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CASE REPORT

Severe insulin resistance in disguise: A familial case of reactive hypoglycemia associated with a novel heterozygous *INSR* mutation

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Aim: Hypoglycemia in childhood is very rare and can be caused by genetic mutations or insulin-secreting neoplasms. Postprandial hypoglycemia has previously been associated with insulin receptor (*INSR*) gene mutations. We aimed to identify the cause of postprandial hypoglycemia in a 10-year-old boy.

Subjects: We studied the symptomatic proband and his apparently asymptomatic mother and elder brother. All of them were lean.

Methods: Metabolic screening of the proband included a 5-hour oral glucose tolerance test (OGTT), angio-magnetic resonance imaging, and ^{18}F-dihydroxyphenylalanine positron emission tomography/computed tomography imaging of the pancreas. *INSR* gene sequencing and in vitro functional studies of a novel *INSR* mutation were also undertaken.

Results: Fasting hyperinsulinemia was detected during metabolic screening, and 5-hour OGTT showed hypoglycemia at 240′ in the proband, his mother, and brother. Pancreatic imaging showed no evidence of neoplasia. Acanthosis nigricans with high fasting insulin levels in the proband suggested severe insulin resistance and prompted *INSR* gene sequencing, which revealed the novel, heterozygous p.Phe1213Leu mutation in the patient and his family members. In vitro studies showed that this mutation severely impairs insulin receptor function by abolishing tyrosine kinase activity and downstream insulin signaling.

Conclusions: The identification of etiological cause of hypoglycemia in childhood may be challenging. The combination of fasting hyperinsulinemia with acanthosis nigricans in a lean subject with hypoglycemia suggests severe insulin resistance and warrants *INSR* gene screening.

KEYWORDS

hypoglycemia, insulin receptor, insulin resistance, mutation

The authors Stefania Innaurato and Gemma V Brierley contributed equally to this study.

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1 | INTRODUCTION

Hyperinsulinemic hypoglycemia (HH) is a rare condition that has multiple causes in adults, including pancreatic beta-cell tumors and circulating insulin or insulin receptor autoantibodies.[1] In neonates, however, HH is more common and generally has a genetic etiology, being associated with mutations in genes involved in the regulation of insulin secretion, such as *ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *SLC16A1*, *HNF4A*, and *HNF1A*.[2] Exercise-induced HH can be caused by mutations in the promoter of monocarboxylate transporter 1 (*MCT1*).[3] Differential diagnosis of HH with onset in childhood or adolescence may be more challenging due to the presence of either genetic[4,5] or tumoral[6–8] etiologies. Dominant-negative, heterozygous mutations in the tyrosine-kinase domain of the insulin receptor gene (*INSR*), conversely, usually cause extreme hyperinsulinemia with either normal or increased blood glucose concentrations. However, scattered reports attest that *INSR* mutations may sometimes also cause familial hyperinsulinemia with postload hypoglycemia.[9,10]

In this report, we describe a lean child who presented with postload, reactive hypoglycemia as the only symptom. However, acanthosis nigricans, along with high levels of fasting insulin with normal plasma glucose, suggested severe insulin resistance. A novel *INSR* mutation (F1213L) was identified in the proband and his mother and brother, who also showed reactive hypoglycemia and prolonged (5 hours) oral glucose.

2 | METHODS

Parental informed consent was obtained for all medical procedures. Clinical studies were performed in accordance with the Declaration of Helsinki.

2.1 | Metabolic investigations

The proband underwent routine investigation for HH, including determination of plasma concentrations of amino acids and urinary organic acids, free fatty acids, beta-hydroxybutyric acid, and carnitine with standard methods. Cortisol, adrenocorticotropic hormone (ACTH) and growth hormone (GH) levels levels, and fasting insulin were also assessed using standard clinical laboratory methods. A 5-h oral glucose tolerance test (OGTT) with assay of glucose, insulin, and C-peptide was performed for the proband and his first-degree relatives with a history of symptoms of postprandial hypoglycemia.

