LETTER TO JMG

Congenital hyperinsulinism and mosaic abnormalities of the ploidy

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Background: Congenital hyperinsulinism and Beckwith-Wiedemann syndrome both lead to β islet hyperplasia and neonatal hypoglycaemia. They may be related to complex genetic/epigenetic abnormalities of the imprinted 11p15 region. The possibility of common pathophysiological determinants has not been thoroughly investigated.

Objective: To report abnormalities of the ploidy in two unrelated patients with congenital hyperinsulinism.

Methods: Two patients with severe congenital hyperinsulinism, one overlapping with Beckwith-Wiedemann syndrome, had pancreatic histology, ex vivo potassium channel electrophysiological studies, and mutation detection of the encoding genes. The parental genetic contribution was explored using genome-wide polymorphism, fluorescent in situ hybridisation (FISH), and blood group typing studies.

Results: Histological findings diverged from those described in focal congenital hyperinsulinism or Beckwith-Wiedemann syndrome. No potassium channel dysfunction and no mutation of its encoding genes (*SUR1*, *KIR6.2*) were detected. In patient 1 with congenital hyperinsulinism and Beckwith-Wiedemann syndrome, paternal isodisomy for the whole haploid set was homogeneous in the pancreatic lesion, and mosaic in the leucocytes and skin fibroblasts (hemihypertrophic segment). Blood group typing confirmed the presence of two erythroid populations (bi-parental *v* paternal only contribution). Patient 2 had two pancreatic lesions, both revealing triploidy with paternal heterodisomy. Karyotype and FISH analyses done on the fibroblasts and leucocytes of both patients were unremarkable (diploidy).

Conclusions: Diploid (biparental/paternal-only) mosaicism and diploid/triploid mosaicism were present in two distinct patients with congenital hyperinsulinism. These chromosomal abnormalities led to paternal disomy for the whole haploid set in pancreatic lesions (with isodisomy or heterodisomy), thereby extending the range and complexity of the mechanisms underlying congenital hyperinsulinism, associated or not with Beckwith-Wiedemann syndrome.

Congenital hyperinsulinism is a cause of profound hypoglycaemia resulting from an excess of insulin secretion. The most common mechanism underlying congenital hyperinsulinism is dysfunction of the pancreatic ATP sensitive potassium channel (K^+_{ATP}), which can be assessed by an ex vivo test. Congenital hyperinsulinism can result from either focal adenomatous pancreatic hyperplasia, histologically characterised by the presence of small β islets (2.5 to 7.5 mm) of hyperplastic cells,[1 2] or functional diffuse pancreatic insulin hypersecretion.[1] These two discrete forms share a similar clinical presentation,[3] but result from different pathophysiological and molecular mechanisms.[4–6] In addition, diffuse congenital hyperinsulinism usually presents as an autosomal recessive disorder, whereas focal congenital hyperinsulinism is sporadic.

The two subunits of the K^+_{ATP} channel are encoded by either the sulphonylurea receptor gene (*SUR1* or *ABCC8*) or the inward rectifying potassium channel gene (*KIR6.2.* or *KCNJ11*), both located in the 11p15.1 region. Focal congenital hyperinsulinism has been shown to result from a paternally inherited mutation of the *SUR1* or *KIR6.2* genes and loss of the maternal 11p15 allele (loss of heterozygosity, LOH). LOH is a somatic event restricted to the pancreatic lesion which leads to tumour inception through disruption of the balance of expression of several imprinted genes located in the 11p15.5 region and controlling cell growth.[7]

Hypoglycaemia may be observed in several overlapping syndromes, such as Beckwith-Wiedemann syndrome,[8] Perlman syndrome,[9] and more rarely in Sotos syndrome.[10] Beckwith-Wiedemann syndrome results from several identified genetic and epigenetic molecular events including paternal isodisomy,[11] abnormal methylation of *IGF2/H19*,[12] chromosomal aberrations involving the 11p15 region,[13] and *CDKN1C* mutation.[14] Hypoglycaemia in patients with Beckwith-Wiedemann syndrome has been associated with paternal uniparental disomy of 11p15 rather than other genetic abnormalities,[8] but the pathophysiological mechanism leading to hyperinsulinaemic hypoglycaemia is still unclear, as no evidence for duplication of *INS, HRAS1*, and *IGF2*[15] or overexpression of the *INS* and *IGF2* genes[16] was found.

Mosaicism for paternal isodisomy restricted to the 11p15 region is well known in Beckwith-Wiedemann syndrome, but neither mosaicism nor abnormalities of ploidy have been reported in congenital hyperinsulinism to date. However, to our knowledge only two cases of mosaicism with genome-wide paternal isodisomy have been described so far, one in a Wilms tumour originating in a patient with Beckwith-Wiedemann syndrome[17] and the other confined to the placenta, with to a healthy female infant.[18]

We here report unprecedented abnormalities of the ploidy in two unrelated patients with congenital hyperinsulinism, one with associated Beckwith-Wiedemann syndrome.

METHODS

Patients

Patient 1 was a girl born at the gestational age of 35 weeks (birth weight 2260 g, length 46 cm, head circumference 32.5 cm) to unrelated white parents. At the age of 14 months hypoglycaemic seizures appeared and right hemihypertrophy

Abbreviations: FISH, fluorescent in situ hybridisation; LOH, loss of heterozygosity

