Chromosome 11p15 Paternal Isodisomy in Focal Forms of Neonatal Hyperinsulinism

L. Damaj, M. le Lorch, V. Verkarre, C. Werl, L. Hubert, C. Nihoul-Fékété, Y. Aigrain, Y. de Keyzer, S. P. Romana, C. Bellanne-Chantelot, P. de Lonlay, and F. Jaubert

Department of Pathology (L.D., V.V., C.W., F.J.), Hôpital Necker-Enfants Malades, Assistance Publique–Hôpitaux de Paris, Service d'Anatomie Pathologique and Tumorotheque, Departments of Genetics (M.l.L., S.P.R.) and Surgery (C.N.-F., Y.A.), Hôpital Necker-Enfants Malades, Assistance Publique–Hôpitaux de Paris, and Centre de Référence des Maladies Métaboliques (L.H., Y.d.K., P.d.L.), Institut National de la Santé et de la Recherche Médicale U-781, Hôpital Necker-Enfants Malades, 75015 Paris, France (Assistance Publique-Hôpitaux de Paris, Université Paris 5 René Descartes); Department of Genetics (C.B.-C.), Groupe hospitalier Pitié-Salpétrière, 75651 Paris, France; and Department of Pediatrics (L.D.), Rennes Teaching Hospital, 35043 Rennes, France

Context: Focal forms of congenital hyperinsulinism are due to a constitutional heterozygous mutation of paternal origin in the *ABCC8* gene, more often than the *KCNJ11* gene, located in the 11p15.1 region. This mutation is associated with the loss of the maternally inherited 11p15.1 to 11p15.5 region in the lesion. We investigated the possible occurrence of a compensatory duplication of the paternal 11p15.1-11p15.5 region.

Materials and Methods: A combined immunohistochemistry and fluorescent *in situ* hybridization study on β-cell interphase nuclei with probes covering two genes located in this region (*ABCC8* and *CDKN1C* genes) was performed in four cases of focal forms of hyperinsulinism.

Results: β-Cells in the lesions of four cases of focal congenital hyperinsulinism were diploid for chromosomes 11 and 13. The 11p15.1 to 11p15.2 and 11p15.4 to 11p15.5 regions containing *ABCC8* and *CDKN1C* genes, respectively, were present with two copies. Loss of the maternal allele was confirmed in these focal lesions with microsatellite markers flanking the *ABCC8* and *CDKN1C* genes, whereas a heterozygous mutation in the *ABCC8* gene was inherited from the father.

Conclusions: There is a duplication of the paternal allele on chromosome 11 in the focal forms of hyperinsulinism lesion. The paternal isodisomy observed rendered the β-cells homozygous for *ABCC8* mutation and harbored a K-channel defect in the lesion similar to that observed in diffuse forms of congenital hyperinsulinism. **(*J Clin Endocrinol Metab* 93: 4941–4947, 2008)**

Congenital hyperinsulinism is a rare metabolic disease with an incidence estimated at 1:50,000 live births in the European population. It is characterized by inappropriate insulin secretion constituting one of the principal causes of hypoglycemia in young children, and causing severe brain damage. Insulin secretion in pancreatic β-cells is controlled by an ATP-sensitive K+ channel and through several enzyme pathways, which explains why congenital hyperinsulinism has numerous causes, ranging from enzymatic deficiency to potassium channel dysfunction. The β-cell ATP-sensitive K+ channel is a hetero-octameric complex composed of two types of subunits: four inward-rectifying potassium channel pore-forming (Kir6.2) subunits; and four high-affinity sulfonylurea receptor 1 (SUR1) subunits, belonging to the ABC transporter family. These K channels are mainly present in the granule membrane inside the cell, and are involved in granule docking and the insulin secretion mechanism. It is demonstrated that Kir6.2 and SUR1 subunits are present on the granule membrane for secretion and are recycled at the cell membrane level. The two subunits of the K^+_{ATP} channel are encoded by the SUR gene (*SUR1* or *ABCC8*) and the inward-rectifying potassium channel gene (*Kir6.2* or *KCNJ11*), both located in the 11p15.1 region. *ABCC8* gene mutations are the cause of 50–60% of congenital hyperinsulinism cases, particularly in neonates. More than 100 distinct mutations, distributed throughout

0021-972X/08/$15.00/0

Printed in U.S.A.

Copyright © 2008 by The Endocrine Society

doi: 10.1210/jc.2008-0673 Received March 26, 2008. Accepted September 10, 2008.

First Published Online September 16, 2008

Abbreviations: AR, Allelic ratio; BAC, bacterial artificial chromosome; DiCHI, diffuse form of congenital hyperinsulinism; dUTP, deoxyuridine 5-triphosphate; FISH, fluorescent *in situ* hybridization; FITC, fluorescein isothiocyanate; FoCHI, focal form of congenital hyperinsulinism; SRS, Silver-Russell syndrome; SUR1, sulfonylurea receptor 1.

Downloaded from https://academic.oup.com/jcem/article/93/12/4941/2627388 by guest on 25 April 2024

the *ABCC8* gene, have already been described. Mutations of the *Kir6.2* gene are less frequent and are responsible for 10–15% of congenital hyperinsulinism only. Most congenital hyperinsulinism forms linked to the potassium channel are resistant to diazoxide.

