Editorial Commentary

Improving biochemical markers for disorders of N-glycosylation

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Comment on: Chen J, Li X, Edmondson A, *et al.* Increased Clinical Sensitivity and Specificity of Plasma Protein N-Glycan Profiling for Diagnosing Congenital Disorders of Glycosylation by Use of Flow Injection-Electrospray Ionization-Quadrupole Time-of-Flight Mass Spectrometry. Clin Chem 2019;65:653-63.

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Identification of new biomarkers for diseases or improved sensitivity of established ones can help identify patients, track their natural history, and monitor the efficacy of potential therapies. All clearly benefit patients. In a recent paper by Chen *et al.* (1), the authors apply a highly sensitive, commercialized method to the analysis of a few patients with several different congenital disorders of glycosylation (CDG). The method significantly expands quantitative glycomic information available for patients. These results are encouraging, however, analysis of many more samples will be required to have sufficient confidence in its general usefulness for CDG patients.

CDGs are a group of 130–140 rare metabolic disorders involving protein glycosylation (2). Patients have a broad and heterogenous set of symptoms that overlap with other rare disorders, making initial diagnosis challenging. For the 70+ disorders that impact the N-glycosylation pathway, initial biochemical diagnosis was almost single-handedly captured by determining the glycosylation status of serum transferrin (Tf) (3). This liver-derived protein contains two N-glycans, whose presence and general structure can be assessed by multiple methods including isoelectric focusing, various chromatographic approaches and, most sensitively, by mass spectrometry. Absence of entire glycans on Tf narrows the potential defects to a limited number of genes. Alternatively, detection of altered glycan structures suggests a different set of genes. A few disorders show both absent glycans and altered glycan structures. In all cases, definitive diagnosis requires gene sequencing and evidence that the variants are pathological. Tf is a very useful tool, but not a CDG "gold standard" because not all CDGs show abnormal Tf, and some afflicted individuals normalize Tf glycosylation, in time, without clinical improvement.

Mass spectrometry can be applied to the analysis of N-glycans cleaved from Tf or to glycans cleaved from total serum glycoproteins using the enzyme PNGaseF. The reaction creates a labile glycosylamine-terminated glycan which can be derivatized with highly fluorescent molecules such as 2-aminobenzamide to provide sensitive quantitation via liquid chromatography (LC). By combining N-glycan cleavage, derivatization, and flow-injection electrospray ionization quadrupole time of flight mass spectrometer (QTOF MS) analysis with an internal standard, it is possible to identify and quantify a series of individual N-glycans (4). In fact, Waters, Inc., has optimized and packaged this technology into a convenient, robust, well-documented, but pricy, kit for release, derivatization and MS analysis. The all-in-one tag uses N-hydroxysuccinimidyl (NHS) carbamate to react with the reducing end of the PNGaseF-released glycans and the quinoline fluorophore provides a highly sensitive readout. In addition, the inclusion of a highly basic tertiary amine greatly enhances MS ionization and sensitivity. This tagging improves detection of even very minor glycans when combined with direct infusion QTOF analysis. The combination of convenience, reproducibility, high throughput and great

MS sensitivity may be worth the price tag for labs doing a large number of samples. It certainly extends the range and options for analysis of samples derived from patients with glycosylation disorders (5). A note of caution is that most of the "assigned structures" have actually not been determined; they are the most reasonable assignments based on the combined knowledge of biosynthesis and other well-known glycans.

Applying this technology to a series of serum samples from 31 normal and 19 patients with 11 types of CDG produced the data for this study. Most, but not all, of the patients had a confirmed molecular diagnosis and 6 of the 11 disorders listed count only a single patient. The most common disorder, PMM2-CDG, presents only 6 patients. It is puzzling that the authors did not analyze more patients to increase overall confidence. Hopefully the procedure will be useful for additional patients with these disorders or those who have the other 60 untested types of CDG.

This method provides the potential for substantially improving resolution for diagnosis and monitoring of patients as part of a natural history or therapeutic studies. The challenge will be analyzing a sufficient number of patients of each CDG type. More important, will changes in very minor components reflect changes that are meaningful for the patients? The answers may be forthcoming since an NIH-funded multi-center clinical study of CDG patients in the US will apply the method to the diagnosis, natural history, and promising therapeutic investigations of over 100 CDG patients. The technology offers quite a significant improvement beyond the traditional methods of Tf or serum glycan analysis. Perhaps further mining will usher in a new CDG biochemical "gold standard".

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Footnote

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