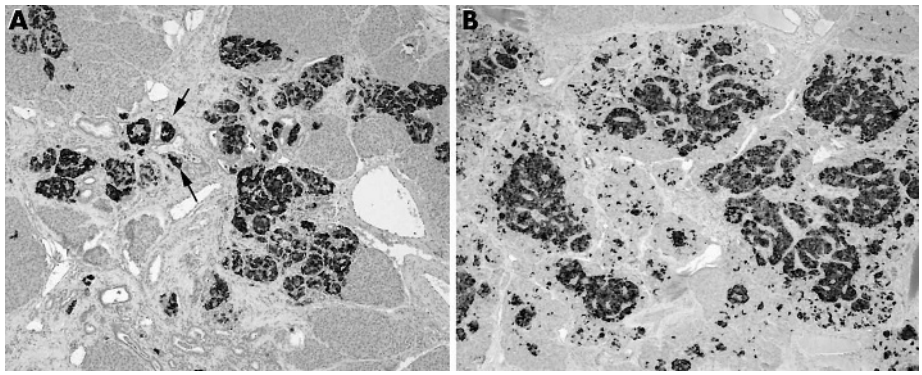

Figure 1 Histology of the pancreatic lesions. (A) Patient 1: Hyperplastic endocrine area with β cell nests of various size within fibrous tissue, sometimes budding off pancreatic ducts (arrows). Insulin immunodetection, original magnification ×5. (B) Patient 2: Proliferation of endocrine cells, isolated or arranged in trabecular structures obscuring the exocrine acini. Chromogranin immunodetection, original magnification ×5.

was observed suggesting Beckwith-Wiedemann syndrome. All diagnostic criteria for congenital hyperinsulinism[3 19] were present, including recurrent fasting and fed hypoglycaemia (<3 mmol/l) with inadequate plasma insulin levels, a positive intravenous glucagon test, and requirements for high rates of intravenous glucose (>10 mg/kg/min). Hypoglycaemia was not controlled by diazoxide or octreotide. Blood ammonia was normal. Intravenous stimulation tests, carried out as previously described,[20] included a normal intravenous glucose tolerance test (IVGTT), a positive tolbutamide test, and no response to intravenous calcium stimulation.

Pancreatic arteriography with calcium stimulation (PACS) showed high insulin response in the gastroduodenal artery, but no insulin increase in the superior mesenteric and splenic arteries, suggesting an excess of insulin secretion from the head of the pancreas.

Partial pancreatectomy of the head was undertaken, and residual low glucose levels (2.5–3 mmol/l) were normalised by the use of raw cornstarch. This patient is now four years old has four meals a day with cornstarch before each meal and at 12 pm. He has a satisfactory fasting glucose tolerance.

Patient 2 was a boy born at the gestational age of 33 weeks (with normal measures for term) to unrelated white parents. Hypoglycaemia was fortuitously detected at birth. All diagnostic criteria for congenital hyperinsulinism[3 19] were present and the rate of glucose infusion required was 18 mg/kg/min. Blood ammonia was normal. Hypoglycaemia could not be controlled with oral diazoxide. He had a coarse facies, a large and bulbous nose with short columella, a smooth philtrum, and a thin upper lip.

Selective pancreatic venous blood sampling by transhepatic catheterisation was uninformative as the patient did not develop hypoglycaemia during this investigation. At surgery, a macroscopically abnormal pancreatic area was visible in the lower half of the body of the pancreas (20×15 mm), with irregular borders. A pancreatic resection including the isthmus, the corpus, and the tail was undertaken at two months of age. Between the ages of two months and two years, the child still had episodes of hypoglycaemia (<2 mmol/l) unresponsive to drug treatment. A second pancreatic catheterisation revealed insulin hypersecretion in the remaining pancreas. Resection of the inferior moiety of the pancreatic head showed a second abnormal area with histological features similar to the first lesion. Because of the unusual histological pattern, which was possibly consistent with malignancy, a complete diagnostic work up including thoracic, abdominal, and pelvic computed tomography (before and after octreotide injection) and cerebral magnetic resonance imaging was undertaken. These explorations revealed hepatic cystic lesions identified as benign hamartomas. Kidneys, heart, and thyroid gland were normal as were all blood tumour markers.

At six years of age, the boy presented with overgrowth (height and weight >2 SD) but neuropsychological development was normal. He still had mild hypoglycaemia (fasting glucose ~2.9 mmol/l) necessitating four meals a day and cornstarch before each meal and at 11 pm. The hepatic lesions remained stable.

Histopathological studies

Formalin fixed, paraffin embedded pancreatic samples were studied by conventional microscopy by and immunohistochemistry with anti-insulin and anti-proinsulin antibodies (Novo Biolabs, Bagsvaerd, Denmark), anti-chromogranin antibody (Boehringer, Mannheim, Germany), and anti-CDKN1C peptide antibody (NeoMarkers, Fremont, California, USA), as previously described.[2]

Electrophysiology and microfluorimetry

Pancreatic samples from patients with hyperinsulinism and cadaver human donor organs were obtained after legal consent. Langerhans islets were isolated and electrophysiological studies were carried out using the cell attached or inside-out recording configurations of the patch-clamp technique, as previously described.[21]

Genotype analysis

The resected pieces of pancreas were investigated by conventional microscopic and histomorphometric studies, and DNA was extracted from the pancreas contiguous to the analysed samples. DNA from leucocytes of patients and their parents, as well as DNA from skin fibroblasts (from the right hemihypertrophic upper segment and left normal upper segment of patient 1), hyperplastic, and normal pancreas, was used for genotyping. Whole genome screening was carried out using microsatellite markers. Genotyping methods used in this study and marker information are available online (http://irinag.club.fr/index.htm).

For both patients, genomic sequence analysis was undertaken on exons and intron–exon boundaries of the *ABCC8* (*SUR1*) and *KCNJ11* (*KIR6.2*) genes, as previously defined.[4]

Karyotype and fluorescent in situ hybridisation

Standard karyotyping using RHG and GTG banding was done on the patients' blood lymphocytes, fibroblasts, and buccal epithelium. Interphase fluorescent in situ hybridisation (FISH) analysis was used to define the ploidy on cultured and non-cultured leucocytes. α-Satellite DNA probes, specific for the centromeric regions of chromosomes 7 (pZ7.5), 9 (pMR9A), and 12 (pBR12), were used. The clones were a generous gift from Dr Mariano Rocchi, University of Bari, Italy.

