The Heterogeneity of Focal Forms of Congenital Hyperinsulinism

Dunia Ismail, **Ritika R. Kapoor**
Departments of Endocrinology, Great Ormond Street, Hospital for Children National Health Service Trust, London, and The Institute of Child Health, University College London, London WC1N 1EH, United Kingdom

Virpi V. Smith,
Surgery, Great Ormond Street, Hospital for Children National Health Service Trust, London, and The Institute of Child Health, University College London, London WC1N 1EH, United Kingdom

Michael Ashworth,
Histology, Great Ormond Street, Hospital for Children National Health Service Trust, London, and The Institute of Child Health, University College London, London WC1N 1EH, United Kingdom

Oliver Blankenstein,
Department of Endocrinology, Charité-University Medicine, 10117 Berlin, Germany

Agostino Pierro
Surgery, Great Ormond Street, Hospital for Children National Health Service Trust, London, and The Institute of Child Health, University College London, London WC1N 1EH, United Kingdom

Sarah E. Flanagan, **Sian Ellard**
Institute of Biomedical and Clinical Science, Peninsula Medical School, University of Exeter, London EX2 5DW, United Kingdom

Khalid Hussain
Departments of Endocrinology, Great Ormond Street, Hospital for Children National Health Service Trust, London, and The Institute of Child Health, University College London, London WC1N 1EH, United Kingdom

Abstract

Background—Congenital hyperinsulinism (CHI) is a cause of persistent hypoglycemia due to unregulated insulin secretion from pancreatic β-cells. Histologically, there are two major subgroups, focal and diffuse. Focal CHI is typically unresponsive to diazoxide and can be cured with surgical removal of the focal lesion.

Aims—We report on three patients with focal CHI to illustrate the marked clinical, genetic, radiological, and histological heterogeneity.

Methods and Results—The first two patients had focal CHI due to a paternal (c.3992–9G→A) *ABCC8* mutation. One of these patients was fully responsive to a small dose (5 mg/kg · d)

of diazoxide, whereas the other patient was medically unresponsive. In both patients, the focal lesions were accurately localized preoperatively by [18F]dihydroxyphenylalanine (DOPA) positron emission tomography (PET) and surgically resected. The third patient had a paternally inherited *ABCC8* (A1493T) mutation, and the initial [18F]DOPA PET scan indicated extensive uptake of DOPA in the body and tail of the pancreas. However, despite surgical resection of the body and tail, this patient continued to have severe CHI. A subsequent [18F]DOPA PET scan now showed markedly increased DOPA uptake in the remaining body and head of the pancreas. This focal lesion occupied virtually the whole of the pancreas.

Conclusions—These three cases illustrate that focal lesions even with the same genotype (c.3992–9G→A) may have a different clinical presentation and that [18F]DOPA PET scans in very large focal lesions may be difficult to interpret. (*J Clin Endocrinol Metab* **97: E94–E99, 2012**)

Congenital hyperinsulinism (CHI) is a cause of persistent hypoglycemia due to unregulated insulin secretion. Histologically, there are two major subgroups, diffuse and focal (1). Diffuse CHI involves the whole pancreas, whereas in focal CHI (focal adenomatous hyperplasia), the abnormality is confined to a single region. Histologically, the focal region is characterized by nodular hyperplasia of islet-like cell clusters with ductuloinsular complexes and scattered giant ß-cell nuclei (1–3). Focal CHI occurs due to the inheritance of a paternal *ABCC8/KCNJ11* mutation followed by the somatic loss of the maternal chromosome 11p15 region within the focal lesion only (4–7). This somatic loss of the maternal 11p15 region leads to hemizygosity of the paternally derived *ABCC8/KCNJ11* allele and also causes an imbalance in the imprinted genes within this chromosomal region.

