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 are 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.

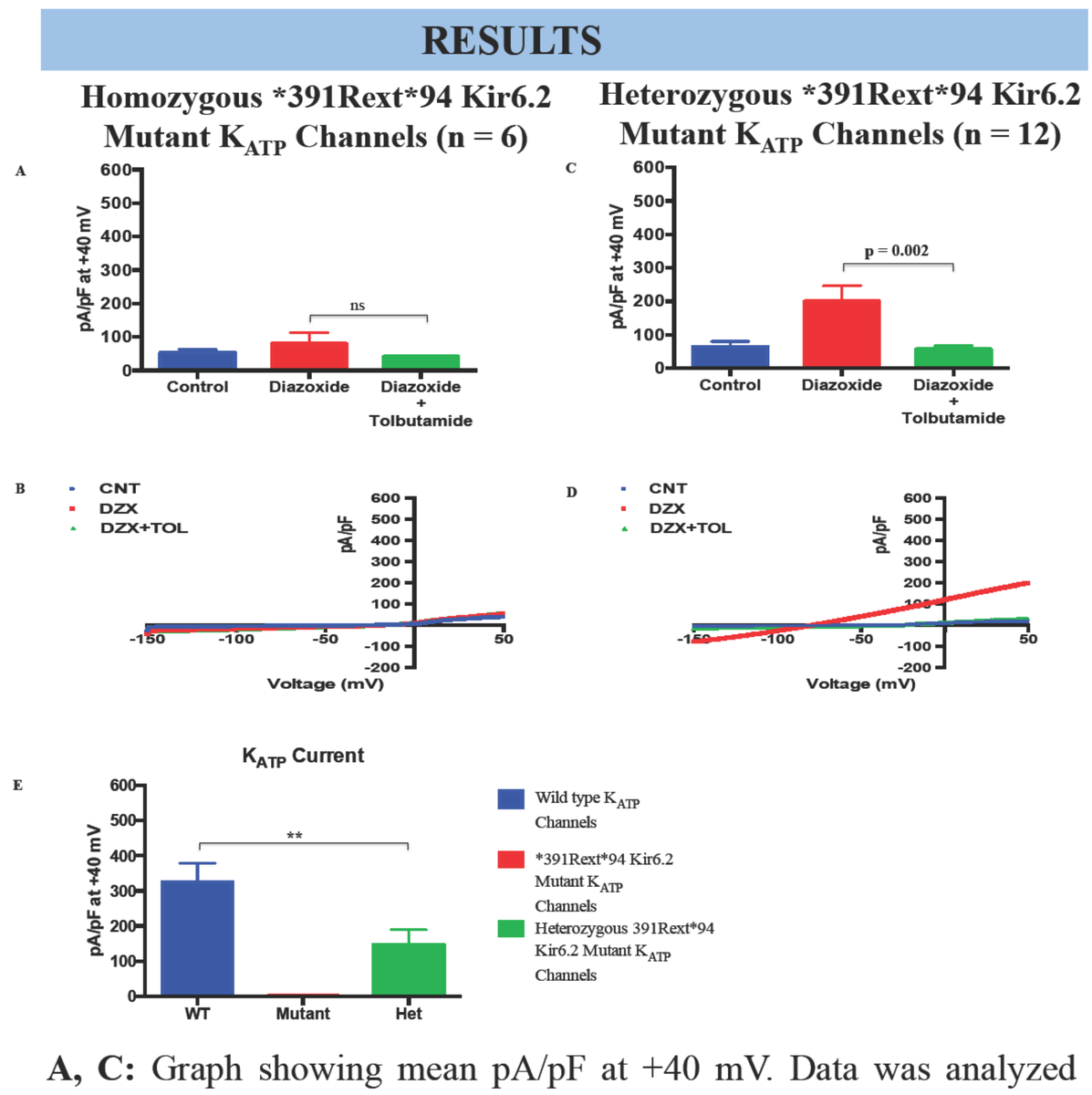
Hypoglycaemia Screen			
Plasma Glucose (mmol/l)	2.5	Serum Insulin (mU/l)	53
Non-esterified fatty acids (mmol/l)	0.4	β-hydroxybutyrate (mmol/l)	<0.1
C-peptide (pmol/l)	459	Serum Cortisol (nmol/l)	628
Acylcarnitine profile	N	Serum ammonia (µmol/l)	37

- Initial medical treatment with diazoxide was unsuccessful.
- Molecular genetic analysis identified a paternally inherited heterozygous *KCNJ11* nonstop mutation (c.1171T>C).
- ^{18}F 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 ^{18}F 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 K_{ATP} currents, and 100 µM DZX and 100 µM Tolbutamide (DZX+TOL) to inhibit K_{ATP} currents.
- Both homogenous and heterozygous expressions of the mutants were studied.

RESULTS



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 *391Rext*94 Kir6.2 Mutant. **E:** Graph showing K_{ATP} 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

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