Morphological Mosaicism of the Pancreatic Islets: A Novel Anatomopathological Form of Persistent Hyperinsulinemic Hypoglycemia of Infancy

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Background: Morphological studies of the pancreas in persistent hyperinsulinemic hypoglycemia of infancy (PHHI) have focused on the diagnosis of focal *vs.* diffuse forms, a distinction that determines the optimal surgical management. *ABCC8* or *KCNJ11* genomic mutations are present in most of them.

Aim: Our aim was to report a new form of PHHI with peculiar morphological and clinical characteristics.

Research Design and Methods: Histopathological review of 217 pancreatic PHHI specimens revealed 16 cases morphologically different from diffuse and focal forms. They were analyzed by conventional microscopy, quantitative morphometry, immunohistochemistry, and *in situ* hybridization.

Results: Their morphological peculiarity was the coexistence of two types of islet: large islets with cytoplasm-rich β-cells and occasional enlarged nuclei and shrunken islets with β-cells exhibiting little cytoplasm and small nuclei. In small islets, β-cells had abundant insulin content but limited amount of Golgi proinsulin. Large islets had low insulin storage and high proinsulin production and were mostly confined to a few lobules. No evidence for K_{ATP} channels involvement or 11p15 deletion was found. Genomic mutations for *ABCC8*, *KCNJ11*, and *GCK* were absent. Patients had normal birth weight and late hypoglycemia onset and improved with diazoxide. Ten were cured by limited pancreatectomy. Six recurred after surgery and were medically controlled.

Conclusion: This new form of PHHI is characterized by a morphological mosaicism. Pathologists should recognize this mosaicism on intraoperative frozen sections because it is often curable by partial pancreatectomy. The currently unknown genetic background does not involve the classical genomic mutations responsible for diffuse and focal PHHI. **(*J Clin Endocrinol Metab* 96: 3785–3793, 2011)**

Persistent hyperinsulinemic hypoglycemia of infancy (PHHI), a syndrome that was first recognized by McQuarrie (1), has been repeatedly attributed to nesidioblastosis, a particular histological feature that is characterized by the presence of numerous small clusters of endocrine cells in the vicinity of the pancreatic ducts (2). Nesidioblastosis was considered a permanent β-cell proliferation, leading to an increased β-cell mass that was responsible for

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Abbreviations: H&E, Hematoxylin and eosin; ISH, *in situ* hybridization; LOH, loss of heterozygosity; PET, positron emission tomography; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; PVS, pancreatic venous sampling; SUR1, sulfonylurea receptor 1.

hyperinsulinism. However, the proliferation index and the fractional volume of β-cells are not higher in the pancreas of hypoglycemic infants than in controls, and nesidioblastosis is not specific to the disease in that it is also observed in the pancreas of normoglycemic neonates and infants (3, 4).

Since the 1990s, histological retrospective analyses of case series led several groups to characterize two forms of PHHI: the focal and the diffuse forms (3–7). In the first form, a small focal endocrine lesion, often invisible to the naked eye, is responsible for insulin hypersecretion. This lesion is an adenomatous hyperplasia made from the confluence of histologically roughly normal islets with few non-β-cells at the periphery of a β-cell central core. Abnormal, large β-cell nuclei are often observed within the lesion but are not seen outside of the focal lesion where the islets exhibit small nuclei and less cytoplasm, with evidence of low proinsulin synthesis. This focal form is curable by a partial pancreatectomy restricted to the lesion. The second form is diffuse and involves the whole pancreas. Its histological hallmark is the presence of abnormally large β-cell nuclei scattered throughout most islets (3–6). In both forms, infants have a high birth weight, hypoglycemia occurs in the neonatal period, and diazoxide is nearly always ineffective (8, 9). Neither clinical nor biological data can be used to differentiate the focal from the diffuse forms. To distinguish the two forms, portal venous catheterization with selective venous sampling for insulin measurements (10) and more recently noninvasive imaging using positron emission tomography (PET) with [^{18}F]fluoro-L-DOPA (^{18}F-PET) are useful (11, 12). Intraoperative frozen sections are essential, not only to confirm the diagnosis made based on imaging but also to determine whether the suspected area effectively corresponds to a focal lesion and is completely resected (13).

