same antibody pairs, and each assay is approved by the Food and Drug Administration for the same clinical indications. This multicenter evaluation shows that the performance of the automated Access Hybritech PSA and free PSA assays is analytically specific, sensitive, linear, accurate, and precise.

This study was funded by Beckman Coulter, Inc.

References

Congenital Disorder of Glycosylation Ia with Deficient Phosphomannomutase Activity but Normal Plasma Glycoprotein Pattern, Thierry Dupre,1 Maryvonne Cuer,1 Sandrine Barrot,1 Anne Barnier,1 Valérie Cornier-Daire,2 Arnold Munnich,2 Geneviève Seta1,4* (1) Laboratoire de Biochimie A, Hôpital Bichat, 75877 Paris Cédex 18, France; (2) Service de Génétique Médicale, INSERM U393 Hôpital Necker, 75015 Paris, France; (3) Faculté de Pharmacie, Université Paris XI, 92296 Châtenay-Malabry Cédex, France; (4) Faculté de Pharmacie, Université Paris V, 75006 Paris, France; * address correspondence to this author at: Laboratoire de Biochimie A, Hôpital Bichat-Claude Bernard, 46, rue Henri Huchard, 75877 Paris Cédex 18, France; fax 33-1-40-25-88-21, e-mail nathalie.seta@bich.ap-hop-paris.fr

Congenital disorders of glycosylation [CDG; previously carbohydrate-deficient glycoprotein syndrome (1)] represent a newly delineated group of inherited diseases (2). The CDG are now clearly classified in two groups including subgroups. CDG I, by far the most common type with >300 patients described in the literature, is characterized by defects in the assembly of dolichol pyrophosphate oligosaccharide and/or in the transfer of oligosaccharide from dolichol pyrophosphate to an Asn residue on the nascent proteins. The other group, CDG II, reflects defects in the processing of protein-bound glycans. Only a few cases have been described (1).

The diagnosis of CDG I is based on biochemical changes involving a unique carbohydrate deficiency observed in serum transferrin (TRF). In healthy subjects, serum TRF is fully glycosylated, containing two N-glycan chains, whereas in CDG I patients, it is partially (one chain) or totally deglycosylated (3). This structural abnormality is associated with different enzyme deficiencies (4). The most common, subtype Ia, is a deficiency of phosphomannomutase (PMM; EC 5.4.2.8) (5) and is present in 70% of CDG I patients. The disease is linked to chromosome 16p13, and numerous missense mutations have been identified in the PMM2 gene (6, 7). The condition is an autosomal recessive multisystemic disorder affecting the nervous system and numerous organs, including the liver, kidney, heart, adipose tissue, bone, and genitalia (4).

The characteristic biochemical abnormalities of CDG can be demonstrated by various methods, including microion-exchange chromatography or isoelectric focusing of TRF (8), based on sialic acid content, and Western-blot analysis of plasma glycoproteins (9), based on variations of protein molecular weight. Fig. 1A shows typical isoelectric focusing patterns for serum from a healthy subject and a CDG I patient; Fig. 1B shows typical Western-blot patterns for serum TRF, α1-antitrypsin, haptoglobin, and α1-acid glycoprotein from a healthy subject and a CDG I patient. The detection limit of the Western-blot method, tested by serial dilution, was <1 ng on the gel regardless of the glycoprotein tested. No discordance was observed between the TRF Western-blot assays and isoelectric focusing when >20 CDG I patient patterns were compared (data not shown).

We report here two cases of CDG Ia for which the condition could not be detected as easily as usual. In the first family (F1), the sibling pair was composed of a 16-year-old girl (F1G) and a 6-year-old boy (F1D). Both have classical clinical features of CDG I, including psychomotor retardation, cerebellar ataxia, strabismus, and cerebellar hypoplasia; the girl also has hypogonadism. When Western blotting of the four different glycoproteins was performed on sera from both children, the results were puzzling. The boy’s results showed a characteristic CDG I pattern (Fig. 1B, lane 4), consistent with the clinical features. By contrast, the pattern of serum glycoproteins of the girl (Fig. 1B, lane 3) was identical to the one of healthy subjects. The serum carbohydrate-deficient transferrin (CDT) was measured for both siblings (reference interval, 10–30 units/L CDT; F1G, 38 units/L CDT; F1D, 148 units/L CDT) and was consistent with the results of
the Western-blot analysis. These results were also confirmed by the isoelectric focusing pattern (Fig. 1A). Three months later, the results were confirmed on new serum samples. During the intervening period, PMM activity was measured according to the method of Van Schaftingen and Jaeken (5) on mononucleated leukocytes and on cultured skin fibroblasts from both children. The results obtained from the two cell types demonstrated undetectable PMM activity for both children. Identical mutations of the PMM2 gene, R141H and T226S as determined by complete sequencing of cDNA, were found in both children.

