Received: 14 April 2023 | Revised: 28 September 2023 | Accepted: 6 October 2023

DOI: 10.1002/prca.202300040

Proteomics Clinical Applications

RAPID COMMUNICATION

"Hide and seek": Misleading transferrin variants in PMM2-CDG complicate diagnostics

Alexandre Raynor[1] | Arnaud Bruneel[1,2] | Pieter Vermeersch[3] | Sophie Cholet[4] | Sebastian Friedrich[5] | Matthias Eckenweiler[6] | Anke Schumann[5] | Simone Hengst[7] | Ali Tunç Tuncel[7] | François Fenaille[4] | Christian Thiel[7] | Daisy Rymen[8]

[1]AP-HP, Biochimie Métabolique et Cellulaire, Hôpital Bichat, Paris, France

[2]INSERM UMR1193, Faculté de Pharmacie, Université Paris-Saclay, bâtiment Henri Moissan, Orsay, France

[3]Clinical Department of Laboratory Medicine, UZ Leuven, Leuven, Belgium

[4]Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), MetaboHUB, Gif sur Yvette, France

[5]Centre for Child and Adolescent Medicine Freiburg, Department of General Paediatrics, Adolescent Medicine and Neonatology, Freiburg, Germany

[6]Department of Neuropediatrics and Muscle Disorders, Centre for Child and Adolescent Medicine Freiburg, Freiburg, Germany

[7]Department 1, Centre for Child and Adolescent Medicine Heidelberg, Heidelberg, Germany

[8]Department of Pediatrics, Center for Metabolic Diseases, University Hospitals Leuven, Leuven, Belgium

Correspondence
Alexandre Raynor, Hôpital Bichat, Biochimie Métabolique et Cellulaire, 46 rue Henri Huchard, 75018 Paris, France.
Email: alexandre.raynor@aphp.fr

Christian Thiel, Centre for Child and Adolescent Medicine Heidelberg, Department 1, Analysezentrum 3, Im Neuenheimer Feld 669, 69120 Heidelberg, Germany.
Email: christian.thiel@med.uni-heidelberg.de

Funding information
E-Rare-3 Joint Transnational Call 2018/EUROGLYCAN-omics; Commissariat à l'Energie Atomique et aux Energies Alternatives, Grant/Award Number: ANR-11-INBS-0010; Deutsche Forschungsgemeinschaft, Grant/Award Numbers: FOR2509, TH1461/7-2; ERA-NET, Grant/Award Number: 643578

Abstract

Purpose: Congenital disorders of glycosylation (CDG) are one of the fastest growing groups of inborn errors of metabolism. Despite the availability of next-generation sequencing techniques and advanced methods for evaluation of glycosylation, CDG screening mainly relies on the analysis of serum transferrin (Tf) by isoelectric focusing, HPLC or capillary electrophoresis. The main pitfall of this screening method is the presence of Tf protein variants within the general population. Although reports describe the role of Tf variants leading to falsely abnormal results, their significance in confounding diagnosis in patients with CDG has not been documented so far. Here, we describe two PMM2-CDG cases, in which Tf variants complicated the diagnostic.

Experimental Design: Glycosylation investigations included classical screening techniques (capillary electrophoresis, isoelectric focusing and HPLC of Tf) and various confirmation techniques (two-dimensional electrophoresis, western blot, *N*-glycome, UPLC-FLR/QTOF MS with Rapifluor). Tf variants were highlighted following neuraminidase treatment. Sequencing of *PMM2 was performed*.

Results: In both patients, Tf screening pointed to CDG-II, while second-line analyses pointed to CDG-I. Tf variants were found in both patients, explaining these discrepancies. *PMM2* causative variants were identified in both patients.

Abbreviations: 2-DE, two-dimensional electrophoresis; AAT, alpha-1-antitrypsin; CDG, congenital disorder(s) of glycosylation; CE, capillary electrophoresis; HPLC, high pressure liquid chromatography; Hpt, haptoglobin; IEF, isoelectric focusing; IFCC, International Federation of Clinical Chemistry; LC-MS, liquid chromatography–mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MRI, magnetic resonance imaging; MS, mass spectrometry; PNGase F, peptide-N-glycosidase F; Tf, transferrin.

Alexandre Raynor and Arnaud Bruneel contributed equally as first co-authors.

Christian Thiel and Daisy Rymen contributed equally as last co-authors.

Conclusion and Clinical Relevance: We suggest that a neuraminidase treatment should be performed when a typical CDG Tf pattern is found upon initial screening analysis.

KEYWORDS
CDG, congenital disorder of glycosylation, PMM2-CDG, transferrin variant

1 | MAIN TEXT

Congenital disorders of glycosylation (CDG) constitute a family of over 150 rare inborn errors of metabolism characterized by defective synthesis of glycan chains, typically associated to poorly specific multivisceral clinical phenotypes [1, 2]. Classically, biochemical CDG screening involves charge-based separation and quantification of the sialylated glycoforms of serum transferrin (Tf) using isoelectric focusing (IEF) or high-pressure liquid chromatography (HPLC) [3]. Indeed, Tf is typically hyposialylated in CDG (although not always; e.g., CDG with fucosylation defects [SLC35C1-CDG, FCSK-CDG, FUT8-CDG], MOGS-CDG…), making it a hallmark of this group of diseases [4–7]. This led to the first CDG classification, defining a type-1 (CDG-I) Tf pattern with decreased 4-sialo Tf and increased 2-sialo and 0-sialo Tf (loss of one/two entire bi-sialylated *N*-glycan chains) and a type-2 (CDG-II) pattern showing decreased 4-sialo Tf and inconstantly increased 3-, 2-, 1- and 0-sialo Tf (maturation defects of *N*-glycan chains) [8]. While CDG classification has since largely evolved, this dichotomy remains widely used [9]. Although Tf glycoforms separation by IEF/HPLC remains the foremost CDG screening test, it is also commonly performed by capillary electrophoresis (CE), which allows easier and faster processing of larger sets of samples [10]. Noteworthy, it should be emphasized that HPLC and CE of Tf are currently considered as leading techniques by the International Federation of Clinical Chemistry (IFCC) for the accurate determination of the 2-sialo Tf fraction (CDT for "carbohydrate deficient transferrin") in the screening of chronic alcohol abuse [11].

