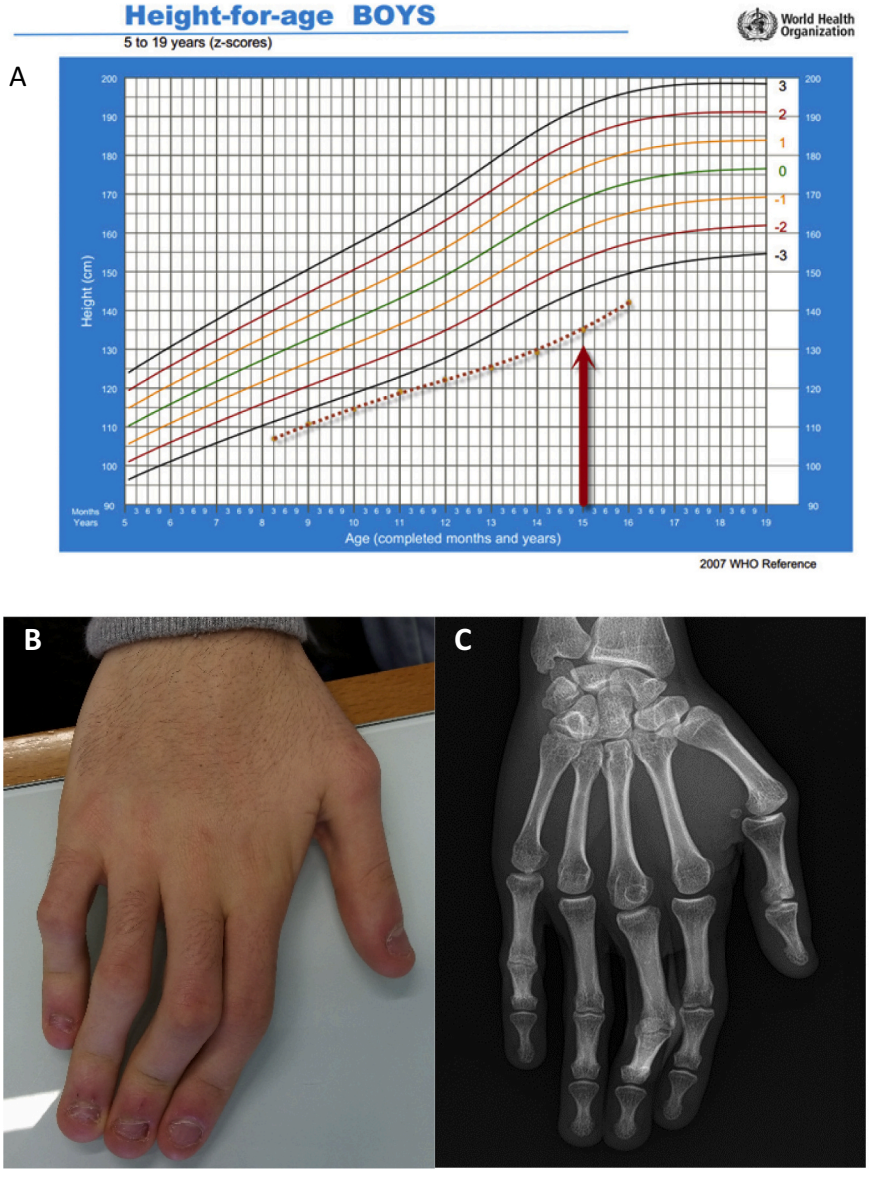

Fig. 1. A- The red arrow shows the start of the recombinant IGF-1 at 15 years, followed by a positive growth response; B and C - Hand deformities observed in the patient: clinodactyly, camptodactyly of the four ulnar fingers, and ulnar deviation of the two distal phalanges of the fourth and fifth fingers at age 13.