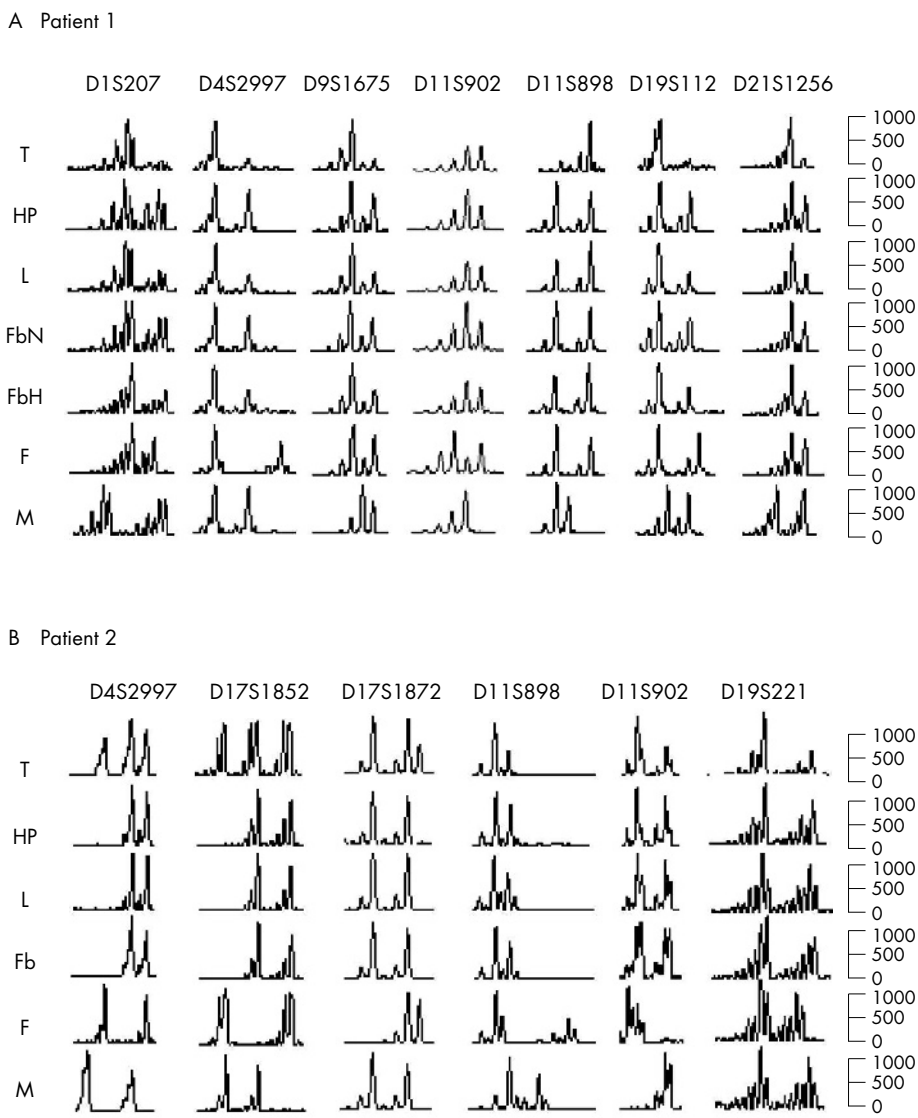

Figure 2 Genotyping by simple fluorescent polymerase chain reaction assay. (A) Patient 1: All the markers showed a loss of the maternal allele in the lesional pancreas and a partial loss of the maternal allele in leucocytes and in the fibroblasts from the hypertrophic upper segment when compared with the healthy pancreas and the fibroblasts from the normal upper segment. (B) Patient 2: In the lesional pancreas the first three markers revealed three alleles (two paternal and one maternal) and three other markers revealed two alleles (one paternal and one maternal) with a paternal/maternal allele intensity about 2 to 1. F, father; FbH, fibroblasts from the hypertrophic upper arm; FbN, fibroblasts from the normal upper arm; HP, healthy pancreas; L, leucocytes; M, mother; T, tumoral pancreas.

Methylation analysis of *LIT1* and *H19*

To identify Beckwith-Wiedemann syndrome, the allelic status of the 11p15 region and the methylation pattern of *LIT1* and *H19* genes were investigated in leucocyte DNA by Southern blot analysis.[22] *LIT1* methylation was assessed by digestion with *Bam*HI and *Not*I (the unmethylated 6 kb *Bam*HI fragment is cut with *Not*I, resulting in a 4.2 kb fragment). The *LIT1* methylation index was determined by scanning autoradiographs and measuring the intensity of the 4.2 kb band relative to the 4.2 kb and 6 kb bands. *H19* methylation was assessed by digestion with *Pst*I and *Sma*I (the unmethylated 1.8 kb *Pst*I fragment is cut with *Sma*I),

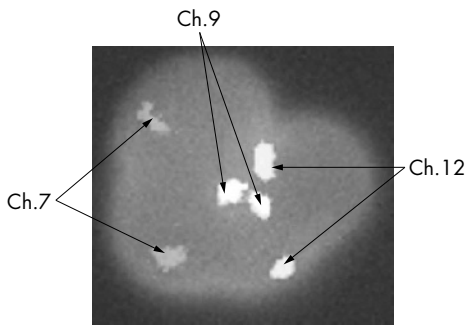

Figure 3 Fluorescent in situ hybridisation (FISH) analysis showing an apparently normal diploid pattern with two spots for each chromosome (Ch) on cells from patient 1.

