Familial Focal Congenital Hyperinsulinism

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Background: Congenital hyperinsulinism (CHI) is a cause of persistent hypoglycemia. Histologically, there are two subgroups, diffuse and focal. Focal CHI is a consequence of two independent events, inheritance of a paternal mutation in ABCC8/KCNJ11 and paternal uniparental isodisomy of chromosome 11p15 within the embryonic pancreas, leading to an imbalance in the expression of imprinted genes. The probability of both events occurring within siblings is rare.

Aim: We describe the first familial form of focal CHI in two siblings.

Patients and Methods: The proband presented with medically unresponsive CHI. He underwent pancreatic venous sampling and Fluorine-18-L-dihydroxyphenylalanine positron emission tomography scan, which localized a 5-mm focal lesion in the isthmus of the pancreas. The sibling presented 8 yr later also with medically unresponsive CHI. An Fluorine-18-L-dihydroxyphenylalanine positron emission-computerised tomography scan showed a 7-mm focal lesion in the posterior section of the head of the pancreas. Both siblings were found to be heterozygous for two paternally inherited ABCC8 mutations, A355T and R1494W. Surgical removal of the focal lesions in both siblings cured the Hyperinsulinaemic hypoglycaemia.

Conclusion: This is the first report of focal CHI occurring in siblings. Genetic counseling for families of patients with focal CHI should be recommended, despite the rare risk of recurrence of this disease. (J Clin Endocrinol Metab 96: 24–28, 2011)
imbalance in the expression of imprinted genes within this region and a growth advantage for the cell that ultimately becomes the focal lesion. The hyperinsulinism results from the paternally inherited ABCC8/KCNJ11 mutation, which is homozygous within the focal lesion due to loss of the maternal allele. Preoperative localization of the focal form with Fluorine-18-L-dihydroxyphenylalanine positron emission-computed tomography ($^{18}$F-DOPA-PET-CT) scan and removal of the focal lesion provides cure for the patient.

The most common region of UPD on chromosome 11 encompasses two areas of interest, the 11p15.5 region (which is imprinted) and the 11p15.1 region (not imprinted). The 11p15.5 region contains several imprinted genes characterized by monoallelic expression. These include maternally expressed genes such as $H19$ (a candidate tumor suppressor gene) and $CDKN1C$ (a negative regulator of cell proliferation and paternally expressed genes such as the $IGF2$). $CDKN1C$ (p57KIP2) encodes for the p57 protein, which is absent in focal CHI by immunohistochemistry (8). The imbalance between imprinted genes (increased $IGF2$ and diminished $H19$ and $CDKN1C$) gives rise to the increase in proliferation of $\beta$-cells.

The probability of a sibling of a child with focal CHI inheriting the paternal ABCC8/KCNJ11 mutation is 50%, but the likelihood of the second sibling also having somatic paternal UPD for chromosome 11p15.5 is very rare, and so far no siblings with focal forms of CHI have been described. We now describe the first familial form of focal CHI.

Subjects and Methods

Clinical history

Index case

The first sibling was born at term with a birth weight of 3.76 kg. At 8 h of age, he was noticed to be cyanosed, and investigations confirmed hyperinsulinism (blood glucose, 1.9 mmol/liter; simultaneous serum insulin, 32.4 mU/liter). He failed to respond to diazoxide (20 mg/kg·d) and octreotide. Transhepatic hepatic portal venous sampling (PVS) was performed (9) as well as an $^{18}$F-DOPA-PET. PVS showed a focal lesion in the isthmus of the pancreas, and the $^{18}$F-DOPA-PET scan also showed increased uptake in the region of the isthmus (Fig. 1, A and B). At operation, a focal lesion was found in the position suggested by the PVS and the $^{18}$F-DOPA-PET scan and was removed. After removal of the focal lesion, the child was cured with no more episodes of hypoglycemia.