The genetic cause of many cases has been identified. In most diffuse forms, hyperinsulinism is due to inactivating mutations in *ABCC8* or *KCNJ11*, which are located on chromosome 11p15 and code for the sulfonylurea receptor 1 (SUR1) and Kir6.2 proteins, respectively, the two subunits of ATP-sensitive K (K_{ATP}) channels, resulting in abnormal channels (14, 15). In the focal forms, in addition to a paternally inherited inactivating mutation in *ABCC8* or *KCNJ11*, there is a loss of heterozygosity in the 11p15 region, leading to the occurrence of focal adenomatous hyperplasia because of an imbalance between imprinted genes involved in cell proliferation (*H19* and *IGF2*) and a loss of the antiproliferative factor p57 (16–20). Infrequent cases of congenital hyperinsulinism unrelated to K_{ATP} channels have also been reported; most of these cases were sensitive to diazoxide. These cases may be due to dominant activating mutations of the *GLUD1* gene, which codes for glutamate dehydrogenase (21, 22), or due to activating mutations of *GCK*, which codes for glucokinase (23, 24); these are two enzymes that are involved in the metabolic control of insulin secretion. Loss-of-function mutations in the *HADH* gene and mutations in *HNF4α* can also produce infantile hyperinsulinism (25–29). The rarity of these forms, which are unrelated to defects of the K_{ATP} channels, explains why exhaustive descriptions of their pathological characteristics are limited.

Among the 217 cases of operated PHHI that we collected over 25 yr, 16 cases (7.4%) did not fit with the usual types. They displayed peculiar pancreatic morphologies and had a distinct clinical presentation, with normal birth weight in the majority of the cases, a late onset of hyperinsulinism, and a relative sensitivity to diazoxide. Furthermore, when genetic analyses were performed in these infants (n = 13), genomic mutations of *ABCC8*, *KCNJ11*, and *GCK* were ruled out, and no similar peculiar pathologies were found in cases with similar mutations. The aim of the present study is to report the histological characteristics of these patients to permit intraoperative recognition and diagnosis of this particular form of PHHI that can often be cured by a limited pancreatectomy.

Materials and Methods

On the basis of their specific histological features alone, we identified 16 cases of the 217 PHHI pancreases collected during surgery in several European hospitals since 1976. This study was approved by the ethical committee of the Université Catholique de Louvain.

Whenever possible and after obtaining the written informed consent of the parents for the genetic testing of their children, genetic analyses were performed to search for *ABCC8*, *KCNJ11*, or *GCK* genomic mutations. The genetic results of four of these 16 patients have recently been published in a large series of 109 patients (30); this previous publication evaluated the incidence and spectrum of *ABCC8* and *KCNJ11* mutations in this cohort of PHHI.

Diagnoses of PHHI were made according to the clinical criteria previously reported (8). The infants were considered to be or to become nonresponsive to diazoxide therapy when 10 mg/kg · d was insufficient to avoid hypoglycemic events (8).

Preoperative radiological evaluation was performed by pancreatic venous sampling (PVS) (10) in 14 cases, by ^{18}F-PET (12, 13, 31) in one case, and with both in the last case.

All samples of partial pancreatectomies were fixed in 4% formalin to study SUR1, Ki67, and p57 and to perform molecular biology investigations, including *in situ* hybridization (ISH) for proinsulin mRNA and/or in Bouin's solution for conventional microscopy on hematoxylin and eosin (H&E)-stained sections and the immunostaining of insulin, proinsulin, somatostatin, and glucagon.

The anomalies were sometimes very small, being limited to one or a few pancreatic lobules and were not detectable with the naked eye. Moreover, the areas of interest were sometimes fixed only in Bouin's solution. All of the analyses were thus not performed in all of the cases but were performed according to the adequacy of the available material.

The sizes of the β-cell nuclei and the β-cell nuclear crowding (number of β-cell nuclei per 1000 μm^2 of β-cell cytoplasm) were

measured in both types of islet (at least 10 islets in each case) according to the method previously reported (6) The area of these islets was measured and used to evaluate the differences between the types of islet (Axiovision, Zeiss, Germany).

Immunoperoxidase staining was performed to detect insulin, proinsulin, and somatostatin as well as SUR-1, Ki67, and p57. The source of the antibodies and their dilution for use are given in Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Quantifications of Golgi proinsulin (diaminobenzidine) and somatostatin cells (Fast Red) were performed on paraffin sections. The total immunostained area of each islet was measured, and the coordinates of each islet were stored by an image analyzer (KS-400 system; Zeiss-Vision, Munich, Germany). On the same slides, insulin was further detected (Fast Red), and the total immunostained area was measured again. After removal of the Fast red staining by washing in alcohol, the Golgi proinsulin area was measured, and the areas of somatostatin (δ-cell area) and insulin (β-cell area) immunolabeling were calculated. Then, the Golgi proinsulin-to-cytoplasmic insulin area ratio that reflects the β-cell synthesis function was calculated as previously reported (18) and related to the δ-cell-to-β-cell area ratio. Islet sections (n = 220) from six age-matched normoglycemic controls, born to nondiabetic mothers and dead from a disease not related to the endocrine pancreas, were compared with 300 islets from six infants with islet mosaicism.

The insulin content of β-cells in both types of islet was quantified on paraffin sections by measuring the specific absorbance (OD) on 10 islets of each type after immunostaining for insulin according to a method of immunodensitometry previously reported (32).