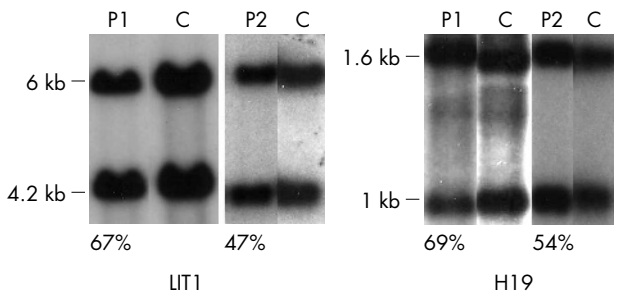

Figure 4 Methylation analysis in Southern Blot of LIT1 and H19 genes. Leucocyte DNA analysis of patient 1 showed a paternal isodisomy of chromosome 11p15 with a partial demethylation (67%) of gene LIT1 and a partial hypermethylation (69%) of gene H19. For patient 2, the methylation analysis of LIT1 and H19 genes was normal at both loci. The methylation index is indicated in the lower part of the figure (calculated as described in Methods). C, control; P1, patient 1; P2, patient 2.

resulting in a 1 kb fragment. The *H19* methylation index was determined by scanning autoradiographs and measuring the intensity of the 1.8 kb band relative to the 1.8 kb and 1 kb bands, as above.

Blood typing
Erythrocyte ABO, Rh, and Duffy antibody detection was carried out using DiaMed® gel according to the manufacturer's specifications.

RESULTS
Histopathological findings
Patient 1
Histological analysis revealed a poorly delineated pancreatic area of hyperplastic tissue which contained endocrine nests of irregular borders and variable sizes scattered within fibrous tissue (fig 1A). In these nests, hyperfunctional cells with large cytoplasm and nuclei were observed. The endocrine cells were mainly β cells with variable staining intensities with regard to insulin, and high labelling for proinsulin, further confirming their hyperactive state. The endocrine nests were surrounded by apparently resting islets. Staining for the CDKN1C peptide (encoded by the maternally expressed $p57^{KIP2}$ gene and located on chromosome region 11p15) was visible in the hyperplastic nests, but the number of CDKN1C positive nuclei was reduced compared with the nuclei of islets at rest. These findings suggest a loss of 11p15 maternal allele expression in some cells from the hyperplastic area.

Patient 2
The first lesion (2 cm in diameter) was localised to the pancreatic corpus and had irregular borders. It was characterised by proliferation of the endocrine component, reaching up to 90% of the pancreatic tissue in some areas. In some fields, endocrine cells were well differentiated and organised as insular structures. However, 60–70% of the lesions were not organised as islets, and the endocrine cells were either isolated or formed large sheets and trabeculae (fig 1B). Their cytoplasm was scarce and their nuclei were irregular. Apoptosis was abundant in some areas and mitoses were common. All cells were chromogranin positive (indicative of their endocrine nature), but only a few were positive for insulin and proinsulin. The second lesion, removed two years later, showed similar histological features.

Electrophysiological investigation of K^+_{ATP} channels
Electrophysiological investigations were undertaken in patient 1 only. Electrophysiology and insulin secretion were studied ex vivo on small fragments of the morphologically normal and abnormal compartments of the pancreas. No significant reduction in the K^+_{ATP} channel peak current values was observed during inside-out patch formation when compared with β cells from control pancreas. Qualitatively similar responses were obtained in both compartments. Glucose-triggered insulin secretion was suppressed by diazoxide, and this suppression was reversed by tolbutamide. Insulin secretion was also increased by a combination of leucine and glutamine. This increase was inhibited reversibly by omission of extracellular Ca^{2+}. These experiments indicate that the basic features of stimulus–secretion coupling (fuel metabolism, K^+_{ATP} channel regulation, and Ca^{2+} sensing) were normal in β cells from all pancreatic regions.

Genotype analysis
Patient 1
Allele loss was detected in all markers studied genome-wide (23 chromosome set) in the lesional pancreas and in some leucocytes and fibroblasts from the right hypertrophic upper segment. All allele losses involved maternally derived markers. No allelic imbalance was detected in normal pancreas or skin fibroblasts from the normal left upper segment of this patient (fig 2A).

Patient 2
Triploidy with two distinct paternal alleles and one maternal allele was observed for half the studied markers in the lesional pancreas. These markers were located on chromosomes 4, 5, 7, 8, 12, 13, 16, 17, 18, 21, and 22. For the remaining markers—located on chromosomes 1, 2, 3, 6, 9, 10, 11, 14, 15, 19, 20, and X—two alleles were found, one of maternal origin and one of paternal origin. However, a 2 to 1 paternal/maternal allele intensity ratio was observed for most markers. In this patient, no allelic imbalance was detected in leucocytes, normal pancreas, or skin fibroblasts (fig 2B).

No mutation of the *ABCC8* and *KCNJ11* genes was detected in either patient, using direct sequencing methods.

FISH and karyotype
Standard blood, skin fibroblast, and buccal epithelium karyotyping was normal for both patients (patient 1: 46, XX; patient 2: 46, XY). Interphase FISH analysis of blood leucocytes showed a normal pattern with two spots for each chromosome on 200 cells analysed for patient 1, confirming diploidy in leucocytes (fig 3).

Methylation analysis of *LIT1* and *H19*
Leucocyte DNA analysis of patient 1 showed paternal isodisomy for the 11p15 region with partial demethylation of the *LIT1* gene and hypermethylation of the *H19* gene. For patient 2, the methylation pattern of *LIT1* and *H19* genes showed two bands of equal intensity, indicating that the two parental alleles were normally methylated at both loci (fig 4).

Blood group typing
Blood group typing was carried out in patient 1. On ABO group typing, patient 1's mother was found to be group A while his father was group B. Some of patient's erythrocytes presented both the A and B epitopes, whereas others presented the B epitope only (fig 5). This indicates the coexistence of two erythrocyte populations in patient 1: one of the AB phenotype with a biparental contribution and another of the B phenotype with a paternal contribution only.

Upon Rh typing, the patient's mother was D+C−E+c+e+ and her father was D+C+E+c+e+ (data not shown). All the patient's erythrocytes presented the C and D antigens, suggesting that the DCe haplotype was inherited from the father and the DcE haplotype from the mother. The presence of a C antigen on all erythrocytes from patient 1, but the lack of an E antigen in some of patient 1's erythrocytes, indicated that one erythrocyte population had an exclusively paternal contribution (fig 5).

