

Persistent Hyperinsulinaemic Hypoglycaemia and Maturity Onset Diabetes of the Young (MODY) due to Heterozygous *HNF4A* Mutations

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Running Title: Heterozygous *HNF4A* Mutations and persistent hypoglycaemia.

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ABSTRACT

Objective: Mutations in the human *HNF4A* gene encoding the hepatocyte nuclear factor 4 alpha (HNF-4 α) are known to cause maturity-onset diabetes of the young (MODY), which is characterized by autosomal dominant inheritance and impaired glucose-stimulated insulin secretion from pancreatic β -cells. HNF-4 α has a key role in regulating the multiple transcriptional factor networks in the islet. Recently heterozygous mutations in the *HNF4A* gene were reported to cause transient hyperinsulinaemic hypoglycaemia associated with macrosomia.

Research Design and Methods: Three infants presented with macrosomia and severe hypoglycaemia with a positive family history of MODY. The hypoglycaemia was confirmed to be due to hyperinsulinism and all three patients required diazoxide therapy to maintain normoglycaemia. Two of the three infants are still requiring diazoxide therapy at 8 and 18 months while one of them had resolution of hyperinsulinaemic hypoglycaemia at 32 months of age.

Results: Sequencing of the *HNF4A* gene identified heterozygous mutations in all three families. In family 1 a frameshift mutation L330fsdel17ins9 (c.987_1003del17ins9; p.Leu330fs) was present in the proband, a mutation affecting the conserved A nucleotide of the intron 2 branch site (c.264-21A>G) was identified in the proband of family 2 and finally a nonsense mutation, Y16X (c.48C>G, p.Tyr16X), was found in the proband of family 3.

Conclusions: Heterozygous *HNF4A* mutations can therefore cause both transient and persistent hyperinsulinaemic hypoglycaemia associated with macrosomia. We recommend that macrosomic infants with transient or persistent hyperinsulinaemic hypoglycaemia should be screened for *HNF4A* mutations if there is a family history of young-onset diabetes.

Hyperinsulinaemic hypoglycaemia (HH) is characterised by the inappropriate secretion of insulin in relation to the blood glucose concentration and can be transient or persistent. Recent studies established that heterozygous mutations in the transcription factor hepatocyte nuclear factor 4 alpha (HNF-4 α encoded by the *HNF4A* gene) are associated with a mild form of transient HH and considerable risk of macrosomia (1,2). HNF-4 α is a transcription factor of the nuclear hormone receptor superfamily and is expressed in liver, kidney, gut, and pancreatic islets (3). It plays a key role in the regulation of pancreatic insulin secretion. Loss-of-function *HNF4A* mutations have been identified in MODY (maturity-onset diabetes of young) families in both coding and regulatory regions of the gene, including the P2 promoter region, which is suggested to be the primary transcriptional start site used in β -cells (4,5). MODY is characterized by an autosomal dominant inheritance pattern and impaired glucose-stimulated insulin secretion from pancreatic β -cells (4).

The finding of transient mild HH is unexpected as heterozygous mutations in the *HNF4A* gene lead to loss of glucose induced insulin secretion with glucose intolerance in these patients. We now extend the observations of two previous studies (1, 2) and report that heterozygous *HNF4A* mutations can cause macrosomia with severe and persistent HH as well as MODY in three families.

CLINICAL CASES

Patient 1. Patient 1 was born at 39 weeks gestation with a birth weight of 5.9 kg following a vaginal delivery. The delivery was complicated with a prolonged second stage and shoulder dystocia. After delivery the baby developed severe symptomatic hypoglycaemia (jitteriness, irritable with a blood glucose concentration of 0.8mmol/L). He required a continuous infusion of 25% dextrose delivering 25mg/kg/min of glucose

as well as an infusion of glucagon to maintain normoglycaemia. Biochemical analysis showed an inappropriately raised level of insulin (103mU/l) during hypoglycaemia (glucose 1.6mmol/l) along with undetectable serum ketone bodies and fatty acids. The hypoglycaemia was responsive to 10mg/kg/day of diazoxide. An ¹⁸Fluoro-L-Dopa Positron Emission Tomography (¹⁸F-DOPA-PET) scan showed intense uptake of tracer throughout the pancreas consistent with increased metabolic activity of the islets (Figure 1).

The child was admitted again at 7 months for a trial off medications. During the 24-hour profile, several hypoglycaemic (blood glucose levels down to 2.1mmol/L) episodes were documented and therefore therapy was re-initiated with a good response. At the age of 8 months, the child remains on 8mg/k/d of diazoxide and 5mg/kg/d of chlorothiazide with normoglycaemia.