ISH to detect proinsulin mRNA was performed with a fluorescein isothiocyanate-labeled probe (final dilution 1:2; NCL-Proins, Novocastra, UK).

For microsatellite analysis, normal and hyperfunctional islets were microdissected from paraffin sections of three cases (cases 8, 12, and 15; Table 1) using a PALM MicroLaser Systems pulsed laser (337 nm; Bernied, Germany) coupled to an Axiovert 200 microscope (Zeiss, Oberkochen, Germany). Although the morphology was maintained using formalin, every sixth section from the consecutive sections was stained with H&E to confirm the identification of normal and hyperfunctional islet areas. A Picopure DNA extraction kit (Arcturus Bioscience, Mountain View, CA) was used to extract DNA from microdissected tissues following the manufacturer's recommendations. Loss of heterozygosity (LOH) was studied with a selection of markers located in the 11p15 region, from telomere to centromere (NCBI database): D11S4046 (0.8 Mb), D11S2351 (3.9 Mb), D11S902 (16.3 Mb), and D11S4114 (19.5 Mb), as described previously (18). PCR products were ana-

TABLE 1. Clinical data

Case	Sex	Birth weight (percentile)	Age at presentation (months)	Preop Dzx treatment (mg/kg · d)	Localization (PVS or ^{18}F-PET)	Surgery	Follow-up
1	F	46	3	6.5	Inconclusive (PVS)	Partial tail	Recurrence controlled by low-dose Dzx
2	F	78	6	10	Focal-corpus (PVS)	Partial corpus and tail	Cured
3	M	94	8	10	Focal (PVS)	Partial corpus and tail	Cured
4	F	4	5	12	Diffuse (PVS)	Partial corpus and tail	Cured
5	F	64	6	15	Focal-tail (PVS)	Partial tail	Cured
6	F	69	3	Sensitive to Dzx	Focal (PVS)	Partial corpus and tail	Cured
7	M	79	4	Transient sensitivity	Tail (PVS)	Tail	Cured
8	M	48	5	15	Focal-head (PVS)	Partial	Recurrence controlled by low-dose Dzx
9	M	7	9	10	Focal tail (PVS)	Partial tail	Cured
10	M	40	5.5	12	Focal tail (PVS)	Partial tail	Recurrence controlled by low-dose Dzx
11	F	51	6	6	Focal tail (PVS)	Partial corpus and tail	Cured
12	F	86	NN	Transient sensitivity	Focal corpus (PVS)	Partial corpus and tail	Cured
13	M	48	4	18	Focal corpus and tail (PVS)	Partial corpus and tail	Cured
14	F	90	NN	15	Inconclusive (PVS)	Head corpus and tail	Recurrence controlled by low-dose Dzx
15	F	Unknown	6	Sensitive to Dzx	Inconclusive (^{18}F-PET)	Partial head and isthmus	Recurrence controlled by low-dose Dzx
16	M	45	7	Transient sensitivity	Inconclusive (PVS and ^{18}F-PET)	Corpus and tail	Recurrence controlled by low-dose Dzx

Cases 4, 6, and 13 were not tested for *ABCC8*, *KCNJ11*, and *GCK* mutations. Mutations in the *GDH* gene have been ruled out in patients 7, 10, and 14. Dzx, Diazoxide; F, female; M, male; Preop, preoperative.

lyzed using ABI Genescan Software. Allelic loss was scored if the area under the allelic peak was reduced by a minimum of 50%. A previously tested focal lesion served as the positive control (case number 7 from Ref. 18).

The statistical evaluation of the results was conducted using the Fisher's exact test for the differences in proportions of infants with high birth weight and by Wilcoxon signed-rank test for all of the other parameters.

Results

Clinical data

The clinical data of the 16 atypical cases retrieved from the patient files are detailed in Table 1. In most of the cases (14 of 16), the hypoglycemic events occurred later in life (median 165 d, range 1–270 d) than those in the classical focal and diffuse forms, which are almost always diagnosed in the neonatal period (8, 9). The birth weights expressed in percentiles were lower than in the forms previously reported (z score = +0.04 *vs.* +1.36). Only one patient (6%) had a percentile higher than 90% *vs.* 46% in a series of 52 classical PHHI cases reported by de Lonlay-Debeney *et al.* (8); this proportion of infants was significantly lower than that previously reported (8) ($P < 0.001$, Fisher's exact test). At the onset of disease, all of the infants were responsive to diazoxide at least for a short period of treatment. Later, the sensitivity to the drug decreased, and the infants required progressive and continuous increasing of the dose, which, in certain infants, finally exceeded the 10 mg/kg · d that is characterized for diazoxide sensitivity (8). Although they experienced marked improvement upon diazoxide treatment, most infants remained prone to occasional hypoglycemic events. This is strikingly different from the classical focal and diffuse forms that are related to K_{ATP} channelopathies, which are almost always insensitive to diazoxide from birth (8, 9). Insulin levels after PVS or ^{18}F-PET were suggestive of focal lesions in 11 cases, diffuse lesions in one case, and inconclusive or difficult to interpret in four other cases.