Upon Duffy typing, the patient's mother was FY-1, FY+2 and her father was FY+1, FY-2 (data not shown). The child had two types of erythrocytes: one with a biparental contribution FY+1, FY+2 and one with a paternal contribution FY+1, FY-2 only (fig 5).

DISCUSSION
We report two patients with the clinical and biological criteria of hyperinsulinaemic hypoglycaemia. Electrophysiological studies carried out ex vivo on isolated β cells from patient 1 did not reveal any K^+_{ATP} channel dysfunction and no mutation was identified in the *SUR1* and *KIR6.2* genes in either patient, suggesting that the pancreatic K^+_{ATP} channel did not harbour any primary defect in these patients. However, in both patients, treatment by diazoxide (a K_{ATP} channel agonist) was not effective, so that secondary K^+_{ATP}

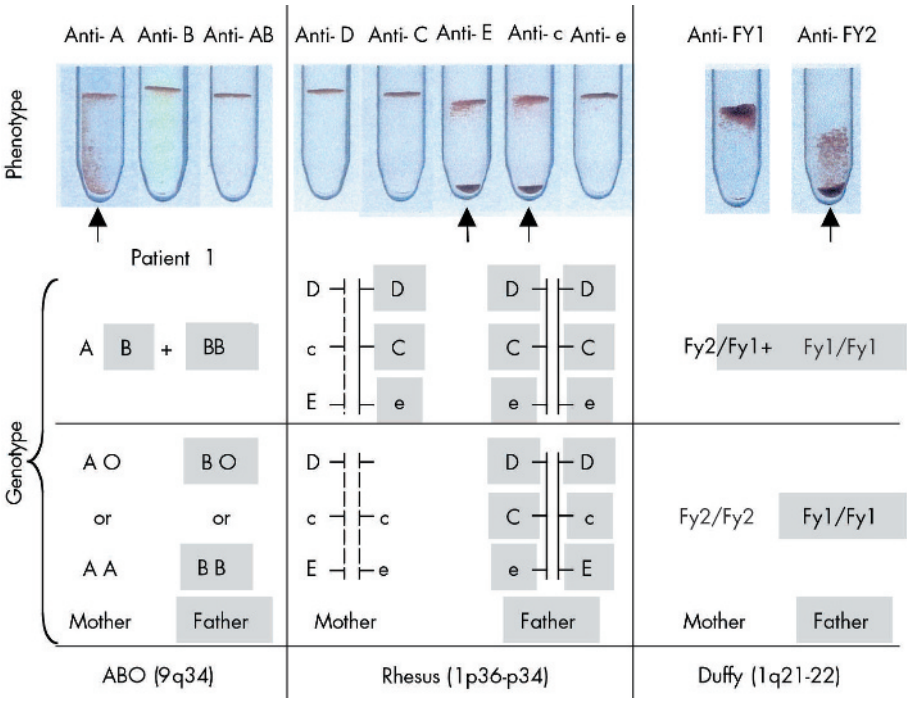

Figure 5 Blood grouping of patient 1 and his parents. Top panels: ABO, Rh, and Duffy blood group phenotypes of patient 1. Arrows indicate mixed field agglutination of antigen–antibody interactions, which are in favour of the presence of two erythroid populations. Bottom panels: schematic representation of the most probable genotype corresponding to the blood group phenotype analysis of patient 1 and her parents. The probability of Rh genotype distribution in the population is taken into account. The localisation of genes coding for ABO, Rh, and Duffy glycoproteins is given in parentheses.

channel dysfunction could not be excluded. Indeed, abnormalities in proteins with a regulatory effect on K^+_{ATP} channel expression (such as HNF3β, a transcription factor)[23 24] could be responsible for a dysfunction of this channel.

Both patients had chromosomal abnormalities involving a whole haploid set (paternal isodisomy or triploidy), restricted or not to the pancreatic tissue. These chromosomal abnormalities could not be readily explained by chimaerism, as no novel maternal allele was observed in hyperplastic pancreas compared with normal pancreas (though in the triploid tissue, heterozygosity for paternal alleles was detected). Therefore mosaicism is suggested. Because in both patients a whole haploid set from a single parent is involved (excess for the paternal haploid set and insufficiency for the maternal haploid set), it is likely that abnormal chromosomal segregation occurred very early during the zygotic stage. Indeed, there is no experimental evidence that the chromosomes can segregate independently according to their parental origin after the fusion of the male and female pronuclei. Thus these segregation abnormalities presumably took place between the fertilisation of the ovum by one or two sperms and the fusion of the pronuclei. Since the early 1990s, several reports have aimed at dissecting the early events occurring between fertilisation and the blastomere stage in a wide variety of species, including mammals. In the hours following fertilisation, the haploid female and male pronuclei undergo a cycle of DNA synthesis resulting in two separate unfused diploid sets. Following DNA synthesis, the two diploid sets converge towards the centre of the blastodisc and fuse to form the zygotic nucleus, before the complete breakdown of the nuclear envelope.[25–27] Any abnormality of these early events, whether owing to the fertilisation by two sperms (diandry), or to abnormal duplication, or to segregation of pronucleic DNA, will therefore possibly result in a homogeneous or mosaic imbalance of the parental haploid sets.