There are two main forms of congenital hyperinsulinism that are identical clinically but differ in histological and genetic terms: diffuse (DiCHI) and focal (FoCHI) (1). DiCHIs are characterized by β-cell hyperactivity throughout the pancreas. They are genetically heterogeneous but mainly due to recessive mutations in genes encoding potassium channel subunits (*ABCC8* or *KCNJ11* genes). By contrast, FoCHIs are characterized by the presence of numerous β-cells in a single area (focal hyperplasia) that can develop throughout the pancreas. Focal hyperplasia is composed of adjacent foci of centrolobular β-cell hyperplasia surrounded by a rim of exocrine acini. Two events, one inherited and the other acquired, explain the focal lesion. The first event is a constitutional heterozygous mutation of paternal origin in *ABCC8* more often than in *KCNJ11* (2), occurring in chromosome 11p15. The second event is somatic and involves the loss of the corresponding maternal segment in the 11p15 region, found in abnormal β-cells only, and extending from 11p15.1 to 11p15.5. This loss of heterozygosity is thought to reduce the β-cell to monosomy for the mutated *ABCC8* gene, altering the potassium channel function and resulting in increased insulin secretion (2). The lost 11p15 region contains several imprinted genes implicated in cell proliferation, such as *CDKN1C* in the 11p15.4 region, and *IGF-II* and *H19* (3) in the 11p15.5 region. *CDKN1C* *(p57KIP2)* encodes for the p57 protein, which is a member of the p21 cyclin-dependent kinase inhibitor family that negatively regulates the cell cycle at the G1 checkpoint. This imprinted gene is normally expressed from the maternal allele. The p57 protein was absent in FoCHIs by immunohistochemistry (4). *H19* is also imprinted and only expressed from the maternal allele. It encodes a biologically active nontranslated mRNA that may act as a tumor suppressor gene and regulates *IGF-II*. By contrast, *IGF-II*, encoding for a growth factor, is paternally expressed. Thus, in FoCHI pathogenesis, it is considered that maternal allele loss results in a loss of *H19* and *CDKN1C* expression, which usually inhibits cell proliferation, whereas *IGF-II* promotes cell growth. This imbalance of several imprinted genes located in the 11p15 region could partly explain the β-cell hyperplasia in FoCHIs (5, 6).

However, in FoCHIs, it is unknown whether the loss of the maternally inherited 11p15 region is due to segmental deletion or whether there is a compensatory duplication of the paternal 11p15 region. Paternal isodisomy of the 11p15 region may modify the imprinted gene expression imbalance and cause the enhanced proliferative lesion, according to many other proliferative and/or tumoral diseases and syndromes resulting in uniparental isodisomy, such as Beckwith-Wiedemann or Silver-Russell syndrome (SRS).

In an attempt to answer the previous questions, chromosome 11 and 13 ploidy and 11p15.1 to 11p15.5 region status were investigated by fluorescent *in situ* hybridization (FISH) on β-cell interphase nuclei with probes covering *ABCC8* or *CDKN1C* loci. Findings were then correlated with PCR amplification of microsatellite markers located upstream, within, or downstream of *ABCC8* and *CDKN1C* in the bacterial artificial chromosome (BAC) probes, on DNA extracted from four FoCHI cases. These results were compared with those of two DiCHI cases used as controls.

Materials and Methods

Four cases of human FoCHIs and two cases of human DiCHIs were derived from partial and subtotal pancreatectomies, respectively. The diagnostic criteria of hyperinsulinism in all cases were typical (7). One *ABCC8* mutation of paternal origin was identified for the four cases of FoCHIs. Only one of the DiCHI cases had a *ABCC8* mutation (Table 1).

Blood lymphocytes were collected from each child and from the parents of three cases of FoCHIs.

FISH

The first step involved indirect immunohistochemistry. Imprints on glass slides were prepared from stored frozen specimens containing mainly β-cells, as a result of there being fewer other endocrine and exocrine cells. Among the pancreatic cells, β-cells were identified by indirect immunohistochemistry, with primary antibodies directed against insulin antigens, at 1:150 (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK), followed by biotinylated secondary antibodies (Dako biotinylated link; DakoCytomation, Glostrup, Denmark). These secondary antibodies were revealed by a complex of avidin labeled with fluorescein isothiocyanate (FITC). On epifluorescence microscopy, the β-cells have a green cytoplasm around a preserved negative central nucleus.

Next, the imprint cytology slides were used for FISH. After the first step of indirect immunohistochemistry to enable β-cell identification, the focal forms and DiCHI slides were fixed with 4% paraformaldehyde for 5 min and then washed in two PBS baths for 5 min each. Nuclear proteins were digested with proteinase K 8 ml at 10 mg/ml. The slides were then denatured in 72% formamid/2 saline sodium citrate (at 72 C for 30 sec), before being dehydrated in ethanol solutions with graded concentrations.

