Case Report

Permanent neonatal diabetes due to a novel insulin signal peptide mutation


We report a rare case of permanent neonatal diabetes (PND) due to insulin (INS) gene mutation in a 51-month-old girl who presented with hyperglycemia in the neonatal period. Mutational analysis of KCNJ11 and INS was performed and this detected a novel heterozygous c.38T>G (p.Leu13Arg) INS de novo mutation. The non-conservative change substitutes the highly conserved L13 residue within the hydrophobic core region of the proinsulin signal peptide. Given the frequent tendency of heterozygous INS mutations to exhibit dominant negative disease pathogenesis, it is likely that the mutant proinsulin perturbed the non-mutant counterpart progression and processing within the β-cells, and this resulted to a permanent form of congenital diabetes.

Suhaimi Hussaina,†, Johari Mohd Allib,†, Muhammad Yazid Jalaludinc and Fatimah Harunc

aDepartment of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia; bDepartment of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; and cDepartment of Pediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
†These authors contributed equally.

Key words: congenital absence of insulin-producing β-cells with diabetes mellitus – insulin – mutation – proinsulin – signal peptide

Corresponding author:
Dr Suhaimi Hussain, Department of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, 16150 Kelantan, Malaysia.
Tel: +60 9 767 6947
Fax: +60 9 765 9057
e-mail: grinfin06@yahoo.com; hsuhami@kb.usm.my

Submitted 29 May 2012. Accepted for publication 2 November 2012

Neonatal diabetes (ND) is a form of monogenic diabetes that is usually defined as overt diabetes diagnosed during the first 6 months of life. A recent study quoted its minimal incidence as 1 in 90,000 (1). ND cases include transient (TND) and permanent (PND) forms of diabetes, which display differing insulin dependency and molecular mechanism of disease pathogenesis (2, 3). Mutations in KCNJ11, ABCC8, and INS are among the major causes of PND (4, 5). KCNJ11 and ABCC8 encode the subunits of the ATP sensitive potassium channel (KATP) of the pancreatic β-cells and activating mutations of these subunits could impair insulin secretions (6, 7). The insulin gene (INS) mutations are associated with PND and a spectrum of other clinical conditions such as type 1b diabetes, MODY, early onset type 2 diabetes, and TND (5, 8). PND cases could also arise from glucokinase (GCK) and insulin promoter factor-1 (IPF-1) mutations (9, 10). Syndromic cases associated with PND include rare mutations in PTF1A, FOXP3, GLIS3, EIF2AK3, NEUROD1, RFX6, NEUROG3, GATA6, and SLC19A2 (11, 12).
Hussain et al.

Case report

The proband is currently 51 months. She is the youngest of four siblings and there was no family history of diabetes. She was born at 36 wk via spontaneous vertex delivery with a birth weight of 1.7 kg (<third percentile), length 44 cm (<third percentile), and head circumference 32 cm (<third percentile). Her Apgar score was good, 9 at 1 min and 10 at 5 min. She was admitted to the neonatal ward in view of her low birth weight. From day 2 of life, she was noted to have persistently high blood sugar ranging from 15.0 to 30.0 mmol/L without ketonuria. There was no fever and her septic parameters were negative. Glutamic acid decarboxylase (GAD-65) antibody was negative and C-peptide was <165 pmol/L (normal range: 297.9–1324). Insulin was given by intravenous continuous infusion at 0.1 U/kg/h. The patient continued to have insulin dependency, but also had multiple episodes of hypoglycemia during intravenous insulin infusion. From day 10 of life, subcutaneous insulin was started to replace intravenous insulin infusion. Achieving a good control of blood sugar was difficult as she was born small for the gestational age and at the same time she was very sensitive to insulin. To get a good match between her insulin and dietary intake, enteral feeding was optimized up to 200 mL/kg/d (130 kcal/kg/d). This regime helped to prevent hypoglycemia. The dose and frequency of insulin was gradually increased to match her requirement. She was discharged on day 33 of life with insulin at a total daily dose of 0.6 U/kg/d. She was regularly followed up at the Pediatric clinic but it was difficult to have good glycemic control. Her hemoglobin A1c (HbA1c) ranged between 7.4 and 14.3%. The diabetic control deteriorated from the age of 2–3 yr with HbA1c reaching as high as 14.3% (Fig. 1). Insulin was then increased to 0.8 U/kg/d, with insulin aspart added in the insulin mixture. With that, she had a variable blood sugar pattern, with rapid drop of blood sugar, especially when insulin aspart is used. Her mother refused to include or use insulin aspart, as the child was very sensitive to it even at a small dosage of 0.5 U/kg/d.