IEF, HPLC and CE CDG screening efficiency can be significantly hampered by the presence of heterozygous Tf variants. These can change the protein's isoelectric point, or even exceptionally lead to the loss of a complete *N*-glycan chain, blurring separation patterns and leading to false-positives [12, 13]. Variants in transferrin are not unusual with ~90 point mutations reported in genetic databases (as NCBI ClinVar, last access 10/2022). In most cases, reanalysis following sample digestion by sialidase/neuraminidase alleviates this issue, yielding profiles with two 0-sialo Tf peaks rather than one in control samples [13].

Furthermore, mass spectrometry (MS)-based methods applied to immunopurified serum Tf [14] or to whole serum/plasma *N*-glycome [8, 15] have more recently been developed for CDG screening and diagnosis. By contrast with enzymatically generated *N*-glycome that is not dependent on the Tf protein sequence, MS analysis of purified intact Tf can allow the simultaneous assessment of Tf glycosylation and variants, especially when using high resolution/high mass accuracy mass spectrometers.

Here, we describe two patients with clinical suspicion of CDG who displayed deceptive type II Tf patterns in initial laboratory investigations, while second-line analyses were more evocative of CDG-I. These discrepancies led to the implementation of an "arsenal" of additional techniques, finally leading to the exceptional diagnoses of PMM2-CDG (CDG-Ia, OMIM #601785) associated with misleading Tf variants.

Patient 1, a boy, was born after a normal pregnancy as the first child of healthy, non-consanguineous Belgian parents. At 6 months, he was placed into foster care because of neglect and possible physical abuse. He displayed axial hypotonia, pressure ulcers, cryptorchidism, strabismus, intermittent nystagmus, and facial features reminiscent of fetal alcohol syndrome. Parental drug and alcohol abuse were suspected. At 2 years, he was referred to our center because of persistent global developmental delay despite adequate calorie intake. Clinically, slight dysmorphic features were present: large, low-set ears, a thin upper lip and a flat philtrum. Brain magnetic resonance imaging (MRI) had been performed at 1 year and was completely normal. In light of these signs, CDG screening was performed.

Transferrin CE profile, performed on a Capillarys 2 Flex Piercing (Sebia) [10] (Figure 1A), showed markedly elevated 3-, 2-, 1- and 0-sialo Tf levels, evocative of CDG-II. Second-line analyses were carried out, as classically conducted in this case. Firstly, we performed two-dimensional electrophoresis (2-DE) of apoC-III [16], to search for an associated Golgi homeostasis defect affecting protein mucin core 1 *O*-glycosylation, found in certain CDG-II. We found a normal apoC-III glycosylation profile (Figure S1), suggesting a specific defect in an enzyme involved in golgian trimming/maturation of *N*-glycans. Secondly, we studied the total serum *N*-glycome by MALDI-TOF mass spectrometry (UltrafleXtreme, Bruker Daltonics, Germany) after treatment with PNGase F [17, 18]. Surprisingly, the profile (Figure S2) did not display any peak corresponding to partially sialylated *N*-glycan structures, which are typically found in CDG-II. Thirdly, we performed a 2-DE analysis of two additional serum *N*-glycoproteins, alpha-1-antitrypsin (AAT) and haptoglobin (Hpt) [19]. This showed a clear and unexpected CDG-I profile with numerous additional spots of lower molecular weight and less acidic charge (indicating the loss of entire *N*-glycan chains) compared to control (Figure S3). In addition, western blotting of Tf, AAT and alpha-1-acid glycoprotein (Figure 1B) [20], unequivocally

corroborated this CDG-I pattern with typical additional bands of lower molecular weight compared to controls.

In view of these conflicting results, which necessitated checks and reanalyzes, we turned to the possibility of a CDG-I associated with a Tf variant to explain the contradictory CE pattern. Therefore, we treated the patient's serum with neuraminidase [21], to remove all terminal sialic acids from Tf and highlight a potential variant. In individuals homozygous for *TF*, all Tf fractions merge into a single 0-sialo Tf peak, while in heterozygous individuals with two isoforms of different isoelectric points, two peaks are present (Figure 1C). Here, after treatment, two 0-sialo Tf peaks were observed (Figure 1C), compatible with Tf heterozygosity. Taken together, the data suggest that the Tf CE pattern of Patient 1 corresponded to the overlay of 4-sialo, 2-sialo, and 0-sialo Tf peaks of the two protein isoforms (Figure S4) where the peaks of the faster-migrating isoform were mistakenly labelled as 3-sialo and 1-sialo, resulting in a deceptive CDG-II pattern. *PMM2* Sanger sequencing (NM_000303.3) revealed compound heterozygosity for the previously described missense mutations c.317A > G (p.Tyr106Cys) and c.422G > A (p.Arg141His), confirming that the patient suffered from PMM2-CDG (CDG-Ia).

