CASE REPORT

Clinical Case Reports Open Access WILEY

Inheritance of a paternal *ABCC8* variant and maternal loss of heterozygosity at 11p15 retrospectively unmasks the etiology in a case of Congenital hyperinsulinism

Caroline M. Joyce[1] 🔵 | Jayne A. Houghton[2] | Domhnall J. O'Halloran[3] | Paula M. O'Shea[4] | Susan M. O'Connell[5]

[1]Department of Clinical Biochemistry, Cork University Hospital, Cork, Ireland

[2]Exeter Genomics Laboratory, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

[3]Department of Endocrinology, Cork University Hospital, Cork, Ireland

[4]Department of Clinical Biochemistry, University College Hospital, Galway, Ireland

[5]Department of Paediatrics and Child Health, Cork University Hospital, Cork, Ireland

Correspondence
Caroline M. Joyce, Department of Clinical Biochemistry, Cork University Hospital, Cork, Ireland.
Email: caroline.joyce@hse.ie

Abstract

Advances in genomics and ^{18}F-DOPA PET-CT imaging have transformed the management of infants with Congenital Hyperinsulinism. Preoperative diagnosis of focal hyperinsulinism permits limited pancreatectomy with improved clinical outcomes while knowledge of the molecular etiology informs genetic counseling and provides a more accurate recurrence risk to families.

KEYWORDS

^{18}F-DOPA, diabetes mellitus, Congenital hyperinsulinism, hypoglycemia, loss of heterozygosity

1 | INTRODUCTION

Congenital Hyperinsulinism (CHI) is a rare condition that presents as severe recurrent neonatal hypoglycemia and can lead to irreversible brain injury if not treated promptly. Focal CHI is usually restricted to a small area of the pancreas and results from inheritance of a paternal *ABCC8* or *KCNJ11* variant together with somatic loss of heterozygosity (LOH) of the maternal allele at 11p15. In contrast, diffuse CHI is spread throughout the pancreas and requires a near-total pancreatectomy. We report the case of a male infant who presented with severe hypoglycemia soon after birth and was diagnosed with CHI. As he was unresponsive to medical therapy, he underwent a subtotal pancreatectomy at 6 weeks of age. At age 16, genetic testing revealed a paternally inherited *ABCC8* nonsense variant in the genomic DNA and maternal LOH in the resected pancreatic tissue confirming focal CHI. Shortly after transition to adult endocrinology services, his HbA1c levels started to rise and he was diagnosed with diabetes mellitus. Had genetic testing and ^{18}F-DOPA-PET-CT imaging been available to guide surgery in 1997, this child would have had a partial pancreatectomy to cure his focal CHI thereby mitigating his risk of developing insulin-dependent diabetes. At presentation, his parents were given a 1:4 recurrence risk which could have been reduced to 1:1200 by genetic testing. This case highlights major changes in medical management of CHI over a 20-year period, which have helped guide surgical options, predict prognosis, and improve genetic counseling for these families.

Congenital hyperinsulinism (CHI; MIM #256450) is characterized by recurrent severe hypoglycemia due to inappropriate insulin secretion from pancreatic ß-cells. CHI is the most frequent cause of hypoglycemia in infancy with an estimated incidence of 1:50,000 live births, rising to 1 per 2,500 in populations with high rates of consanguinity. [1,2] CHI may be transient or permanent and inherited in both autosomal recessive and dominant forms. The *ABCC8* and *KCNJ11* genes coding for the sulphonylurea receptor (SUR1) and potassium channel subunit (Kir6.2), respectively, account for 82% of variants in diazoxide-unresponsive patients and 45% of all cases of CHI.[3,4] Both SUR-1 and KIR6.2 are subunits of the ATP-sensitive potassium (K_{ATP}) channel, which regulates insulin secretion. Histologically, two subgroups are apparent; diffuse and focal CHI. Both types may be unresponsive to diazoxide treatment and are difficult to differentiate clinically. Diffuse CHI is usually inherited as an autosomal recessive disorder with abnormal pancreatic β-cells throughout the pancreas. Atypical diffuse forms of CHI exist due to mosaic uniparental disomy (UPD) in patients with dominantly inherited *ABCC8* or *KCNJ11* gene variants.[5] In focal CHI, the abnormal β-cells are confined to an isolated lesion in the pancreas and minimal surgery can be curative.

