GCS-H PROTEIN: DUAL ROLE

The GCS-H protein has a dual role in the mitochondrion (moonlighting function). First, the GCS-H protein has a central role in lipote synthesis. The post-translational translocation of the 2-ketoadipate dehydrogenases (2KDH) is essential in the bioenergetics metabolism of eukaryotes. The GCS-H protein, the lipoylated subunit of the glycin cleavage system, has been proposed as the only acceptor of the lipote before transferring to other 2KDH in mammals.

Second, the GCS-H protein represents that H-protein of the glycin cleavage enzyme system. Genetic defects of the glycin cleavage enzyme system cause nonketotic hyperglycinemia. Thus far, only pathogenic variants in GLDC and AMT have been reported, and (although long time suspected) this is the first report of variants in the GCS-H gene. Nonketotic Hyperglycinemia (NKH) is an autosomal-recessive disorder, with a neurological presentation and biochemically characterized by a large accumulation of glycin in body fluids including cerebrospinal fluid (CSF). Data from the impact of nucleotide variations in GCS-H on human health has been recently reported.

We present data on nucleotide changes identified in the GCS-H gene of six patients resulting from a worldwide collaborative study to decipher the GCS-H role in human health. We describe the phenotype, provide evidence for pathogenicity, and illustrate how it affects the dual role of the GCS-H protein.

**The Patients**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Outcome</th>
<th>Plasma GCS (µM)</th>
<th>CSF GCS (µM)</th>
<th>CSF GCS plasma GCS ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Severe</td>
<td>1.44</td>
<td>2.78</td>
<td>0.52</td>
</tr>
<tr>
<td>2</td>
<td>Severe</td>
<td>1.40</td>
<td>2.88</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>Severe</td>
<td>1.15</td>
<td>6.5</td>
<td>0.39</td>
</tr>
<tr>
<td>5</td>
<td>Severe</td>
<td>0.12</td>
<td>6.5</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>Severe</td>
<td>0.88</td>
<td>6.5</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Patients had normal CSF lactate and clinical presentations with a fatal outcome similar to severe classic NKH or to milder courses similar to attenuated classic NKH but with some bioenergetic features.

**FUNCTIONAL ASSAYS**

**In vivo studies in fibroblasts**

Immunohistochemical staining with antibodies for lipote (red) and 148P (green) shows a clear reduction in protein lipoylation in patient 5 fibroblasts.

**GCS-H knockdown COS7 cells**

We generated a GCS-H knockdown model in COS7 cells. GCS-H wild-type and mutant constructs were then transiently co-transfected with GCS-F wild-type protein, and the presence, size, and functional capacity of the protein were studied.

**GCS enzyme activity**

GCS enzyme activity was decreased in patient 5 fibroblasts compared to healthy controls.

**Biochemistry: pure protein studies**

Recombinant GCS-H activity

Pathogenic variants relative to concorrent wild-type showed decreased GCS activity of purified recombinant lipoylated GCS-H protein in vitro by the glycine exchange assay, including p.T148P. They showed reduced lipote transfer by L1P1 from H-protein to the L2 of PDH.

**Structural Biology**

Homology Modeling

Interaction of GLDC (blue) and GCH(F1ellow) variants. Location of H77 and T148. H77 interacts with D148 and E149 located in a region with many aromatic residues. F148 is predicted to bind H-bond with Tyrl64. In H77 the H-bond and the hydrophobic interactions would be lost.

**CONCLUSIONS**

- Our studies confirm the pathogenicity of the listed variants; the missense variants were shown to impair both the GCS and the lipote synthesis in COS7 and yeast. p.H57R and p.T148P showed decreased glycine exchange reaction in vitro and decreased GCS activity in a liver biopsy.
- The clinical picture resembles classic NKH but has some features of bioenergetic defects such as dystonia.
- Several patients show deficiency in lipoylated proteins illustrating the dual role of GCS-H.
- This study for the first time describes a range of patients with GCS-H pathogenic variants causing a variant form of NKH combining deficient GCS complex functioning resulting in NKH, and deficient lipoylation causing some bioenergetic defects due to alterations in 2KDH protein lipoylation. The severity of the clinical picture relates to the residual activity.

**GCS-encoding variants in GCS-H encoding the H-protein cause a variant form of nonketotic hyperglycinemia**