Focal CHI is generally medically unresponsive and requires surgery to remove the focal lesion. Accurate pre-operative localization and complete resection of the focal lesion can cure the patient. [18F]Dihydroxyphenylalanine (DOPA) positron emission tomography (PET) scanning allows accurate preoperative localization of focal lesions before any planned surgery (8), although in some population, it may not be so accurate (9). We report on three patients with focal CHI to illustrate the marked heterogeneity of this disease. We show that patients who have focal CHI due to the same genetic mutation can have markedly different clinical presentations and that [18F]DOPA PET scanning may be difficult to interpret in very large focal lesions which involve virtually the entire pancreas.

Materials and Methods

Ethics

This study was approved by the Ethics Committee of Great Ormond Street Children's Hospital and the Institute of Child Health.

[18F]DOPA PET scan

The PET/computed tomography (CT) scans (Siemens Biograph, Berlin, Germany) were performed as described previously (10).

Histology

Routine histological procedures were performed as previously reported (11).

Genetics

DNA was extracted from peripheral leukocytes and the *KCNJ11* and *ABCC8* genes amplified and sequenced as previously described (11). Sequences were compared with published sequences (NM_000525 and NM_000352.2) using Mutation Surveyor software (SoftGenetics, State College, PA). Parental samples were tested for mutations identified in the probands, and microsatellite analysis confirmed *de novo* mutations. To determine the chromosome of origin of a *de novo* mutation, primers were designed to amplify a single copy of the *ABCC8* gene using a primer complementary to an informative single-nucleotide polymorphism, S1370A;c.4108T→G, which was within close proximity to the mutation (primers available on request).

Loss of heterozygosity (LOH) within the focal lesion was investigated by microsatellite analysis. Ten markers spanning chromosome 11p15 were amplified using DNA extracted from the focal lesion, from surrounding normal pancreatic tissue when available, and from the patient and parents' peripheral leukocytes. The allele peak heights were compared using GeneMarker software (SoftGenetics).

Clinical cases

Clinical and biochemical details are summarized in Table 1.

Results

Patient 1

Genetics—Sequencing analysis identified a previously reported heterozygous *ABCC8 de novo* mutation, c.3992-9G→A (12). Microsatellite analysis confirmed family relationships, and allele-specific PCR demonstrated that the mutation had arisen on the paternal chromosome.

[18F]DOPA PET scanning—This patient underwent an [18F]DOPA PET/CT scan of the pancreas that confirmed the presence of a focal lesion located in the cranial part of the body positioned more to the backside (dorsal) measuring $4 \times 8 \times 7$ mm. The patient underwent surgery, and the focal lesion was removed, curing the patient.

Histology—The histology of the focal lesion showed nodules of endocrine tissue with large nuclei. The lesion measured 0.8 cm in diameter, and the remaining pancreas showed normal resting islets.

LOH studies—Microsatellite analysis of 10 markers spanning the differentially methylated region and the *ABCC8* gene demonstrated a loss the maternal allele in the pancreatic lesion when compared with the leukocyte DNA.

Patient 2

Genetics—A previously reported heterozygous *ABCC8* gene splicing mutation c.3992-G→A was identified (12), and family member testing identified the mutation in the unaffected father.

[^{18}F]DOPA PET scanning—The [^{18}F]DOPA PET showed a large focal lesion in the head of the pancreas (standard uptake value ratio of 2.3) with nearly no tracer uptake in the body and tail of the pancreas. The operation involved resection of the head of the pancreas and pancreato-jejunostomy. Postoperatively, the patient was cured with no postoperative complications.

Histology—A focal lesion consisting of nodules of endocrine tissue with large endocrine nuclei was identified and measuring 0.7 cm in diameter. Histology of the pancreas outside the focal lesion showed normal resting islets.

LOH studies—Microsatellite analysis of 10 markers spanning the differentially methylated region and the *ABCC8* gene demonstrated a reduced peak height for the maternal allele in the pancreatic lesion when compared with the leukocyte DNA.