For one or several reasons (persistence of hypoglycemic events, progressive loss of diazoxide sensitivity, or major hypertrichosis and /or because PVS was suggestive of a localized form), the patients finally underwent surgery. After partial pancreatectomy, 10 were definitively cured. In the six others, hypoglycemic events reoccurred in the first days or weeks after surgery and were medically treated (Table 1).

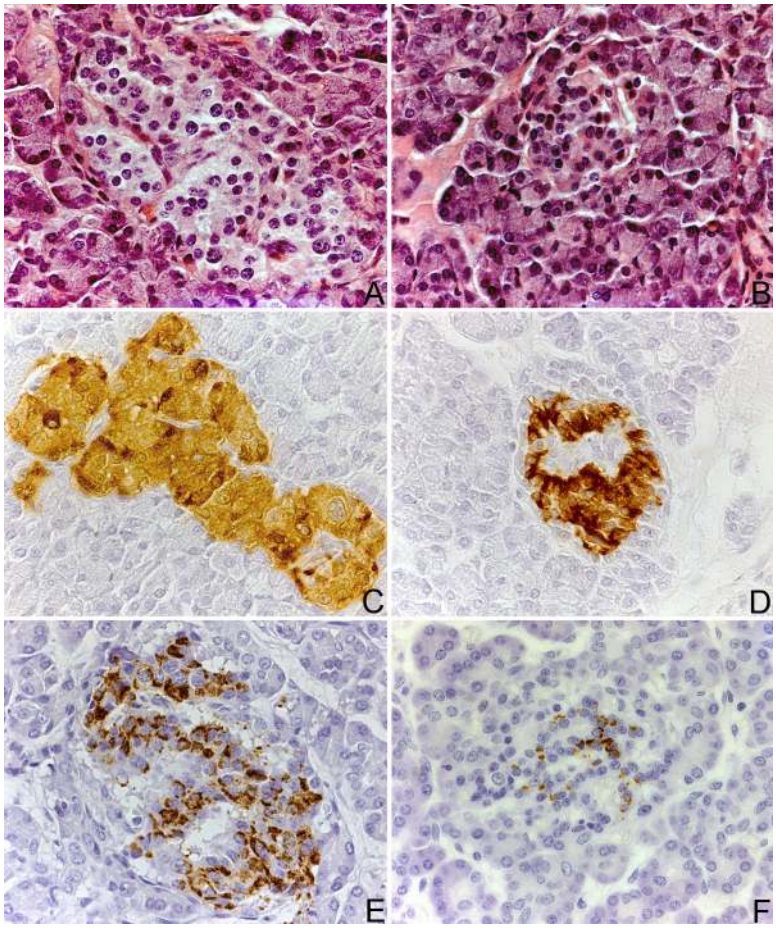

FIG. 1. Islet mosaicism. A and B, On H&E-stained sections, the typical feature of this new form of PHHI was the presence of both large hyperplastic islets with cytoplasm-rich β-cells exhibiting some enlarged nuclei (A) as well as small shrunken islets that are 2-fold smaller (B); C and D, after the immunodetection of insulin, the large islets showed a faint diffuse homogeneous staining (C), whereas the small islets had strong polarized labeling (D); E, the staining for proinsulin was stronger in large islets, suggesting a high level of function; F, this staining was limited to a small dot in the small shrunken islets.

Morphology

Conventional microscopy

Macroscopically, the pancreas appeared normal, and no lesions were identified macroscopically by the surgeon during surgery. Several biopsies (from four to 20) were thus taken by the surgeon in the area suspected by PVS or ^{18}F-PET or in different areas of the gland when the preoperative localization techniques were inconclusive, and frozen sections were made. After recognition of the abnormal area, biopsies were taken to ensure that the limits of the resection were healthy. The typical characteristic of the lesions, already recognizable on frozen sections, were confirmed after H&E staining; these typical characteristics were the coexistence of two strik-