Family history: Our patient is the first child born to non-consanguineous parents. There is a strong history of diabetes mellitus that is summarised in figure 2 (pedigree 1). The patient's father was diagnosed with diabetes mellitus at the age of 26 years and was treated with oral hypoglycaemic agents. Interestingly, he was born macrosomic at 26 weeks gestation with a birth weight of 2.79 kg. The paternal grandmother was diagnosed with diabetes mellitus at the age of 17 years and was commenced on subcutaneous insulin therapy.

Patient 2. Patient 2 was born at 37 weeks gestation with a birth weight of 4.2 kg via a ventouse delivery. Delivery was difficult and associated with a right-sided Erb's palsy. She developed hypoglycaemia (blood glucose 2.5mmol/l) at 2 days of age with a seizure. The child required a continuous intravenous infusion of 20% glucose solution delivering 12.5 mg/kg/min of glucose and an intravenous infusion of glucagon to maintain normoglycaemia. A diagnosis of hyperinsulinaemic hypoglycaemia was made and her

hypoglycaemia responded well to 10 mg/kg/d of diazoxide and 10 mg/kg/d of chlorothiazide. An attempt at stopping the diazoxide at 18 months of age resulted in further hypoglycaemic episodes and therapy had to be re-initiated.

Family History: There was a strong family history of young-onset diabetes mellitus (Fig. 2, pedigree 2). The child's father was diagnosed with diabetes mellitus at the age of 23 years which was initially controlled by diet. He was started on oral hypoglycaemic agents at the age of 25 and had been on subcutaneous insulin since the age of 37. Two of the paternal uncles and the paternal grandfather were also diagnosed with diabetes in their 20s. Interestingly, one of the uncles had a birth weight of more than 5.4 kg. The father and both the uncles required neonatal intensive care, but further details were not available. There is further history of young onset diabetes in the extended family shown in figure 2 (pedigree 2). An *HNF4A* mutation, Y16X, had previously been identified in the proband's cousin (1).

Patient 3. Patient 3 was born at 36 weeks gestation via vacuum extraction with a birth weight of 4.05kgs. Delivery was complicated by shoulder dystocia. The child developed symptomatic hypoglycaemia (blood glucose concentration 1.4mmol/l) soon after birth. She required a continuous intravenous infusion of glucose delivering 11mg/kg/min as well as intravenous glucagon infusion. Investigations confirmed hyperinsulinism (blood glucose 1.1mmol/l with a simultaneous serum insulin 105mU/L). The hypoglycaemia responded to diazoxide therapy at 6mg/kg/d. The patient was readmitted at 18 months of age to attempt withdrawal of diazoxide, but experienced frequent hypoglycaemic episodes and therefore therapy was continued. She was eventually weaned off diazoxide at 2 years and 8 months of age.

Family History: There was a strong family history of diabetes mellitus following an autosomal dominant pattern of inheritance (Fig. 2, pedigree 3). The child's mother

developed gestational diabetes at the age of 31 years and later went on to require subcutaneous insulin treatment for diabetes at the age of 33 years (changed to sulphonylureas following the results of molecular genetics). The maternal grandfather and great-grandfather were also diagnosed to have diabetes at the ages of 33 and in late 70s respectively.

DNA SEQUENCE ANALYSIS

Sequencing of the *HNF4A* gene using previously described primers (1) identified heterozygous mutations in all three infants. In family 1 a frameshift mutation L330fsdel17ins9 (c.987_1003del17ins9; p.Leu330fs) was present in the proband, his father and paternal grandmother. This mutation has not been reported previously. A mutation affecting the conserved A nucleotide of the intron 2 branch site (c.264-21A>G) was identified in the proband, father and paternal grandfather of family 2. *In silico* splicing predictor software (www.fruitfly.org) predicted that this novel mutation would result in the creation of a cryptic splice acceptor site 20 nucleotides upstream from the normal splice acceptor site. An EBV-transformed lymphoblastoid cell was established from the grandfather and mRNA analysis confirmed this prediction by the identification of an aberrant transcript with 20 additional bases from intron 2 leading to a premature termination codon in exon 3. A nonsense mutation, Y16X (c.48C>G, p.Tyr16X), was found in the proband of family 3 and her mother. This mutation is located in exon 1D and is described according to the reference sequence AY680697 with the A of the ATG start codon as c.1.