2.2 | Pancreatic imaging

Angio-magnetic resonance imaging (angio-MRI) and ^{18}F-dihydroxyphenylalanine (^{18}F-DOPA) positron emission tomography/computed tomography (PET/CT; GE Healthcare 2.1753) were performed in the proband according to standard protocols.

2.3 | Genetic screening

DNA sequencing of *INSR* gene was performed as described previously.[11]

2.4 | Assessment of INSR expression and signaling

Site-directed mutagenesis was used to introduce the L1213 mutation into the C-terminal myc-tag wild-type INSR expression vector pCDNA5/FRT/TO/hINSR. Chinese hamster ovary (CHO) Flp-In cells (Invitrogen) were transfected with either pCDNA5/FRT/TO/hINSR or pCDNA5/FRT/TO/hINSR-L1213 and pOG44 using Lipofectamine 2000 (Invitrogen). The polyclonal populations surviving Hygromycin B selection were used for experiments. To assess insulin receptor expression, CHO Flp-In cells stably overexpressing either wild type or F1213L mutant human INSR were lysed, and 25 μg of total protein was resolved by SDS-PAGE before transfer to nitrocellulose and Western blotting for INSR β-subunit (Santa Cruz Biotech: sc-711) and β-actin (Cell Signaling Technology: 4970). Cell-surface expression of the insulin receptor was determined by flow cytometry using anti-INSR α-subunit antibody 83-14 (a gift from Professor Ken Siddle, University of Cambridge), with negative isotype Immunoglobulin G (IgG; Sigma)-labeled cells as a negative control. Finally, to evaluate insulin-induced receptor autophosphorylation CHO Flp-In cells overexpressing either wild-type or F1213L, human INSR were serum-starved overnight prior to stimulation with increasing amounts of insulin for 10 minutes. Cells were lysed, and myc-tagged INSR were immunocaptured onto 96-well plates with anti-myc antibody 9E10 (a gift from Professor Ken Siddle, University of Cambridge) before incubation with europium-labeled PY20 antibody (Perkin Elmer: AD0038) to detect phosphorylated tyrosine residues. DELFIA-enhancement solution (Perkin Elmer) was added and time-resolved fluorescence measured (Ex: 340 nm/Em: 615 nm).

3 | RESULTS

3.1 | Clinical presentation

The proband was born at 38 weeks of gestation with a birthweight of 2780 g (25th centile). At the age of 10, the patient experienced

TABLE 1 5-h OGTT data in the proband and his family members carrying the INSR/F1213L

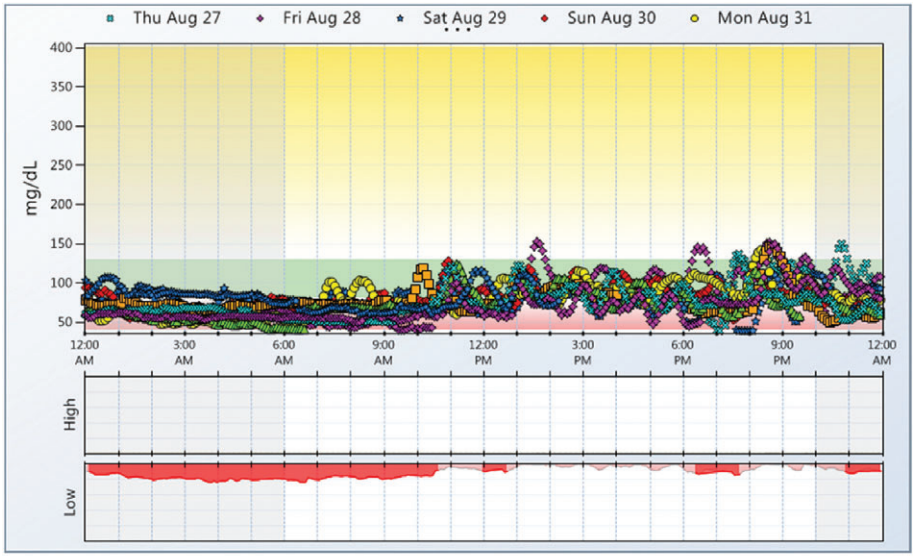
Proband 11 y—BMI 19.8 (66th centile)						
Time (min)	0	60	120	180	240	270
Plasma glucose (mg/mL)	81	98	111	100	55	56
Serum insulin (mU/mL)	23,9	265	304	215	86,2	24,7
Mother 36 y—BMI 19						
Time (min)	0	60	120	180	240	270
Plasma glucose (mg/mL)	90	156	102	73	35	67
Serum insulin (mU/mL)	25,2	173	264	198	42,6	16,5
Brother 19 y—BMI 17						
Time (min)	0	60	120	180	210	270
Plasma glucose (mg/mL)	77	130	125	89	40	51
Serum insulin (mU/mL)	27,2	253	>300	>300	84,7	34,7

