

A homozygous nonsense variant in the oligosaccharyltransferase complex gene, *RPN1*, causes a congenital disorder of glycosylation

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Summary

Congenital disorders of glycosylation (CDGs) are a phenotypically diverse group of genetic conditions arising from pathogenic variants in various glycosylation pathways. The most prevalent are N-glycosylation disorders. Here, we present clinical and biochemical data on two siblings with a neurodevelopmental disorder and a pathogenic homozygous nonsense variant in ribophorin I (*RPN1*), an essential component of the oligosaccharyltransferase (OST) complex. Both affected individuals showed a classical type I serum transferrin profile, while lymphoblasts revealed that the variant resulted in a truncated *RPN1* protein with reduced levels. The protein stability of other essential OST complex components, including STT3 OST complex catalytic subunit A (STT3A), *RPN2*, and dolichyl-diphosphooligosaccharide (DDOST), was also significantly reduced. Structural modeling of both OST-A and OST-B complexes shows that *RPN1* truncation eliminates a C-terminal four-helix bundle, which interacts with the translating ribosome. This interaction is necessary and specific for the co-translational activity of the OST-A complex. Supporting this observation, hypoglycosylation of an OST-A-specific substrate protein was observed, while OST-B-specific substrates were unaffected. These data convey that a rare loss-of-function *RPN1* variant causes an autosomal recessive CDG characterized by neurodevelopmental deficits.

Introduction

The hetero-oligomeric oligosaccharyltransferase (OST) complex resides in the endoplasmic reticulum (ER) and consists of seven to eight subunits required for protein N-glycosylation.¹ Its primary function is to transfer a preassembled lipid-linked oligosaccharide (LLO) of 14 sugars onto the asparagine residues using the consensus sequence Asn-X-Ser/Thr (where X ≠ Pro).^{1,2} The catalytic subunit, STT3, is highly conserved.² In humans, two isoforms exist: STT3 OST complex catalytic subunit A (STT3A) (MIM: 601134) and STT3B (MIM: 608605), which form distinct OST complexes with shared and unique subunits.^{1,3}

The STT3A-containing complex (OST-A) is responsible for co-translational glycosylation by interacting with the ribosome via its cytoplasm-facing domain, and it enables the addition of N-glycans to nascent polypeptides.^{4,5} In addition to STT3A, OST-A includes OST complex non-catalytic subunit (OSTC; MIM: 619023), keratinocyte-associ-

ated protein 2 (KRTCAP2; MIM: 619029), OST complex subunit 4, non-catalytic (OST4; MIM: 618932), ribophorin I (*RPN1*; MIM: 180470), *RPN2* (MIM: 180490), defender against cell death 1 (*DAD1*; MIM: 600243), dolichyl-diphosphooligosaccharide (DDOST; MIM: 602202), and transmembrane protein 258 (*TMEM258*; MIM: 617615).³

In contrast, the STT3B-containing complex (OST-B) functions in post-translational glycosylation, independently of the translation process and without ribosomal interactions.⁵ OST-B comprises STT3B and magnesium transporter 1 (*MAGT1*; MIM: 300715), tumor suppressor candidate 3 (*TUSC3*; MIM: 601385), OST4, *RPN1*, *RPN2*, *DAD1*, DDOST, and *TMEM258*.³ While both complexes share core subunits, OST-A uniquely contains OSTC and KRTCAP2, whereas OST-B contains *MAGT1* and *TUSC3*.³ These combinations contribute to the functional differences in co- versus post-translational glycosylation. Shared subunits, including *RPN1*, *RPN2*, OST4, *DAD1*, and DDOST, play a crucial role in structural stability and functional efficiency of the OST complex.⁶ Although

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many proteins can utilize either OST complex, certain proteins exhibit a strong preference for one over the other. For instance, N-glycosylation of prosaposin (PSAP; MIM: 176801) is particularly reliant on STT3A, while β -glucuronidase (GUSB; MIM: 611499) and sex hormone binding globulin (SHBG; MIM: 182205) are solely dependent on the STT3B-containing OST complex for proper N-glycosylation.^{7,8}

