Understanding individual mutations underlying Niemann-Pick disease type C using CRISPR/Cas9-mediated base editing and haploid human cell models

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Mutations in NPC1 underlie the lysosomal storage disorder Niemann-Pick disease type C

Niemann-Pick Disease Type C
- Autoimmune recessive disorder caused by mutations in the NPC1 gene
- Progressive liver disease and exocrine pancreatic insufficiency
- Symptoms result from a lysosomal accumulation of cholesterol and other lipids
- More than 200 disease-causing mutations have been identified to date and patients with NPC often present as compound heterozygotes with at least one private mutation

Figure 1. Filipin staining of NPC patient-derived fibroblasts. Patient-derived fibroblasts have a high abundance of unesterified cholesterol accumulated intracellularly - a clinical hallmark of NPC. Healthy age-matched controls show a relative absence of staining.

Figure 2. Most NPC1 mutations are novel or rare. The mutational spectrum of NPC1 is characterized by many novel or rare mutations. Each point represents one mutation – the size and colour of each point corresponds to the number of occurrences of each mutation. Data retrieved from gnomAD and encompasses all documented coding variants.

Figure 3. The majority of NPC1 mutations documented on ClinVar are variants of uncertain significance. The relative proportion of each clinical variant interpretation of NPC1 is illustrated.

NPC1 null HAP1 cells are a viable model of the NPC cellular phenotype

CRISPR/Cas9-mediated base editing allows for targeted single nucleotide substitutions

Table 1. Efficiency of CRISPR/Cas9-mediated base editing

<table>
<thead>
<tr>
<th>Variant</th>
<th>Base editor</th>
<th>Positive clones/total clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPC1 p.D946N</td>
<td>Cytosine</td>
<td>3/7</td>
</tr>
<tr>
<td>NPC1 p.R1077X</td>
<td>Cytosine</td>
<td>4/19</td>
</tr>
<tr>
<td>NPC1 p.D1007T</td>
<td>Cytosine</td>
<td>3/15</td>
</tr>
<tr>
<td>NPC1 c.3591+2G&gt;T</td>
<td>Adenine</td>
<td>3/14</td>
</tr>
</tbody>
</table>

CRISPR/Cas9-mediated single base editing generates nucleotide substitutions.

Haploid cell models of NPC allow for individual variant characterization

Haploid cell models of NPC allow for the clinical interpretation of individual mutations

Figure 4. Lack of functional NPC1 induces intracellular accumulation of unesterified cholesterol in HAP1 cells. (A) Knockout of NPC1 expression was confirmed by Western Blot. (B) Filipin staining of NPC1 knock out HAP1 clones reveals discrete foci of intracellular accumulations of unesterified cholesterol. Scale bars: 6.3 µm.

Figure 5. CRISPR/Cas9-mediated single base editing generates nucleotide substitutions.

Figure 6. Example of positive sequencing chromatograph following CRISPR/Cas9-mediated base editing. For each NPC1 variant modified, at least three independent clones were isolated. Positive nucleotide substitution was confirmed via Sanger Sequencing.

Figure 7. Unique disease mechanisms underlie individual NPC1 mutations. (A) The expression of NPC1 mRNA is unaltered compared to wildtype in all modeled variants, except NPC1 c.3591+2G>T where there is a significant reduction in NPC1 mRNA expression. (B) Western blot analysis shows a reduction in NPC1 protein expression in all modeled variants compared to wildtype.

Figure 8. Staining for cholesterol accumulation can be used to clinically interpret novel NPC1 variants. Each NPC1 variant modeled displayed a distinct intracellular accumulation of free cholesterol, visualized using filipin staining. This phenotype is a biochemical hallmark of NPC. Each variant modeled can thus be designated as pathogenic, showing the utility of this system in clinical variant interpretation.

Figure 9. The majority of NPC1 mutations can be modeled using CRISPR/Cas9-mediated base editing.

The majority of NPC1 mutations are nucleotide transitions. CRISPR/Cas9-mediated base editing allows for the efficient introduction of nucleotide transition mutations. In Niemann-Pick disease type C, the majority of mutations are single point nucleotide transitions. Accordingly, CRISPR/Cas9-mediated base editing can be utilized to investigate the pathogenicity and specific disease mechanisms of the majority of NPC1 variants.