There are at least seven genes associated with monogenic forms of CHI (*ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH1*, *HNF4A,* and *HNF1A*) as well as several syndromic genetic forms of CHI (eg, Beckwith-Wiedemann, Kabuki, and Turner syndromes).[6] Focal CHI exhibits a non-Mendelian mode of inheritance with K_{ATP} channel dysfunction and beta-cell hyperplasia that results in CHI.[7] In this condition, a paternal *ABCC8* or *KCNJ11* germline mutation is inherited in combination with somatic loss of heterozygosity (LOH) of a maternally derived region within a pancreatic progenitor cell.[8] Somatic loss of the maternal 11p15 region appears to be the causative factor for focal hyperplasia.[9] There are at least two maternally expressed tumor suppressor genes (*H19* and *CDKN1C*) and a paternally expressed insulin-like growth factor II gene (*IGF2*) in this region.[8] The paternally derived mutation is reduced to somatic homozygosity by maternal LOH, and there is an imbalance in imprinted genes in the lesion.[10] Preoperative use of ^{18}F-Fluorine-18-L-dihydroxyphenylalanine positron emission-computerized tomography (^{18}F-DOPA-PET-CT) scans has proven useful for locating focal lesions of islet adenomatosis.[11,12] The clinical challenge is to adequately manage patients unresponsive to medical therapy as severe persistent hypoglycemia can potentially lead to permanent brain damage and developmental delay. This case highlights changes in medical management of patients with hyperinsulinism over a 20-year period.

2 | CASE PRESENTATION

A male infant born at term with macrosomia (4.2 kg, SDS + 1.38) presented with seizures and profound neonatal hypoglycemia soon after birth. There was no family history of hypoglycemia or maternal diabetes. He had a good glycemic response to glucagon and demonstrated inappropriately elevated insulin and C-peptide at time of hypoglycemia. Free fatty acids and ketones were suppressed, ammonia and cortisol were within normal limits, and a metabolic screen was negative. He was diagnosed with CHI and started on diazoxide to limit insulin release but was unresponsive to both diazoxide and octreotide. As a result of his ongoing intractable hypoglycemia, a decision was made to proceed to surgery. At 6 weeks of age, he had a subtotal pancreatectomy (95% removed) in a tertiary pediatric surgical unit. Histological examination revealed multifocal adenomatosis of islet cells, in keeping with focal CHI. He initially required insulin for 6 weeks postoperatively, but made a full recovery and passed a provocative fast at 1 year of age. He did not have genetic testing at diagnosis but his parents were given a 1:4 recurrence risk for CHI.

3 | OUTCOME AND FOLLOW-UP

The child had follow-up in the pediatric endocrinology service in Cork University Hospital. He had normal development, growth, puberty, and academic performance. At 16 years of age, his HbA1c was 41-44 mmol/mol (Reference Interval 20-42), he was asymptomatic, and he was not on any medication. At this stage, the proband consented to genetic testing to determine the etiology of his CHI and to provide a recurrence risk for his family.

Sanger sequencing of the proband revealed a heterozygous nonsense variant, p.(Trp998Ter), (c.2994G > A) in exon 25 of the *ABCC8* gene. Parental analysis showed paternal inheritance of the p.(Trp998Ter) variant. A second variant was not found in *ABCC8* or *KCNJ11,* and the possibility of a focal lesion inherited through maternal LOH was considered. Genomic DNA was extracted from the formalin-fixed paraffin-embedded (FFPE) block of resected pancreatic tissue. The archival DNA was analyzed for twelve microsatellite markers spanning chromosome 11p15.5-11p.15.1, which includes the differentially methylated region (*IGF2, H19*) plus *KCNJ11* and *ABCC8* genes. Peripheral leukocyte DNA from the proband and parents was also analyzed for microsatellite markers to facilitate haplotyping. Genetic analysis of eight informative microsatellite markers on chromosome 11, (D11S2071, D11S1984, D11S4046, D11S1318, D11S1901, D11S1397, D11S1981, and D11S14138) confirmed somatic maternal LOH by showing a reduction in the maternal allele in the pancreatic tissue compared with peripheral leukocyte DNA (Figure 1).