Cryo-electron microscopy (cryo-EM) of both OST complexes reveals clear structural differences. In particular, there is a C-terminal four-helix bundle within RPN1, which adopts a distinct orientation in each complex. In OST-A, interactions between STT3A and TMEM258 position the bundle to remain exposed within the cytoplasm so that it can interact with ribosomal protein L28 (RPL28; MIM: 603638), thereby facilitating co-translational N-glycosylation in cooperation with the translocon complex.^{3,9} In OST-B, this region is reconfigured to eliminate ribosome binding, which aligns with its post-translational N-glycosylation function.³

Pathogenic variants in several OST subunits, *STT3A* (MIM: 615596 and 619714), *STT3B* (MIM: 615597), *DDOST* (MIM: 614507), *OSTC* (MIM: N/A), *TUSC3* (MIM: 611093), and *MAGT1* (MIM: 301031 and 300853) cause clinically diverse types of congenital disorders of glycosylation (CDGs) with autosomal recessive and X-linked modes of inheritance and display significant disruptions in the N-linked glycosylation process.^{10–16}

Here, we characterize a CDG attributed to the disruption of RPN1 function. Two siblings with a neurodevelopmental disorder were found to harbor a rare homozygous nonsense variant in *RPN1*, c.1654C>T (GenBank: NM_002950.4) (p.Gln552*), accompanied by an abnormal carbohydrate-deficient transferrin (CDT) profile. Analysis of biological samples confirmed the pathogenicity of the c.1654C>T (p.Gln552*) variant, which results in a dysfunctional OST complex with proven glycosylation deficiencies, further expanding the number of known N-linked glycosylation disorders.

Subjects and methods

Human subject research statement

This study was approved by the institutional review boards of Boston Children's Hospital (protocol 05-05-076R) and Kuwait Medical Genetics Center. Subjects were identified and evaluated in a clinical setting, and biological samples were collected for research purposes after obtaining written informed consent.

Whole-exome sequencing

Whole blood was collected from consenting participants according to standard clinical practices, and DNA was extracted using standard methods. Whole-exome sequencing (WES) was performed at the Broad Institute using an Agilent Sure-Select Human All Exon v.2.0 capture kit and sequenced on an Illumina HiSeq2000 sequencer. Samples were aligned to GRCh37 using BWA v.0.5.9, and variant calling was performed with GATK

v.2.6. 87.969% (MC33301), 85.7735% (MC33302), 87.1519% (MC33305), and 87.8726% (MC33306) of the targeted sequence had 20× or greater coverage.

CDT and serum glycomics

CDT and serum glycomics were performed as previously described.¹⁷

Tissue culture

Lymphoblasts were cultured in RPMI-1640 (Corning 10-040-CV) supplemented with 12% FBS (SIGMA F0926-Lot 24A338), 1× L-glutamine (Corning 25-005-CL), and 1× penicillin-streptomycin (Corning 30-002-CL). HEK293 (ATCC CRL-1573) cells were cultured in 1 g/L glucose DMEM (Corning 10-014-CV), 10% FBS, 1× L-glutamine, and 1× penicillin-streptomycin.

Western blotting

Western blotting was performed as previously described.¹⁰ Antibodies used are provided in Table S3.

CRISPR base-editing HEK293 cells

HEK293 cells were co-transfected with pGuide-EF1a-GFP (OriGene GE100044) containing the guide RNA sequence 5'-AATGCAGAAGCTGGATGCAC-3' targeting exon 10 of *RPN1* and the base editor pcmv-BE4max (Addgene #112093). Transfected cells were recovered for 48 h, and subsequently GFP-positive cells were sorted into 96-well plates using a FACSaria IIu instrument (BD Biosciences) for isogenic clones. Clones were expanded and screened for successful genome editing by Sanger sequencing.

