C2 Centre for Chronic
D2 Diseases & Disorders

Developing a method for CDG testing from dried blood spots

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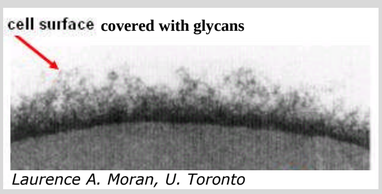
1. Summary

Congenital glycosylation disorders (CDGs) form a family of rare diseases. Clinical diagnosis of these disorders is very difficult due to the large set of possible pleiotropic symptoms. A very reliable biochemical test is available though, which relies on the analysis of a marker protein from serum. Unfortunately, serum samples from potential patients – mostly small children – are difficult to request without a good clinical case. For this reason diagnostic efficiency of the disease remains low, and the true numbers suffering from the disease are unknown.

This project will adapt the CDG-test for use with dried blood spots (DBS). DBS have he advantage that they are generated for every new-born, and therefore do not pose an additional burden on potential patients. Thus DBS should be useful for determining the true abundance of the disorders, and should increase diagnostic efficiency. An important value for the latter point is the possible treatment of about 5% of all CDG cases, these patients will get direct benefit from the new testing.

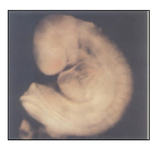
2. Glycosylation

Glycosylation is a universal protein modification generating sugar polymers called glycans. Every cell surface is covered by glycans. Glycans are important for many developmental and physiological processes



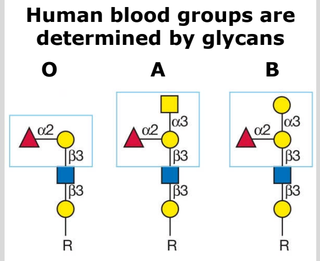
Laurence A. Moran, U. Toronto

Mgat1$^{-/-}$ mice lack one class of glycans and die at embryonic day 9.5



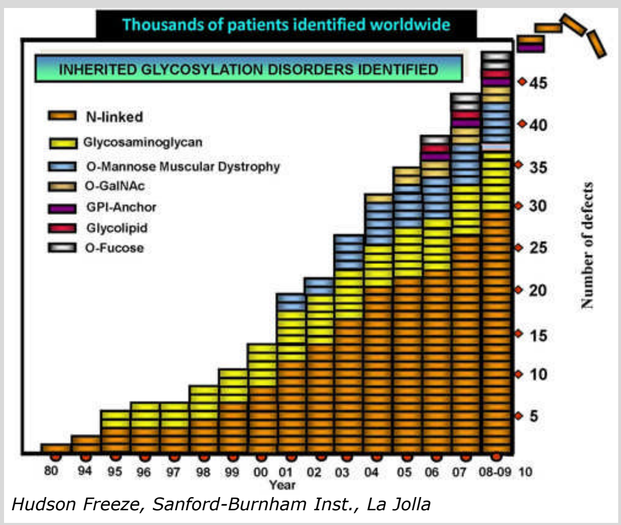
Metzler et al (1994) EMBO J., 13:2056

Human blood groups are determined by glycans



3. Congenital Disorders of Glycosylation – CDG

Group of 30+ autosomal recessive disorders. Currently with a little more than 1000 patients world-wide. Clinical diagnostics very difficult due to the large number of wide ranging symptoms that can affect any organ.



Hudson Freeze, Sanford-Burnham Inst., La Jolla

Some common symptoms

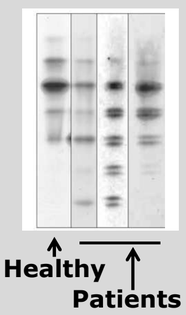
- Hypotonia
- Failure to thrive
- Developmental delay
- Coagulopathy
- Seizures
- Cerebellar hypoplasia
- Ataxia
- Retinitis pigmentosa
- Scoliosis
- Esothropia
- …

Two subtypes are treatable:
CDG-Ib (5% of all CDGs): treated by administering the sugar mannose
CDG-IIc (1%): half of the patients respond well to fucose-therapy