DISCUSSION

This case series illustrates that heterozygous *HNF4A* mutations can cause severe and persistent HH associated with macrosomia in addition to the mild, transient HH (1,2). Macrosomia is known to be associated with HH due to the growth stimulating actions of insulin during the

fetal period. All the affected infants with heterozygous *HNF4A* mutations were macrosomic (birth weight >4kg). The father of patient 1 was macrosomic (birth weight 2.79 kg, 99th centile) at 26 weeks gestation.

HH is clinically a very heterogeneous condition in terms of severity of disease, persistence of disease and responsiveness to medical therapy (6). All our patients had severe HH requiring large amounts of intravenous glucose and subcutaneous glucagon infusions to maintain normoglycaemia. Diazoxide therapy was effective in all our patients. However withdrawal of therapy at 7 months in patient 1 and at 18 months in patients 2 and 3 resulted in persistence of HH suggesting continued unregulated insulin secretion. Due to the severity of hypoglycaemia, this patient underwent an ¹⁸F-DOPA-PET scan. The degree of uptake of tracer reflects the metabolic activity in the islets (7). The ¹⁸F-DOPA-PET showed an intense uptake of the ¹⁸Fluoro-L-Dopa throughout the pancreas indicating increased metabolic activity of the islets as observed in patients with HH due to mutations in the *ABCC8* and *KCNJ11* genes.

In comparison to the study by Pearson et al (1) our patients presented with severe and persistent HH. The difference in severity and extent of investigations of the HH may be explained by the fact that Pearson et al carried out a retrospective review of case notes. This retrospective review of case notes was carried out once the diagnosis of *HNF4A* MODY was made in the family. In contrast in two of the families in this study the *HNF4A* mutations were first identified in infants presenting with HH. This then subsequently lead to the identification of family members with *HNF4A* MODY. The third case was born into a family where the mutation had previously been identified, but her severe hypoglycaemia (with a seizure at 2 days of age) prompted detailed medical investigation. It is likely that haploinsufficiency of *HNF4A* results in a variable phenotype ranging from macrosomia without HH detected, to

macrosomia with transient HH and then persistent neonatal HH. It is unlikely that the differences in phenotypes result from characteristics of the mutation as there is no documented history of HH in other family members.

HNF-4α has a key role in regulating the multiple transcriptional factor networks in the islet and in combination with other hepatocyte nuclear factors (such as *HNF-1α*) has been proposed to form a functional regulatory loop in the adult β-cell (8,9). *HNF-4α* interacts with regulatory elements in promoters and enhancers of genes whose products are involved in diverse function, including cholesterol, fatty acid, amino acid, and glucose metabolism, as well as liver development and differentiation (10, 11, 12). The Y16X mutation is located in exon 1D which is only present in the *HNF4A*7-9 isoforms expressed from the P2 promoter. The identification of neonatal hypoglycaemia and/or macrosomia in 4 of 5 patients with this mutation suggests that P2 derived *HNF-4α* isoforms are involved in the hypersecretion of insulin *in utero* and early infancy.

Since loss of *HNF-4α* function leads to multiple defects in glucose stimulated insulin secretion (13) it is unclear how heterozygous *HNF4A* mutations can also cause HH in the newborn period. Using the conditional Cre-loxP-based inactivation system and deleting the *HNF4A* gene in β-cells Gupta et al (14) were able to show that fasted and fed mice were hyperinsulinaemic but paradoxically also displayed impaired glucose tolerance. These mice showed a 60% reduction in expression of the potassium channel subunit Kir6.2 with cotransfection assays demonstrating that the *Kir6.2* gene is a transcriptional target of *HNF-4α*. However two further studies have reported no change in the expression of Kir6.2 in *Hnf4a*- deficient mice (1, 15). This suggests that the reduction in expression of the potassium channel subunit Kir6.2 may not be the only mechanism responsible for the HH in *Hnf4a*- deficient mice.

HNF-4 α has also been shown to have an interaction with the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR α) with low levels of PPAR α reported in HNF-4 α deficient β -cells (14, 16). Given the postulated role of PPAR α in the regulation of β -cell lipid metabolism, it is possible that the lower level of PPAR α in the HNF-4 α mutants partially contributes to the elevated basal insulin levels (17). In support of this hypothesis PPAR α null mice develop fasting HH suggesting that PPAR α is important for regulated insulin secretion during fasting (18). HNF-4 α binds to the promoters of 11% of islet genes and it is quite likely that HNF-4 α deficiency probably exhibits its phenotype via abnormal expression of one or more of these target islet genes (9). Hence, further studies

are required to study the effect of HNF-4 α deficiency on these genes. Also, a long term prospective study is required to completely assess the phenotype of these children with *HNF4A* mutations and HH.