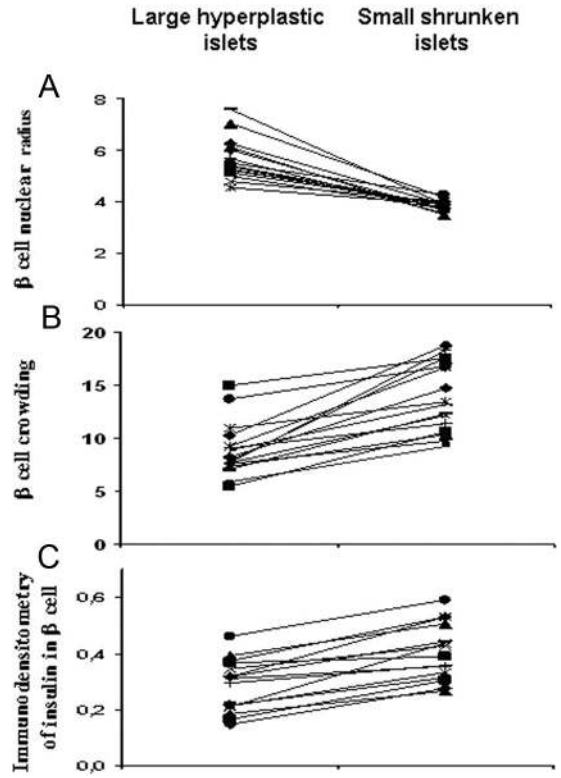

FIG. 2. Nuclear radius, nuclear crowding, and densitometry of insulin. The *two symbols linked by a line* represent the mean value of the parameter measured in large hyperplastic islets (*left*) and in small shrunken islets (*right*) in the same patient. A, Mean radius of 50 selected β-cell nuclei (micrometers) in large hyperplastic and small shrunken islets; B, β-cell nuclear crowding (number of β-cell nuclei/1000 μm^2 of β-cell cytoplasm); C, specific absorbance (OD) of insulin labeling (immunodensitometry); means of 10 islets in each islet type and patients.

ingly different types of islet, resulting in a mosaic pattern (Fig. 1, A–F).

Morphometry

Profiles from islets of the first type were 2.06-fold larger than the second type of islet, with a mean value of 11,407 μm^2 ($P < 0.01$). They contained numerous β-cells with abundant cytoplasm and sometime large nuclei. However, these nuclei were rarely as large as those in the classical diffuse form of the disease (Fig. 1A). The radius of the β-cell nuclei and the β-cell crowding have been evaluated on both types of islet (Fig. 2) The β-cell nuclear mean radius was constantly larger in the hyperplastic type 1 islets than in the small type 2 islets (5.66 ± 0.83 *vs.* 3.85 ± 0.21 μm, $P < 0.01$) (Fig. 2A). The β-cell nuclear crowding was higher in small type 2 than in hyperplastic type 1 islets (13.91 ± 3.27 *vs.* 8.88 ± 2.56) (Fig. 2B). The hyperplastic type 1 islets were not observed throughout the whole pancreas but were confined to one or some adjacent lobules of the pancreas (Fig. 3). Certain lobules contained only this type of islet, but other lobules showed both types of islet. The islets of the second type were small and appeared

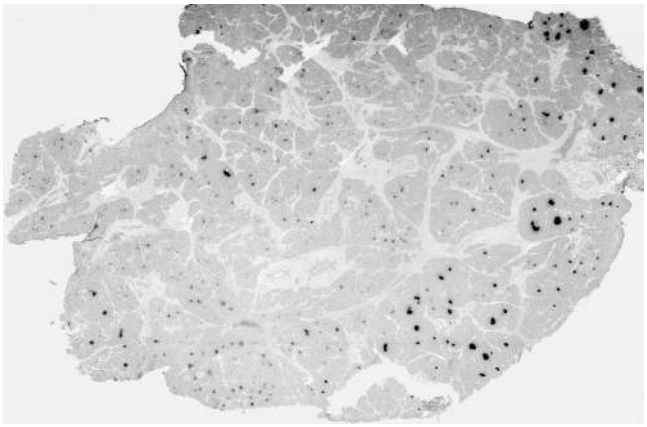

FIG. 3. Lobular distribution of large hyperplastic islets. At low magnification, the lobular topography of hyperplastic-hyperactive islets was already recognizable (proinsulin immunodetection).

hypotrophic. They contained β-cells with scanty cytoplasm and small nuclei and appeared suppressed (Fig. 1B). This second type of islet was distributed throughout the whole pancreas.

Immunocytochemistry, ISH, and microsatellite analysis

Immunocytochemistry also revealed striking differences between both types of islet (Fig. 1, C–F). In the first type (large islets), the outlines of the islets labeled with insulin antibody were very regular, and the cytoplasm of the β-cells was only faintly stained at a very uniform level (Fig. 1C), whereas the proinsulin antibody gave a strong labeling, which marked a large Golgi area (Fig. 1E). The insulin mRNA content, which was demonstrated by ISH, was also abundant, and only a few somatostatin and glucagon cells were present at the periphery of these islets (Fig. 4A). In the second type of islet (small shrunken islets), the staining with the antiinsulin antibody was strong, and the outlines of the islets were irregular (Fig. 1D), but the proinsulin antibody labeled only a small dot corresponding to the Golgi area (Fig. 1F). Insulin mRNA labeling was slightly lower in the islets, and the somatostatin and glucagon cells were much more numerous than in the first type of islet (Fig. 4B).

p57 was present in both types of islet (Fig. 4, C and D), and SUR1 immunolabeling had a similar pattern and intensity of staining in both types of islet, appearing cytoplasmic with a perimembranous reinforcement (Fig. 4, E and D).