Results

MC33300 is a consanguineous family of Kuwaiti origin (Figure 1A). Two brothers (subjects MC33301 and MC33302) presented for clinical evaluation with mild intellectual disability, developmental delay, and dysmorphic features of unknown origin. Clinical testing was unrevealing, and the family was offered the opportunity to participate in research to further investigate a genetic etiology.

Subject MC33302

MC33302, now 19 years old, is the second child of parents who are second cousins. He was born at full-term following a pregnancy complicated by maternal bleeding in the first trimester and polyhydramnios at 7 months of gestation. Motor and speech delay, as well as cognitive deficits, were all noted in childhood. At 10 years, he had a narrow-based gait with toes pointed inward. At age 18 years, he displayed an imbalance, a left-sided shuffling gait, and fine tremors in both hands. Dysmorphic features include deep, sunken eyes; long eyelashes; thick eyebrows; broad nasal root; beaked nose; short philtrum; thick upper and lower lips; widened oral aperture; mottling of skin all over the body, especially the hands; and arachnodactyly. A brain MRI at 6 years, 1 month showed mild hypoplasia of the cerebellar vermis,

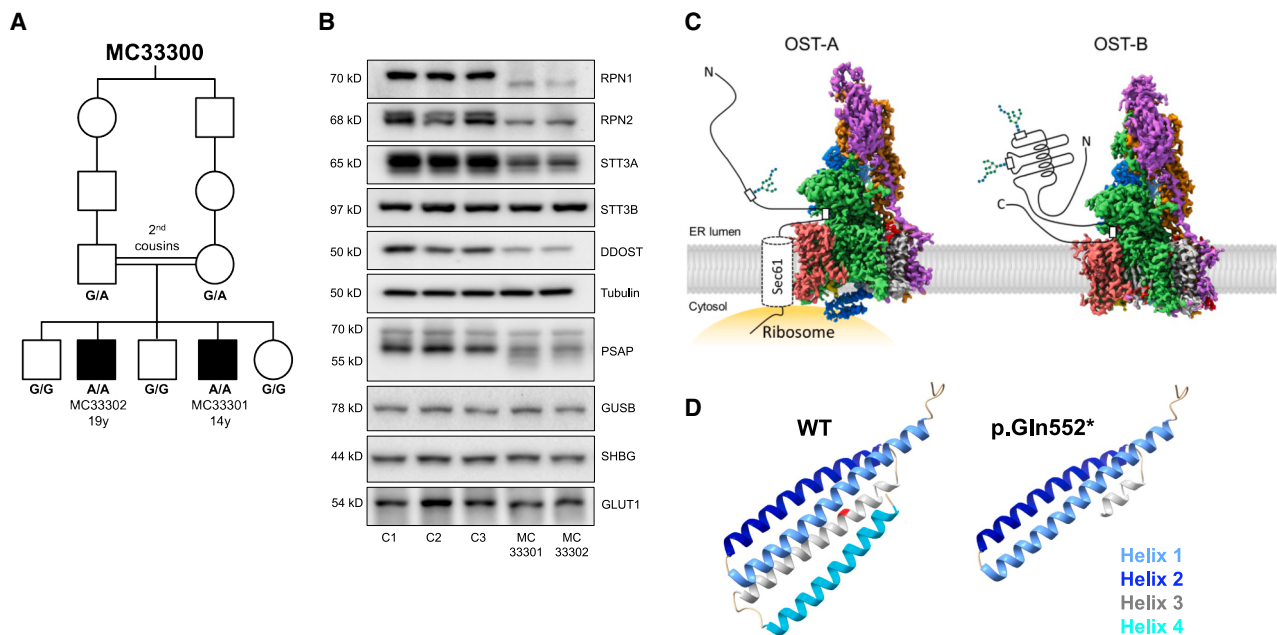


Figure 1. Identification of a homozygous c.1654C>T (p.Gln552*) variant in *RPN1*

(A) Pedigree of family MC33300 harboring the c.1654C>T (GenBank: NM_002950.4) (p.Gln552*) nonsense variant in *RPN1* (chr3:128620581G>A).