TABLE 1. Four focal and two diffuse forms of neonatal hyperinsulinism are studied

Case	CHI type	Gene	Location	Nucleotide change	Protein effect
1	FoCHI	*ABCC8*	Exon 33	c.4040_4045del	p.Ile1347_Gln1348del
2	FoCHI	*ABCC8*	Exon 8	c.1331A>G	p.Gln144Arg
3	FoCHI	*ABCC8*	Intron 32	c.3992-9G>A	p.?, aberrant splicing
4	FoCHI	*ABCC8*	Exon 4	c.536A>G	p.Tyr179Cys
5	DiCHI	*ABCC8*	Exons 12 and 20	[c.1792C>T]+[c.2425C>T]	[p.Arg598ter]+[p.Gln809X]
6	DiCHI	NA			

ABCC8 mutation was proven for the four focal form cases and one of the diffuse form cases. All sequence information is based on GenBank reference sequence NM_000352.2 incorporating the alternate exon 17 (GenBank L78208, L78224), which contains an additional amino acid. CHI, Congenital hyperinsulinism; NA, not available.

Downloaded from https://academic.oup.com/jcem/article/93/12/4941/2627388 by guest on 25 April 2024

FISH studies

Dual-color FISH experiments were performed with probes prepared from BAC RP11-481E11 (spanning the CDKN1C locus) labeled with spectrum green-deoxyuridine 5-triphosphate (dUTP) and from BAC RP11-976K8 (spanning the ABCC8 locus) labeled with spectrum orange-dUTP.

BAC RP11-410I24 (labeled with spectrum green-dUTP) located on 11qter and BAC RP11-569D9 (labeled with DEAC-dUTP) located on 13qter were concomitantly used to determine cell ploidy. BAC DNA was extracted with QIAfilter Plamid Maxi-Kit (QIAGEN, Inc., Valencia, CA) and labeled by nick translation (Vysis, Inc., Downers Grove, IL) following the manufacturer's instructions. Hybridization was performed using a Slide Booster (Advalytix; Olympus America Inc., Concord, MA) for 24 h at 37 C. After washing, the slides were counterstained with 4′,6-diamidino-2-phenylindole, and the images were captured with a Sensys camera (Photometrics, Ltd., Tucson, AZ) and processed with QFISH software (Leica Microsystems GmbH, Wetzlar, Germany).

Loss of allele: PCR with microsatellite markers flanking *ABCC8* and *CDKN1C*

The microsatellite markers were D11S 921 (11p15.1) upstream and D11S 902 (11p15.2) within intron 3 of the *ABCC8* gene, and D11S 4146 (11p15.4) upstream and D11S 4088 (11p15.5) downstream of the *CDKN1*C gene. PCR assay, based on the fluorescent method, was performed on DNA extracted from circulating lymphocytes of children and parents when available, and on DNA extracted from frozen samples of the FoCHIs lesion containing mainly β-cells. Allele separation was achieved on an electropherogram and defined by the migration site expressed by a number. The two allele peaks obtained from tissue sections were compared using the allelic ratio (AR). The AR is calculated by AR = (peak height 1)/(peak height 2), as described in the article by Murakami *et al.* (8).

In our study, peak height 1 was the height of the peak of the maternally inherited allele in the lesion, and peak height 2 was the height of the peak of the maternally inherited allele in the child's constitutional lymphocytes.

Downloaded from https://academic.oup.com/jcem/article/93/12/4941/2627388 by guest on 25 April 2024