Patient 3

Genetics—A previously reported heterozygous *ABCC8* mutation, A1493T; c.4477G→A (13) was found. Mutation testing in the unaffected parents showed the proband had inherited the mutation from her father.

[^{18}F]DOPA PET scanning—The results of the initial [^{18}F]DOPA PET/CT scan showed intense tracer uptake in the tail and parts of the body (Fig. 1, A and B). Because the affected region was unusually large (32 × 25 × 15 mm), this was interpreted either as a very large focus or as an atypical form of CHI. Based on the [^{18}F]DOPA PET scan findings, the child underwent a laparoscopic pancreatectomy involving the removal of the tail and the body of the pancreas.

However, despite the removal of the tail and body of the pancreas, the child still had hypoglycemia. She was restarted on octreotide, but once again could not be weaned off iv dextrose. Six weeks after the first [^{18}F]DOPA PET scan, the patient underwent a repeat scan. On this occasion, the remaining head of the pancreas showed markedly increased tracer uptake involving virtually the whole of the head (Fig. 1D). Based on these findings, the patient thus underwent a second pancreatectomy to remove the remaining head of the pancreas. However, despite this second pancreatectomy, the patient's blood glucose levels could only be stabilized on continuous gastrostomy feeds.

Histology

First resection: (*see* Fig. 1, C, E, and F). Macroscopically, there was no focal lesion. Microscopically frozen sections from all specimens labeled A, B, C, and D showed nodules of endocrine tissue with large endocrine nuclei. In specimen E, however, in addition to lesion tissue, occasional islets of Langerhans with small resting endocrine nuclei (suggesting

normal tissue) were also seen. The lesion was widespread, being present in virtually every segment and reminiscent of a giant focal lesion previously reported (14, 15).

Second resection: The second resection involved removing the major portion of the head of the pancreas. Once again, macroscopically, the pancreas head appeared normal. However, microscopically, the portion F had nodules of irregular islet tissue with occasional large endocrine nuclei, whereas the remaining portion G showed normal endocrine histology.

LOH studies—Microsatellite analysis demonstrated that four markers (D11S2071, D11S1397, D11S1888, and D11S902) had a reduced peak height for the maternal allele in the pancreatic lesion when compared with the normal tissue and leukocyte DNA. These results confirmed LOH from 11p15.5 to 11p15.1, which encompasses the differentially methylated region and the *ABCC8* gene, within the focal lesion of the pancreas. Sections A, B, C, D, and F all showed evidence of LOH for the maternal 11p15 allele (Fig. 1F) Using the information from all the histological samples and the data from the LOH studies for the maternal 11p15 allele, we were able to construct a model of the focal lesion as depicted in Fig. 1F. The whole pancreas was virtually composed of the focal lesion except a small segment of normal pancreatic tissue in the superior aspect of the head of the pancreas.

Discussion

These three cases illustrate the complex heterogeneity of focal CHI in terms of clinical presentation, histology, and imaging with [^{18}F]DOPA PET/CT scan. Typically focal CHI does not respond to treatment with diazoxide and requires surgery. The first two patients were heterozygous for the previously reported paternally inherited *ABCC8* splicing mutation, c.3992-9G→A. In both patients, a focal lesion was radiologically confirmed preoperatively by the [^{18}F]DOPA PET scan. Histologically, both focal lesions were approximately the same size (0.7 and 0.8 cm), but their location was different. However, their clinical response to treatment with diazoxide was very different with the first patient responding to a small dose of diazoxide, whereas the second patient had no response at all. These observations suggest that unknown genetic or environmental factors may be influencing the expression of the phenotype, and not all focal lesions present with severe medically unresponsive CHI.

Two frequent mutations in the *ABCC8* gene, c.3992–9G→A and p.F1388del, are associated with 88% of CHI in the Ashkenazi Jewish population (12). In this patient group with these two mutations, there is marked intra- and interfamilial phenotypic heterogeneity. This clinical heterogeneity may be due to modifying genes or other unknown factors that influence the expression of the CHI phenotype. Previous studies have documented that most Ashkenazi Jewish patients with CHI can be managed medically without the need for a pancreatectomy (16). Our study suggests that some patients of Ashkenazi Jewish origin with paternally inherited c.3992–9G→A mutation in the *ABCC8* gene might have mild focal CHI, thus explaining the response to medical therapy with diazoxide and or octreotide.