Mutational analysis of KCNJ11 and INS

Peripheral blood lymphocyte DNA was extracted using standard methods. Primers for all coding exons of KCNJ11 and INS were designed to include ∼50–100 bp of intron–exon junctions. Purified polymerase chain reaction (PCR) products were directly sequenced. No sequence change was detected in KCNJ11 but a heterozygous p.L13R INS mutation was found (Fig. 2A), that resides within the hydrophobic core region (HCR) of proinsulin (PI) signal peptide (SP). Direct DNA sequencing did not detect this mutation in the parental DNA. The mutation created a novel BstUI site.

A 1476-bp fragment was generated using the primer pair GATTCCAGGGTGGCTGGAC and CCTGGCCGGCGTTGGCACC, which flanked the mutation site and was then subjected to BstUI digestion (Fig 2C, D). The variant was not detected in dbSNP (Build 137) and 162 control chromosomes derived from apparently healthy volunteers. The parent–proband biological relationship was positively confirmed (probability >99.99%) and this was deduced through DNA profiling using the following 15 STR loci: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, D7S820, TH01, D13S317, D16S539, D2S1338, D13S317, D16S539, D19S433, vWA, TPOX, D18S51, D5S818, and FGA.

Discussion

PND due to INS mutations was first reported in 2007 (4). Most cases are due to de novo mutations,
Fig. 2. Analysis of INS p.L13R mutation. (A) Electropherogram for c.38T>G (p.L13R) heterozygous INS mutation (top) and the normal DNA sequence (bottom) for comparison. Boxed numbers indicate codon positions. (B) N-terminal Signal Peptide-alignment from representative chordates (mammals, bird, reptile, amphibian, and fish). Boxes indicate the archetypal N–H–C regions of an SP as described by von Heijne (20), where the hydrophobic core region (HCR) contains a minimum of seven hydrophobic residues interrupted by no more than one residue of either Gly, Pro, Ser, or Thr. The conserved L13 residue is highlighted. (C) BstUI map of 1476 bp PCR fragment amplified using flanking primers (arrows). The normal allele has only one BstUI site, whereas the p.L13R mutation created a novel BstUI site in exon 2. (D) PCR-RFLP analysis: the patient (PT) shows 922+196 bp BstUI RFLP associated with p.L13R mutation. M/F, maternal/paternal DNA; N1–N5, normal DNA; U, undigested control DNA. (E) Kyte–Doolittle plot (27) shows a significant drop in HCR hydrophobicity score (dashed line) upon p.L13R substitution.

although some show autosomal dominant inheritance. Heterozygous INS mutations are quite common as have been reported by various groups (8), and they can be dominantly acting and cause among others non-mutant PI misfolding, endoplasmic reticulum (ER)-retention/stress, abnormal insulin secretion, and β-cell apoptosis (13–15).

The proband’s undetectable C-peptide, hyperglycemia, and growth retardation features which occurred very early in life suggested severe insulin deficiency, which have already begun in utero. The absence of GAD and ICA antibodies suggested a non-autoimmune disorder. The proband has thus far showed an appropriate developmental age and has caught up in her physical growth, without evidence of neuropsychological or neuromotor dysfunctions, features that have been observed in some PND cases with K_ATP channel mutations (16).

