Reduced temperature and the chemical chaperone 4-phenylbutyrate enhance stability of 21-OHD mutations

Yiqing Chen, Kerstin Schaefers, Maria Gasteiger, Angela Taylor, Wiebke Arlt, Nils Krone, Soeren Gersting, Nicole Reisch

1 Department of Endocrinology, Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, München
2 Department of Molecular Pediatrics, Dr. von Hauner’sches Kinderspital, Klinikum der Universität München, München
3 School of Clinical & Experimental Medicine, Institute of Metabolism and Systems Research, University of Birmingham, UK
4 Academic Unit of Child Health, Department of Oncology and Metabolism, University of Sheffield, UK

Introduction
Congenital adrenal hyperplasia (CAH) is one of the most common autosomal recessive metabolic disorders characterized by a complex imbalance of adrenal steroids. The most common form, 21-hydroxylase deficiency (21-OHD) due to CYP21A2 mutations accounts for more than 90% of all cases of CAH and constitutes a life-threatening disease. The current therapeutic situation is unsatisfying and demands novel treatment approaches. In silico modelling suggests protein misfolding and intracellular retention to play a significant role in the pathogenesis of CAH. The study aimed to investigate protein misfolding due to CYP21A2 mutations and explore chemical chaperones as potential therapeutic tools for CYP21A2 mutants.

Methods and materials
Clinically relevant CYP21A2 mutations where in silico modelling suggested protein misfolding to play a role in the pathogenesis of CAH were selected and subcloned into pcDNA6-V5/His expressing vectors. Residual activity of variant CYP21A2 proteins was determined in living cells using an enzyme activity assay with LC/MSMS based analysis of steroids. The effect of mutations on protein half-life (susceptibility to proteinase K) was measured comparing wild-type and variant CYP21A2. The influence of 4-phenylbutyrate (4-PBA) and reduced temperature on protein half-life was investigated.

Results

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Mean (min)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>8.35</td>
<td>0.62</td>
</tr>
<tr>
<td>P30L</td>
<td>1.40</td>
<td>0.04</td>
</tr>
<tr>
<td>P30Q</td>
<td>1.00</td>
<td>0.07</td>
</tr>
<tr>
<td>G90V</td>
<td>0.95</td>
<td>0.17</td>
</tr>
<tr>
<td>R483Q</td>
<td>2.37</td>
<td>0.05</td>
</tr>
<tr>
<td>R483W</td>
<td>0.93</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table 1. Half-life of wild-type (WT) and variants CYP21A2 at 37°C. Degradation of the WT and mutant proteins were probed by western-blotting analyses. Densiometric analysis was performed using ImageQuant software. The calculated half-life of WT and mutations are given in mean ± SEM of n=3 independent experiments.

Compared with WT, all mutations showed reduced half-life, indicating that CYP21A2 mutations caused protein instability.

Figure 1. A. Representative Western blot images of WT and G90V against limited proteolysis by Proteinase K at 37°C. B. Degradation curve of WT and G90V according to densitometric analysis of each time point. The value of each time point is given in mean ±SEM of n=3 independent experiments.

WT showed a longer half-life because of the lowest rate of degradation. In contrast, G90V had a rapid decay rate compared with WT so that the half-life was much shorter.

Figure 2. Half-life of WT and mutant proteins at 37°C and 30°C. Half-life determinations were from three independent experiments at 37°C and 30°C. The bar graph represents mean ±SEM.

The half-life of WT at 37°C and 30°C did not show any difference, while 30°C enhanced half-life of all tested mutants.

Conclusion
Our preliminary data substantiate the hypothesis of protein misfolding with loss-of-function as a relevant molecular mechanism in CAH that can be addressed by structural stabilization of CYP21A2. These strategies above may provide new avenues in 21-OHD treatment.

References