Patient 2, a German girl, is the third child of a non-consanguineous family. She was born at 40+5 weeks of gestational age after birth induction. Her two older brothers are healthy. The family reported on two miscarriages. At 5 months, the girl displayed psychomotor developmental delay and failure to thrive and was referred to our hospital for further clarification. Physical examination revealed axial muscular hypotonia, strabismus with reduced fixation, inverted nipples, orange peel skin and abnormal fat distribution. Persistent diarrhea in the absence of infection and minor pericardial effusion were observed. Hyperechogenicity of the liver tissue was observed at ultra-sound investigation. Laboratory investigation revealed lactic acidosis, normochromic anemia, moderate hyperammonemia, elevated liver enzymes, lowered levels of antithrombin, protein C, protein S and hypogonadism. In light of these signs, CDG screening was performed.

HPLC analysis of Tf (Waters Alliance HPLC e2695 with PDA detector 2998, Waters, USA) was performed [3] and revealed a highly suspicious pattern with markedly lowered proportion of 4-sialo (24.7%; normal value > 80%) and elevated levels of peaks co-eluting with 5- (30.3%; normal value < 15%), 3- (19.5%; normal value 1%–6%) and 2-sialo (19.9%; normal value 1%–3%) Tf standards. Mono-sialo- (4.2%; normal value 0%) and 0-sialo (1.4%; normal value 0%) Tf were mildly increased as well (Figure 2A). Due to these abnormal results, a CDG-II defect was assumed. Thus, second-line analyses were performed. Tf IEF was performed on a Phast System (GE Healthcare) [22], and displayed a clearly abnormal pattern marked by elevated 2-sialo Tf and 0-sialo Tf, in combination with reduced 4-sialo Tf bands in double form (Figure 2B). This was indicative of a CDG-I defect. To find out whether the double bands arose due to a variant in the Tf protein backbone, the patient's sample was treated with neuraminidase and the IEF was repeated. In contrast to the control which then presented with a single 0-sialo Tf band, Patient 2 showed an additional band, which confirmed existence of a Tf variant (Figure 2C). As both isoforms were (hypo)glycosylated, this led to the initial suspicion that the patient suffers from a CDG-II disease. To learn more about this CDG-I defect, we next performed an ultrasensitive LC-MS analysis of *N*-glycans from whole serum glycoproteins (BioAccord integrated UPLC-FLR/QTOF MS system with Rapifluor [Waters]) [15]. Analysis identified 38 fluorescence-labelled glycans in Patient 2 and 35 glycans in the control pool (Figure 3) (Table S1). The three additional glycans found in the patient sample were the high mannose glycans Man3GlcNAc2 and Man4GlcNAc2 together with the *N*-tetrasaccharide Neu5Ac1Gal1GlcNAc2 ("N-tetra"). All three fluorescence-labelled sugar moieties were confirmed by MS. In a recent study with 111 patients suffering from different CDGI, several abnormal structures and ratios of *N*-glycans were identified which helped identify the patient's gene defect [15]. In the case of Patient 2, the presence of the N-Tetra glycan and a high Man3/N-Tetra ratio together with her clinical presentation clearly hinted to PMM2-CDG. Interestingly, the RapiFluor LC-MS technique applied to Patient 2 proved more sensitive than the MALDI-TOF MS method applied to Patient 1 for evidencing lower-mass *N*-glycans of interest, as it revealed three minor *N*-glycans species that orientated towards a specific CDG subtype. *PMM2* Sanger sequencing (NM_000303.3) ascertained compound heterozygosity for c.574T > C (p.Cys192Arg) and c.710C > T (p.Thr237Met) in the *PMM2* gene. While p.Thr237Met is an already known variant (class V), p.Cys192Arg is undescribed so far. We classify p.Cys192Arg as a class IV mutation.

Therefore, both patients suffer from ascertained PMM2-CDG (CDG-Ia) and not from a CDG-II disorder, as initially suspected because of misleading Tf variants.

With the development of newer laboratory techniques such as MS analysis of whole serum/plasma *N*-glycans or immunopurified Tf, traditional CDG screening techniques may appear obsolete. However, they retain strong competitive advantages. Equipment and operating expenses are low, while the techniques remain sensitive and specific.

Significance Statement

Biochemical diagnosis of Congenital disorders of glycosylation (CDG), an important group of inborn errors of metabolism, mainly relies on the study of transferrin glycoforms by capillary electrophoresis, isoelectric focusing or high-performance liquid chromatography. Transferrin variants, which are not rare, have previously been described by many authors as a cause of false-positive CDG screening. However, we here describe for the first time two cases of patients with combined transferrin variants and type I CDG, with the variants complicating the diagnoses. We suggest that, upon discovery of a type II CDG transferrin glycoforms profile, samples should be reanalyzed after treatment by neuraminidase/sialidase, to exclude a co-occurring variant, and allow a time- and cost-efficient diagnosis.