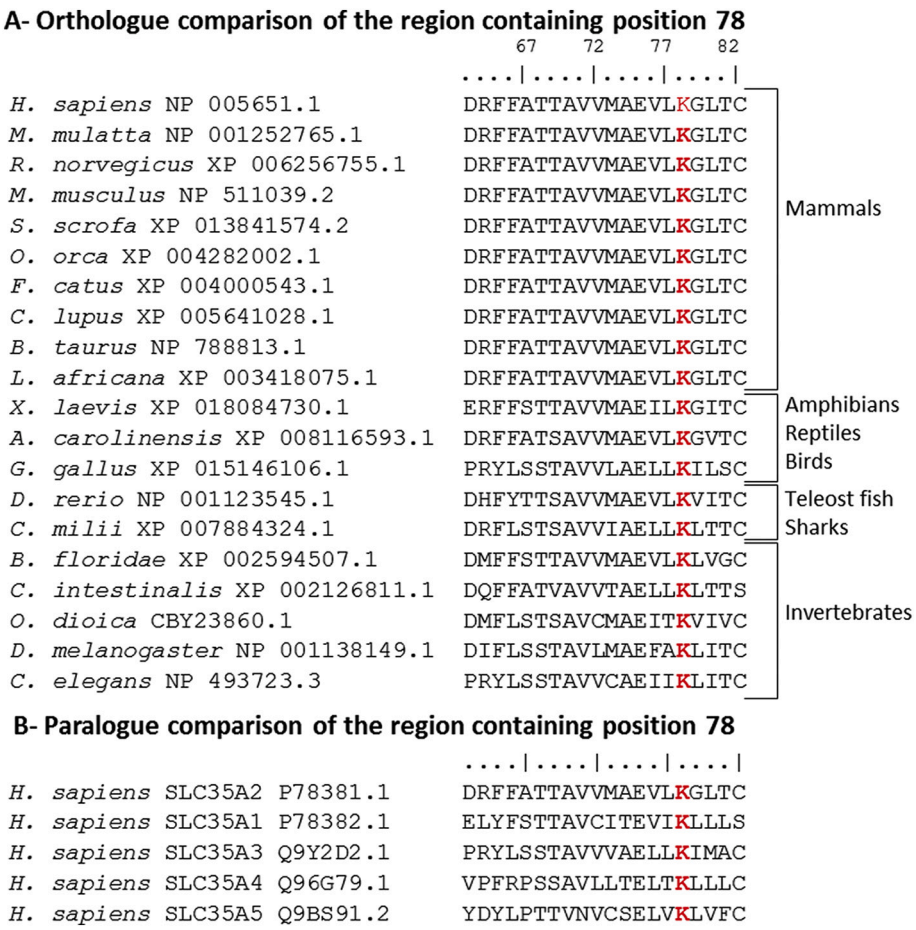

Fig. 2. Multiple sequence alignment (MSA) analysis. A- MSA of 20 SLC35A2 orthologous around position 78 (red). B- MSA of SLC35A2 and human paralogues focusing on the region containing position 78.

to be conserved at the homologous position in all five proteins (Fig.2 B). Altogether, this information points towards a deleterious effect of the lysine to arginine replacement at position 78 in the SLC35A2 transporter.

We next gathered the information regarding the previously reported germline *SLC35A2* variants in order to compare the type of inheritance and prediction scores using CADD with the novel variant at position 78 (Table 2). Noteworthy is the observation that almost all variants occur *de novo*. This indicates a strong selection against mutations at this gene, which impairs their transmission through generations, resulting in a scenario where most of the pathological diversity observed is novel. In accordance, it is expected that most of the variants described thus far should be predicted as deleterious by the bioinformatics tools. CADD scores for all previously reported missense and frameshift variants revealed values that are undoubtedly indicators of deleteriousness [33].

Considering Combined Annotation Dependent Depletion (CADD) scores obtained for previously reported pathogenic variants as well as from the present patient variant c.233A > G (p.Lys78Arg) we find that the present variant scores (26.6) well within the range of other pathogenic variants with clearly associated phenotypes.

Also the positioning of missense variants presented in Table 2 in the paralogous proteins SLC35A2, SLC35A1 and SLC35A3 revealed that many pathogenic variants are localized in positions conserved within them (Fig. 3).