The proband was transitioned to adult endocrinology services for ongoing medical follow-up. The clinical concern

for the patient at this stage was the high probability of him developing diabetes, as there is a higher incidence of diabetes (75%-85%) reported in children after pancreatectomy for CHI, and he was counseled on this.[13] During routine surveillance, at 18 years of age, his HbA1c was elevated at 107 mol/mol, without symptoms, and he was started on insulin therapy.

4 | DISCUSSION

This case highlights retrospectively how changes in best practice and clinical management for CHI over a 20-year period (1997-2017) could have provided a different long-term outcome for this patient. Children with diffuse CHI require a near-total (80%-95%) pancreatectomy and are postoperatively
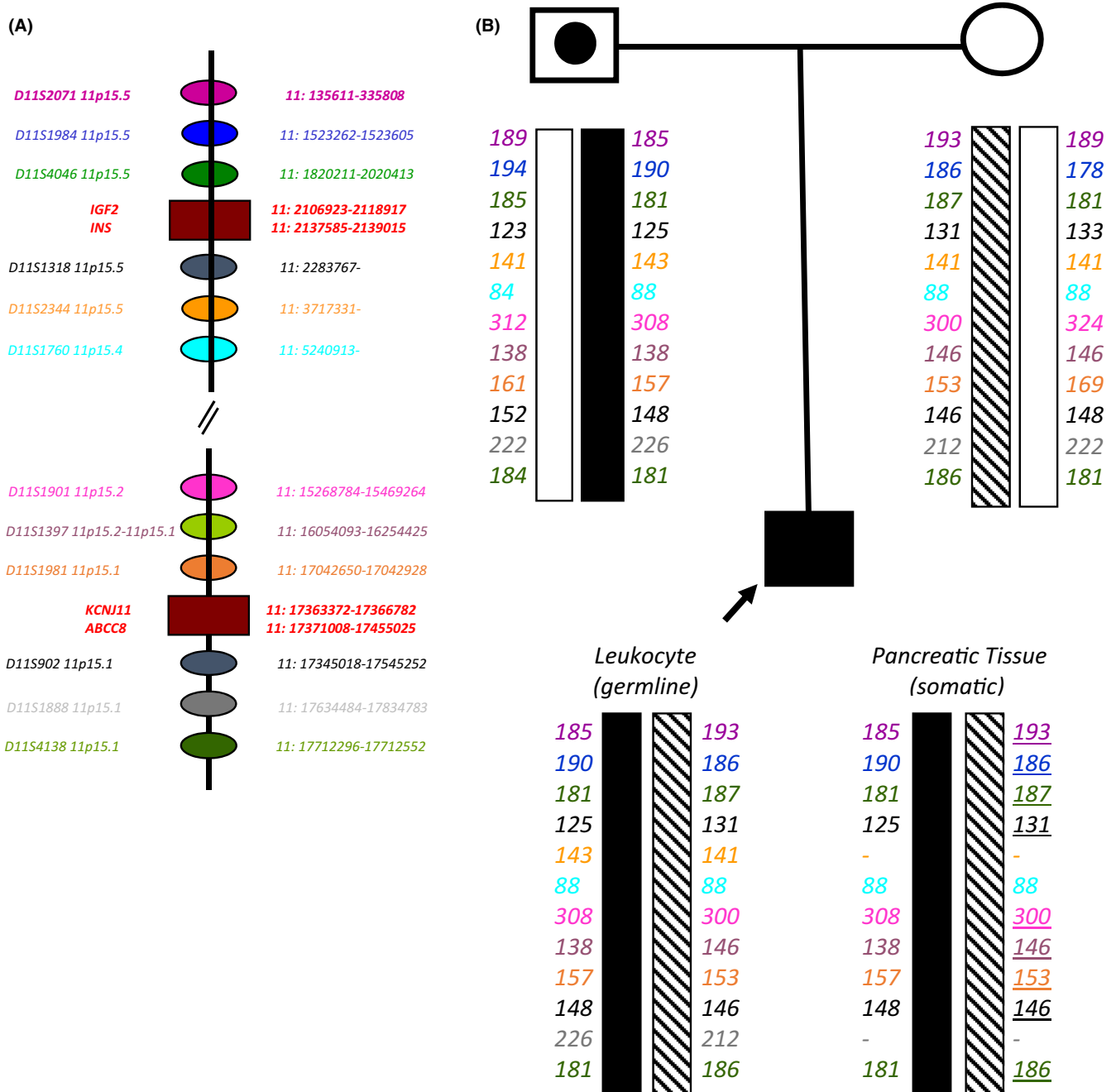