To conclude, we report that heterozygous mutations in the *HNF4A* gene can cause macrosomia with severe and persistent HH that is responsive to diazoxide therapy in the newborn period. We recommend that *HNF4A* gene is sequenced in children with transient or persistent HH where there is a family history of diabetes or macrosomia.

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LEGENDS

Table 1. Clinical Characteristics of the patients with hyperinsulinism

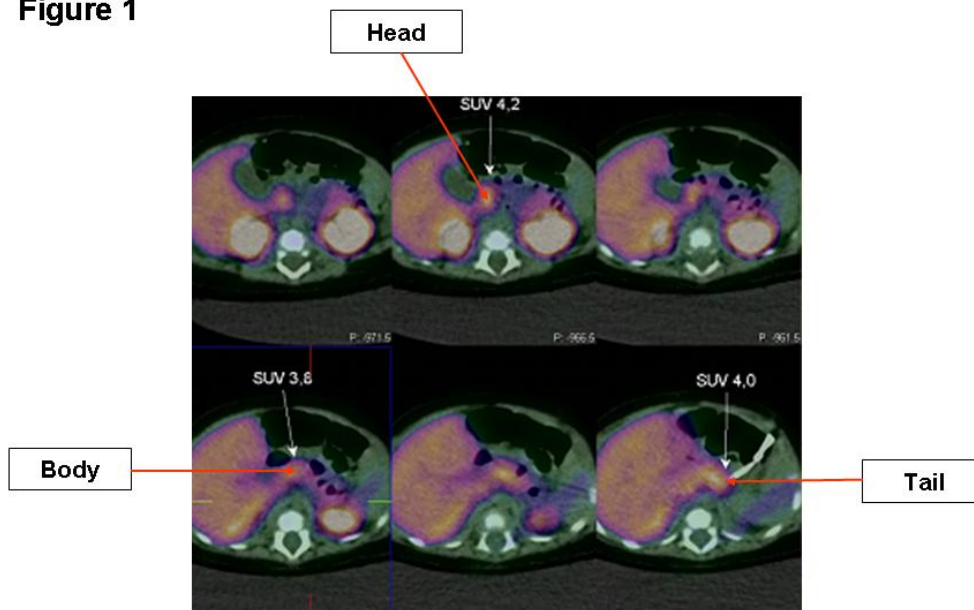
Figure 1. ^{18}F -L-DOPA-PET/CT scan showing uptake of the ^{18}F -L-Dopa throughout the whole of the pancreas in patient 1. The principle of this test is that, pancreatic islets take up L-3, 4-dihydroxyphenylalanine (L-DOPA), and convert it to dopamine by dopa decarboxylase, present in the islet cells. The SUV (standard uptake value) reflects the intensity of uptake of DOPA. The ^{18}F -L-DOPA-PET/CT scan in this patient shows uniformly (in the head, body and tail of pancreas) increased uptake of ^{18}F -L-Dopa reflecting increased metabolic activity of the islets. The intensity of uptake of the ^{18}F -L-Dopa by the islets was equivalent to that observed in patients with defects in pancreatic K_{ATP} channels (7). This fits in with the clinical observation of severe hyperinsulinaemic hypoglycaemia.

Figure 2. Family pedigrees; proband's with hyperinsulinism are shaded with bold diagonal stripes and family members with diabetes are coloured black. The mutation status (NM = heterozygous *HNF4A* mutation, NN = No mutation), birth centile, age at diagnosis of diabetes and treatment of diabetes/hyperinsulinism (INS- insulin, OHA- oral hypoglycaemic agents) is shown.

TABLE 1. Clinical characteristics of the patients with hyperinsulinism.

	Patient 1	Patient 2	Patient 3
Birth Weight (grams)	5900	4200	4055
Gestation age (weeks)	39/40	37/40	36/40
Age of presentation (days)	1	2	1
Maximum Glucose Infusion Rate (mg/kg/min)	25	12.5	11
Glucagon Infusion Required	Yes	Yes	Yes
Diazoxide responsive (dose mg/kg/day)	10	10	6
Family history of diabetes	Yes	Yes	Yes
Attempted withdrawal of diazoxide	Not successful at 7 months	Not successful at 18 months	Successful at 32 months

Figure 1



SUV: Standard uptake values of ¹⁸F-DOPA in head, body and tail of pancreas