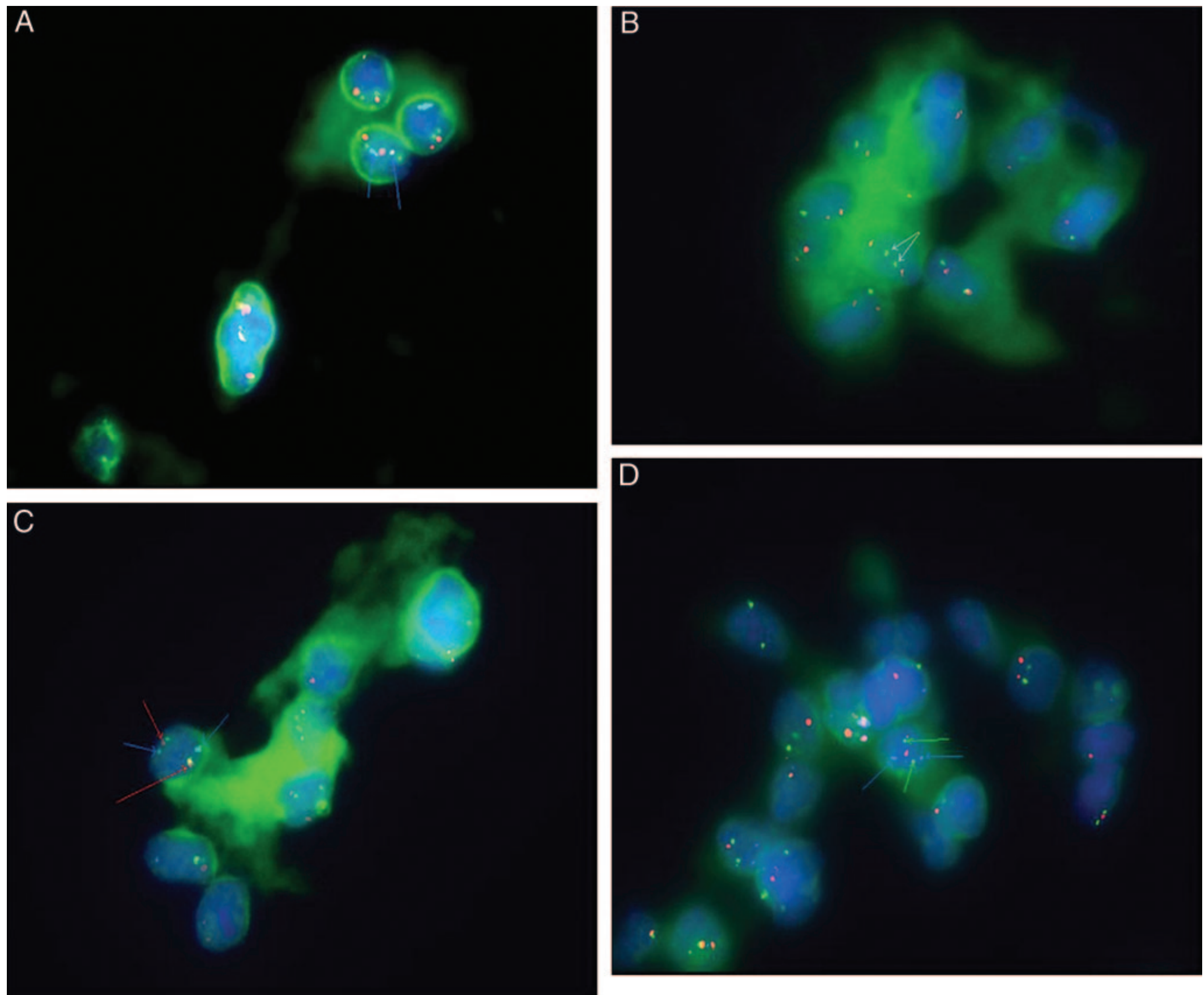


FIG. 1. FoCHI nuclei stained with 4′,6-diamidino-2-phenylindole: β-cell cytoplasm stained with FITC (*green*). A, FoCHI cell imprints. The combined identification of β-cells by insulin cytoplasmic immunofluorescence (*green*), and nuclear 11q probes by FISH, shows two *green* signals (*green arrows*) reflecting the presence of two chromosome 11s in each β-cell nucleus (diploidy) (×100). B, FoCHI. 13q probes detected by FISH; the two *blue* signals (*blue arrows*) also show the presence of two chromosome 13s per β-cell nucleus (insulin: *green* cytoplasm) confirming their diploidy (×100). C, FoCHI. FISH of β-cells (insulin: *green* cytoplasm). The diploid cells for 13q probes (*blue arrows*) also have two copies of the *ABCC8* gene (BAC RP 11 976K8) (*red arrows*) eliminating a chromosome 11 microdeletion (×100). D, FoCHI. FISH of β-cells (insulin: *green* cytoplasm). The cells are diploid as shown by the two *blue dots* (*blue arrows*) for chromosome 13q probes and also two copies of the *CDKN1C* gene (BAC RP 11 481 E11) (*green arrows*) eliminating a chromosome 11 microdeletion (×100).

A ratio of less than 0.30 was considered as significant of a loss of heterozygosity, given that there is always non-β-cell contamination of unknown quantity.

Results

β-Cell indirect immunohistochemistry

Indirect staining with the avidin-FITC complex was used to identify β-cells among the cells obtained by imprint cytology. In the four FoCHI cases, the majority of β-cells were organized into aggregates. They adhered to each other, and their cytoplasm limits were sometimes ill defined. There were also a few enlarged nuclei. There were 50 nuclei counted on each slide.

β-Cell ploidy

β-Cell ploidy was determined by FISH according to the number of copies of the telomeric 11q probe (green fluorescence) (Fig. 1A) and 13q probe (blue fluorescence) (Fig. 1B) observed in each nucleus. For FoCHIs and DiCHIs, two green signals and two blue signals were observed per nucleus in 50 nuclei of each focal and diffuse forms sample. This indicated that the FoCHI and DiCHI cells were diploid for chromosomes 11 and 13.

ABCC8 gene FISH detection (Fig. 1C)

A probe spanning the *ABCC8* gene locus and labeled with spectrum orange-dUTP was hybridized on the β-cell interphase nuclei in the four focal forms and two DiCHI samples. It generated two red signals per nucleus in the 50 nuclei analyzed. We deduced that these cells had two copies of the *ABCC8* gene.

CDKN1C (*p57KIP2*) gene FISH detection (Fig. 1D)

A specific probe spanning the *CDKN1C* gene locus and labeled with spectrum green-dUTP resulted in two signals in 50 nuclei in the four focal forms and two DiCHI samples. We deduced that the focal forms β-cells had two copies of the *CDKN1C* gene.