The intensity of the insulin immunolabeling was measured in both types of islet by immunodensitometry. The OD of insulin labeling was always higher in small suppressed islets than in hyperplastic-hyperactive islets ($0.403 + 0.101$ *vs.* $0.288 + 0.093$, $P < 0.01$) (Fig. 2C).

The proinsulin in the Golgi of β-cells, a reflection of β-cell proinsulin synthesis, was measured in the islets from normoglycemic patients ($n = 6$) and from six cases with

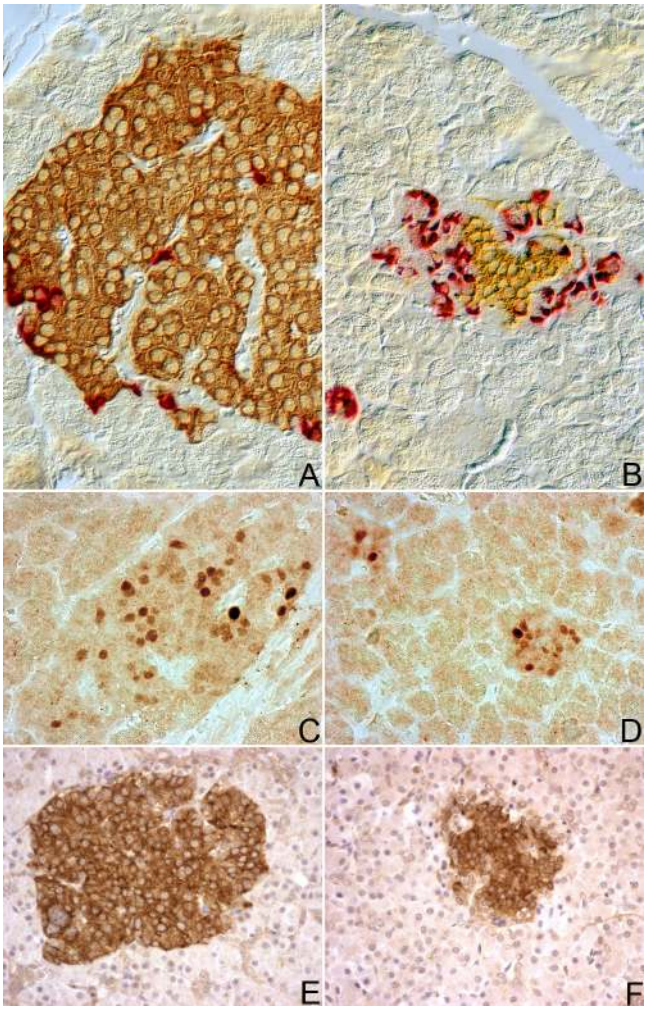

FIG. 4. Insulin mRNA and somatostatin cells, p57, and SUR1. A and B, Insulin mRNA and somatostatin cells in large hyperplastic islets (A) and in small shrunken islets (B). Insulin mRNA labeling was darker in the large hyperplastic islets, whereas δ-cells were less numerous (ISH/immunohistochemistry, differential interference contrast of Nomarski). C and D, p57 immunodetection in large hyperplastic islets (C) and in small shrunken islets (D). There were no differences in the numbers of positive cells or in the intensities of the labeling. E and F, SUR1 immunodetection showed no difference between the large hyperplastic islets (E) and the small shrunken islets (F).

islet mosaicism, and these measurements were compared with the amount of δ-cells in these islets. For this purpose, the δ-cell-to-β-cell area ratio (area percentage) was measured in each individual islet and related to the proinsulin in the Golgi area (expressed as proinsulin-to-β-cell area percentage) in the same islets. In the controls (Supplemental Fig. 1A), the δ-cell-to-β-cell area ratio varied from 2.4–119.0%. The Golgi proinsulin-to-insulin area ratio showed weak variations (0–3.3%). By contrast, in hypoglycemic infants with islet mosaicism (Supplemental Fig. 1B), the δ-cell-to-β-cell area ratio markedly varied within the islets (from 0–250%) as did their Golgi proinsulin to insulin area (from 0–21.6%). In this series of infants, many islets with low δ-cell-to-β-cell area ratios had large Golgi proinsulin areas, whereas other infants with high δ-cell-to-β-cell area ratios had normal or small Golgi proinsulin areas (the small and shrunken islets).

The proliferation index of β-cells was counted in both types of islet and was found to range 1.12–1.18% of β-cells in suppressed and hyperplastic islets (not significant).