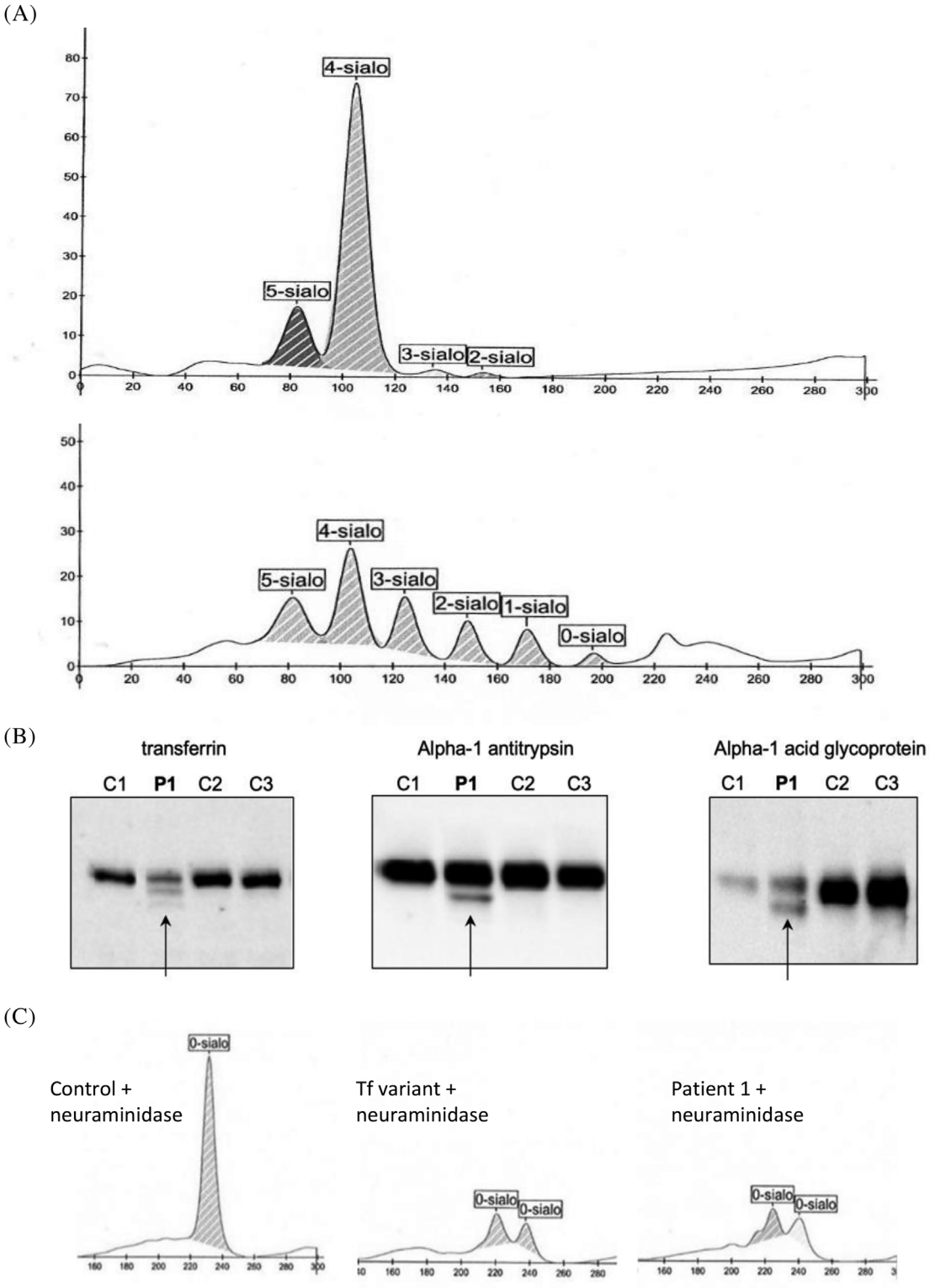

FIGURE 1 CE of transferrin and western blots of serum *N*-glycoproteins of Patient 1. (A) Compared to control, the capillary electrophoresis (CE) transferrin (Tf) profile of Patient 1 showed decreased level of 4-sialo Tf and elevated levels of 3-sialo to 0-sialo Tf, which is highly evocative of CDG -II. (B) Compared to controls (C1, C2, C3), western blot of transferrin (Tf), alpha-1-antitrypsin (AAT) and alpha-1 acid glycoprotein in Patient 1 (P1), showed numerous additional bands of lower molecular weight (arrows), highly evocative of CDG-I. (C) In a control without Tf variant, neuraminidase treatment generated one peak corresponding to one 0-sialo Tf protein isoform. In a control with a heterozygous Tf variant and in Patient 1, two peaks, corresponding to two differentially charged 0-sialo Tf protein isoforms can be separated by CE.