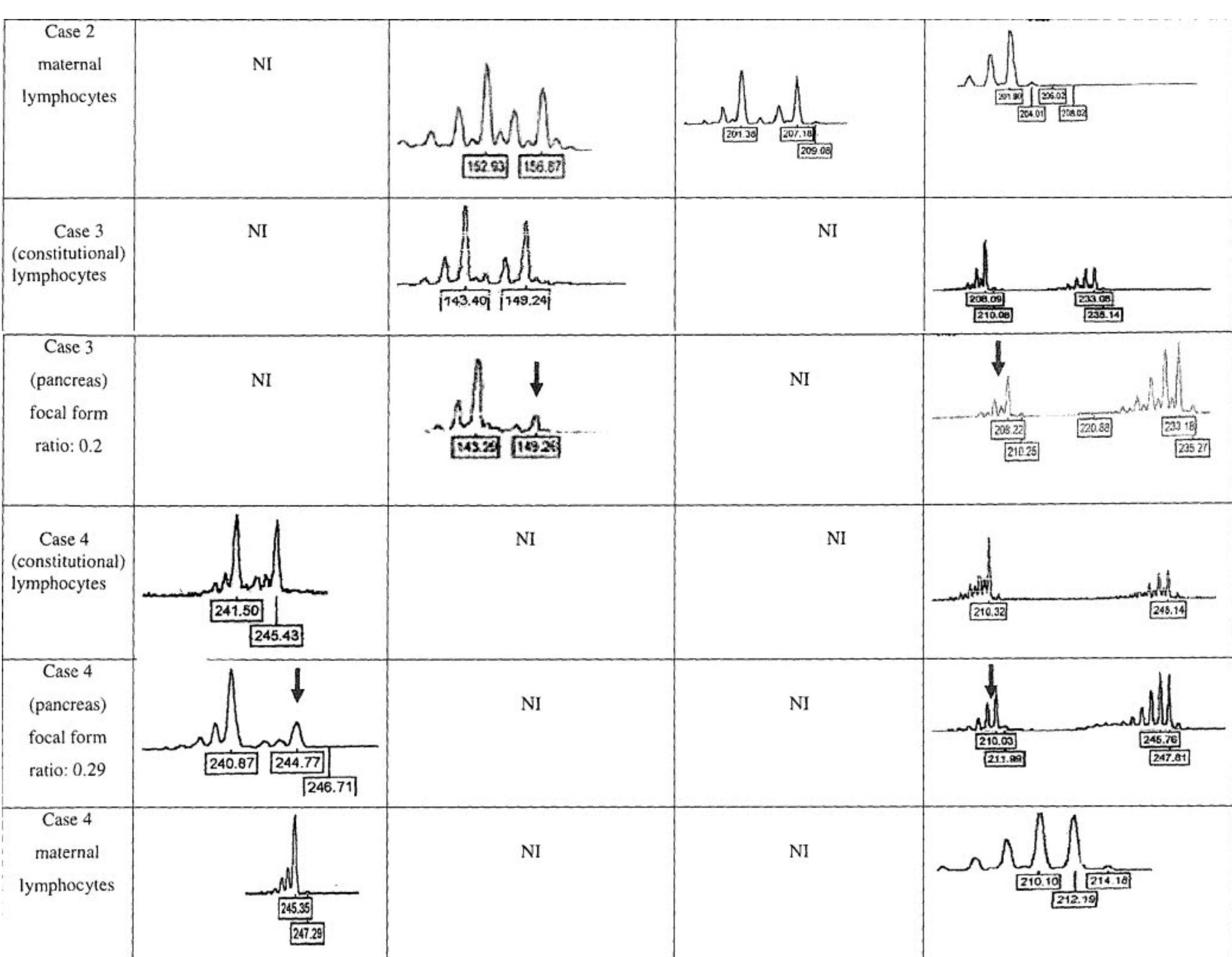
TABLE 2. PCR of *ABCC8* gene microsatellite markers

	D11S921 upstream 11p15.1	D11S902 within ABCC8 11p15.2	D11S4146 upstream 11p15.4	D11S4088 downstream 11p15.5
Case 1 (constitutional) lymphocytes	NI	147.22; 151.07	NI	194.47; 203.98; 214.10; 212.11; 221.50; 219.48
Case 1 (pancreas) focal form ratio: 0.30	NI	141.56; 147.29; 154.08; 151.17	NI	204.00; 214.06; 208.00; 212.06
Case 1 maternal lymphocytes	NI	147.18	NI	206.27; 212.19
Case 2 (constitutional) lymphocytes	NI	145.17; 156.84	197.84; 199.57; 207.25; 209.14	202.08; 206.04; 208.11
Case 2 (pancreas) focal form ratio: 0.05	NI	145.29	197.68	
Case 2 paternal lymphocytes	NI	145.25; 147.92; 154.90	197.40; 205.18; 207.18	197.40; 205.78; 207.18

(*Continues*)

Downloaded from https://academic.oup.com/jcem/article/93/12/4941/2627388 by guest on 25 April 2024

TABLE 2. (*Continued*)



Case 2 maternal lymphocytes	NI	152.93, 156.87	201.38, 207.18, 209.08	201.99, 204.01, 206.03, 208.02
Case 3 (constitutional) lymphocytes	NI	143.40, 149.24	NI	208.09, 210.08, 233.08, 235.14
Case 3 (pancreas) focal form ratio: 0.2	NI	143.25, 149.26	NI	208.22, 210.25, 220.88, 233.18, 235.27
Case 4 (constitutional) lymphocytes	241.50, 245.43	NI	NI	210.32, 245.14
Case 4 (pancreas) focal form ratio: 0.29	240.87, 244.77, 246.71	NI	NI	210.03, 211.99, 245.76, 247.81
Case 4 maternal lymphocytes	245.35, 247.29	NI	NI	210.10, 212.19, 214.18