4. Biochemical diagnosis of CDG

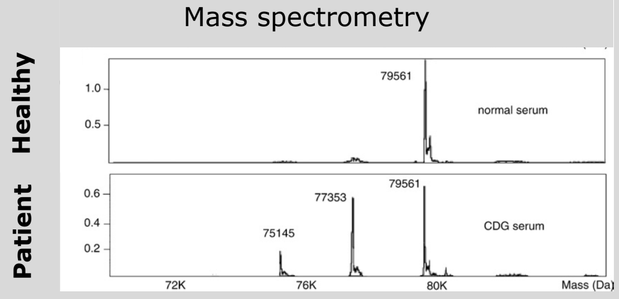
The serum glycoprotein transferrin is analysed. Healthy individuals have two fully sialylated bi-antennary glycans on transferrin. These glycans change upon disease.

Isoelectric focussing



Easy instrumentation, good diagnostic value, but cannot really distinguish subtypes

Mass spectrometry



Instrumentation becoming more routine now, allows precise diagnostics, can often distinguish subgroups of the various subtypes.

BUT: require serum samples – these are only available from suspected patients

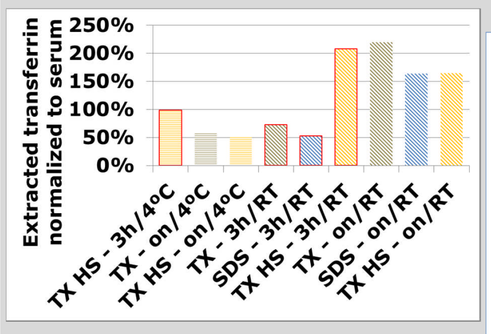
Frequency of disease not known

5. Using DBS for CDG testing

DBS are readily available for all new-borns. Should be ideal for determining the true frequency of CDG. Would also make screening for CDG early on possible.

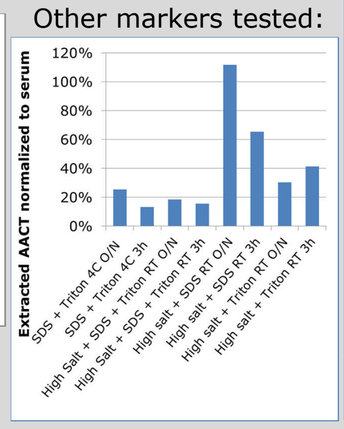
Difficulties:

- Glycoprotein marker needs to be extracted from filter paper
- Glycan profile of marker protein could be in transition between fetal and child/adult form
- Biomarker may not be stable on stored DBS

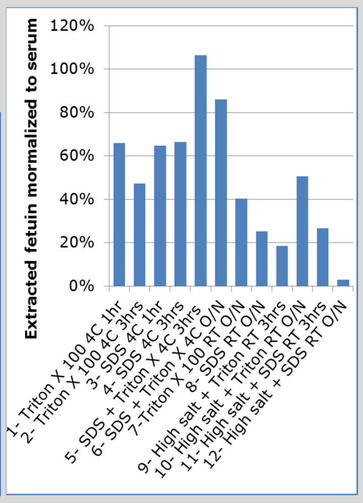


Transferrin is better extracted by Triton X-100 (TX) than SDS. High salt concentration (HS) speeds up extraction.

Other markers tested:



α-1-antichymotrypsin (AACT) is best extracted by high salt and SDS



Fetuin is best extracted by a combination of Triton and SDS

Three different biomarkers could be successfully extracted with high efficiency, albeit using very different conditions

6. Conclusions and future directions

DBS can supply CDG biomarkers. The suitability of these marker for diagnostics needs to be further tested.

Extracted biomarkers will be analysed by ESI-MS (with David Ashford, Biology Technology Facility) to determine their suitability for versatile CDG diagnostics.

Currently used DBS is from a healthy adult volunteer – samples from healthy new-borns (obtained with consent) and subsequently from CDG patients will be tested next.

We thank Charles Lacey for his help in obtaining blood samples from healthy adult volunteers.

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