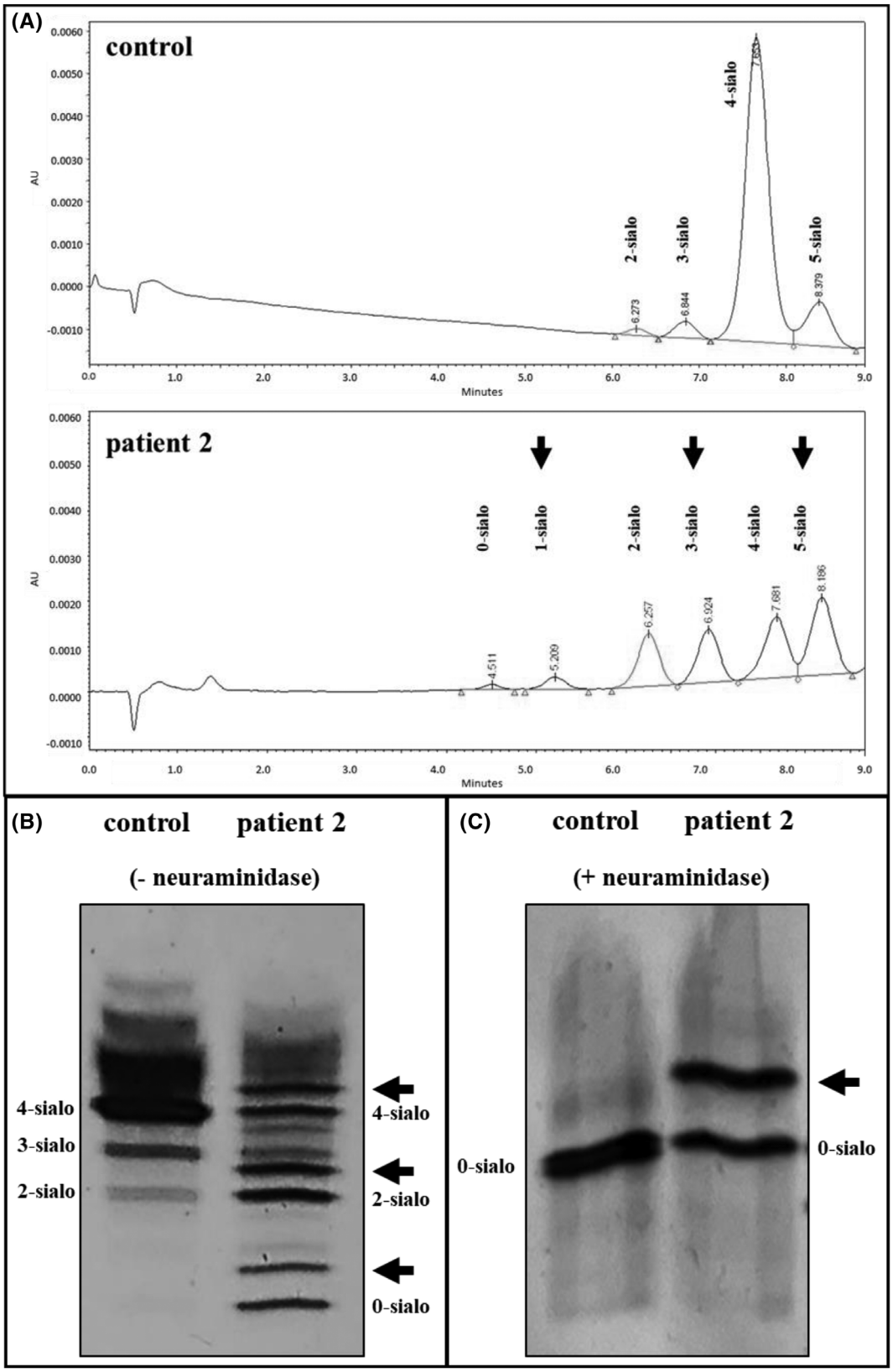

FIGURE 2 HPLC and isoelectric focusing of serum Tf of Patient 2. HPLC analysis (A) of the sample of Patient 2 revealed an abnormal pattern which was classified as CDG-II due to elevated amounts of 2-sialo, 3-sialo and 5-sialo Tf combined with a reduced level of 4-sialo Tf. Mono-sialo and 0-sialo Tf were elevated as well, although in lower proportions. Isoelectric focusing (B) also showed an abnormal pattern, however more indicative of a CDG-I defect marked by elevated 2-sialo and 0-sialo Tf bands in double presence. Neuraminidase treatment (C) proved existence of a Tf protein variant. Arrows indicate peaks and bands which emerged due to the variant Tf protein.