Typical focal forms of CHI are small (2–10 mm in diameter) and well circumscribed, and if completely resected surgically, the patient is cured. However, some focal forms

are not so well defined and have branches (or tentacles); yet others can be large in size (14, 15). The size of the focal lesion is determined by the timing of the paternal uniparental isodisomy event (15). In the first two patients, it is likely that the loss of maternal heterozygosity occurred late in gestation, whereas in the third patient, the loss of the maternal heterozygosity must have occurred earlier.

Patient 3 had a focal lesion occupying virtually the whole pancreas. The first [^{18}F]DOPA PET scan showed tracer uptake in the tail and body, but after surgical removal of the tail and body, there was marked uptake of [^{18}F]DOPA tracer in the remaining head region. It is unclear why the [^{18}F]DOPA tracer uptake in the head of the pancreas was suppressed during the first PET scan. The two different [^{18}F]DOPA PET scan findings point toward mechanisms that might result in incomplete tracer uptake in different regions of the pancreas in very large focal lesions.

The principle of the [^{18}F]DOPA PET scan depends on DOPA being taken up by the β-cells and being converted to dopamine by aromatic L-amino acid decarboxylase (AADC). Pancreatic β-cells have high AADC activity, but the role of this enzyme in insulin secretion is still unknown. Some studies have shown that L-DOPA accumulates within the β-cell secretory granules and inhibits insulin secretion (17, 18), yet other studies in patients with CHI have shown no effect on insulin secretion when administered an AADC inhibitor (19). However, there are no data to suggest that different regions of the pancreas take up DOPA at different rates.

In summary, focal lesions with the same genotype can have very different clinical presentations, and [^{18}F]DOPA PET scans may be difficult to interpret in patients with large focal lesions that occupy virtually the entire pancreas. More case studies of patients with large focal lesions are required to better understand the role of [^{18}F]DOPA PET in this group of patients.

Acknowledgments

Abbreviations

AADC	L-Amino acid decarboxylase
CHI	congenital hyperinsulinism
CT	computed tomography
DOPA	dihydroxyphenylalanine
LOH	lossof heterozygosity
PET	positron emission tomography

References

1. Rahier J, Guiot Y, Sempoux C. Persistent hyperinsulinaemic hypoglycaemia of infancy: a heterogeneous syndrome unrelated to nesidioblastosis. Arch Dis Child Fetal Neonatal Ed. 2000; 82 :F108–F112. [PubMed: 10685982]

2. Goossens A, Gepts W, Saudubray JM, Bonnefont JP, Nihoul-FeketeHeitz PU, Klöppel G. Diffuse and focal nesidioblastosis. A clinicopathological study of 24 patients with persistent neonatal hyperinsulinemic hypoglycemia. Am J Surg Pathol. 1989; 13 :766–775. [PubMed: 2669541]

3. Klöppel G, Reinecke-Lüthge A, Koschoreck F. Focal and diffuse β-cell changes in persistent hyperinsulinemic hypoglycemia of infancy. Endocr Pathol. 1999; 10 :299–304. [PubMed: 12114766]

4. Verkarre V, Fournet JC, de Lonlay P, Gross-Morand MS, Devillers M, Rahier J, Brunelle F, Robert JJ, Nihoul-Fékété C, Saudubray JM, Junien C. Paternal mutation of the sulfonylurea receptor (SUR1) gene and maternal loss of 11p15 imprinted genes lead to persistent hyperinsulinism in focal adenomatous hyperplasia. J Clin Invest. 1998; 102 :1286–1291. [PubMed: 9769320]