ABCC8 gene microsatellite marker electropherograms obtained by PCR for the four focal forms of neonatal hyperinsulinism: the two AR is given for the pancreatic sample that contains predominantly β-cells with some remaining normal cells due to contamination. Comparison of the allele peaks between blood lymphocytes and pancreatic foci is used to determine the reduced allele migration number. Comparison with blood lymphocytes from the parents determines maternal or paternal inheritance. Allele loss is observed for the four cases studied, but its maternal origin is proven for three cases only. *CDKN1C* gene microsatellite marker electropherograms obtained by PCR for the four focal forms of neonatal hyperinsulinism: the two AR is given for the pancreatic sample that contains predominantly β-cells with some remaining normal cells due to contamination. Comparison of the allele peaks between blood lymphocytes and pancreatic foci is used to determine the reduced allele migration number. Comparison with blood lymphocytes from the parents determines maternal or paternal inheritance. Allele loss is observed for the four cases studied, but its maternal origin is proven for three cases only. NI, Non informative.

Microsatellites flanking *ABCC8* and *CDKN1C* genes: PCR analysis results (Table 2)

The study of the microsatellite markers flanking *ABCC8* (Table 2) showed that D11S 921 was uninformative in cases 1–3 but was lost in the pancreatic lesion in case 4 (ratio 0.29). The D11S 902 marker was lost in the pancreatic lesion in cases 1 (ratio 0.30), 2 (ratio 0.05), and 3 (ratio 0.20). Microsatellite marker D11S921 loss was of maternal origin in the pancreatic lesion in case 4, and this was also the case for the loss of the microsatellite marker D11S902 in cases 1 and 2. The origin of D11S902 marker loss in the lesion was indeterminate in case 3.

The study of *CDKN1C* flanking markers (Table 2) showed that D11S 4146 was informative only in case 2, with a loss of the maternal allele in the pancreatic lesion (ratio 0.10). By contrast, D11S 4088 was informative in three cases (cases 1, 2, and 4), with a loss of the maternal allele in the lesions. The DNA of the parents of case 3 was not available, so the parental origin of the loss of heterozygosity observed could not be determined.

The ARs in the pancreas reflect the respective quantity of the normal and abnormal cells in the tissue sample.

Discussion

The combined use of insulin immunofluorescent detection with FISH on interphase nuclei is a new method of analyzing the β-cell genome *in situ* in congenital hyperinsulinism. It demonstrated that abnormal β-cells are diploid for chromosomes 11 and 13, and that

Downloaded from https://academic.oup.com/jcem/article/93/12/4941/2627388 by guest on 25 April 2024

TABLE 3. Synthesis of results for the PCR and FISH studies of *ABCC8* and *CDKNIC* genes

Chromosome 11	FISH	Microsatellite	FISH	Microsatellite
11p15.1, 11p15.5 β-cell	*ABCC8*: (BAC RP11-976 K 8)	D11S 921 and D11S902 (*ABCC8* gene)	*CDKN1C*: (BAC RP 11-481 E11)	D11S 4146 and D11S 4088 (*CDKN1C* gene)
Case 1	Diploid	Maternal LOH	Diploid	Maternal LOH
Case 2	Diploid	Maternal LOH	Diploid	Maternal LOH
Case 3	Diploid	LOH	Diploid	LOH
Case 4	Diploid	Maternal LOH	Diploid	Maternal LOH

The four focal forms of neonatal hyperinsulinism have diploid β-cells and two copies of the *ABCC8* and *CDKN1C* genes detected by FISH. By contrast, loss of heterozygosity (LOH) shows a loss of maternal material for three cases resulting in the conclusion that there is a duplication of paternal chromosome 11p15.1 to 11p15.5 (paternal disomy).

they have two copies of the *ABCC8* and *CDKN1C* genes. Our results and the previous results for loss of heterozygosity of chromosome 11 of the maternal allele open up the discussion on an underlying chromosome rearrangement, contrary to DiCHIs, which are the consequence of two inherited mutations.

The theory of a microscopical chromosome 11 deletion involving segments 11p15.1 to 11p15.5 of maternal origin in focal congenital hyperinsulinism, suggested by loss of heterozygosity results, was not confirmed. Two BAC probes from the Human Genome Project, including the regions 11p15.1 to 11p15.2 with the *ABCC8* gene and 11p15.4 to 11p15.5 with the *CDKN1C* gene, showed two dots in all β-cell interphase nuclei. Consequently, there was no focal deletion on chromosome 11, but duplication of the paternal 11p15.1 to 11p15.5 segment (Table 3). Indeed, a paternal chromosome 11 segmental duplication and crossover to the maternal one is the only explanation for the results obtained. The resulting paternal isodisomy renders the β-cell K-channel defect biallelic in the abnormal foci. By contrast, in DiCHIs containing diploid cells, both K-channel alleles are mutated with both paternal and maternal origins. Consequently, the mechanism of insulin secretion dysregulation is similar for both forms of the disease and is due to a biallelic K-channel mutation.