In the other family (F2), the sibling pair was composed of two adult men (F2L and F2T) with nonprogressive cerebellar ataxia. The Western-blot pattern of the serum glycoproteins from F2T (Fig. 1B, lane 6) was typical for CDG I. In contrast, fewer bands or paler lower bands were found for F2L (Fig. 1B, lane 5). Similarly, the isoelectric

![Isoelectric focusing patterns](image)

**Fig. 1.** Isoelectric focusing patterns of serum TRF (A) and Western blots of serum glycoproteins (B) from a healthy subject (lane 1), a CDG Ia patient with a typical electrophoretic pattern (lane 2), and the CDG Ia patients F1J (lane 3), F1D (lane 4), F2L (lane 5), and F2T (lane 6).

(A), AAT, α1-antitrypsin; HPT, haptoglobin; AGP, α1-acid glycoprotein.
focusing patterns showed a characteristic CDG I profile for F2T but only a partially abnormal one for F2L (Fig. 1A). PMM activity measured in the leukocytes of both patients was undetectable, corresponding to an identical double mutation on the PMM2 gene, R141H and C9Y as determined by complete sequencing of cDNA.

Until now, the diagnosis of CDG I has been based on clinical features and confirmed by the presence of abnormally glycosylated serum glycoproteins. Considering our results, we are facing a new situation: patients who have clinical CDG I features and belong to families in which other relatives are clinically and biologically CDG I patients, but who have either intermediate electrophoretic patterns corresponding to glycoproteins lacking fewer glycan chains, or even non-CDG patterns corresponding to normally glycosylated serum glycoproteins. In the first family, the patient F1J with the normal pattern is almost an adult, and the results can be related to those observed in adult patients (10). Stibler et al. (10) reported that concentrations of CDT are profoundly increased in all patients but tend to be lower in adults than in patients younger than 15 years, with a loss of correlation with age in older patients. The normalization of the glycoprotein glycan content could reflect an adaptation to the metabolic abnormalities. In the case of the 16-year-old patient, the adaptation could be complete although the PMM activity was deficient. In the other family, age does not explain the findings of a typical CDG I pattern in one sibling and, in the other, a pattern with fewer or paler lower bands for all glycoproteins tested, despite similar clinical presentations for both subjects.

In conclusion, we have seen at least one clinically confirmed CDG Ia patient with normal serum glycoproteins. The diagnosis of CDG Ia presently based on the evidence of abnormal glycosylation of serum glycoproteins, whatever the method used, might lack sensitivity when applied to teenagers or adults. Biologists who are involved in the diagnosis of CDG should be aware of the possibility of false-negative results.

References


A Precaution in the Detection of Heterozygotes by Sequencing: Comparison of Automated DNA Sequencing and PCR-Restriction Fragment Length Polymorphism Methods, Mehmet Simsek,1* Musbah O.M. Tanira,2 Khalid A. Al-Baloushi,3 Haneeda S. Al-Barwani,3 Khulood M. Lavatia,4 and Riad A.L. Bayoumi1 (Departments of 1Biochemistry and 2Pharmacology, Sultan Qaboos University, College of Medicine, Muscat, Oman; * address correspondence to this author at: Sultan Qaboos University, College of Medicine, Department of Biochemistry, PO Box 35, Al-Khod, Postal code 123, Muscat Sultanate of Oman; fax 968-513-419, email mssimsek@omantel.net.om)

Single-nucleotide polymorphisms have been detected by various methods, including allele-specific oligonucleotide hybridization (1), allele-specific amplification (2), and restriction fragment length polymorphism (RFLP) analysis (3). In some cases, sequencing of amplified DNA has been used as a direct method (4, 5). RFLP-based methods appear to be superior in genotyping studies. In fact, recently, Cascori and Roots (6) recommended the use of RFLP in preference to other methods for single-nucleotide polymorphism detection in the N-acetylgalactosamine-transferase-2 (NAT2) gene. Some groups have used more than one method in their analyses, and when discrepancies occurred between two methods, they used DNA sequencing as a gold standard (7). In this report, we describe a comparison of RFLP methods with automated DNA sequencing for the detection of three different mutations in the NAT2 gene. Our results indicate that caution is needed in the use of automated sequencing to detect heterozygous mutations accurately at some polymorphic sites in the NAT2 gene.

Established PCR-RFLP methods were used to detect NAT2 mutations at the C282Y, T341C, and C481T sites. The C-to-T mutations at the 282 and 481 sites were detected with RFLP analysis of a 360-bp DNA fragment with FokI or KpnI digests, respectively (3). Two different RFLP methods were used for detection of the T341C mutation: a previously described Ddel method (3), and a new complementary NcoI-RFLP. For both methods, a seminested PCR was performed to amplify a 360-bp DNA fragment using a 998-bp DNA fragment as a template. The latter fragment was preamplified as described previously by Hickman and Sim (8), using their Nat-Hul14 and Nat-Hul16 primers. The seminested PCR mixture (50 μL) contained 2 μL of 100-fold diluted 998-bp DNA, 200 μM each dNTP, 1.0 μM primers, 1.25 U of Taq DNA polymerase (Life Technologies), and a buffer consisting of 10 mM