Patient 1 presented with hemihypertrophy and mosaic paternal 11p15 isodisomy in leucocytes, consistent with the diagnosis of Beckwith-Wiedemann syndrome. Pancreatic lesions were reminiscent of ''nesidioblastosis''[28] and the overall histological findings departed from classical Beckwith-Wiedemann syndrome in that the β islets were abnormally large and confluent because of massive proliferation instead of well organised structures.[29] Two discrete diploid biparental and paternal-only cell populations were found in the pancreas, blood cells and fibroblasts, consistent with mosaicism. As the paternal-only cell populations is isodisomic, the excess haploid set must have arisen and segregated before fusion of pronuclei. As suggested by Makrydimas *et al*, this could have occurred following the normal fertilisation by a single haploid sperm, the initial male pronucleus having undergone an endoduplication which resulted in two genetically identical male pronuclei, mimicking the configuration of three pronuclei, one female and two male.[18] This configuration would have generated a first cell contingent resulting from the fusion of the female pronucleus with one of the male pronuclei, and developing to form the majority of the embryonic tissues. The remaining male pronucleus would then have undergone a new round of DNA replication and division to generate the isodisomic cell contingent, contributing to a limited portion of the embryo only (fig 6A).

Patient 2 was operated on for two adenomatous hyperplastic lesions which differed from classical focal adenomatous hyperplasia[2] by their size and histology. Both diploid (normal) and triploid (lesional) cells were found in his pancreatic tissue. In this patient, two hypotheses can be proposed to explain the presence of two genetically different paternal alleles and one maternal allele in the hyperplastic pancreas. First, triploidy may result from dispermy (simultaneous fertilisation by two sperms), or from diploid sperm fertilisation. The resulting ovum with three pronuclei might produce two kinds of blastomeres, one with a triploid set, and the other one with a normal diploid set (fig 6B). This cytogenetic phenomenon is known as ''post-zygotic diploidisation of triploids'' and was well described by Golubovsky.[30] Second, the delayed incorporation of the second sperm pronucleus into one the two blastomeres, after the diploid zygote has been formed,[31 32] is a less probable alternative.

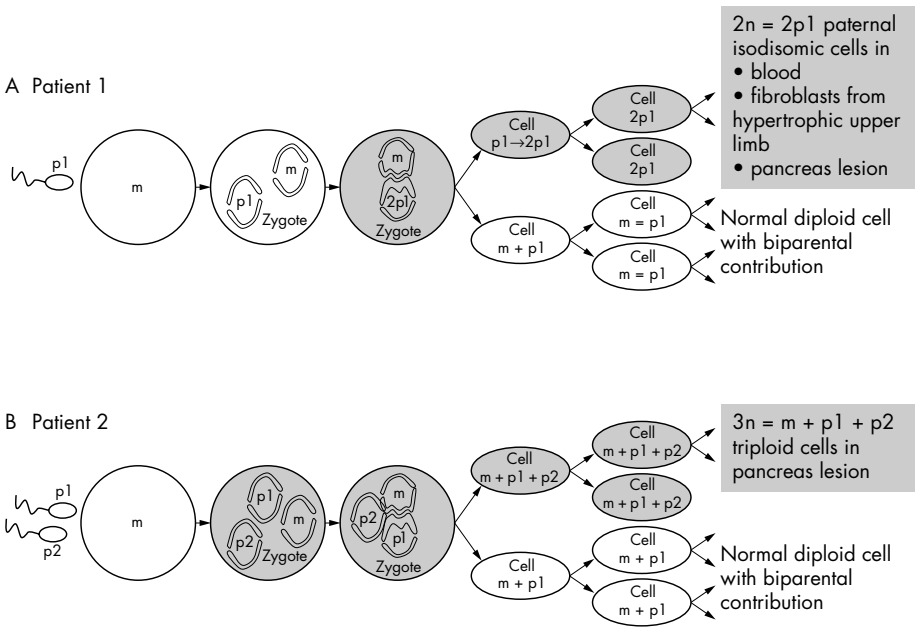

Figure 6 Schematic representation of the most probable mechanism responsible for the mosaicism in patient 1 (A) and patient 2 (B): m, haploid maternal genome; n = 23 chromosomes; p1, a haploid paternal genome; p2, a haploid paternal genome distinct from p1; p1→2p1, the haploid paternal genome perform a DNA replication in order to generate a diploid cell, which is isodysomic 2p1 = 2n.

In patient 2, a triploid population was detected from two lesional foci of pancreatic islet cells, but not from normal pancreas, blood cells, fibroblasts, or buccal epithelium. Unfortunately, no sample from the hepatic hamartoma was available for molecular analysis. In non-mosaic triploid fetuses, development beyond 10 weeks is unusual, and an occasional survival past 20 weeks would lead to stillbirth.[33] Thus mosaic triploidy owing to elimination of most of the triploid cells during early embryogenesis is the most credible explanation for the development and survival of patient 2.

A single observation described the pancreas of four non-mosaic triploid fetuses, one being diandric (two paternal alleles) and three digynic (two maternal alleles). Cytomegaly located particularly to the periphery of the islets was observed in all four fetuses. However, this feature was more outstanding in two of the digynic fetuses than in the other two. Based on immunohistochemistry, these cells were positive for somatostatin but negative for insulin, glucagon, and pancreatic peptide.[33] As somatostatin inhibits insulin secretion, islet cell triploidy is unlikely to be the sole cause of hyperinsulinism in patient 2. In contrast, paternal isodisomy, present in both patients, could be causative through the following possible mechanisms. First, paternal isodisomy could unmask a mutation in the paternally derived allele in different chromosomal settings. In the hyperplastic diploid isodysomic cells, as observed in patient 1, a paternally derived recessive mutation would be upheld to homozygosity (autozygosity).[34] In patient 2, triploidy would result in two copies of a dominant negative paternal mutation overriding a single copy of the maternal allele. Second, paternal isodisomy might deregulate the expression of genes located in chromosomal regions undergoing parental imprinting, leading to genetic imbalance and disease, as observed for imprinted genes located in 11p15, a cause of focal congenital hyperinsulinism[7] and Beckwith-Wiedemann syndrome.[8] Furthermore, the observations underline the genetic overlap of Beckwith-Wiedemann syndrome and congenital hyperinsulinism and the possibility of common pathophysiological determinants, but also extend the complexity of the mechanisms of hyperinsulinism. Indeed, a large proportion of patients with congenital hyperinsulinism have a less severe form of hyperinsulinism which is efficiently treated by diazoxide and does not require pancreatectomy. Some of these patients do not have an aetiological diagnosis for their hyperinsulinism. If the mosaic 11p15 paternal isodisomy (related to the Beckwith-Wiedemann syndrome) is limited to the pancreatic tissue, one can speculate that it could be responsible for isolated hypoglycaemia. Unfortunately, the absence of available pancreatic tissue from these patients precluded verification of this hypothesis. To broaden the scope of these findings, it is specially remarkable that the parental imbalance involved the whole haploid set in both patients, whatever the exact underlying genetic/epigenetic mechanism involved. This might suggest that loci lying elsewhere than 11p15 could also be involved as other known imprinted regions of the human genome equally show imbalance in those patients.