5. de Lonlay P, Fournet JC, Rahier J, Gross-Morand MS, Poggi-Tra-vert F, Foussier V, Bonnefont JP, Brusset MC, Brunelle F, Robert JJ, Nihoul-Fékété C, et al. Somatic deletion of the imprinted 11p15 region in sporadic persistent hyperinsulinemic is specific of focal adenomatous hyperplasia and endorses partial pancreatectomy. J Clin Invest. 1997; 100 :802–807. [PubMed: 9259578]

6. Damaj L, le Lorch M, Verkarre V, Werl C, Hubert L, Nihoul-Fékété C, Aigrain Y, de Keyzer Y, Romana SP, Bellanne-Chantelot C, de Lonlay P, et al. Chromosome 11p15 paternal isodisomy in focal forms of neonatal hyperinsulinism. J Clin Endocrinol Metab. 2008; 93 :4941–4947. [PubMed: 18796520]

7. Bellanné-Chantelot C, Saint-Martin C, Ribeiro MJ, Vaury C, Verkarre V, Arnoux JB, Valayannopoulos V, Gobrecht S, Sempoux C, Rahier J, Fournet JC, et al. ABCC8 and KCNJ11 molecular spectrum of 109 patients with diazoxide-unresponsive congenital hyperinsulinism. J Med Genet. 2010; 47 :752–759. [PubMed: 20685672]

8. Otonkoski T, Nänto-Salonen K, Seppanen M, Veijola R, Huopio H, Hussain K, Tapanainen P, Eskola O, Parkkola R, Ekström K, Guiot Y, et al. Non-invasive diagnosis of focal hyperinsulinism of infancy with [18F]-DOPA positron emission tomography. Diabetes. 2006; 55 :13–18. [PubMed: 16380471]

9. Masue M, Nishibori H, Fukuyama S, Yoshizawa A, Okamoto S, Doi R, Uemoto S, Tokumi T, Kasai T, Yorifuji T. Diagnostic accuracy of [18F]fluoro-L-DOPA PET scan for persistent congenital hyperinsulinisminJapan. ClinEndocrinol (Oxf). 2011; 75 :342–346.

10. Mohnike K, Blankenstein O, Christesen HT, De Lonlay J, Hussain K, Koopmans KP, Minn H, Mohnike W, Mutair A, Otonkoski T, Rahier J, et al. Proposal for a standardized protocol for 18F-DOPA-PET (PET/CT) in congenital hyperinsulinism. Horm Res. 2006; 66 :40–42. [PubMed: 16710094]

11. Ismail D, Smith VV, de Lonlay P, Ribeiro MJ, Rahier J, Blankenstein O, Flanagan SE, Bellanné-Chantelot C, Verkarre V, Aigrain Y, Pierro A, et al. Familial focal congenital hyper-insulinism. J Clin Endocrinol Metab. 2011; 96 :24–28. [PubMed: 20943779]

12. Nestorowicz A, Wilson BA, Schoor KP, Inoue H, Glaser B, Landau H, Stanley CA, Thornton PS, Clement JP 4th, Bryan J, Aguilar-Bryan L, et al. Mutations in the sulonylurea receptor gene are associated with familial hyperinsulinism in Ashkenazi Jews. Hum Mol Genet. 1996; 5 :1813–1822. [PubMed: 8923011]

13. Kassem SA, Ariel l, Thornton PS, Hussain K, Smith V, Lindley KJ, Aynsley-Green A, Glaser B. p57(KIP2) expression in normal islet cells and in hyperinsulinism of infancy. Diabetes. 2001; 50 :2763–2769. [PubMed: 11723059]

14. Suchi M, MacMullen CM, Thornton PS, Adzick NS, Ganguly A, Ruchelli ED, Stanley CA. Molecular and immunohistochemical analyses of the focal form of congenital hyperinsulinism. Mod Pathol. 2006; 19 :122–129. [PubMed: 16357843]