Abbreviation: BMI, body mass index.

frequent episodes of tremors occurring about 2 hours after meals and resolving quickly on consumption of simple carbohydrates. When in her 20s, the proband's mother, now 38 years old, had experienced symptoms suggestive of hypoglycemia, including cold sweating and trembling also occurring 1 to 2 hours postprandially, particularly after simple carbohydrate-based food. Maternal grandparents were diagnosed with type 2 diabetes, but neither the mother nor the maternal grandparents showed signs of insulin resistance.

The proband was first evaluated metabolically when he was 11 years old. His weight and height were 35.8 kg and 136.5 cm, respectively; his body mass index was 19.8; and Tanner stage was 1. The presence of acanthosis nigricans in the axillae and neck was recorded. The patient's fasting insulin concentration was 97 µU/mL at first determination, and on 5-hour OGTT, he developed symptoms of hypoglycemia with a plasma glucose concentration of 55 mg/dL at 240 minutes (Table 1) and an insulin peak value of 304 µU/mL at 120 minutes (Table 1). Counter-regulatory hormones measured during OGTT-induced hypoglycemia showed no secretory deficit (cortisol 574 nmol/L; reference values: 138-690; ACTH 136 ng/L; reference values 10-50; GH 4,21 µg/L; reference values 0-8). non esterified fatty acids (NEFA) 3OH-butyric acid, carnitine, acylcarnitines, plasma amino acids, and urinary organic acids were all normal. An angio-MRI and an ^{18}F-DOPA PET/CT scan, performed to detect/exclude insulinoma, a rare cause of recurrent hypoglycemia in childhood, did not reveal any abnormality of the pancreas (Figure S1).

The patient was given a diet devoid of simple carbohydrates, reducing the frequency of hypoglycemia to less than 1 per month; however, continuous glucose monitoring (Dexcom G4) showed that glucose levels were below 80 mg/dL for 60% of the period observed, with values down to 50 mg/dL at night. Concomitant blood glucose concentration on self-monitoring was 60-78 mg/dL; Figure 1). The combination of fasting hyperinsulinemia and acanthosis nigricans in a lean subject with anecdotal evidence of hypoglycemia in the mother prompted screening of the *INSR* gene and identified the heterozygous



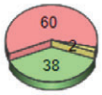
Statistics	
Average Glucose	78 mg/dL
Sensor Usage	10 of 30 Days
Calibrations / day	2.6
Standard Deviation	± 20 mg/dL
	2 % High
	38 % Target
	60 % Low
Target Range	80 - 130 mg/dL

FIGURE 1 A 5-day recording of glucose values by CGM, each identified by a different color. Note that low glucose values are consistently seen at night (10:00 PéM—6:00 AM)

c.3637T>C [p.Phe1213Leu (F1213L)] mutation. This variant was not present in the HGMD database or the ExAC browser and was found in the proband's mother and brother, who also showed fasting hyperinsulinemia and low plasma glucose concentration at 240 minutes of OGTT (35 and 40 mg/dL, respectively; Table 1). However, neither the patient's mother nor the brother showed any clinical symptoms or signs of hypoglycemia. A repeat 5-hour OGTT, performed in the proband 2 years later, confirmed fasting hyperinsulinemia (27 mU/L) and low glucose levels at the end of the test (300′: 51 mg/dL).