Conclusion

We report the second case of Beckwith-Wiedemann syndrome with genome-wide paternal isodisomy (the first having been described in a Wilms tumour[17]), and suggest that mosaic and genome-wide paternal isodisomy are likely to be underdiagnosed in patients with clinical signs of Beckwith-Wiedemann syndrome or congenital hyperinsulinism. Thus, in disorders known to relate to imprinted regions of the human genome, mosaic chromosomal anomalies should be the focus of further in-depth diagnostic efforts.

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Conflicts of interest: none declared

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REFERENCES

1 **Rahier J**, Sempoux C, Fournet JC, Poggi F, Brunelle F, Nihoul-Fekete C, Saudubray JM, Jaubert F. Partial or near-total pancreatectomy for persistent neonatal hyperinsulinaemic hypoglycaemia: the pathologist's role. *Histopathology* 1998;**32**:15–19.
2 **Sempoux C**, Guiot Y, Dahan K, Moulin P, Stevens M, Lambot V, de Lonlay P, Fournet JC, Junien C, Jaubert F, Nihoul-Fekete C, Saudubray JM, Rahier J. The focal form of persistent hyperinsulinemic hypoglycemia of infancy: morphological and molecular studies show structural and functional differences with insulinoma. *Diabetes* 2003;**52**:784–94.
3 **de Lonlay-Debeney P**, Poggi-Travert F, Fournet JC, Sempoux C, Vici CD, Brunelle F, Touati G, Rahier J, Junien C, Nihoul-Fekete C, Robert JJ, Saudubray JM. Clinical features of 52 neonates with hyperinsulinism. *N Engl J Med* 1999;**340**:1169–75.
4 **Nestorowicz A**, Wilson BA, Schoor KP, Inoue H, Glaser B, Landau H, Stanley CA, Thornton PS, Clement JP, Bryan J, Aguilar-Bryan L, Permutt MA. Mutations in the sulonylurea receptor gene are associated with familial hyperinsulinism in Ashkenazi Jews. *Hum Mol Genet* 1996;**5**:1813–22.
5 **Glaser B**, Kesavan P, Heyman M, Davis E, Cuesta A, Buchs A, Stanley CA, Thornton PS, Permutt MA, Matschinsky FM, Herold KC. Familial hyperinsulinism caused by an activating glucokinase mutation. *N Engl J Med* 1998;**338**:226–30.
6 **Stanley CA**, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, Perlman K, Rich BH, Zammarchi E, Poncz M. Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med* 1998;**338**:1352–7.
7 **Fournet JC**, Mayaud C, de Lonlay P, Gross-Morand MS, Verkarre V, Castanet M, Devillers M, Rahier J, Brunelle F, Robert JJ, Nihoul-Fekete C, Saudubray JM, Junien C. Unbalanced expression of 11p15 imprinted genes in focal forms of congenital hyperinsulinism: association with a reduction to homozygosity of a mutation in ABCC8 or KCNJ11. *Am J Pathol* 2001;**158**:2177–84.
8 **DeBaun MR**, Niemitz EL, McNeil DE, Brandenburg SA, Lee MP, Feinberg AP. Epigenetic alterations of H19 and LIT1 distinguish patients with Beckwith-Wiedemann syndrome with cancer and birth defects. *Am J Hum Genet* 2002;**70**:604–11.
9 **Henneveld HT**, van Lingen RA, Hamel BC, Stolte-Dijkstra I, van Essen AJ. Perlman syndrome: four additional cases and review. *Am J Med Genet* 1999;**86**:439–46.
10 **Baujat G**, Rio M, Rossignol S, Sanlaville D, Lyonnet S, Le Merrer M, Munnich A, Gicquel C, Cormier-Daire V, Colleaux L. Paradoxical NSD1 mutations in Beckwith-Wiedemann syndrome and 11p15 anomalies in Sotos syndrome. *Am J Hum Genet* 2004;**74**:715–20.
11 **Henry I**, Bonaiti-Pellie C, Chehensse V, Beldjord C, Schwartz C, Utermann G, Junien C. Uniparental paternal disomy in a genetic cancer-predisposing syndrome. *Nature* 1991;**351**:665–7.
12 **Weksberg R**, Shen DR, Fei YL, Song QL, Squire J. Disruption of insulin-like growth factor 2 imprinting in Beckwith-Wiedemann syndrome. *Nat Genet* 1993;**5**(2):143–50.
13 **Okano Y**, Osasa Y, Yamamoto H, Hase Y, Tsuruhara T, Fujita H. An infant with Beckwith-Wiedemann syndrome and chromosomal duplication 11p13-pter. : correlation of symptoms between 11p trisomy and Beckwith-Wiedemann syndrome, *Jinrui Idengaku Zasshi* 1986;**31**:365–72.
14 **Hatada I**, Ohashi H, Fukushima Y, Kaneko Y, Inoue M, Komoto Y, Okada A, Ohishi S, Nabetani A, Morisaki H, Nakayama M, Niikawa N, Mukai T. An imprinted gene p57KIP2 is mutated in Beckwith-Wiedemann syndrome. *Nat Genet* 1996;**14**:171–3.
15 **Henry I**, Jeanpierre M, Barichard F, Serre JL, Mallet J, Turleau C, de Grouchy J, Junien C. Duplication of HRAS1, INS, and IGF2 is not a common event in Beckwith-Wiedemann syndrome. *Ann Genet* 1988;**31**:216–20.