15. Giurgea I, Sempoux C, Bellanné-Chantelot C, Ribeiro M, Hubert L, Boddaert N, Saudubray JM, Robert JJ, Brunelle F, Rahier J, Jaubert F, et al. The Knudson's two-hit model and timing of somatic mutation may account for the phenotypic diversity of focal congenital hyperinsulinism. J Clin Endocrinol Metab. 2006; 91 :4118–4123. [PubMed: 16882742]

16. Glaser B, Hirsch HJ, Landau H. Persistent hyperinsulinemic hypoglycemia of infancy: long-term octreotide treatment without pancreatectomy. J Pediatr. 1993; 123 :644–650. [PubMed: 8410523]

17. Raffo A, Hancock K, Polito T, Xie Y, Andan G, Witkowski P, Hardy M, Barba P, Ferrara C, Maffei A, Freeby M, et al. Role of vesicular monoamine transporter type 2 in rodent insulin secretion and glucose metabolism revealed by its specific antagonist tetrabenazine. J Endocrinol. 2008; 198 :41–49. [PubMed: 18577569]

18. Ericson LE, Håkanson R, Lundquist I. Accumulation of dopamine in mouse pancreatic B-cells following injection of l-DOPA Localization to secretory granules and inhibition of insulin secretion. Diabetologia. 1977; 13 :117–124. [PubMed: 404204]