Clinical history of sibling

The sibling was born 8 yr after the index case first presented. She was born at term with a weight of 4.2 kg. She presented soon after birth symptoms of lethargy and poor feeding. A blood glucose concentration of 0.5 mmol/liter was documented with a

![FIG. 1. A, Pancreatic venous sampling. B, $^{18}$F-DOPA-PET [with magnetic resonance imaging (MRI)] scan for the index case. Both the pancreatic venous sampling and the $^{18}$F-DOPA-PET scan show the focal lesion to be located in the isthmus region of the pancreas (arrow). C, $^{18}$F-DOPA-PET-CT scan of the sibling. The standard uptake value (SUV) was highest in the head of the pancreas.](https://academic.oup.com/jcem/article-abstract/96/1/24/2833188)
simultaneous serum insulin level of 10 mU/liter. She failed to respond to 20 mg/kg · d of diazoxide and octreotide. An 18F-DOPA-PET-CT scan showed a focal lesion superiorly located in the posterior section of the head of the pancreas (measuring 5 × 4 × 7 mm; Fig. 1C), although intraoperatively the lesion was more inferior. The pancreatic head was excised, and then a distal pancreatic-jejunostomy was performed. There were no postoperative complications, and the child was normoglycemic.

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

Histology
Intraoperative frozen section diagnosis was done on hematoxylin and eosin (and toluidine blue) stained frozen sections, after which the specimens were fixed in buffered formalin and processed into paraffin wax. Paraffin sections from the lesion were immunostained as previously described (10).

Genetics
Genomic DNA was extracted from peripheral leukocytes. The 39 exons of the ABCC8 gene were amplified in the female sibling by the PCR (PCR primer sequences are available on request). Unidirectional sequencing was performed using universal M13 primers and a Big Dye Terminator Cycler Sequencing Kit v3.1 (Applied Biosystems, Warrington, UK) according to manufacturer’s instructions. Reactions were analyzed on an ABI 3730 Capillary sequencer (Applied Biosystems), and sequences were compared with the reference sequences (NM_000525 and NM_000352.2) using Mutation Surveyor v3.24 (SoftGenetics, State College, PA). Mutation testing was undertaken for the affected brother and unaffected parents.

Loss of heterozygosity was investigated in the female sibling by microsatellite analysis of DNA extracted from paraffin-embedded pancreatic tissue and peripheral leukocytes. Six markers (D11S2071, D11S1964, D11S419, D11S1397, D11S1888, and D11S138) were amplified by PCR, and allele peak heights were compared using GeneMarker v1.85 (SoftGenetics). Primer sequences are available on request.

Results
Histology
In the index case, a small focal lesion (5 mm of large axis) was found at the junction of the corpus and the tail of the pancreas. It was characterized by nodular hyperplasia of islet-like cell clusters, including ductuloinsular complexes and scattered giant β-cell nuclei (characteristic of focal adenomatous hyperplasia; Fig. 2B). Outside the focal lesion, the islets were small, with reduced cytoplasm and crowded nuclei (Fig. 2A). p57KIP2 was expressed in these resting islets but not in the endocrine tissue from the focal lesion, demonstrating a loss of heterozygosity of the 11p15 region. Because the lesion was small, no lesional tissue was available in the paraffin block to perform microsatellite marker analysis. In the sibling, a focal lesion (measuring 7 mm) was found in the inferior section of the head of the pancreas with histological features typical of focal adenomatous hyperplasia (Fig. 2C).

Genetics
ABCC8 sequencing analysis identified two heterozygous mutations, A355T (c.1063G>A) and R1494W (c.4480C>T). The R1494W mutation is a loss of function mutation that has been identified in at least six unrelated probands with CHI to date (11). In contrast, the A355T mutation is novel, and although it affects a residue that is well conserved across species, its pathogenicity is currently uncertain. Family member testing demonstrated that the two ABCC8 mutations were in cis, with both affected siblings having inherited the two mutations from their unaffected father. An ABCC8 mutation of maternal origin was not identified.