The link between paternal isodisomy of chromosome 11 and neonatal hyperinsulinism is already known in Beckwith-Wiedemann syndrome (9–11), certain rare forms of hyperinsulinism (unclassified β-cell proliferation) (12), and recently atypical cases of hyperinsulinism due to *ABCC8* mutation (13). Diabetes is also related to *ABCC8* mutation associated with isodisomy (14). Segmental chromosome duplication and isodisomy can also be found in other diseases such as SRS because approximately 10% of SRS cases showed maternal uniparental isodisomy for chromosome 7. Embryonal rhabdomyosarcoma complicating Costello syndrome (15) also contains uniparental isodisomy in chromosome 11p15.5 followed by HRAS mutations. Segmental isodisomy is also found in lymphoma (16). The mechanism of maternal allele loss remains unexplained, but microsatellite instability is a well-known predisposing factor in tumor genesis and facilitates duplications (17, 18).

The β-cell growth observed in FoCHIs can be explained by chromosome 11 disomy. Indeed, in one focal lesion *IGF-II* FISH detection showed two dots in interphase nuclei, probably of paternal origin according to the loss of the maternal allele (data not shown). This gene disomy counterbalances the loss of the maternal copy of the maternally expressed *H19* gene, which usually controls *IGF-II* activity. On the other hand, p57 loss reported in focal forms may also contribute to β-cell accumulation through apoptosis reduction with a decrease in the cell death rate (4). By contrast, in malignant tumors, gene amplification such as N-Myc produces a higher number of copies that are routinely detected by specific FISH probes (double minute) resulting in multiple dots on FISH interphase nuclei (19) and HSR (when remaining inside the chromosome) in cell cultures or metaphases. Many studies concerning *IGF-II* have been published, with conflicting results in terms of the level of RNA synthesis and protein turnover (20). The underlying mechanism remains to be determined because crossover remains a physiological mechanism during meiosis (21–23) but has not been reported during mitosis. However, stem cells are akin to germ cells and share the same initial asymmetrical division. Consequently, they may have retained meiotic properties from their first division. FoCHIs could result from a focally acquired β-cell stem cell pathology (24).

In conclusion, we report on chromosome 11p15 isodisomy of paternal origin in FoCHIs, explaining the numerous β-cells in the lesion.

Acknowledgments

We thank the Necker-Enfants Malades Tumorotheque for its support.

Address all correspondence and requests for reprints to: Professor F. Jaubert, Department of Pathology, Hôpital Necker-Enfants Malades, Assistance Publique–Hôpitaux de Paris, Service d'Anatomie Pathologique and Tumorotheque, 149 rue de Sèvres, 75015 Paris, France. E-mail: francis.jaubert@nck.aphp.fr.

This work was funded by the Groupe d'Intérêt Scientifique-Institut des Maladies Rares.

Disclosure Summary: The authors have nothing to declare.

References

1. **Delonlay P, Galmiche-Rolland L, Giurgea I, Verkarre V, Aigrain Y, Santiago-Ribeiro MJ, Polak M, Robert JJ, Bellanne-Chantelot C, Brunelle F, Nihoul-Fekete C, Jaubert F** 2007 Neonatal hyperinsulinism: clinicopathologic correlation. Hum Pathol 38:387–399
2. **Verkarre V, de Lonlay P, Gross-Morand MS, Devillers M, Rahier J, Brunelle F, Robert JJ, Nihoul-Fékété C, Saudubray JM, Junien C** 1998 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 102:1286–1291
3. **Shuman C, Steele L, Ray PN, Clericuzio C, Zackai E, Parisi MA, Meadows AT, Kelly T, Tichauer D, Squire JA, Sadowski P, Weksberg R** 2006 Constitutional UPD for chromosome 11p15 in individuals with isolated hemihyperplasia is

Downloaded from https://academic.oup.com/jcem/article/93/12/4941/2627388 by guest on 25 April 2024

associated with high tumor risk and occurs following assisted reproductive technologies. Am J Med Genet A 140:1497–1503