4. Discussion

We present a novel patient showing a type 2 serum sialoTf pattern (persisting until the actual age of 20 years) suggesting thus a defect in the glycan remodeling pathway. Whole exome sequencing showed in *SLC35A2* a novel variant (c.233A > G; p.Lys78Arg) that was absent in the mother (*de novo*). This variant was also present in the patient's fibroblasts. It is not present in gnomAD. *In silico* analysis using CADD predicts the novel variant (c.233A > G; p.Lys78Arg) as a deleterious substitution. Additionally, our extended MSA analysis of SLC35A2 orthologs and paralogs showed that that Lys78 is an invariant residue.

The patient has only mild psychomotor disability, no hypotonia, no epilepsy, and no brain abnormalities on MRI. He thus represents the mildest presentation described to date. A short stature has been reported in other SLC35A2-CDG patients [12,14,24]. IGF1 deficiency has not been reported in this CDG but it has been reported in PMM2-CDG, MPI-CDG and PGM1-CDG [36,37]. All reported patients showed psychomotor disability. The following symptoms were present in half or less of the patients: skeletal abnormalities, ocular/visual abnormalities, microcephaly, a Rett syndrome-like phenotype, cerebral and cerebellar abnormalities, and other symptoms.

As to the serum transferrin IEF, three situations have been observed: most with a normal pattern [16], a persisting type 2 pattern [12,14,38] as in the patient here reported, and a type 2 pattern in infancy that normalized in late childhood [10,17].

A type 2 pattern was reported in 22/66 patients (7/8 males and 15/58 females) including adults [13,23,24]. This observation is not in accordance with normalization of transferrin glycosylation with age, probably due to normal intake of dietary galactose as proposed by Dörre [12] and later by Yates [20]. Considering that the presence of a functional allele is probably required for survival [20], mosaicism could explain a normal glycosylation pattern in male patients but that was not reported in male patients reported so far.

Table 2
Genetic information, biochemical screening result and gender of the 65 reported patients and the present patient.