(B) Western blot analysis of the core OST complex proteins (RPN1, RPN2, STT3A, STT3B, and DDOST) and OST-A- (PSAP) and OST-B- (GUSB, SHBG, and GLUT1) specific substrates showing glycosylation status in apparently healthy controls and both affected individuals.

(C) Cryo-EM structure of human oligosaccharyltransferase complex OST-A and OST-B as reported by Ramírez et al.³

(D) An expanded view of the four-helix bundle, with the left image showing the wild-type structure (the p.Gln552 residue is colored in red) and the right image showing the predicted truncated structure due to the c.1654C>T (GenBank: NM_002950.4) (p.Gln552*) variant.

brainstem and pons, and persistent cavum septum pellucidum (Table 1; Figure S1).

Subject MC33301

MC33301 is 14 years old and the fourth of five siblings. He is the younger brother of MC33302 and similarly has mild intellectual disability, developmental delay, and dysmorphic features. He was born at 36 weeks of gestation following a pregnancy complicated by maternal hypertension, intrauterine growth retardation, and polyhydramnios that was noted at 5 months of gestation. Concerns for hypotonia and floppiness at 6 months of age were noted. At about 4.5 years, he was not speaking, nor was he social, and cyanotic attacks were reported but without loss of consciousness or known occurrence of seizures. At that time, his gait was slightly wide with toes pointed inward. Dysmorphic features include deep, sunken eyes; left ptosis; thick eyebrows; broad nasal root; beaked nose; shallow glabella; short philtrum; thick upper and lower lips; arachnodactyly; and six café au lait spots (2–2.5 cm). His brain MRI at 3 years, 4 months showed mild hypoplasia of the corpus callosum, brainstem and pons, and persistent cavum septum pellucidum (Table 1; Figure S1).

Without a definitive clinical diagnosis for either sibling, WES was performed and identified a homozygous

nonsense variant, c.1654C>T (GenBank: NM_002950.4) (p.Gln552*), in *RPN1*, a core component of the OST complex (Table 1). Sanger sequencing verified the homozygous variant in both affected siblings, while both healthy parents were carriers and the three unaffected siblings were homozygous reference (Figure S2). The variant was absent from public variant databases, including gnomAD v.4.1.0, All of Us, and the UK Biobank. Furthermore, this *RPN1* nonsense variant was the only homozygous coding variant identified by WES to be shared by the affected brothers. All other shared recessive variants (compound heterozygous or X-linked) of high genotype quality with an allele frequency less than 0.01 in gnomAD v.4.1.0 are missense, synonymous, or non-coding variants of uncertain significance in seven other genes that are either associated with discordant phenotypes or not known to cause human disease (Table S1).

Given the role of RPN1 in glycosylation, serum samples from each parent and both affected siblings were analyzed for CDT, a commonly used biomarker for CDG. CDT can often confirm a diagnosis of CDG but not which gene is defective. Serum transferrin (Tf) (MIM: 190000) has two N-glycans referred to as “di-glycosylated” but can also have either a single “mono-glycosylated” or an “A-glycosylated” version, which is missing both chains.¹⁷ Determining the ratios between these three glycosylated forms

Table 1. Summary of clinical findings for siblings homozygous for a nonsense variant in *RPN1*