4. **Kassem SA, Thornton PS, Hussain K, Smith V, Lindley KJ, Aynsley-Green A, Glaser B** 2001 p57(KIP2) expression in normal islet cells and in hyperinsulinism of infancy. Diabetes 50:2763–2769
5. **Giurgea I, Ribeiro M, Hubert L, Sempoux C, Robert JJ, Blankenstein O, Hussain K, Brunelle F, Nihoul-Fékété C, Rahier J, Jaubert F, de Lonlay P** 2006 Molecular mechanisms of neonatal hyperinsulinism. Horm Res 66:289–296
6. **Fournet JC, de Lonlay P, Rahier J, Brunelle F, Robert JJ, Nihoul-Fékété C, Saudubray JM, Junien C** 1998 Loss of imprinted genes and paternal SUR1 mutations lead to hyperinsulinism in focal adenomatous hyperplasia. Ann Endocrinol (Paris) 59:485–491
7. **Giurgea I, Ribeiro MJ, Boddaert N, Touati G, Robert JJ, Saudubray JM, Jaubert F, Bellanne-Chantelot C, Brunelle F, Nihoul-Fekete C, de Lonlay P** 2005 [Congenital hyperinsulinism in newborn and infant]. Arch Pediatr 12: 1628–1635 (French)
8. **Murakami I, Gogusev J, Fournet JC, Glorion C, Jaubert F** 2002 Detection of molecular cytogenetic aberrations in Langerhans cell histiocytosis of bone. Hum Pathol 33:555–560
9. **Itoh N, Reeve AE, Morison IM** 2000 Proportion of cells with paternal 11p15 uniparental disomy correlates with organ enlargement in Wiedemann-Beckwith syndrome. Am J Med Genet 15:111–116
10. **Grati FR, D'Ajello P, Ruggeri A, Miozzo M, Bracalente G, Baldo D, Laurino L, Boldorini R, Frate E, Surico N, Larizza L, Maggi F, Simoni G** 2007 Chromosome 11 segmental paternal isodisomy in amniocytes from two fetuses with omphalocoele: new highlights on phenotype-genotype correlations in Beckwith-Wiedemann syndrome. J Med Genet 44:257–263
11. **Cooper WN, Macdonald F, Maher ER** 2007 Mitotic recombination and uniparental disomy in Beckwith-Wiedemann syndrome. Genomics 89:613–617
12. **Giurgea I, Fournet JC, Sempoux C, Bellanné-Chantelot C, Touati G, Hubert L, Groos MS, Brunelle F, Rahier J, Henquin JC, Dunne MJ, Jaubert F, Robert JJ, Nihoul-Fékété C, Vekemans M, Junien C, de Lonlay P** 2006 Congenital hyperinsulinism and mosaic abnormalities of the ploidy. J Med Genet 43: 248–254
13. **Hussain K, Smith VV, Ashworth M, Day M, Pierro A, Ellard S** 2008 An ABCC8 gene mutation and mosaic uniparental isodisomy resulting in atypical diffuse congenital hyperinsulinism. Diabetes 57:259–260
14. **Shield JP, Mackay DJ, Harries LW, Proks P, Girard C, Ashcroft FM, Temple IK, Ellard S** 2008 Mosaic paternal uniparental isodisomy and an ABCC8 gene mutation in a patient with permanent neonatal diabetes and hemihypertrophy. Diabetes 57:255–258
15. **Kratz CP, Niemeyer CM, Schlegelberger B, Koscielniak E, Kontny U, Zenker M** 2007 Uniparental disomy at chromosome 11p15.5 followed by HRAS mutations in embryonal rhabdomyosarcoma: lessons from Costello syndrome. Hum Mol Genet [Erratum (2007) 16:2781] 16:374–379
16. **Nielaender IM, Wagner F, Martínez-Climent JA, Siebert R** 2006 Partial uniparental disomy: a recurrent genetic mechanism alternative to chromosomal deletion in malignant lymphoma. Leukemia 20:904–905
17. **Stark JM** 2003 Extensive loss of heterozygosity is suppressed during homologous repair of chromosomal breaks. Mol Cell Biol 23:733–743
18. **Russo S, Recalcati MP, Ferraiuolo S, Cogliati F, Dalla Bernardina B, Tibiletti MG, Agosti M, Sala M, Bonati MT, Larizza L** 2006 Molecular and genomic characterisation of cryptic chromosomal alterations leading to paternal duplication of the 11p15.5 Beckwith-Wiedemann region. J Med Genet 43:e39
19. **Mathew P, Bowman LC, Rowe ST, Nash MB, Valentine VA, Cohn SL, Castleberry RP, Brodeur GM, Look AT** 2001 Detection of MYCN gene amplification in neuroblastoma by fluorescence in situ hybridization: a pediatric oncology group study. Neoplasia 3:105–109
20. **Sempoux C, Dahan K, Moulin P, Stevens M, Lambot V, de Lonlay P, Fournet JC, Junien C, Jaubert F, Nihoul-Fekete C, Saudubray JM, Rahier J** 2003 The focal form of persistent hyperinsulinemic hypoglycemia of infancy: morphological and molecular studies show structural and functional differences with insulinoma. Diabetes 52:784–794
21. **Ward JO, Motley WW, Niswander LM, Deacon DC, Griffin LB, Langlais KK, Backus VL, Schimenti KJ, O'Brien MJ, Eppig JJ, Schimenti JC** 2007 Mutation in mouse hei10, an e3 ubiquitin ligase, disrupts meiotic crossing over. PLoS Genet 3:e139
22. **Terasawa M, Tsukamoto Y, Shinohara M, Shirahige K, Kleckner N, Ogawa T** 2007 Meiotic recombination-related DNA synthesis and its implications for cross-over and non-cross-over recombinant formation. Proc Natl Acad Sci USA 104:5965–5970
23. **Cromie GA** 2007 Branching out: meiotic recombination and its regulation. Trends Cell Biol 17:448–455
24. **Spence JR, Wells JM** 2007 Translational embryology: using embryonic principles to generate pancreatic endocrine cells from embryonic stem cells. Dev Dyn 236:3218–3227

Downloaded from https://academic.oup.com/jcem/article/93/12/4941/2627388 by guest on 25 April 2024