Molecular Characterization of a Novel Non-Stop KCNJ11 Mutation Associated with a Dual Focal and Diffuse Hyperinsulinaemic Hypoglycaemia Phenotype

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BACKGROUND

- Hyperinsulinaemic hypoglycaemia (HH) results from unregulated insulin secretion from pancreatic β-cells.
- There are 2 main histological subtypes: diffuse (60-70 %) and focal (30-40 %).
- Diffuse HH is most commonly due to recessive or dominant ABCC8/KCNJ11 mutations.
- Focal HH results due to somatic loss of the maternal 11p allele involving the ABCC8 and KCNJ11 region in patients with paternally inherited ABCC8 or KCNJ11 mutation.

OBJECTIVE

To molecular characterize a novel non-stop KCNJ11 mutations associated with a unique dual clinical phenotype of focal and diffuse HH.

PATIENT

- The proband, born to non-consanguineous Caucasian parents at 40 weeks’ gestation with a birth weight of 4.6 kg, presented with hypoglycaemic convulsion at 9 months of age.
- She required high intravenous glucose infusion (10 mg/kg/min) to maintain euglycaemia.

<table>
<thead>
<tr>
<th>Hypoglycaemia Screen</th>
<th>Plasma Glucose (mmol/l)</th>
<th>Serum Insulin (mU/l)</th>
<th>Non-esterified fatty acids (mmol/l)</th>
<th>β-hydroxybutyrate (mmol/l)</th>
<th>C-peptide (pmol/l)</th>
<th>Serum Cortisol (nmol/l)</th>
<th>Acylecarnitine profile</th>
<th>Serum ammonia (μmol/l)</th>
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<tbody>
<tr>
<td></td>
<td>2.5</td>
<td>53</td>
<td>0.4</td>
<td>&lt;0.1</td>
<td>459</td>
<td>628</td>
<td>N</td>
<td>37</td>
</tr>
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- Initial medical treatment with diazoxide was unsuccessful.
- Molecular genetic analysis identified a paternally inherited heterozygous KCNJ11 nonstop mutation (c.1171T>C).
- 18F DOPA-PET CT scan identified a focal lesion in the tail of the pancreas, which was laparoscopically resected.
- Histological and microsatellite marker analysis on the resected pancreatic tissue confirmed the diagnosis of focal disease.
- Post-surgery, persistence of HH resulted in a repeat 18F DOPA-PET CT scan, which suggested diffuse disease.
- A partial pancreatectomy around the site of focal lesion showed diffuse disease on histology with no resolution of HH. However, HH was responsive to diazoxide.

METHODS

- Site-directed mutagenesis was used to create the KCNJ11 non-stop point mutation in pcDNA3.1-human Kir6.2 cDNA construct.
- HEK293 cells were transfected with WT hamster SUR1 cDNA and WT/mutant human Kir6.2 cDNA using FuGENE.
- Functional properties of channels were studied using whole-cell patch-clamp recordings.
- After attaining whole-cell configuration, cells were voltage-clamped.
- The voltage-clamp protocol consisted of a holding potential of -80 mV, after which the cells were ramped from -150 mV to 50 mV over 1 second (200mV/s) and then stepped back to -80 mV.
- Cells were superfused with 5 K+ bath solution (CNT), followed by 100 μM DZX to activate KATP currents, and 100 μM DZX and 100 μM Tolbutamide (DZX+TOL) to inhibit KATP currents.
- Both homogenous and heterozygous expressions of the mutants were studied.

RESULTS

<table>
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<tr>
<th>Homozygous <em>391Rex5</em>94 Kir6.2 Mutant KATP Channels (n = 6)</th>
<th>Heterozygous <em>391Rex5</em>94 Kir6.2 Mutant KATP Channels (n = 12)</th>
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<tr>
<td><img src="image1" alt="" /></td>
<td><img src="image2" alt="" /></td>
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A, C: Graph showing mean pA/pF at ±40 mV. Data was analyzed using Wilcoxon matched-pairs signed rank test. B, D: Representative trace from whole-cell patch-clamp recordings for *391Rex5*94 Kir6.2 Mutant. E: Graph showing KATP current at ±40 mV from WT, homozygous and heterozygous mutant channels. Data is presented as Mean ± SEM, and was analyzed using Mann-Whitney test, **p = 0.03, n = 6-10 cells.

CONCLUSIONS

- This study describes the first reported dual focal and diffuse HH phenotype with KCNJ11 mutations.
- Molecular characterization supports the observed clinical phenotype.

The authors have nothing to disclose.