Because the purity of the cell population was critical in LOH detections, both types of islet were microdissected from the same histological sections, and microsatellite analysis was performed separately in the three cases (cases 8, 12, and 15). None of these showed an 11p15 LOH involving imprinted genes in 11p15.5 and *ABCC8*, *IGF2* or *H-19*, and *KCNJ11* (Fig. 5). In patient 8, one marker was not informative (D11S902); in patient 12, the four markers were informative; and in patient 15, one marker was not informative (D11S4114). To confirm that the laser microdissection did not reduce the sensitivity for the detection of LOH in small microdissected tissues by microsatellite analysis, a similar procedure was applied in a case of focal adenomatous hyperplasia, and this procedure confirmed the 11p15 deletion that was previously demonstrated (case number 7 from Ref. 18) (Fig. 5).

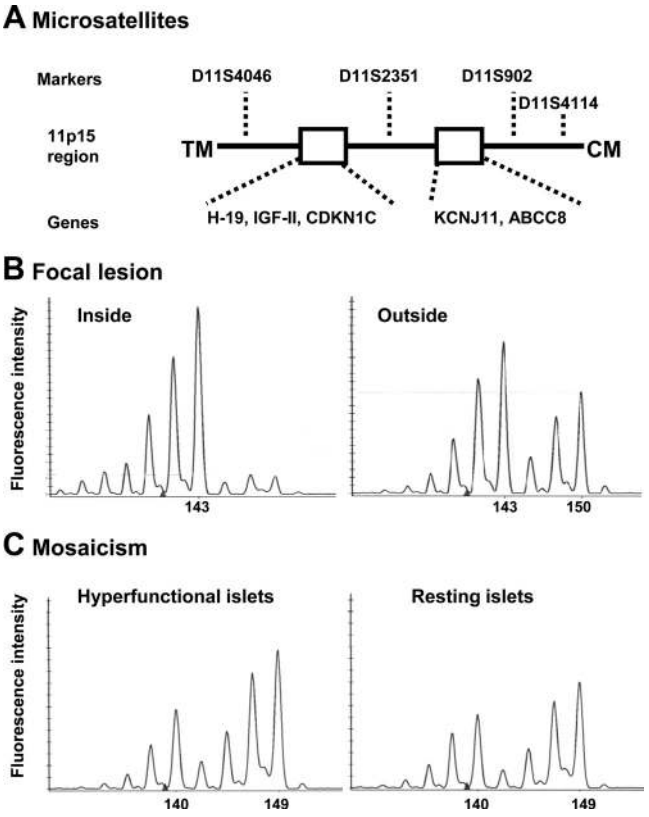

FIG. 5. Microsatellite analysis. A, Markers used and their distribution in the 11p15 region; B and C, tracing corresponding to marker D11S2351. In contrast to a classical K_{ATP}-related focal lesion used as positive control (B), no LOH was observed in patients with histological mosaicism (C, case 8). TM, Telomere; CM, centromere.

Discussion

From our careful review of the series of 217 PHHI patients, we identified 16 cases (7.4%) with a unique pancreatic histology characterized by the coexistence of two different types of islet, a striking mosaic aspect never described in normoglycemic infants or in infants suffering from the diffuse form of PHHI related to *ABCC8* or *KCNJ11* mutations; unlike those with the mosaicism, these pancreases have islets that have the same appearance, with numerous small poorly formed islets and large β-cell nuclei and cytoplasm (3, 5, 6). The novel mosaic pattern differs from that of the focal form because of the absence of adenomatous hyperplasia, but the islets of the second type (with small nuclei and reduced cytoplasm) resemble the normal resting islets located outside the focus of insulin hypersecretion (3, 5, 6, 7, 18).

In the mosaic forms that we analyzed, the hyperactive islets were located in one or some adjacent lobules. This focal distribution explains why many cases were cured by partial pancreatectomy.

The distinct aspects of the two types of islet suggest major functional differences. The islets of the first type contain β-cells with large nuclei and cytoplasm, characteristic of hyperactive β-cells. Their proinsulin antibody-labeled Golgi were large, reflecting a high rate of hormone synthesis, as confirmed by a stronger labeling for insulin mRNA by ISH. By contrast, the faint labeling of the antiinsulin antibody suggested that insulin is not stored for very long but is rapidly released. The islets of the second type showed the opposite characteristics: small nuclei, shrunken cytoplasm, and proinsulin-stained Golgi area restricted to a punctate labeling but strong antiinsulin antibody staining, indicating that these β-cells are resting with a low production of proinsulin but a high storage of insulin. This morphological aspect suggested that persistent hypoglycemia inhibits insulin secretion and synthesis in these β-cells, which implies that they remain normally regulated.