Our proband represents the first PND case with heterozygous INS mutation that occurred within the HCR of PI SP and resulted in an early onset of diabetes. Other SP-residing INS pathogenic mutations include heterozygous p.R6C/H and p.A24D (4, 5, 14), but these mutations seem to show lesser disease severity compared to p.L13R. The p.R6C/H carriers had milder clinical course, normal birth weight, and a later age of diabetes onset, consequently them being classified as MODY cases. The p.R6H substitution is a conservative change and only caused mild ER-stress without significant disruption of the mutant PI release in HEK293 cells, while functional analysis using MIN6 β-cells indicated p.R6C/H substitution did not impair mutant-PI vesicular targeting (14). Patients with p.A24D mutation showed variable disease presentation and a later age-at-diagnosis ranging from 4 wk to 7 yr (5). Functional assay using MIN6 β-cells indicated p.A24D mutant is retained in the ER and causes ER-stress, but it did not significantly affect wild type insulin secretion (15).

The p.L13R substitution is likely to compromise insulin SP functions. SignalP 4.0 analysis indicated the authentic cleavage site score for p.L13R is reduced from ~0.9 to ~0.5 (17) (Fig. 3). Such indication seems to agree that HCR modifications can affect signal peptidase cleavage activity as previously reported (18). PolyPhen-2 and SIFT analysis (19) predicted p.L13R mutation as likely to be pathogenic. Such mutation is a non-conservative change, substituting a neutral-hydrophobic residue into a polar or charged-hydrophilic amino acid. This introduced an
abnormality as polar and charged amino acids are virtually absent in the HCR (20). An introduction of a charged residue in the HCR is functionally disruptive and not tolerated (21). An intact HCR is crucial for the SP interaction with signal recognition particle and the ER-membrane, so that the co-translational translocation process of insulin synthesis could occur correctly (22). Other reports have shown that similar HCR disruptions were functionally detrimental. The same missense substitution (L→R) in the HCR was able to block the peripheral export of the affected secretory proteins in the prokaryotic system (23, 24). A heterozygous p.L25R COL5A1 mutation within the HCR resulted to the classic Ehlers-Danlos syndrome (25). A homozygous substitution p.L15R in the bilirubin UDP-glucurononitrangerase (B-UGT) HCR causes the development of type II Crigler-Najjar disease (26). The mutant B-UGT translation and processing were markedly impaired in the presence of the microsomal fraction, indicating a detrimental interaction of the mutant B-UGT with the ER-machinery. The mutation may have impaired the enzyme ER-translocation, causing defective processing and its eventual degradation. Although B-UGT is membrane bound and insulin is instead a secretory protein, their common ER-targeting and processing requirement may be similarly impacted by the similar HCR disruption, albeit a dominant acting disease pathogenesis is expected for INS p.L13R mutation. This is based on the typical behavior of numerous heterozygous INS mutations reported to date (8, 13, 16). Such scenario is conceivable as our proband had an undetectable C-peptide, which strongly suggests that, the non-mutant PI processing or secretion is impaired through the usual dominant negative disease mechanism.

The case presented here suggests that a heterozygous INS mutation which disrupts the HCR can cause severe insulin deficiency with very early onset of diabetes and in utero growth retardation. A detailed functional analysis is warranted, as it could shed more insights on how such HCR disruption negatively affects insulin trafficking, maturation, and secretion in β-cells and eventually leading to a severe disease development. The identification of specific genetic cause contributing to PND guides us in the treatment decision, as patients with INS mutations may experience severe β-cells dysfunction that requires supplemental insulin for the rest of their life.

Acknowledgement
This study is partially funded by University of Malaya Research Grant RG197/10HTM.

References
5. Edghill EL, Flanagan SE, Patch AM et al. Insulin mutation screening in 1,044 patients with diabetes:


