Persistent Hyperinsulinemic Hypoglycemia and Maturity-Onset Diabetes of the Young Due to Heterozygous *HNF4A* Mutations

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OBJECTIVE—Mutations in the human *HNF4A* gene encoding the hepatocyte nuclear factor (HNF)-4α 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-4α 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 hyperinsulinemic hypoglycemia associated with macrosomia.

RESEARCH DESIGN AND METHODS—Three infants presented with macrosomia and severe hypoglycemia with a positive family history of MODY. The hypoglycemia was confirmed to be due to hyperinsulinism, and all three patients required diazoxide therapy to maintain normoglycemia. Two of the three infants are still requiring diazoxide therapy at 8 and 18 months, whereas one of them had resolution of hyperinsulinemic hypoglycemia 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 hyperinsulinemic hypoglycemia associated with macrosomia. We recommend that macrosomic infants with transient or persistent hyperinsulinemic hypoglycemia should be screened for *HNF4A* mutations if there is a family history of youth-onset diabetes. ***Diabetes* 57: 1659–1663, 2008**

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^{18}F-DOPA-PET, ^{18}fluoro-L-Dopa positron emission tomography; HNF, hepatocyte nuclear factor; MODY, maturity-onset diabetes of the young; PPAR, peroxisome proliferator–activated receptor.

Hyperinsulinemic hypoglycemia is characterized 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 (HNF)-4α (encoded by the *HNF4A* gene) are associated with a mild form of transient hyperinsulinemic hypoglycemia and considerable risk of macrosomia (1,2). HNF-4α 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 maturity-onset diabetes of the young (MODY) 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 hyperinsulinemic hypoglycemia is unexpected, since 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 hyperinsulinemic hypoglycemia as well as MODY in three families.

RESEARCH DESIGN AND METHODS

Patient 1. Patient 1 was born at 39 weeks' gestation with a birth weight of 5.9 kg after a vaginal delivery. The delivery was complicated with a prolonged second stage and shoulder dystocia. After delivery, the baby developed severe symptomatic hypoglycemia (jitteriness and irritability with a blood glucose concentration of 0.8 mmol/l). He required a continuous infusion of 25% dextrose delivering 25 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} glucose, as well as an infusion of glucagon to maintain normoglycemia. Biochemical analysis showed an inappropriately raised level of insulin (103 mU/l) during hypoglycemia (glucose 1.6 mmol/l) along with undetectable serum ketone bodies and fatty acids. The hypoglycemia was responsive to 10 mg $\cdot$ kg^{-1} $\cdot$ day^{-1} of diazoxide. An ^{18}fluoro-L-Dopa positron emission tomography (^{18}F-DOPA-PET) scan showed intense uptake of tracer throughout the pancreas consistent with increased metabolic activity of the islets (Fig. 1).

The child was admitted again at 7 months for a trial off medications. During the 24-h profile, several hypoglycemic

TABLE 1
Clinical characteristics of patients with hyperinsulinism

	Patient 1	Patient 2	Patient 3
Birth weight (g)	5,900	4,200	4,055
Gestational age (weeks)	39/40	37/40	36/40
Age of presentation (days)	1	2	1
Maximum glucose infusion rate (mg · kg^{-1} · min^{-1})	25	12.5	11
Glucagon infusion required	Yes	Yes	Yes
Diazoxide responsive dose (mg · kg^{-1} · day^{-1})	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

(blood glucose levels down to 2.1 mmol/l) episodes were documented, and therefore therapy was reinitiated with a good response. At the age of 8 months, the child remains on 8 mg · kg^{-1} · day^{-1} diazoxide and 5 mg · kg^{-1} · day^{-1} chlorothiazide with normoglycemia.

Family history. Our patient is the first child born to nonconsanguineous parents. There is a strong family history of diabetes, which is summarized in Fig. 2 (pedigree 1). The patient's father was diagnosed with diabetes at age 26 years and was treated with oral hypoglycemic 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 at age 17 years and was started 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 hypoglycemia (blood glucose 2.5 mmol/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^{-1} · min^{-1} glucose and an intravenous infusion of glucagon to maintain normoglycemia. A diagnosis of hyperinsulinemic hypoglycemia was made and her hypoglycemia responded well to 10 mg · kg^{-1} · day^{-1} diazoxide and 10 mg · kg^{-1} · day^{-1} chlorothiazide. An attempt at stopping the diazoxide at 18 months of age resulted in further hypoglycemic episodes, and therapy had to be reinitiated.

Family history. There was a strong family history of young-onset diabetes (Fig. 2, pedigree 2). At age 23 years, the child's father was diagnosed with diabetes, which was initially controlled by diet. He was started on oral hypoglycemic agents at age 25 years and had been on subcutaneous insulin since age 37 years. 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 >5.4 kg. The father and both the uncles required neonatal intensive care, but further details were not available. There is further history of youth-onset diabetes in the extended family shown in Fig. 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 4.05 kg. Delivery

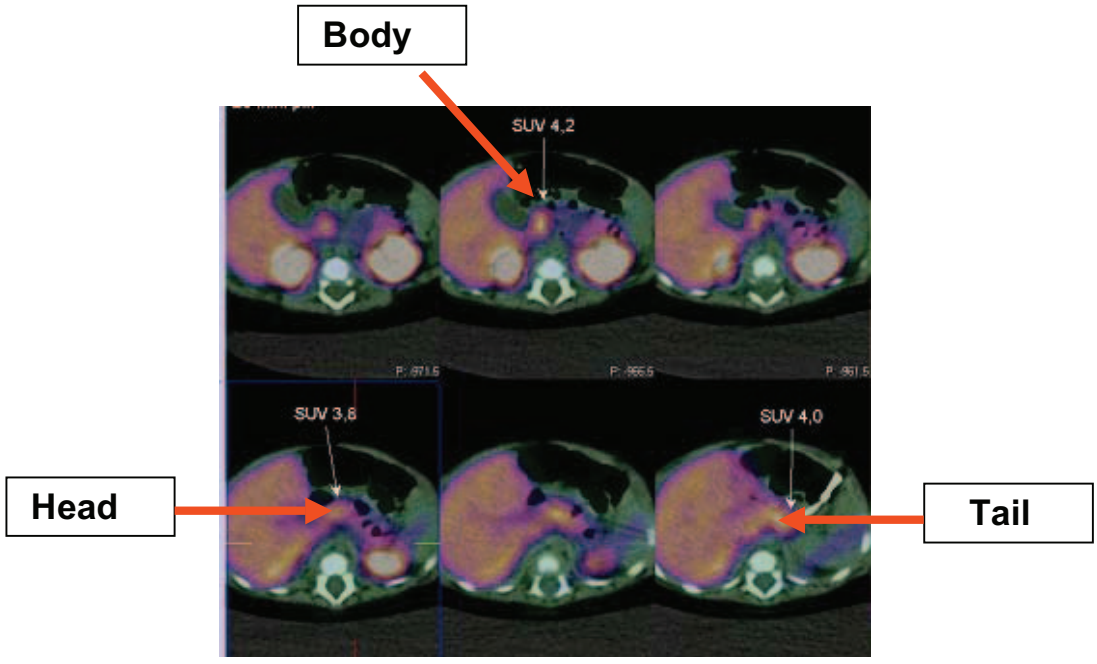

FIG. 1. ^{18}F-DOPA-PET/CT scan showing uptake of the ^{18}F-L-Dopa throughout the whole of the pancreas in patient 1. The principle of this test is that pancreatic islets take up L-3,4-dihydroxyphenylalanine (L-DOPA) and convert it to dopamine by Dopa decarboxylase, present in the islet cells. The standard uptake value (SUV) reflects the intensity of uptake of DOPA. The ^{18}F-DOPA-PET/CT scan in this patient shows uniformly (in the head, body, and tail of the 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 ATP-sensitive K+ (K_{ATP}) channels (7). This fits in with the clinical observation of severe hyperinsulinemic hypoglycemia.

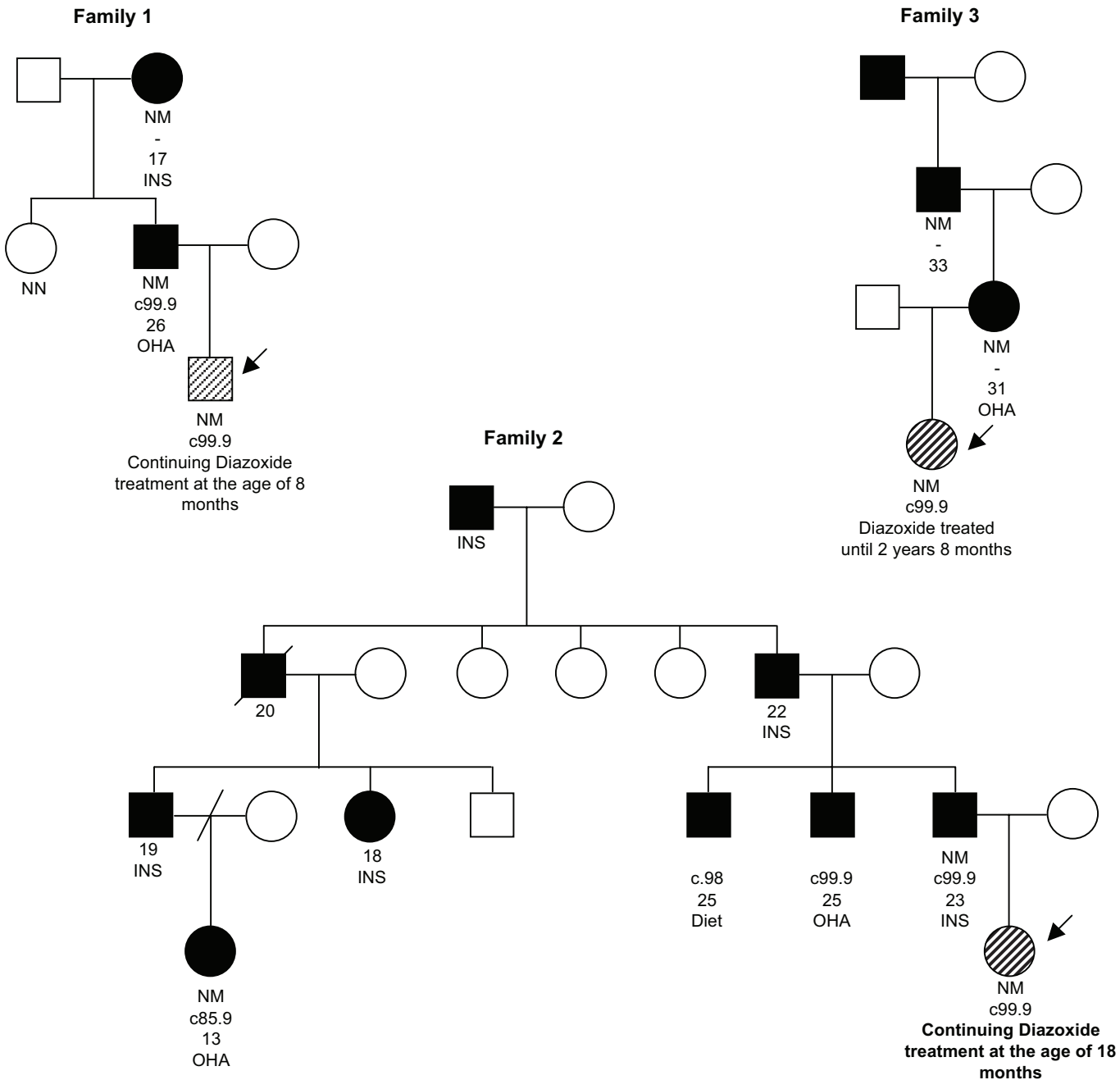

FIG. 2. Family pedigrees. Probands with hyperinsulinism are shaded with bold diagonal stripes, and family members with diabetes are colored black. The mutation status (NM, heterozygous *HNF4A* mutation; NN, no mutation), birth centile, age at diagnosis of diabetes, and treatment of diabetes/hyperinsulinism (INS, insulin; OHA, oral hypoglycemic agents) are shown.

was complicated by shoulder dystocia. The child developed symptomatic hypoglycemia (blood glucose concentration 1.4 mmol/l) soon after birth. She required a continuous intravenous infusion of glucose (11 mg · kg^{-1} · min^{-1}) as well as intravenous glucagon infusion. Investigations confirmed hyperinsulinism (blood glucose of 1.1 mmol/l with a simultaneous serum insulin of 105 mU/l). The hypoglycemia responded to diazoxide therapy at 6 mg · kg^{-1} · day^{-1}. The patient was readmitted at 18 months of age to attempt withdrawal of diazoxide but experienced frequent hypoglycemic 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 after an autosomal-dominant pattern of inheritance (Fig. 2, pedigree 3). The child's mother developed gestational diabetes mellitus at age 31 years and later went on to require subcutaneous insulin treatment for diabetes at age 33 years (changed to sulfonylureas after the results of molecular genetics). The maternal grandfather and great-grandfather were also diagnosed as having diabetes at ages 33 years and in their 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 the 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.

RESULTS AND DISCUSSION

This case series illustrates that heterozygous *HNF4A* mutations can cause severe and persistent hyperinsulinemic hypoglycemia associated with macrosomia in addition to the mild transient hyperinsulinemic hypoglycemia (1,2). Macrosomia is known to be associated with hyperinsulinemic hypoglycemia because of the growth-stimulating actions of insulin during the fetal period. All the affected infants with heterozygous *HNF4A* mutations were macrosomic (birth weight >4 kg). The father of patient 1 was macrosomic (birth weight 2.79 kg, 99th centile) at 26 weeks' gestation.

Hyperinsulinemic hypoglycemia 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 hyperinsulinemic hypoglycemia requiring large amounts of intravenous glucose and subcutaneous glucagon infusions to maintain normoglycemia. 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 hyperinsulinemic hypoglycemia, suggesting continued unregulated insulin secretion. Because of the severity of hypoglycemia, this patient underwent an ^{18}F-DOPA-PET scan. The degree of uptake of tracer reflects the metabolic activity in the islets (7). The ^{18}F-DOPA-PET showed an intense uptake of the ^{18}fluoro-L-Dopa throughout the pancreas, indicating increased metabolic activity of the islets, as observed in patients with hyperinsulinemic hypoglycemia 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 hyperinsulinemic hypoglycemia. The difference in severity and extent of investigations of the hyperinsulinemic hypoglycemia 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 hyperinsulinemic hypoglycemia. 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 hypoglycemia (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 hyperinsulinemic hypoglycemia detected, to macrosomia with transient hyperinsulinemic hypoglycemia and then persistent neonatal hyperinsulinemic hypoglycemia. It is unlikely that the differences in phenotypes result from characteristics of the mutation, since there is no documented history of hyperinsulinemic hypoglycemia 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 (10–12). The Y16X mutation is located in exon 1D, which is only present in the HNF4A7–9 isoforms expressed from the P2 promoter. The identification of neonatal hypoglycemia and/or macrosomia in four of five patients with this mutation suggests that P2-derived HNF-4α isoforms are involved in the hypersecretion of insulin in utero and early infancy.

Because 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 hyperinsulinemic hypoglycemia 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 hyperinsulinemic 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 hyperinsulinemic hypoglycemia in *Hnf4a*-deficient mice.

HNF-4α has also been shown to have an interaction with the nuclear receptor peroxisome proliferator–activated receptor (PPAR)-α, with low levels of PPAR-α reported in HNF-4α–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-4α mutants partially contributes to the elevated basal insulin levels (17). In support of this hypothesis, PPAR-α null mice develop fasting hyperinsulinemic hypoglycemia, suggesting that PPAR-α is important for regulated insulin secretion during fasting (18). HNF-4α binds to the promoters of 11% of islet genes, and it is quite likely that HNF-4α 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-4α deficiency on these genes. Also, a long-term prospective study is required to completely assess the phenotype of these children with *HNF4A* mutations and hyperinsulinemic hypoglycemia.

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

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REFERENCES

1. Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, Ellard S, Ferrer J, Hattersley AT: Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med* 4:e118, 2007
2. Fajans SS, Bell GI: Macrosomia and neonatal hypoglycaemia in RW pedigree subjects with a mutation (Q268X) in the gene encoding hepatocyte nuclear factor 4alpha (HNF4A). *Diabetologia* 50:2600–2601, 2007
3. Sladek FM, Zhong WM, Lai E, Darnell JE Jr: Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Genes Dev* 4:2353–2365, 1990
4. Yamagata K, Furuta H Oda N, Kaisaki P, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). *Nature* 384:458–460, 1996
5. Silander K, Mohlke KL, Scott LJ, Peck EC, Hollstein P, Skol AD, Jackson AU, Deloukas P, Hunt S, Stavrides G, Chines PS, Erdos MR, Narisu N, Conneely KN, Li C, Fingerlin TE, Dhanjal SK, Valle TT, Bergman RN, Tuomilehto J, Watanabe RM, Boehnke M, Collins FS: Genetic variation near the hepatocyte nuclear factor-4 alpha gene predicts susceptibility to type 2 diabetes. *Diabetes* 53:1141–1149, 2004
6. Hussain K: Congenital hyperinsulinism. *Semin Fetal Neonatal Med* 10:369–376 2005
7. Otonkoski T, Nanto-Salonen K, Seppanen M, Veijola R, Huopio H, Hussain K, Tapanainen P, Eskola O, Parkkola R, Ekstrom K, Guiot Y, Rahier J, Laakso M, Rintala R, Nuutila P, Minn H: Noninvasive diagnosis of focal hyperinsulinism of infancy with [18F]-DOPA positron emission tomography. *Diabetes* 55:13–18, 2006
8. Boj SF, Parrizas M, Maestro MA, Ferrer J: A transcription factor regulatory circuit in differentiated pancreatic cells. *Proc Natl Acad Sci U S A* 98:14481–14486, 2001
9. Odom DT, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI, Young RA: Control of pancreas and liver gene expression by HNF transcription factors. *Science* 303:1378–1381, 2004
10. Mietus-Snyder M, Sladek FM, Ginsburg GS, Guo CF, Ladias JA, Darnell JE Jr, Karathanasis SK: Antagonism between apolipoprotein AI regulatory protein 1, *Ear3/COUP*-TF, and hepatocyte nuclear factor 4 modulates apolipoprotein CIII gene expression in liver and intestinal cells. *Mol Cell Biol* 12:1708–1718, 1992
11. Hall RK, Sladek FM, Granner DK: The orphan receptors COUP-TF and HNF-4 serve as accessory factors required for induction of phosphoenolpyruvate carboxykinase gene transcription by glucocorticoids. *Proc Natl Acad Sci U S A* 92:412–416, 1995
12. Chen WS, Manova K, Weinstein DC, Duncan SA, Plump AS, Prezioso VR, Bachvarova RF, Darnell JE Jr: Disruption of the HNF-4 gene, expressed in visceral endoderm, leads to cell death in embryonic ectoderm and impaired gastrulation of mouse embryos. *Genes Dev* 8:2466–2477, 1994
13. Wang H, Maechler P, Antinozzi PA, Hagenfeldt KA, Wollheim CB: Hepatocyte nuclear factor 4alpha regulates the expression of pancreatic beta-cell genes implicated in glucose metabolism and nutrient-induced insulin secretion. *J Biol Chem* 275:35953–35959, 2000
14. Gupta RK, Vatamaniuk MZ, Lee CS, Flaschen RC, Fulmer JT, Matschinsky FM, Duncan SA, Kaestner KH: The MODY1 gene HNF-4alpha regulates selected genes involved in insulin secretion. *J Clin Invest* 115:1006–1015, 2005
15. Miura A, Yamagata K, Kakei M, Hatakeyama H, Takahashi N, Fukui K, Nammo T, Yoneda K, Inoue Y, Sladek FM, Magnuson MA, Kasai H, Miyagawa J, Gonzalez FJ, Shimomura I: Hepatocyte nuclear factor-4alpha is essential for glucose-stimulated insulin secretion by pancreatic beta-cells. *J Biol Chem* 281:5246–5257, 2006
16. Prentki M, Joly E, El-Assaad W, Roduit R: Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes. *Diabetes* 51:S405–S413, 2002
17. Sugden MC, Holness MJ: Potential role of peroxisome proliferator-activated receptor-alpha in the modulation of glucose-stimulated insulin secretion. *Diabetes* 53:S71–S81, 2004
18. Gremlich S, Nolan C, Roduit R, Burcelin R, Peyot ML, Delghingaro-Augusto V, Desvergne B, Michalik L, Prentki M, Wahli W: Pancreatic islet adaptation to fasting is dependent on peroxisome proliferator-activated receptor alpha transcriptional up-regulation of fatty acid oxidation. *Endocrinology* 146:375–382, 2005