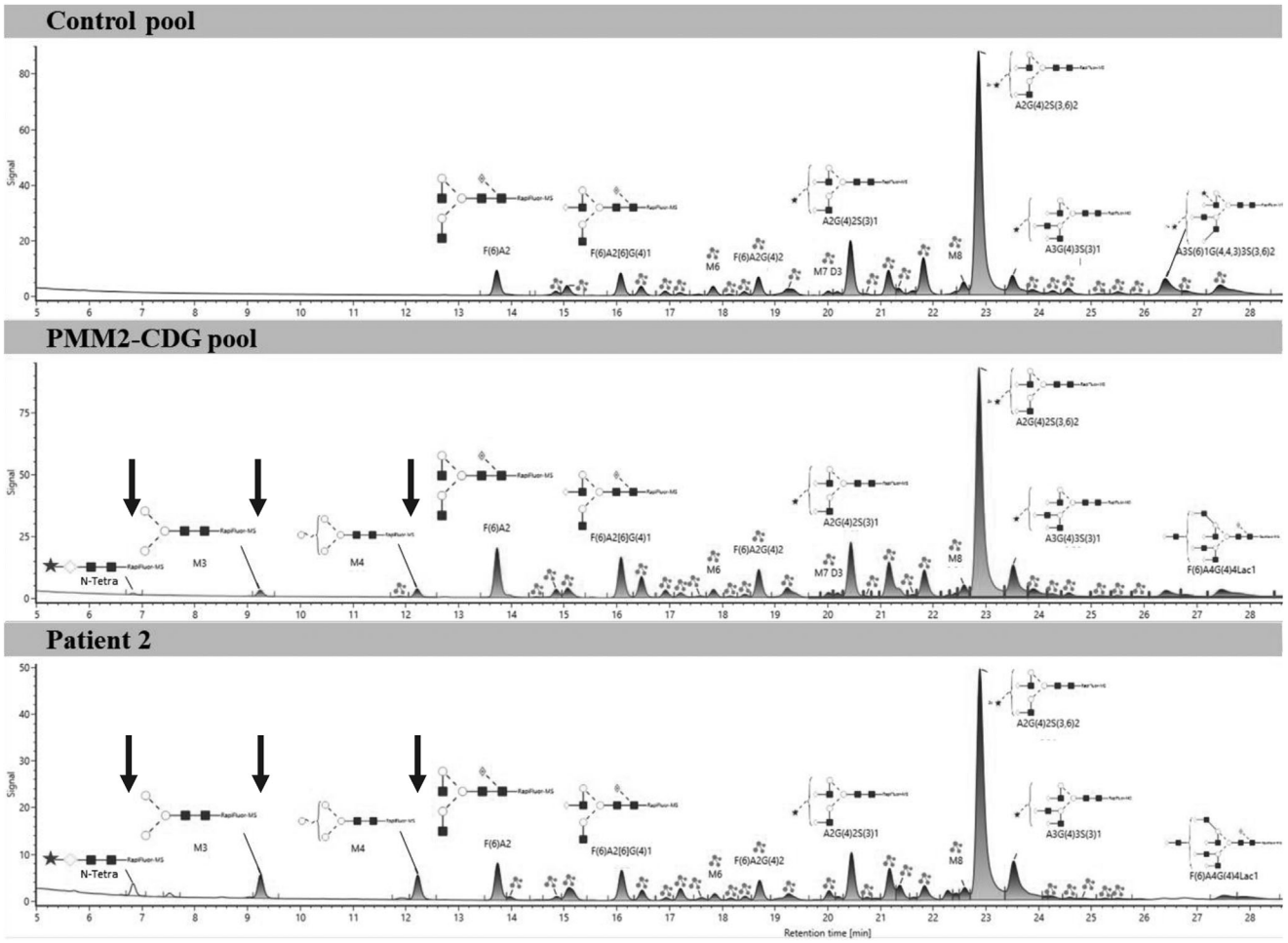

FIGURE 3 RapiFluor labeled *N*-glycan analysis of total serum glycoproteins by LC-MS. Compared to a control pool, clearly abnormal patterns were detected in the sample of Patient 2 and the PMM2-CDG pool which were characterized by presence of the *N*-Tetrasaccharide (N-Tetra) and the mannose-rich structures Man 3 (M3) and Man 4 (M4) (arrows), hereby indicating PMM2-CDG (CDG-Ia) for Patient 2.

Furthermore, the equipment is easy to use and can be applied to other purposes (e.g., serum proteins electrophoresis, hemoglobin studies). Finally, these techniques are recognized as "gold-standards". Therefore, laboratories worldwide remain dependent on these techniques for the time being. We here describe for the first time a pitfall in CDG diagnostics due to Tf variants which led to initial misclassification of Tf patterns in two PMM2-CDG (CDG-Ia) affected individuals. A time- and consumable-costly "arsenal" of second-line techniques was implemented, finally allowing proper diagnosis. However, retrospective analysis showed that simple digestion of samples by neuraminidase, followed by reanalysis using conventional techniques, would have highlighted the co-occurrence of a CDG-I Tf pattern combined to a variant early in the process.

Some laboratories perform genetic analyses directly upon discovery of an abnormal Tf profile. While this option is now viable, thanks to the emergence of cost-effective CDG multi-panels covering the most commonly affected genes, the development of highly sensitive and specific techniques (and in particular "omics") strengthen the place of second-line biochemical analyses in the context of CDG diagnosis. These can prevent diagnostic confusion in case of discrepancies between first-line biochemical results and genetics (as is the case here), or help document new variants of unknown significance (as is also the case here).

Therefore, we suggest that a simple neuraminidase treatment should be systematically performed in samples when a typical CDG Tf pattern is found upon CE/IEF/HPLC analysis, to ensure the appropriate interpretation of biochemical test results and the delivery of useful advice to the clinicians.

2 | EXPERIMENTAL PROCEDURES

2.1 | Subjects

Parents or representatives of the two patients included in this study provided written consent through each primary medical physician. Patient 1 and Patient 2 were referred to specialized medical centers in Belgium (University Hospitals of Leuven) and Germany (University Hospitals of Freiburg and Heidelberg), respectively. Sera were obtained from venous blood samples collected in tubes without antico-

agulant to allow clot formation. For DNA extraction, venous blood was collected in tubes with EDTA.

2.2 | Glycosylation analyses

Tf capillary electrophoresis (Patient 1) was performed on a Capillarys 2 Flex Piercing using the Capillarys CDT kit (Sebia), as previously described [10].

HPLC analysis of Tf (Patient 2) was carried out from 100 μL serum on a Waters Alliance HPLC e2695 with PDA detector 2998 (Waters), as previously described [3].

Isoelectric focusing (IEF) of Tf (Patient 2) was carried out on a Phast System (GE Healthcare) with 2.5 μg of whole serum proteins [22].

For the assessment of Tf protein variant (Patients 1 and 2), sialidase (neuraminidase) treatment of serum glycoproteins was performed as previously described [13], before reanalysis using CE or IEF.

For Patient 1, two-dimensional electrophoresis (2-DE) of *O*-glycosylated apolipoprotein C-III (apoC-III), and western blot and 2-DE of various serum *N*-glycoproteins, were conducted as previously described [16, 19, 20].

For Patient 1, profile of total serum *N*-glycans (starting from 5 μL) was obtained by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) following *N*-glycan cleavage by peptide *N*-glycosidase F (PNGase F), glycan purification by solid-phase extraction and permethylation as previously described [17, 18]. Mass spectra were obtained on an UltrafleXtreme mass spectrometer operated in the reflectron positive ion mode (Bruker Daltonics, Bremen, Germany). Manual assignment of *N*-glycans was done from MS and MS/MS data based on previously identified structures [17] and using the GlycoWorkBench software.

