Introduction

- **Epileptic encephalopathy (EE):** a classification of epilepsy characterized by severe seizures with onset during infancy or childhood with developmental delay and regression.
- Many cases stem from *de novo* mutations: mutations present only in the child, not the mother or father.
- Currently *intractable* to antiepileptic drugs.
- Through **targeted next-generation sequencing**, a cohort clinically diagnosed with EE, was screened for mutations within genes known to be causative of EE.
- **Sanger sequencing** was used to:
  - Determine if the mutation was *de novo* and therefore likely to be pathogenic.
  - Ensure the mutation is not a false-positive.

Methods

- **Primer Design**
- **PCR**
- **Gel Electrophoresis**
- **Sanger Sequencing**
- **Sequence Analysis**

Results

- **WDR45 c.C611T**
- **Missense Mutation**
  - Single nucleotide change results in a codon for a different amino acid.
  - Sanger Sequencing validated: GTC GTG
  - Acquired Sequence: CCG

Mutation Validations

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mutation</th>
<th>3' 5' Coordinates</th>
<th>Gene</th>
<th>Mutation</th>
<th>Amino Acids</th>
<th>Validation</th>
<th>Inheritance</th>
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</thead>
<tbody>
<tr>
<td>e0776-5</td>
<td>c.A1141G</td>
<td>2:156170236</td>
<td>SDNA</td>
<td>missense</td>
<td>MET&gt;VAL</td>
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<td>e0507-1</td>
<td>c.G4068G</td>
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</table>

Conclusion

- From the total of 14 variants identified:
  - **Validated:** 3
  - **De novo:** 1
  - **False Positives:** 4
  - **Require Further Investigation:** 7

**Results confirm that validation by Sanger sequencing is an important step to ensure that the mutation identified by next-generation sequencing is truly present.**

**WDR45 c.C611T** is now considered a solved mutation. The other validated mutations remain unknown because inheritance is indefinite.

**The data obtained will be used to enhance current knowledge of EE and to further the development of potential treatment.**

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References