Testing variants of uncertain significance in a HEK293T model for very long-chain acyl-CoA dehydrogenase deficiency

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Background
- Newborn screening (NBS) identifies inborn errors of metabolism. Upon identification of a positive NBS screen, DNA sequencing is used to confirm the diagnosis. [1]
- Sequencing often identifies variants of uncertain significance (VUS). Patients have different combinations of mutations, resulting in different phenotypic severity and requiring different therapeutic strategies.
- Patient fibroblast samples can be used to perform functional testing, but this is expensive, inefficient, and invasive.
- Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is an autosomal recessive disease resulting from mutations in the ACADVL gene. Patients with VLCADD cannot utilize energy from long fatty acid chains and present with a variety of symptoms, including hypoglycemia, cardiomyopathy, and recurrent rhabdomyolysis. [2]
- VLCADD has over 300 VUS. We characterized a HEK293T ACADVL knockout model for the purpose of efficient functional testing for VUS in VLCADD.

Methods
- CRISPR/Cas9 genome editing was used to ablate the ACADVL gene in HEK293T cells with dual guide RNAs targeting exons 12-16, including the catalytic site in exon 15. Cells were flow-sorted into single wells and grown until confluent in complete DMEM.
- Genomic DNA was extracted from clones. Droplet digital PCR (ddPCR) was used to screen candidate genome-edited clones and confirm the deletion of the catalytic site.
- mRNA was isolated from A1-7 clones transfected with variant plasmids and converted to cDNA. RT-PCR was performed to determine mRNA presence.
- Western blot was used to probe for the presence of the VLCAD protein.
- Electron transfer flavoprotein (ETF) fluorescence reduction enzymatic assay to determine residual VLCAD protein enzymatic activity.

Results
Figure 1: ddPCR identified four candidate genome-edited clones with deletion of the catalytic site in exon 15.

Figure 2: Western blot of genome-edited clones confirms absence of VLCAD protein.

Table 1: Plasmids used to test variants of uncertain significance in ACADVL knockout model

<table>
<thead>
<tr>
<th>Plasmid Name</th>
<th>Mutation</th>
<th>Cell Line Origin</th>
<th>ClinVar Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACADVL-1619</td>
<td>c.1619T&gt;C, p.Leu540Pro</td>
<td>FB671</td>
<td>Uncertain significance (not reported)</td>
</tr>
<tr>
<td>ACADVL-1707</td>
<td>c.1707_1715dup, p.Asp570_Ala572dup</td>
<td>FB671</td>
<td>Uncertain significance</td>
</tr>
<tr>
<td>ACADVL-848</td>
<td>c.848T&gt;C, p.Val283Ala</td>
<td>FB782</td>
<td>Pathogenic (common mutation)</td>
</tr>
<tr>
<td>ACADVL-1248</td>
<td>c.1248A&gt;C, p.Ile420Leu</td>
<td>FB782</td>
<td>Uncertain significance (not reported)</td>
</tr>
<tr>
<td>ACADVL-1217</td>
<td>c.1217A&gt;C, p.Gln406Pro</td>
<td>FB904</td>
<td>Uncertain significance</td>
</tr>
</tbody>
</table>

Discussion
- We generated an ACADVL null HEK293T that has no residual VLCAD protein and reduced enzyme activity towards C21 and C16 substrates.
- Transfection of plasmids with variant ACADVL allows us to perform functional studies to examine variant pathogenicity without the need for invasive skin biopsies.

References