16 **Spritz RA**, Mager D, Pauli RM, Laxova R. Normal dosage of the insulin and insulin-like growth factor II genes in patients with the Beckwith-Wiedemann syndrome. *Am J Hum Genet* 1986;**39**:265–73.
17 **Hoban PR**, Heighway J, White GR, Baker B, Gardner J, Birch JM, Morris-Jones P, Kelsey AM. Genome-wide loss of maternal alleles in a nephrogenic rest and Wilms' tumour from a BWS patient. *Hum Genet* 1995;**95**:651–6.
18 **Makrydimas G**, Sebire NJ, Thornton SE, Zagorianakou N, Lolis D, Fisher RA. Complete hydatidiform mole and normal live birth: a novel case of confined placental mosaicism: case report. *Hum Reprod* 2002;**17**:2459–63.
19 **Aynsley-Green A**, Hussain K, Hall J, Saudubray JM, Nihoul-Fekete C, De Lonlay-Debeney P, Brunelle F, Otonkoski T, Thornton P, Lindley KJ. Practical management of hyperinsulinism in infancy. *Arch Dis Child Fetal Neonatal Ed* 2000;**82**:F98–107.
20 **Giurgea I**, Laborde K, Touati G, Bellanne-Chantelot C, Nassogne MC, Sempoux C, Jaubert F, Khoa N, Chigot V, Rahier J, Brunelle F, Nihoul-Fekete C, Dunne MJ, Stanley C, Saudubray JM, Robert JJ, de Lonlay P. Acute insulin responses to calcium and tolbutamide do not differentiate focal from diffuse congenital hyperinsulinism. *J Clin Endocrinol Metab* 2004;**89**:925–9.
21 **Cosgrove KE**, Antoine MH, Lee AT, Barnes PD, de Tullio P, Clayton P, McCloy R, De Lonlay P, Nihoul-Fekete C, Robert JJ, Saudubray JM, Rahier J, Lindley KJ, Hussain K, Aynsley-Green A, Pirotte B, Lebrun P, Dunne MJ. BPDZ 154 activates adenosine 5′-triphosphate-sensitive potassium channels: in vitro studies using rodent insulin-secreting cells and islets isolated from patients with hyperinsulinism. *J Clin Endocrinol Metab* 2002;**87**:4860–8.
22 **Gaston V**, Le Bouc Y, Soupre V, Burglen L, Donadieu J, Oro H, Audry G, Vazquez MP, Gicquel C. Analysis of the methylation status of the KCNQ1OT and H19 genes in leukocyte DNA for the diagnosis and prognosis of Beckwith-Wiedemann syndrome. *Eur J Hum Genet* 2001;**9**:409–18.
23 **Sund NJ**, Vatamaniuk MZ, Casey M, Ang SL, Magnuson MA, Stoffers DA, Matschinsky FM, Kaestner KH. Tissue-specific deletion of Foxa2 in pancreatic beta cells results in hyperinsulinemic hypoglycemia. *Genes Dev* 2001;**15**:1706–15.
24 **Wang H**, Gauthier BR, Hagenfeldt-Johansson KA, Iezzi M, Wollheim CB. Foxa2 (HNF3beta) controls multiple genes implicated in metabolism-secretion coupling of glucose-induced insulin release. *J Biol Chem* 2002;**277**:17564–70.
25 **Schatten H**, Schatten G, Mazia D, Balczon R, Simerly C. Behavior of centrosomes during fertilization and cell division in mouse oocytes and in sea urchin eggs. *Proc Natl Acad Sci USA* 1986;**83**:105–9.
26 **Szollosi D**, Szollosi MS, Czolowska R, Tarkowski AK. Sperm penetration into immature mouse oocytes and nuclear changes during maturation: an EM study. *Biol Cell* 1990;**69**:53–64.
27 **Sathananthan AH**, Kola I, Osborne J, Trounson A, Ng SC, Bongso A, Ratnam SS. Centrioles in the beginning of human development. *Proc Natl Acad Sci USA* 1991;**88**:4806–10.
28 **Heitz PU**, Kloppel G, Hacki WH, Polak JM, Pearse AG. Nesidioblastosis: the pathologic basis of persistent hyperinsulinemic hypoglycemia in infants. Morphologic and quantitative analysis of seven cases based on specific immunostaining and electron microscopy. *Diabetes* 1977;**26**:632–42.
29 **Stefan Y**, Bordi C, Grasso S, Orci L. Beckwith-Wiedemann syndrome: a quantitative, immunohistochemical study of pancreatic islet cell populations. *Diabetologia* 1985;**28**:914–19.
30 **Golubovsky MD**. Postzygotic diploidization of triploids as a source of unusual cases of mosaicism, chimerism and twinning. *Hum Reprod* 2003;**18**:236–42.
31 **Dewald G**, Alvarez MN, Cloutier MD, Kelalis PP, Gordon H. A diploid-triploid human mosaic with cytogenetic evidence of double fertilization. *Clin Genet* 1975;**8**:149–60.
32 **Daniel A**, Wu Z, Darmanian A, Collins F, Jackson J. Three different origins for apparent triploid/diploid mosaics. *Prenat Diagn* 2003;**23**:529–34.
33 **Rowlands CG**, Hwang WS. Cytomegaly of pancreatic D cells in triploidy. *Pediatr Pathol Lab Med* 1998;**18**:49–55.
34 **Ledbetter DH**, Engel E. Uniparental disomy in humans: development of an imprinting map and its implications for prenatal diagnosis. *Hum Mol Genet* 1995;**4**(spec No):1757–64.