The effect of the mutation on receptor expression and function was examined in CHO cells. Protein expression of the L1213 mutant receptor was similar to that of the wild-type (F1213) receptor (Figure 2A), and moreover, a mutant insulin receptor was detectable at the cell surface at the same level as wild-type receptor (Figure 2B). In contrast, autophosphorylation of tyrosine residues in the receptor beta subunit was abolished in L1213 INSR, even at maximal insulin concentrations (Figure 2C, triangles), indicating that phenylalanine 1213 is fundamental for downstream signaling by the insulin receptor.

4 | DISCUSSION

HH in childhood encompasses congenital HH (of mostly genetic etiology)[2,6,7] admixed with a low prevalence of more "adult" HH entities, such as insulinoma.[1] Unfortunately, criteria based on the temporal relationship of hypoglycemia to meals is not useful in excluding insulinoma, a condition that is exceedingly rare in children.[6,7] When an insulin-secreting tumor is among the possible diagnoses, [18] an F-DOPA PET-CT scan is recommended to localize the lesion.[6,12] Once insulinoma is excluded, as in our case, a genetic cause may be considered. Our proband experienced hypoglycemic episodes in childhood, well beyond what is usually observed in congenital and recessive forms of HH.[2] In addition, his mother reported that she had symptoms highly suggestive of postprandial hypoglycemia. While these findings were suggestive of dominant forms of HH, such as that caused by glucokinase (GCK) mutations,[13,14] individuals with activated *GCK* mutations characteristically present with fasting, not postprandial, hypoglycemia, and they do not have signs of insulin resistance.[13,14]

Heterozygous mutations of INSR have previously been linked to familial, autosomal-dominant HH.[9,10] In both previously reported families, the index case was a lean female with postprandial hypoglycemia, but only 1 showed typical clinical signs of severe insulin resistance, such as hirsutism, acanthosis nigricans, and acne, despite a biochemical profile consistent with severe insulin resistance.[10,15,16] Thus, the hallmark of autosomal-dominant "HH" associated with dominant-negative *INSR* mutations seems to be postload hypoglycemia (either symptomatic or not), with biochemical evidence of severe insulin resistance but low clinical expression of that severe insulin resistance, rendering HH the sentinel clinical presentation [[9,17]i, present study].

The precise mechanism of hypoglycemia in individuals with insulin-receptor mutations is still debated. Paradoxical fasting hypoglycemia is a feature of 2 congenital severe insulin resistance (SIR) syndromes, namely Donohue syndrome (erstwhile "leprechaunism") and Rabson-Mendenhall syndrome, both caused by biallelic mutations of INSR.[15,18] In a milder form of SIR, known as type A insulin resistance (Type A IR), usually associated with dominant-negative, heterozygous *INSR* mutations, hypoglycemia commonly occurs 3 to 4 hours after meals.[18] Type A IR is typically diagnosed in adolescent females with acanthosis nigricans, hirsutism, and menstrual cycle disturbances,[11,16–18] whereas it is probably underdiagnosed in males in the absence of signs of hyperandrogenism.[17,18] In all types of SIR,