For Patient 2, LC-MS analysis of Rapifluor-labelled *N*-glycans from whole serum glycoproteins was performed. For this, 22.5 μg of proteins of a control pool ($n = 120$), a PMM2-CDG pool ($n = 50$) and of patient 2 were used, respectively. Deglycosylation with PNGase F, labelling and clean-up of the *N*-glycans were conducted by using the GlycoWorks RapiFluor-MS N-Glycan-Kit (Waters). Analysis and evaluation of the sugar structures were performed on an Integrated UPLC-FLR/QTOF MS system with integrated software (BioAccord, Waters) [15].

2.3 | Genetics

Based on clinical presentation and biochemical findings, mutation analysis of the *PMM2* gene (NM_000303.3; NG_009209.1) was performed in both patients by Sanger sequencing (primers available upon request).

ACKNOWLEDGMENTS

This work was supported by the European Union's Horizon 2020 research and innovation program under the ERA-NET cofund action n° 643578 (A.R., A.B.), the Commissariat à l'Energie Atomique et aux Energies Alternatives and the MetaboHUB infrastructure (ANR-11-INBS-0010) (S.C., F.F.). This study was further supported by the Deutsche Forschungsgemeinschaft (FOR2509: TH1461/7-2) and the European Commission (E-Rare-3 Joint Transnational Call 2018/EUROGLYCAN-omics) in association with the Deutsche Forschungsgemeinschaft (C.T.).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

For Patient 1, all MALDI-TOF MS glycomics data are available on https://glycopost.glycosmos.org/ (Watanabe Y. et al., Nucleic Acids Res 2021, PMID: 33174597) under the accession number GPST000372. For Patient 2, MS glycomics data are available as a supplementary material. Analyses were performed following the manufacturer's instructions. Additional technical details are available upon request to the author CT.

ORCID

Alexandre Raynor https://orcid.org/0000-0003-2651-650X

REFERENCES

1. Péanne, R., de Lonlay, P., Foulquier, F., Kornak, U., Lefeber, D. J., Morava, E., Pérez, B., Seta, N., Thiel, C., Van Schaftingen, E., Matthijs, G., & Jaeken, J. (2018). Congenital disorders of glycosylation (CDG): Quo vadis? *European Journal of Medical Genetics*, *61*(11), 643–663.
2. Ondruskova, N., Cechova, A., Hansikova, H., Honzik, T., & Jaeken, J. (2021). Congenital disorders of glycosylation: Still "hot" in 2020. *Biochimica et Biophysica Acta (BBA) – General Subjects*, *1865*(1), 129751.
3. Quintana, E., Montero, R., Casado, M., Navarro-Sastre, A., Vilaseca, M. A., Briones, P., & Artuch, R. (2009). Comparison between high performance liquid chromatography and capillary zone electrophoresis for the diagnosis of congenital disorders of glycosylation. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, *877*(24), 2513–2518.
4. Ng, B. G., Dastsooz, H., Silawi, M., Habibzadeh, P., Jahan, S. B., Fard, M. A. F., Halliday, B. J., Raymond, K., Ruzhnikov, M. R. Z., Tabatabaei, Z., Taghipour-Sheshdeh, A., Brimble, E., Robertson, S. P., Faghihi, M. A., & Freeze, H. H. (2020). Expanding the molecular and clinical phenotypes of FUT8-CDG. *Journal of Inherited Metabolic Disease*, *43*(4), 871–879.
5. Sadat, M. A., Moir, S., Chun, T. W., Lusso, P., Kaplan, G., Wolfe, L., Memoli, M. J., He, M., Vega, H., Kim, L. J. Y., Huang, Y., Hussein, N., Nievas, E., Mitchell, R., Garofalo, M., Louie, A., Ireland, D. C., Grunes, C., Cimbro, R., … Rosenzweig, S. D. (2014). Glycosylation, hypogammaglobulinemia, and resistance to viral infections. *New England Journal of Medicine*, *370*(17), 1615–1625.
6. Post, M. A., de Wit, I., Zijlstra, F. S. M., Engelke, U. F. H., van Rooij, A., Christodoulou, J., Tan, T. Y., Le Fevre, A., Jin, D., Yaplito-Lee, J., Lee, B. H., Low, K. J., Mallick, A. A., Ounap, K., Pitt, J., Reardon, W., Vals, M. A., Wortmann, S. B., Wessels, H. J. C. T., … Lefeber, D. J. (2023). MOGS- CDG: Quantitative analysis of the diagnostic Glc 3 Man tetrasaccharide and clinical spectrum of six new cases. *Journal of Inherited Metabolic Disease*, *46*(2), 313–325.
7. Lefeber, D. J., Morava, E., & Jaeken, J. (2011). How to find and diagnose a CDG due to defective N-glycosylation. *Journal of Inherited Metabolic Disease*, *34*(4), 849–852.
8. Bruneel, A., Cholet, S., Tran, N. T., Mai, T. D., & Fenaille, F. (2020). CDG biochemical screening: Where do we stand? *Biochimica et Biophysica Acta General Subjects*, *1864*(10), 129652.