Clinical features (shared HPO terms)	MC33301	MC33302
Biological sex	male	male
Pregnancy history (HP0001561)	maternal hypertension, polyhydramnios noted at 5 m gestation, intrauterine growth retardation (HP0001511)	maternal bleeding 1st trimester, polyhydramnios noted at 7 m gestation
Birth history	planned repeat Cesarean section, neonatal ICU for 12 d	planned repeat Cesarean section
Gestational age at birth (weeks)	36	full-term
Birth weight	1.7 kg (−2.43 SD for 36 wga)	2.9 kg (−1.06 SD)
Birth length	N/A	N/A
Birth head circumference	N/A	N/A
Age at last evaluation (#y#m)	13y3m	18y8m
Head circumference (cm, SD)	52 cm, −1.48 SD	55 cm, −0.07 SD
Weight (kg, SD)	33 kg, −1.85 SD	51 kg, −1.99 SD
Height (cm, SD)	144.5 cm, −1.48 SD	161 cm, −2.04 SD
Intellectual disability (Y/N, degree) (HP0001249)	Y, mild, followed simple commands at 4y11m	Y, mild, IQ testing at 3y3m: 55, understood simple commands at 4 y
Developmental delay (Y/N, describe) (HP0001263)	Y, mild, walked unassisted at 13 m, no speech and noted to be socially delayed at 4y11m	Y, sat at 8 m, walked unassisted at 18 m, first words at 5.5 y and used 3–4 word sentences at 10 y, no concerns regarding social skills
Dysmorphic features (Y/N, list) (HP0000574, HP0000527, HP0000431, HP0000322, HP0012471, and HP0000490)	Y, deep sunken eyes, long eyelashes, thick eyebrows, broad nasal root, beaked nose, shallow glabella, short philtrum, thick upper and lower lips, about 6 café au lait spots (HP0007565, 2.5–3 cm on the back)	Y, deep sunken eyes, long eyelashes, thick eyebrows, broad nasal root, beaked nose, short philtrum, thick upper and lower lips, wide mouth (HP0000154), mottling of skin all over the body, especially hands
Visual/ocular abnormalities	normal visual evoked potentials at 5 m, left ptosis (HP0007687)	strabismus (HP0000486)
Oromotor dysfunction	N/A	slurred speech
Neurologic, other features	cyanotic attacks with upward eye rolling but no loss of consciousness reported around 4.5 y	fine tremors in both hands, brisk tendon reflexes
Musculoskeletal (HP0001166)	generalized mild hypotonia (HP0001290) and muscular wasting, weak hand grip bilaterally, mild winging of right scapula, slight kyphoscoliosis, arachnodactyly, receding 5th toe, prominent malleolus bilaterally	muscle weakness (left side > right side), arachnodactyly
Movement/gait disorders (HP0001288)	gait slightly wide with toes pointed inward (4y11m)	narrow-based gait with toes pointed inward (10y6m), imbalance and shuffling gait more on left side (18y10m)
Cardiac abnormalities	echocardiogram (<6 m): PFO (HP0001655) with tiny L-R shunt and small closing PDA (HP0001643)	N/A
GI/GU abnormalities	N/A	constipation (HP0002019)
Other diagnoses	epistaxis (HP0000421)	N/A
Clinical laboratory investigations	transferrin: 242 mg/dL (ref. 200–400), Trans SAT: 23% (ref. 20–40), ferritin: 31.43 (ref. 15–400 µg/L), CBC: mild decrease in RBC count, Hb: 117 (ref. 130–170 g/L), neutropenia, lymphocytosis, monocytosis, eosinophilia; normal hearing test	transferrin: 248 mg/dL (ref. 200–400), Trans SAT: 22%, ferritin: 52.17 (ref. 15–400 µg/L), elevated direct bilirubin: 8 µmol/L (ref. 0–4 µmol/L), lymphocytosis, monocytosis; normal karyotype, fragile X DNA, serum and plasma amino acids, urine organic acids, ammonia, lactate and serum creatine kinase, hearing test
Brain MRI (HP0002365, HP0012110, and HP0002389)	3y4m: mild hypoplasia of the corpus callosum (HP0002079), brainstem and pons, and persistent cavum septum pellucidum	6y1m: mild hypoplasia of the cerebellar vermis (HP0001320), brainstem and pons and persistent cavum septum pellucidum

(Continued on next page)

