Synapse Glycosylation Drives CDG Neurological Outcomes: Insights from an Animal Model

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Sugars heavily decorate cell surfaces and extracellular spaces in the nervous system.
The Glycosylated Synaptomatrix

- **NMJ**: heavily glycosylated synaptomatrix.

- **Glycosylated synaptomatrix**: crucial role in synaptic organization, adhesion, signaling and transmission (Martin P, J Neurocytol 2003; Yamaguchi Y, Bioch Bioph Acta 2002).
CDG and neurological outcomes

- >100 Congenital Disorders of Glycosylation (CDG) disease states have been identified over the last 20 years and this list is rapidly growing.
- Characterized by severe neurological defects including movement defects, developmental delay and intellectual disability.
- Cellular and molecular mechanisms underlying these disorders are not well understood.
- Effective treatments are overall not available, imposing a huge financial burden on healthcare costs.

<table>
<thead>
<tr>
<th>Number of patients known</th>
<th>CDG-Ia</th>
<th>CDG-Itb</th>
<th>CDG-Ic</th>
<th>CDG-ID</th>
<th>CDG-Id</th>
<th>CDG-Ie</th>
<th>CDG-IIa</th>
<th>CDG-IIb</th>
<th>CDG-IIc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychomotor retardation</td>
<td>+→+++</td>
<td>−</td>
<td>+/++</td>
<td>+++</td>
<td>+++</td>
<td>−→+++</td>
<td>+++</td>
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<td>+++</td>
</tr>
<tr>
<td>Seizures</td>
<td>−→++</td>
<td>±</td>
<td>−→+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>−</td>
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<tr>
<td>Axial hypotonia</td>
<td>+++</td>
<td>±</td>
<td>++/+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Strabismus</td>
<td>+++</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Cerebellar hypoplasia</td>
<td>+++</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
</tr>
<tr>
<td>Dysmorphia <em>(fat pads, inverted nipples)</em></td>
<td>−→+++</td>
<td>−</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Liver disease</td>
<td>+</td>
<td>+++</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
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<td>Coagulopathy</td>
<td>+/++++</td>
<td>+/+++</td>
<td>+++</td>
<td>−</td>
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<tr>
<td>Protein-losing enteropathy</td>
<td>±</td>
<td>+++</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>microcephaly</td>
<td>microcephaly</td>
<td>stereotype behavior</td>
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<tr>
<td>Other</td>
<td>multorgan involvement</td>
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</tbody>
</table>
GLYCOSYLATION DEFECTS may account for neurodevelopmental complications in CG patients:

- locomotion problems and tremor,
- speech delay,
- learning disability.
Drosophila neuromuscular junction (NMJ) as model organism for CG

Drosophila wandering L3

Drosophila

sg2

sg3

BOUTON

Antibody

Cell surface

Dani and Broadie, 2012
**Drosophila melanogaster** resembles the CG patient phenotype

- Galactose metabolic pathway is conserved in fruit flies.

- GALT-null flies survive to adult in the absence of dietary galactose, but failed to do so following galactose exposure.

- Like patients, flies exhibit LOCOMOTOR IMPAIRMENTS independent of galactose exposure.

- **GALT** was identified as a glycan-related gene linked to a significant increase in bouton number at the NMJ (Dani et al., PLoS Genet 2012).
SUGAR COMPOSITION OF GLYCOSYLATED SYNAPTOMATRIX IN CG

Galactose-related glycosylation changes: terminal galactose and N-acetyl galactosamine.
Striking loss of terminal galactose and N-acetyl galactosamine at NMJ in CG

Jumbo-Lucioni et al., DMM 2014
The Glycosylated Synaptomatrix

- NMJ: heavily glycosylated synaptomatrix.
Loss of \textit{dGALT}: increased synaptic growth and structural overelaboration
IS THERE A BEHAVIORAL CONSEQUENCE ASSOCIATED TO THESE CHANGES IN THE SYNAPSE?
Impaired coordinated movement in CG *Drosophila* model rescued by human *GALT*
GALT is part of a highly interactive network of proteins: GALK as potential therapeutic target.
\textit{dGALK} co-removal corrects glycosylation losses in the \textit{Drosophila} CG model
**dGALK** co-removal corrects movement defects and NMJ structure

![Graph and images related to movement time and bouton number comparisons between control, dGALK, dGALT, and dGALK; dGALT conditions.](image-url)

*Jumbo-Lucioni et al., DMM 2014*
GALT is part of a highly interactive network of proteins: GALE as potential therapeutic target
dGALE co-removal worsens sugar loss in the *Drosophila* CG model
*dGALE* co-removal worsens movement defects and NMJ structure

**Bar graphs:**
- **Y-axis:** Movement Time
- **X-axis:** treatment groups (control, dGALT, dGALE, dGALT; dGALE)
- **Graphs:**
  - Y-axis: Bouton number
  - X-axis: treatment groups (control, dGALT, dGALE, dGALT; dGALE)

**Images:**
- Comparison of control and *dGALT* conditions for bouton number and HRP-DLG staining.
New model of CG neurodevelopmental disorder

galactose $\rightarrow$ gal 1-P

ATP ADP

UDP galNAC

UDP glucuronate

UDP UDP UDP UDP

glc 1-P

gal

glc

sgl CG10072

CG5268

CG12660
dGALT; dGALE

PRESYNAPTIC

EXTRACELLULAR MATRIX

POSTSYNAPTIC
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