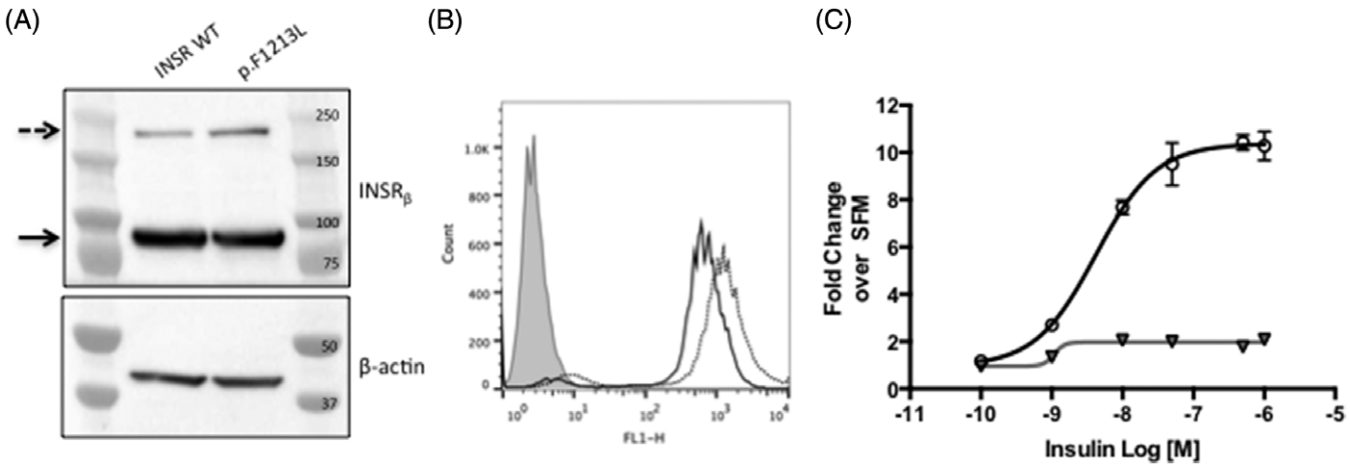


FIGURE 2 Expression and autophosphorylation of INSR F1213 (WT) and L1213 variant. A, Western blot of lysates from CHO Flp-In cells stably expressing F1213 (WT) or L1213 INSR variant as indicated. In INSR β-subunit blot, upper bands are pro-INSR, and lower bands are mature processed β-subunits, as indicated. B, Cell surface expression of INSR in CHO Flp-In cells expressing either F1213 (solid line) or L1213 (dashed line) labeled with anti-INSR antibody 83-14. Negative isotype control IgG-labeled cells are depicted in the gray shaded area. A shift to the right of the negative control peak indicates positive cell surface expression of INSR. C; CHO Flp-In cells stably expressing F1213 (black line, circles) or L1213 INSR variant (gray line, triangles) were serum-starved overnight prior to stimulation with increasing amounts of insulin for 10 minutes. Cells were then lysed and myc-tagged INSR immunocaptured onto 96-well plates and then incubated with europium-labeled PY20 antibody to detect phosphorylated tyrosine residues using time-resolved fluorescence. Error bars are shown when greater than the size of the symbols, and data points are the mean ± SEM from duplicate samples from 3 independent experiments. EC_{50} insulin-stimulated INSR F1213: 4.2 nM (95% CI 2.6-6.7 nM); EC_{50} insulin-stimulated INSR L1213: unable to be determined

an impairment of hepatic insulin clearance due to the *INSR* defect (or to hepatic steatosis) and consequent hyperinsulinemia appear to be likely contributors to the pathogenesis of hypoglycemia. In our patient, in a previous case,[10] Continuous Glucose Monitoring System (CGMS) documented low glucose levels during the night. Thus, we hypothesize that impaired hepatic glycogen synthesis may contribute to less efficient hepatic glucose production during the fasting state, although no formal proof of this mechanism has been provided in our study. To date, 5 mutations in the INSR tyrosine kinase domain (Arg1201Gln, Arg1201Trp, Pro1205Leu, Phe1213Leu, and Trp1273Ter, according to nomenclature that includes 27 amino acids of signal peptide) (References [9–11,17], present study, [19]) have been found to be associated with postprandial hypoglycemia with presentation during childhood and adulthood, but no studies have been performed to address the question of INSR structure-function specificity of these mutations in causing this condition.

In conclusion, in evaluating childhood HH, the possibility of insulin resistance should be considered, and clinical and biochemical evidence should be sought. *INSR* screening should be considered if postprandial/postload hypoglycemia with inappropriately high insulin levels are found in an otherwise apparently healthy and lean subject.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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