Analysis of microsatellite markers across the chromosome 11p15.1–11p15.5 region showed loss of heterozygosity of the maternal allele in DNA extracted from the focal lesion when compared with leukocyte DNA for the female sibling. The heterozygous germ line mutations (A355T; R1494W) are therefore likely to be homozygous within the focal lesion.

Discussion
This is the first report of focal CHI occurring in two siblings. In both siblings, the focal lesions were relatively
small (5 and 7 mm in the widest diameter). In the index case, the lesion was located in the isthmus, whereas in the sibling the lesion was located inferiorly in the head of the pancreas. The focal lesions were completely resected in both patients, providing complete cure from the hyperinsulinaemic hypoglycaemia. Using microsatellite markers in the second sibling, we were able to demonstrate loss of heterozygosity for maternal 11p15.1–11p15.5, which is the typical feature of focal lesions. In the index case, the loss of maternal heterozygosity was shown by the lack of p57kip2 using immunohistochemistry.

Both siblings inherited two heterozygous mutations in ABCC8, A355T (c.1063G>A) and R1494W (c.4480C>T). These mutations were both inherited from their unaffected father and therefore are in cis. The R1494W mutation is a loss of function mutation that has been identified in at least six unrelated probands with CHI to date (11). In contrast, the A355T mutation is novel, and although it affects a residue that is well conserved across species, its pathogenicity is currently uncertain. Thus, it is very likely that one or both of these mutations is pathogenic and the cause of the CHI.

The somatic loss of maternal heterozygosity resulting from paternal UPD of 11p15 is a rare event occurring during fetal development. In one series of 31 patients (7) and in another study looking at 56 pancreatectomy specimens (12), no families or siblings with focal CHI were described. The maternal loss of heterozygosity occurs during a narrow time frame in fetal pancreatic development. The timing of the loss of maternal heterozygosity determines the relative size of the focal lesion (7). Because the lesions were small in our patients, this indicates that the maternal heterozygosity for 11p15 event occurred late and involved few pancreatic lobules. If maternal heterozygosity for 11p15 event occurs early in pancreatic development, then this usually leads to large focal lesions (13).

The location of the focal lesions was different in both siblings (isthmus and head of pancreas). During development, the pancreas originates from two buds on the dorsal and the ventral side of the duodenum (14). The dorsal bud develops about 26 days after conception, and the ventral bud develops a few days later (15). The dorsal pancreatic bud gives rise to the superior half of the head, body, and tail of the pancreas, whereas the ventral pancreatic bud gives rise to the inferior half of the head and the uncinate process. In the index case, the focal lesion was located in the region between the body and tail of the pancreas, whereas in the sibling the focal lesion was located in the posterior section of the head of the pancreas. Thus, this suggests that the somatic 11p15.5 UPD events probably occurred in the dorsal and ventral buds of the male and female siblings, respectively.

The paternally inherited ABCC8 mutation and the somatic paternal UPD for 11p15 occurring in the pancreatic β-cell are two independent genetic events. Germline mutations in ABCC8 are not expected to increase the risk of the somatic event occurring unless by chance only. The frequency of CHI in outbred communities is about 1 in 40,000 to 1 in 50,000. This equates to about 1 in 90,000 focal cases and 1 in 90,000 diffuse cases. Because the carrier frequency is around 1 in 150 and because the number of focal cases is roughly the same as diffuse cases, the risk of paternal UPD occurring in a cell within the embryonic pancreas is around 1 in 600. The chance of one sibling being affected with focal CHI is 1 in 90,000. For the second sibling, the risk of inheriting the paternal mutation is 1 in 2, and the risk of paternal UPD is 1 in 600. Hence, the recurrence risk for future siblings of a child with focal CHI is 1 in 1200.

In conclusion, this is the first report of focal CHI in siblings, and it suggests that focal CHI can occur in siblings. We would recommend genetic counseling for families of patients with focal CHI, despite the very rare risk of recurrence of this disease.

Acknowledgments

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