19. de Lonlay P, Simon-Carre A, Ribeiro MJ, Boddaert N, Giurgea I, Laborde K, Bellanné-Chantelot C, Verkarre V, Polak M, Rahier J, Syrota A, et al. Congenital hyperinsulinism: pancreatic [18F] fluoro-L-dihydroxyphenylalanine (DOPA) positron emission tomography and immunohistochemistry study of DOPA decarboxylase and insulin secretion. J Clin Endocrinol Metab. 2006; 91 :933–940. [PubMed: 16403819]
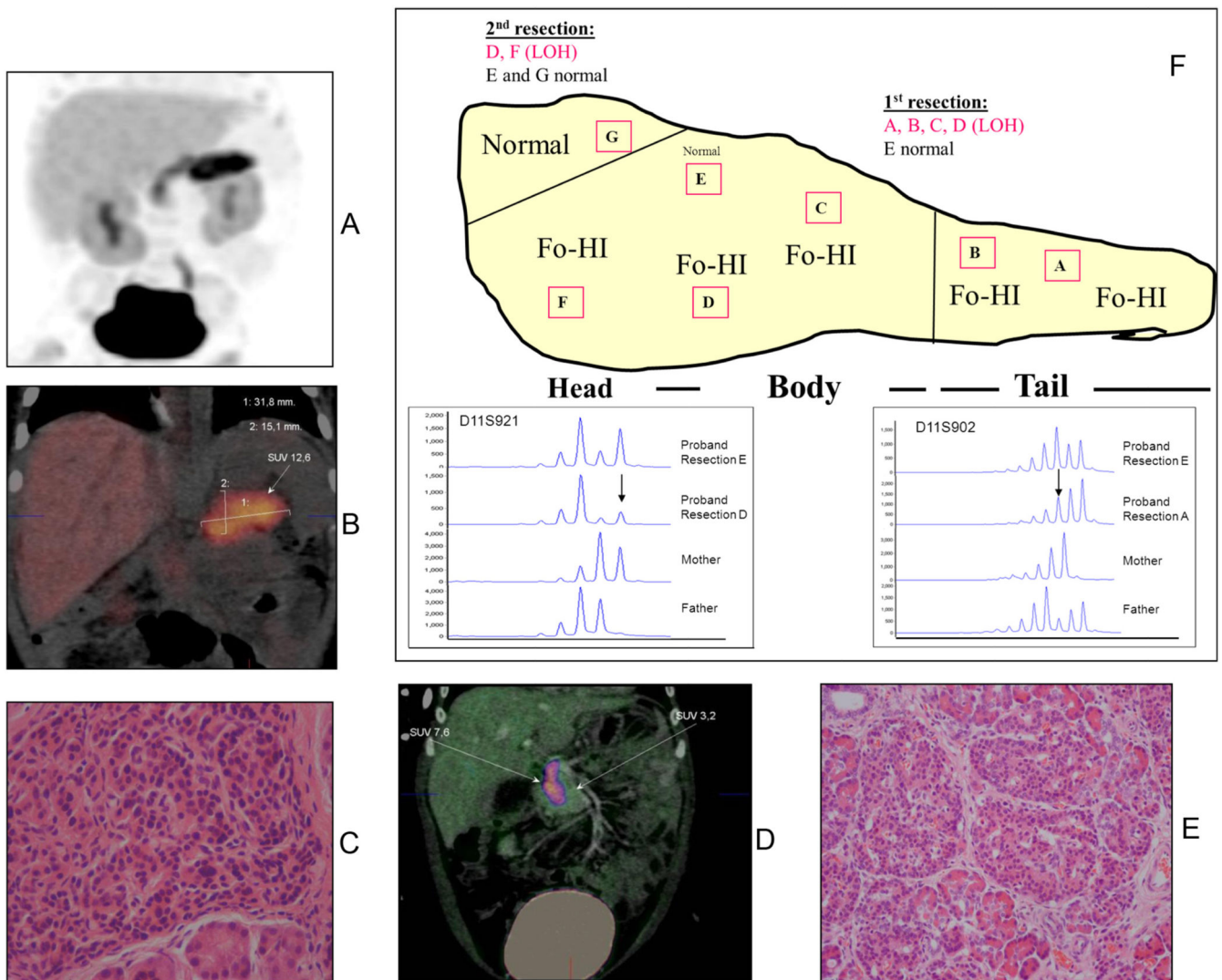
Fig. 1.
Panels A and B, First [18F]DOPA PET/CT scan showing uptake of [18F]DOPA in tail and body of pancreas in patient 3. Panel C, Hematoxylin and eosin staining of the resected pancreas showing histological changes of focal disease. Panel D, Second [18F]DOPA PET/CT scan now showing uptake of [18F]DOPA in remaining head of pancreas in patient 3. Panel E, Hematoxylin and eosin staining of the resected pancreatic head region with histological changes of focal CHI. Panel F, Reconstruction of the extent of the focal lesion based on regions of abnormal histology and loss of maternal heterozygosity for 11p15 in patient 3. Electropherograms demonstrating the result of microsatellite analysis for two of the informative markers on chromosome 11, D11S902 and D11S921, are provided for the proband and her parents. Alleles of the same size (base pairs) occur at the same position on the x-axis. The y-axis indicates the product quantity (arbitrary units). The results for the markers amplified from unaffected tissue (resection E) show normal biparental inheritance as demonstrated by the preferential amplification of the smaller product. In contrast, the

results for the markers amplified from affected tissue (resections D and A) illustrate LOH for the maternal allele (indicated by *arrow*). Fo-HI, Focal hyperinsulinism.

Table 1

Summary of the clinical, genetic, and biochemical aspects of the patients

	Patient 1	Patient 2	Patient 3
Gestational age (wk)	39	39	40
Birth weight (kg)	4.3	5.09	4.8
Age at presentation (h)	14	1	5
Symptoms of hypoglycemia	Lethargy and floppiness	Seizures and cyanosis	Seizures
Maximum glucose infusion rate (mg/kg · min)	11	20	21
Blood glucose (mmol/liter)	2.6	1.6	1.1
Insulin (mU/liter)	3.4	10	126
Nonesterified fatty acids (mmol/liter)	<0.05	<0.05	<0.05
Ketone bodies (mmol/liter)	<0.05	<0.05	<0.05
Diazoxide response	Yes (5 mg/ kg · d)	No	No
ABCC8 gene mutation	c.3992-9G→A	c.3992-9G→A	c.4477G→A