In most cases, hyperactive islets contain only a few δ-cells compared with resting islets. Our quantitative analysis of the mosaic cases indicated that proinsulin synthesis is higher in β-cells from islets with a low δ-cell-to-β-cell area ratio. The absence of δ-cells may have a role in infantile hyperinsulinism (33) but has never been demonstrated in the islets of K_{ATP} hyperinsulinemic infants. The meaning of the decrease in δ-cell number in the hyperactive islets of mosaic cases remains unclear. Suggested previously but not yet demonstrated (34), intra-insular somatostatin secretion may play a role in the control of insulin secretion. Furthermore, because the low δ-to-β ratio also observed in certain islets from normoglycemic controls was not linked to proinsulin overproduction in these controls, it is unlikely that the hyperproduction of proinsulin observed in hyperactive islets is caused by a relative lack of δ-cells.

A lack of expression of the antiproliferative factor p57 has been implicated in the hyperplasia of classic focal lesions (17). Obvious expression of p57 in hyperfunctional as well as resting islets of the mosaic form clearly indicates that 11p15 LOH is not the cause of the lesion. This observation was confirmed in three cases using microsatellite analysis. This new mosaic form of PHHI is thus distinct and unrelated to the focal form previously described. Large nuclei in the hyperactive islets should not be mistaken for a diffuse pathology (which would lead to aggressive management) because they are restricted to a few lobules and are mixed with small resting islets, a feature never observed in the diffuse form of PHHI.

Interestingly, the clinical characteristics of these patients are also different from those of diffuse or focal PHHI patients. Birth weight, corrected for gestational age and sex, was indeed lower than that of infants suffering from diffuse or focal K_{ATP} channelopathy (8, 9). This could suggest that the disease was not present before birth or did not cause major hyperinsulinism *in utero*. The observation that the first symptoms of hypoglycemia occurred or were recognized only later in life in most cases reinforced this interpretation. This late onset may also result from the small size of the lesion or from transient compensation by physiological adaptive mechanisms.

The patients with mosaic histology shared some clinical characteristics with infants presenting a loss-of-function mutation of the *HADH* gene: a later onset in comparison with K_{ATP}-deficient cases and a certain sensitivity to diazoxide. Although we cannot definitely rule out a role for the *HADH* gene for now, no particular protein sensitivity has been found in our mosaic cases, and the decrease in diazoxide sensitivity with time that we observed has not been reported in *HADH*-mutated patients.

In this series, 10 infants were definitively cured by a partial pancreatectomy, but six were not cured. This could suggest that their lesion was not localized or less localized than expected and incompletely resected. In these six cases, the observation of pancreatic lobules that contain only healthy islets means that the lesion sparing at least some lobules is thus a true localized lesion and does not correspond to a classical diffuse form. It is likely that the extension of the lesion, and then the difficulty of its resection, varies according to the chronology of the somatic mutation occurrence.

When genetic analyses were performed, genomic *ABCC8* or *KCNJ11* mutations were not found, indicating that this mosaic form was also genetically different from

those related to K_{ATP} channel abnormalities. This is in agreement with the persistence of a response (although incomplete) to diazoxide in these cases as well as with the normal and similar SUR1 immunohistochemical expression we found in both types of islet. The observation of this insular mosaicism, together with the lobular distribution of the abnormalities, could be explained by the occurrence of specific somatic mutations during the embryonic development of the endocrine pancreas. These mutations have so far not been identified in this series.

Rare cases of PHHI with histology that did not fit with the classical description of focal or diffuse forms have already been reported (35, 36). Whether the particular histological features could result from a longer evolution of the K_{ATP} channel deficiency in children were operated on later in life is unlikely because the usual K_{ATP}-deficient children who are operated on later in life still present numerous abnormal nuclei disseminated throughout the pancreas and never show a mosaic pattern. The relative sensitivity to diazoxide and the absence of demonstrated genomic *ABCC8* or *KCNJ11* mutations in these patients also do not favor this hypothesis.

Pancreatic β-cell nuclear enlargement that is found only in some sections of the pancreas has been recently reported in a hypoglycemic infant by Hussain *et al.* (37). That case, however, completely differs from ours by its mosaic uniparental paternal disomy, with the interstitial region of the uniparental paternal disomy encompassing the K_{ATP} channel genes and including an *ABCC8* mutation, which was not found in the 13 patients we analyzed for genetic mutations. Furthermore, the patient had a neonatal onset of hypoglycemia and an immediate and total insensitivity to diazoxide.

We have identified a new form of PHHI that is characterized by a morphological mosaicism, with particular histological, immunohistochemical, and clinical characteristics. The abnormal hyperfunctional islets of this form are most often confined to a few adjacent lobules. The genetic background of these cases remains unclear, but the diagnosis of morphological mosaicism should be suspected from the peculiar clinical presentation and can be confirmed during surgery using intraoperative surgical frozen sections, with the knowledge that partial pancreatectomy may often be sufficient to cure the infant.

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