Table 1. Continued

Clinical features (shared HPO terms)	MC33301	MC33302
CDT (reference range: mono/di < 0.05, a/di < 0.04)	mono/di: 2.02, a/di: 0.28	mono/di: 1.04, a/di: 0.17

d, day; L-R, Left-Right; m, month; PDA, patent ductus arteriosus; PFO, patent foramen ovale; Trans SAT, transferrin saturation; WGA, weight for gestational age; y, year.

of Tf is the basis of a CDT assay. A type 1 CDT is characterized by the absence of either one or both of the entire N-glycans, suggesting defects in the assembly or transfer to a nascent protein.¹⁷ A type 2 CDT indicates remodeling of the N-glycan after the OST complex has transferred it to a protein.¹⁷ The CDT assay showed that MC33301 had a type 1 pattern with a mono/di-2.02 and A/Di-0.28 and that MC33302 had mono/di-1.04 and A/Di-0.17, both well beyond the reference range: mono/di < 0.05 and A/Di < 0.04. Both unaffected parents were within the normal reference range (Table 1). The type 1 CDT profile confirmed that both affected brothers have a CDG. Additionally, serum glycomics analysis showed similar N-glycan changes to two other OST-CDG defects, STT3B-CDG and DDOST-CDG.¹⁸ Briefly, both affected brothers had increases in the abundances of high mannose (Man) N-glycans Man5 and Man6, with increased ratios of Man5/Man8, Man6/Man8, and Man6/Man8 (Table S2). Thus, RPN1 was prioritized for further cellular biochemical investigation.

Using lymphoblast lines derived from both affected brothers, we confirmed the effects of the variant on RPN1 protein, the OST complex, and the associated translocon complex. When compared to three unrelated non-diseased controls, both affected lymphoblast lines showed substantially reduced levels of a truncated RPN1 protein (Figure 1B). The observed molecular weight of the truncated RPN1 is in line with the predicted size of the p.Gln552* variant product and supports the prediction that the variant transcript escapes nonsense-mediated decay (NMD). Affected lymphoblasts also exhibited reduced levels of essential core complex proteins STT3A, RPN2, and DDOST (Figure 1B). No differences were seen in other OST complex components (STT3B and DAD1), the translocon complex proteins SSR1–4 (MIM: 600868, 600867, 606213, and 300090), SEC61B (MIM: 609214), or RPL28 (Figures 1B and S3A).

Next, we sought to determine if there were detectable glycosylation deficiencies in specific OST-A or OST-B substrates. Western blot analysis showed significant N-glycosylation abnormalities for PSAP (as evidenced by several lower migrating bands), a known OST-A substrate, with no apparent change to the OST-B substrates, GUSB, SHBG, or GLUT1 (Figure 1B). Western blotting of RPN2 showed two bands of different molecular weight in all control samples; however, in the affected brothers' samples, the upper, slower migrating band was absent, while the lower, faster migrating band remained (Figure 1B).

RPN2 is an N-glycosylated membrane-bound protein, and it is likely that the mass differences are due to the loss of an N-glycan that is transferred co-translationally and thus an OST-A substrate.

The reported cryo-EM structure of the OST-A complex was used to assess how the p.Gln552* variant would affect the four-bundle helix region of RPN1. The p.Gln552* variant is expected to delete the last 55 amino acids of RPN1, which is half of helix 3 and all of helix 4, but would still leave a portion of the bundle possibly capable of interacting with the translocon and ribosome (Figures 1C and 1D).

Fibroblasts from the family were unavailable, but to ensure our lymphoblast results were specifically due to the c.1654C>T (GenBank: NM_002950.4) (p.Gln552*) variant in *RPN1*, we used CRISPR-Cas9 base editing to generate a HEK293 cell line carrying the same variant. Importantly, this homozygous p.Gln552* knockin line recapitulated similar biochemical deficiencies as the lymphoblasts from the affected individuals (Figure S3B).