9. Jaeken, J., Hennet, T., Matthijs, G., & Freeze, H. H. (2009). CDG nomenclature: Time for a change! *Biochimica Et Biophysica Acta*, *1792*(9), 825–826.
10. Parente, F., Ah Mew, N., Jaeken, J., & Gilfix, B. M. (2010). A new capillary zone electrophoresis method for the screening of congenital disorders of glycosylation (CDG). *Clinica Chimica Acta, the International Journal of Clinical Chemistry*, *411*(1-2), 64–66.
11. Schellenberg, F., Wielders, J., Anton, R., Bianchi, V., Deenmamode, J., Weykamp, C., Whitfield, J., Jeppsson, J. O., & Helander, A. (2017). IFCC approved HPLC reference measurement procedure for the alcohol consumption biomarker carbohydrate-deficient transferrin (CDT): Its validation and use. *Clinica Chimica Acta, the International Journal of Clinical Chemistry*, *465*, 91–100.
12. Guillard, M., Wada, Y., Hansikova, H., Yuasa, I., Vesela, K., Ondruskova, N., Kadoya, M., Janssen, A., Van den Heuvel, L. P. W. J., Morava, E., Zeman, J., Wevers, R. A., & Lefeber, D. J (2011). Transferrin mutations at the glycosylation site complicate diagnosis of congenital disorders of glycosylation type I. *Journal of Inherited Metabolic Disease*, *34*(4), 901–906.
13. Zühlsdorf, A., Park, J. H., Wada, Y., Rust, S., Reunert, J., DuChesne, I., Grüneberg, M., & Marquardt, T. (2015). Transferrin variants: Pitfalls in the diagnostics of Congenital disorders of glycosylation. *Clinical Biochemistry*, *48*(1-2), 11–13.
14. van Scherpenzeel, M., Steenbergen, G., Morava, E., Wevers, R. A., & Lefeber, D. J. (2015). High-resolution mass spectrometry glycoprofiling of intact transferrin for diagnosis and subtype identification in the congenital disorders of glycosylation. *Translational Research: The Journal of Laboratory and Clinical Medicine*, *166*(6), 639–649.e1.
15. Abu Bakar, N., Ashikov, A., Brum, J. M., Smeets, R., Kersten, M., Huijben, K., Keng, W. T., Speck-Martins, C. E., de Carvalho, D. R., de Rizzo, I. M. P. O., de Mello, W. D., Heiner-Fokkema, R., Gorman, K., Grunewald, S., Michelakakis, H., Moraitou, M., Martinelli, D., van Scherpenzeel, M., Janssen, M., … Lefeber, D. J. (2022). Synergistic use of glycomics and single-molecule molecular inversion probes for identification of congenital disorders of glycosylation type-1. *Journal of Inherited Metabolic Disease*, *45*(4), 769–781.
16. Yen-Nicolaÿ, S., Boursier, C., Rio, M., Lefeber, D. J., Pilon, A., Seta, N., & Bruneel, A. (2015). MALDI-TOF MS applied to apoC-III glycoforms of patients with congenital disorders affecting O-glycosylation. Comparison with two-dimensional electrophoresis. *Proteomics – Clinical Applications*, *9*(7-8), 787–793.
17. Bruneel, A., Cholet, S., Drouin-Garraud, V., Jacquemont, M. L., Cano, A., Mégarbané, A., Ruel, C., Cheillan, D., Dupré, T., Vuillaumier-Barrot, S., Seta, N., & Fenaille, F. (2018). Complementarity of electrophoretic, mass spectrometric, and gene sequencing techniques for the diagnosis and characterization of congenital disorders of glycosylation. *Electrophoresis*, *39*(24), 3123–3132.
18. Goyallon, A., Cholet, S., Chapelle, M., Junot, C., & Fenaille, F. (2015). Evaluation of a combined glycomics and glycoproteomics approach for studying the major glycoproteins present in biofluids: Application to cerebrospinal fluid. *Rapid Communications in Mass Spectrometry RCM*, *29*(6), 461–473.
19. Bruneel, A., Habarou, F., Stojkovic, T., Plouviez, G., Bougas, L., Guillemet, F., Brient, N., Henry, D., Dupré, T., Vuillaumier-Barrot, S., & Seta, N. (2017). Two-dimensional electrophoresis highlights haptoglobin beta chain as an additional biomarker of congenital disorders of glycosylation. *Clinica Chimica Acta, the International Journal of Clinical Chemistry*, *470*, 70–74.
20. Seta, N., Barnier, A., Hochedez, F., Besnard, M. A., & Durand, G. (1996). Diagnostic value of Western blotting in carbohydrate-deficient glycoprotein syndrome. *Clinica Chimica Acta, the International Journal of Clinical Chemistry*, *254*(2), 131–140.
21. Sakhi, S., Cholet, S., Wehbi, S., Isidor, B., Cogne, B., Vuillaumier-Barrot, S., Dupré, T., Detleft, T., Schmitt, E., Leheup, B., Bonnet, C., Feillet, F., Muti, C., Fenaille, F., & Bruneel, A. (2021). MAN1B1-CDG: Three new individuals and associated biochemical profiles. *Molecular Genetics and Metabolism Reports*, *28*, 100775.
22. Thiel, C., Meßner-Schmitt, D., Hoffmann, G. F., & Körner, C. (2013). Screening for congenital disorders of glycosylation in the first weeks of life. *Journal of Inherited Metabolic Disease*, *36*(5), 887–892.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Raynor, A., Bruneel, A., Vermeersch, P., Cholet, S., Friedrich, S., Eckenweiler, M., Schumann, A., Hengst, S., Tuncel, A. T., Fenaille, F., Thiel, C., & Rymen, D. (2023). "Hide and seek": Misleading transferrin variants in PMM2-CDG complicate diagnostics. *PROTEOMICS – Clinical Applications*, 2300040. https://doi.org/10.1002/prca.202300040