cDNA position	Protein position	CADD	Glycosylation screening	Inheritance	Gender	Reference
c.1A > G	p.Met1Val	23.2	Normal	*de novo*	F	[23]
c.3G > A	p.Met1Ile	23.6	Type 2	*de novo*	F	[10]
c.15_91 + 48 delinsA	p.Gly8Serfs*9		Type 2	*de novo*	M	[10]
c.124del	p.Val42Cysfs*53	29.8	Type 2	*de novo*	F	[24]
c.164G > C	p.Arg55Pro	26.7	Normal	*de novo*	F	[23]
		31				[24]
c.168C > A	p.Tyr56*	22.2	Normal	*de novo*	F	[24]
c.193_204del	p.Phe65_Thr68del		Normal	*de novo*	F	[23]
c.195C > A	p.Phe65Leu	24.4	Normal	*de novo*	F	[20]
c.211G > A	p.Val71Met	26.9	Normal	*de novo*	F	[23]
c.233A > G	p.Lys78Arg	26.6	Type 2	*de novo*	M	Present work
c.245G > T	p.Cys82Phe	28.5	Normal	*de novo*	F	[23]
c.262G > C	p.Ala88Pro	24.2	Type 2	*de novo*	F	[24]
						[17]
c.274 + 1G > A		31	Type 2	*de novo*	F	[22]
c.274 + 2 T > C		33	Type 2	*de novo*	F	[23]
c.302 T > C	p.Leu101Pro	26.1	Normal	*de novo*	F	[23]
c.327 T > G	p.Tyr109*	36	Normal	*de novo*	F	[20]
c.346G > C	p.Ala116Pro	26.6	N/A	*de novo*	F	[23]
c.348del	p.Val117Cysfs*27		Normal	*de novo*	F	[23]
c.353C > G	p.Pro118Arg	27	Normal	*de novo*	F	[23]
c.389A > G	p.Tyr130Cys	28.1	Abnormal	*de novo*	F	[23]
		26.9	Type 2			[24]
c.433_434del	p.Tyr145Profs*76		Normal	*de novo*	F	[9]
c.466_468delTCC	p.Ser156del	22	Normal	*de novo*	F	[21]
c.497_501dup	p.Gln168Glyfs*183		NM	*de novo*	F	[23]
c.502C > T	p.Gln168*	36	Normal	*de novo*	F	[23]
			ND			[11]
c.515 T > C	p.Leu172Pro	27.3	Normal	*de novo*	F	[20]
c.523_525del	p.Leu175del	N/A	N/A	*de novo*	F	[23]
c.523C > T	p.Leu175Phe	26.6	Abnormal	*de novo*	F	[23]
c.547C > T	p.Gln183*	36	Normal	*de novo*	F	[23]
c.562G > A	p.Gly188Ser	8.6	N/A	*de novo*	F	[23]
c.569dup	p.Gly191Argfs*31		Normal	*de novo*	F	[23]
c.617del	p.Val206Alafs*143		Normal	*de novo*	F	[23]
c.638C > T	p.Ser213Phe	25.1	Normal	*de novo*	F	[9]
c.670C > T	p.Leu224Phe	24.6	Type 2	*de novo*	M	[14]
c.683C > A	p.Ser228*	36	NM	*de novo*	F	[11]
c.695G > A	p.Trp232*	36	NM	*?*	F	[18]
c.698 T > C	p.Leu233Pro	27.8	Abnormal	*de novo*	F	[23]
		25.8	Type 2			[24]
c.747_757dup	p.Ala253Glyfs*100		Normal	*de novo*	F	[23]
c.753del	p.Trp251Cysfs*98	32	Normal	*de novo*	F	[24]
c.772G > A	p.Val258Met	24.4	Normal	*inherited*	M	[34]
c.795del	p.Phe265Leufs*84		Normal	*de novo*	F	[24]
c. 797G > T	p.Gly266Val	26.1	Type 2	*de novo*	F	[13]
c.800A > G	p.Tyr267Cys	24.5	Normal	*de novo*	F	[35]
c.816G > A	p.Trp272*	38	Normal	*de novo*	F	[23]
c.818G > A	p.Gly273Asp	23.3	Normal	*de novo*	F	[23]
		16.66	Abnormal		M	[24]
c.831C > G	p.Asn277Lys	22.8	Type 2	*inherited M*	F	[15]
c.841G > A	p.Gly281Ser	25.2	Abnormal	*de novo*	F	[24]
c.841G > C	p.Gly281Arg	24.5	Normal			
c.856del	p.Ala286Leufs*63		NM	*de novo*	F	[23]
c.884G > C	p.Gly282Arg	24.1	NM	*de novo*	F	[13]
c.889A > G	p.Lys297Glu	28.1	NM	*de novo*	F	[20]
c.908 T > C	p.Leu303Pro	31	Normal	*de novo*	F	[23]
c.923C > T	p.Ser308Phe	26.7	NM	*de novo*	F	[20]
			Abnormal	*de novo*	M	[24]
c.935C > A	p.Ser312Tyr	26.6	Normal	*de novo*	F	[23]
c.944 T > C	p.Leu315Pro	27.4	Abnormal	*de novo*	M	[23]
c.950delG	p.Gly317Alafs*32		Normal	*de novo*	F	[16]
c.972delT	p.Phe324Leufs*25		Normal	*de novo*	F	[9]
c.991G > A	p.Val331Ile	26.4	Normal	*de novo*	F	[23]
			Type 2		M	
			MS slight abnormal		F	[19]
		26.3	Abnormal		F	[24]

N/A: not available; ND: not done; NM: not mentioned; *MS: mass spectrometry.

As reported by Ng et al. in 2013 [10], serum transferrin IEF showed a type 2 pattern when performed at 5–7 months of age, but showed a nearly normal/normal pattern when performed 3–5 years later in the same patients,without clinical improvement. This normalization suggests that SLC35A2-CDG patients could have a limited abnormal Tf diagnostic window, and accordingly, individuals suspected of having a CDG should be tested by Tf IEF as early in life as possible. From the data collected in this paper this seems particularly relevant for the diagnosis of female patients.