Discussion

Here, we present biochemical data confirming the pathogenicity of a homozygous nonsense variant in *RPN1* and describe its human disease association as a recessive CDG. We identified affected siblings with a homozygous c.1654C>T (GenBank: NM_002950.4) (p.Gln552*) *RPN1* variant, with asymptomatic carrier parents, and suggest that the nonsense variant behaves as a hypomorph rather than a complete loss-of-function (LoF) variant. Studies using affected lymphoblasts and base-edited cell lines are consistent with the prediction that the c.1654C>T (GenBank: NM_002950.4) variant transcript, terminating in the last exon, escapes NMD and produces a truncated RPN1 protein, p.Gln552*. The degree of truncation within the C-terminal four-bundled helix region of RPN1 is within helix 3 and is expected to delete the last 55 amino acids of RPN1, removing half of helix 3 and all of helix 4. Since we still see residual truncated RPN1 protein, this suggests that helices 1 and 2 are still intact, which could provide enough complex stability and ribosome interaction for the p.Gln552* to have partial activity. This supports the notion that this nonsense variant is hypomorphic rather than a complete LoF.

We did not identify additional individuals with biallelic variants in *RPN1* through the Matchmaker

Exchange (MME). *RPN1* is constrained to LoF variation, having a pLI score of 1 (gnomAD v.4.1.0), and the few predicted LoF variants in gnomAD do not occur in homozygosity. Together, this suggests that complete loss of *RPN1* is likely incompatible with life and may explain the apparent extreme rarity of this condition.

One limitation of our study is that we did not pursue rescuing via cDNA complementation in the affected lymphoblast cell lines. Rather, we generated a base-edited cell model to characterize the *RPN1* nonsense variant instead of overexpressing the wild-type protein in affected cells. Our experiences with overexpressing transmembrane domain proteins (such as *RPN1*) have revealed undesired effects, including the activation of ER-associated degradation pathways. While both cell-based models support the expected effect of the *RPN1* variant, the degree of reduced OST subunit levels between affected lymphoblasts and HEK293 cells did vary. We believe these differences are attributable to the different tissue types having varying degrees of OST complex requirements for proper N-glycosylation. Another limitation is that we opted not to perform RNA sequencing (RNA-seq) or investigate additional OSTA/B substrates outside those presented. Given half of the four-helix bundle remains, we suspect that some client proteins might require a complete bundle, while others may be more lenient. Further investigation into either of the above may or may not yield additional beneficial information.

The OST complex plays a role in every developmental process, evidenced by the numerous multisystem disorders caused by variants in OST complex genes. CRISPR-based screens for essential genes have shown that *RPN1* and the core OST complex proteins *RPN2*, *DDOST*, *DAD1*, and *TMEM258* are essential for cellular survival, while *OSTC*, *STT3A*, and *STT3B* have reduced growth but survive.¹⁹ We now provide both clinical and biochemical evidence to support the classification of an additional type of autosomal recessive CDG, known as *RPN1*-CDG, bringing the number of known OST complex disease-associated genes to eight.

Data and code availability

WES data generated in this study are available in dbGaP accession #phs001272.v3.p1. This study did not generate or analyze code.

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Declaration of interests

H.H.F. is a consultant for Glycomine, Inc.

Web resources

All of Us, <https://databrowser.researchallofus.org/>
ChimeraX, <https://www.cgl.ucsf.edu/chimerax/>
dbGaP, <https://dbgap.ncbi.nlm.nih.gov/home/>
GenBank, <https://www.ncbi.nlm.nih.gov/genbank/>
GeneMatcher, <https://genematcher.org/>
gnomAD v.4.1.0, <https://gnomad.broadinstitute.org/>
HPO, <https://hpo.jax.org>
OMIM, <https://www.omim.org>
UK Biobank Allele Frequency Browser, <https://afb.ukbiobank.ac.uk/>

Supplemental information

Supplemental information can be found online at <https://doi.org/10.1016/j.xhgg.2026.100604>.

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