SSIEM Official Satellite Symposia
“The 2nd World Conference on Congenital Disorders of Glycosylation (WCCDG) for Families and Professionals: a challenging story of sugar trees”, 28 to 30 August 2015, in Lyon (France)

This conference is part of the Educational Program of Excellence on CDG created by the Portuguese Association for CDG (APCDG, www.apcdg.com).

It is organized in partnership with several associations and/or country CDG patient advocates: CDG Australia, CDG Brazil, CDG Czech Republic, CDG Denmark, Foundation of Glycosylation (the FoG) Canada, CDG Denmark, CDG Italy/Ireland, CDG Israel, Les ptits CDG France, CDG Spain, CDG Sweden, CDG USA, CDG UK charity and CDG The Netherlands.

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Towards the structural analysis of aberrant glycosylation: 

Focus on CDGs

Pakanová Z.1, Šalingová A.2, Behúlová D.2, Hlavatá A.3, Paschinger K.4, Wilson I.4, Mucha J.1

1 Institute of Chemistry, Centre of Excellence in Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovakia
2 Centre of Inherited Metabolic Diseases, Department of Laboratory Medicine, Children´s Faculty Hospital, Limbová 1, Bratislava, Slovakia
3 2nd Children’s Clinic, Faculty of Medicine of Comenius University and Children’s Faculty Hospital, Limbová 1, Bratislava, Slovakia
4 Department für Chemie, Universität für Bodenkultur, Muthgasse 18, Wien, Austria
SLOVAKIA 2015:
- 5 500 000 citizens
- 53 000 births/year

Centre of Excellence for Glycomics:
- Founded in 2010
- Focused on GLYCO-conjugates and analysis of their structure
- Glycosylation of proteins found CHANGED in:
  - Cancer
  - Neurodegenerative diseases
  - Immunological diseases
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• Glycosylation of proteins found CHANGED in:
  • Cancer
  • Neurodegenerative diseases
  • Immunological diseases
• **CDGs**!
Diagnostics of CDG in Slovakia

• 2012: selective CDG screening method based on IEF Tf established in Children´s Faculty Hospital Bratislava

• Up to this date, **956 suspected patients tested**
  
  • 3 **individuals** manifested aberrant IEF Tf pattern
  
  • Tf polymorphism detected in **5 children**
  
  • secondary causes of abnormal glycosylation excluded

• further solving of a specific subtype:
  
  cooperation between research institute and hospitals is **essential**
Glycoprofile

• Serum glycoprofile = all the glycan structures found in serum

• Everyone has their own GLYCO-fingerprint

• Glycoprofile is DYNAMICAL

• could be changed in two principles:
  a) Minor changes: *aging, infection*, ...
  b) Major changes: more pathological conditions, such as
    • *cancer* = ↑ sialylation
    • *CDGs* = single step in glycan synthesis is disrupted
      all the regarding proteins are affected
    • ...
N-glycosylation pathway

>80 known CDG subtypes due to specificity of glycosylation enzymes and transporters involved
N-glycosylation pathway

1. Analysis of LLO – CDG I

2. Analysis of released glycoproteins – CDG

>80 known CDG subtypes due to specificity of glycosylation enzymes and transporters involved

Edited from Grunewald 2005
IEF can separate transferrin isoforms in the term of number of sialic acids, but **can not provide any information about structure of N-linked glycans**

→ Unable to determine a specific enzyme/transporter defect

→ Structural analysis is essential!
Analytical workflow
1. Analysis of LLO

SAMPLE (ANY sonified cells/tissue)
↓
Proteolytic digest
↓
Extraction of LLOs (CHCl₃/MeOH/H₂O)
↓
Reduction with NaBH₄
↓
Release of oligosaccharides from pyrophosphate (with HCl)
↓
Non-porous graphitised carbon column – elution with 40%ACN
2. Analysis of released Gps

SAMPLE (ANY serum/body liquids/isolated glycoprotein,..)

↓

Proteolytic digest

↓

Isolation of glycopeptides (anion exchange chromatography)

↓

Release of N-linked oligosaccharides from glycopeptides (PNGase F digest)

↓

Isolation of N-glycans (anion exchange chromatography)

↓

Non-porous graphitised carbon column – fractionation:
  • neutral fraction
  • charged/sialylated fraction

↓

Clean-up (C18), fluorescent labelling (2-PA)

LC-MS (HIAX + MALDI TOF/TOF)

Confirmation of linkages by
- $^1$H NMR
- specific ethyl esterification
- specific exoglycosidase digests

DB search
HIAX LC-MS of sialylated, PA-labeled serum N-glycans: „healthy“ vs. patient

Combination of HI + AX allows separation by charge, linkage, size and composition

Dionex IonPac As11

uV(x100,000)

Data1: HIAAser11_CDGIIx_ACNTFA_80_%_2.4.2015.Id Detactor A:Ex:320nm,Em:400nm
Data2: Zuzana_HIAX_healthySerum.Id Detactor A:Ex:320nm,Em:400nm

CDG IIx

CDG IIx

healthy

mono-sialo
di-sialo
tri-sialo
tetra-sialo

QUANTITY
HIAX LC-MS of sialylated, PA-labeled serum N-glycans: „healthy“ vs. patient

MAJOR changes in glycoprofile

Increased asialo and hybrid structures

Mono-sialo

Di-sialo

Completely absent tri- and tetra-sialylated structures

Although antennae are built correctly

Tri-sialo

Tetra-sialo

Healthy
HIAX LC- MS of sialylated, PA-labeled serum N-glycans: „healthy“ vs. patient

Increased asialo and hybrid structures

Mono-sialo

Di-sialo

Completely absent tri- and tetra-sialylated structures

Although antennae are built correctly

CMP-Sialic acid transporter?

→ mutation analysis
Each peak is analyzed by MS/MS

Combination of mass spectrometry and HPLC chromatography enables both
- quantification
- determination of every N-glycan structure
The number of known CDG subtypes is still increasing NOT because these disorders have not been existing so far, but because of still more sensitive analytical technologies available.
Conclusion

• The cooperation between research laboratories and hospitals is *essential* in evolution of further diagnostics.

• Completing the available information about glycoconjugate structures *can help to understand the molecular basis* of many still not completely understood diseases and thus can lead to development of new diagnostic methods in various pathological conditions.
Thank you for your attention

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The Foundation Glycosylation (FoG) founded by Duncan Webster (Canada), is the official sponsor of the videos of all oral session that will be given during the conference. This material will be available in the Youtube channel dedicated to “SSIEM Official Satellite Symposia – Second World Conference on Congenital Disorders of Glycosylation (CDG): a challenging story of sugar trees” at:

For more information about the work of this organization which is focused on research to ALG9 -CDG (CDG-1L), visit the following link: [http://www.thefog.ca/main.html](http://www.thefog.ca/main.html)

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