Ng et al. [10] hypothesized that normalized Tf profiles could result

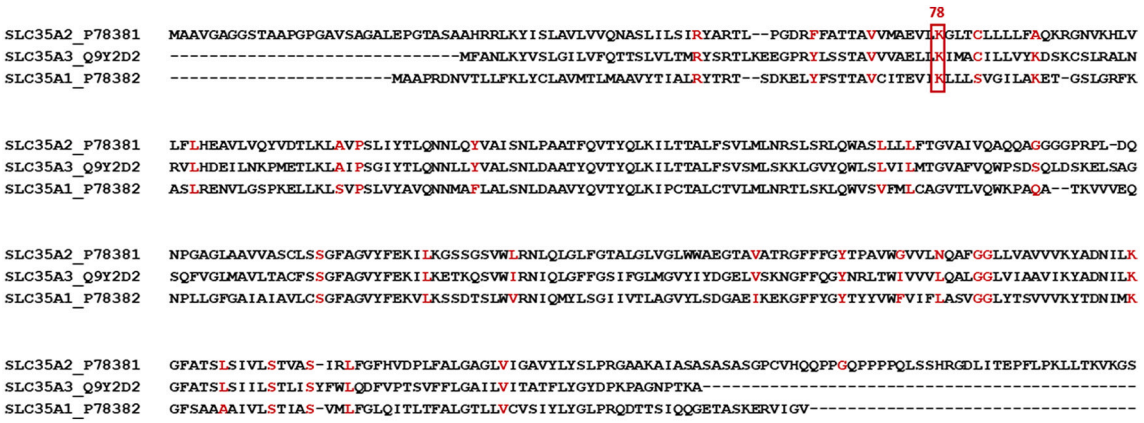

Fig. 3. MSA of SLC35A2 paralogues showing the residues previously reported to be involved in CDG-SLC35A2 (Table 2) and the corresponding position in paralogues proteins. The novel variant identified in this work is highlighted.

from the fact that cells carrying the mutant allele are selected against the normal allele during infancy.

The effect of dietary galactose supplementation was investigated in three patients. There were positive neurological and growth effects, and improvement to normalization of the transferrin IEF pattern [12,14,38,39]. In the present patient, galactose supplementation, has been started 2 years ago (dosis 1.5 g/kg/day) and resulted in improved glycosylation but no clinical effect.

5. Conclusions

This is the first reported SLC35A2-CDG presenting the novel variant c.233A > G (p.Lys78Arg), only minor neurological involvement and a short stature due to IGF1 deficiency.

An explanation for his mild phenotype is not evident. One possibility is related to the location of this variant at the N-terminal of the protein, different from that of the reported patients (Table 2). Mosaicism might be another explanation, although the variant was present in two embryologically different tissues (leukocytes and fibroblasts). Still another mechanism could be the presence of a putative protective genetic background in this patient.

Ethics approval and consent to participate

The authors performed this study in accordance with the Declaration of Helsinki of the World Medical Association.

Authors did not request ethical approval from the local ethics committee due to the nature of the study.

This article does not contain any studies with animal subjects.

Consent for publication

Written informed consent for publication was obtained from the patient's parents.

Availability of data and material

DNA samples and data are available on request. The data hereby presented is available in public databases.

Authors' contributions

Dulce Quelhas – writing and collection of published information, initial laboratory screening for CDG.

Joana Correia – clinical evaluation, data collection and writing.

Jaak Jaeken – collaboration in paper writing and critical review.

Luísa Azevedo – collaboration in writing genetic information and critical review.

Anabela Bandeira – clinical evaluation and data collection.

Mónica Lopes-Marques –sequence collection, sequence analyses of orthologs and paralogs, collaboration in writing.

Liesbeth Keldermans – molecular screening for CDG genes.

Gert Matthijs – collaboration in writing and molecular screening for CDG genes.

Luisa Sturiale - serum transferrin glycan analysis.

Esmeralda Martins – supervised patient clinical information and critical review.

Declaration

All authors of the manuscript have read and agreed to its content and are accountable for all aspects of the accuracy and integrity of the manuscript in accordance with MGMR criteria.

Financial support

This work is supported by National Funds through the Fundação para a Ciência e a Tecnologia (Portuguese national funding agency for science, research and technology) in the frameworks of the UID/Multi/00215/2013 project–Unit for Multidisciplinary Research in Biomedicine–UMIB/ICB AS/UP.

Declaration of Competing Interest

All authors hereby declare that they have no financial or personal relationships with other people or organizations that could inappropriately influence (bias) this work.

Acknowledgments

The authors thank the patient and his family for their collaboration.

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