FIGURE 1 Haplotype Mapping of Chromosome 11. Panel A is a graphical representation of the position of twelve microsatellite markers on chromosome 11 from 11p15.5 to 11p15.1 shown relative to imprinted loci (IGF2, INS) and candidate gene clusters (KCNJ11, ABCC8). Panel B shows the family pedigree and haplotypes based on the results of microsatellite analysis using markers from panel A. Allele sizes for each marker are shown in base pair (bp) and colors correspond to the marker locus in panel A. Analysis was performed on leukocyte DNA (germline) in family members and on somatic DNA from the proband's archival pancreatic tissue. Eight informative markers showed a deletion of maternal alleles (underlined) and revealed loss of heterozygosity in the region spanning the ABCC8 locus

at risk of persistent hypoglycemia or the development of diabetes mellitus in later life.[14] Conversely, children with focal disease may be cured by resection of their hyperplastic lesion and the risk for postoperative diabetes is low.[13] Lord et al recently reported that 94% of children who had a limited pancreatectomy for focal CHI were euglycemic and required no further treatment for blood glucose abnormalities.[15] Some infants with CHI have recognizable clinical features of prenatal hyperinsulinism such as fetal overgrowth.[16] Hoegsbery et al reported hyperinsulinism in a cohort of macrosomic infants from nondiabetic mothers.[17] Our patient was born with macrosomia and presented with hyperinsulinism soon after birth.

The clinical challenge with CHI is to differentiate between diffuse and focal CHI subtypes as management strategies and clinical outcomes differ significantly. Focal CHI lesions are rarely identifiable at time of surgery, and intraoperative assessment of frozen sections is difficult.[18] Prior to 2005, pancreatic venous sampling (PVS) and selective pancreatic arterial calcium stimulation with hepatic venous sampling (ASVS) were used to differentiate between the two subtypes.[3,19] With the advent of ^{18}F-DOPA PET-CT scans in 2005, it became possible to locate focal lesions preoperatively and minimize the amount of pancreatic tissue resected..[11] The availability of ^{18}F-DOPA diagnostic imaging is often limited to specialist centers with expertise in scan interpretation and surgical intervention. However, the high cure rate (94%) associated with partial pancreatectomy for focal CHI justifies referral to these expert centres.[15]

When CHI was first diagnosed in our patient in 1997, ^{18}F-DOPA PET-CT scans were not available and the genetics of CHI was not well understood. Our patient had a subtotal pancreatectomy and histological examination revealed focal CHI. In 2013, when he presented again to endocrinology services, there was concern about his risk of developing secondary diabetes. Diabetes is well recognized in dominant genetic forms of CHI such as *HNF4A* and some *ABCC8* variants as a long-term complication of pancreatectomy for diffuse CHI.[20] Our patient developed diabetes in adulthood as a consequence of the extensive pancreatic surgery he underwent in 1997.

Genetic testing of the proband identified a paternally inherited nonsense variant, p.(Trp998Ter), (c.2994G > A) in the *ABCC8* gene. This variant was previously reported as pathogenic in association with persistent hyperinsulinism and hypoglycemia of infancy.[3] The presence of a monoallelic recessive K_{ATP} variant is reported to predict focal CHI with high sensitivity (97%) and specificity (90%).[21] However, another study demonstrated that heterozygosity for a paternal *ABCC8* or *KCNJ11* variant in diazoxide-unresponsiveness CHI is not synonymous with a focal lesion.[22] Hence, diagnosis of focal CHI must be based on clinical features combined with the results of genetic testing and ^{18}F-DOPA PET-CT scanning.[15]

Microsatellite analysis of the resected pancreatic tissue of the proband showed maternal LOH in the region spanning chromosome 11p15.5-11p15.1, which includes the *ABCC8* locus. The inheritance of a paternal *ABCC8* nonsense mutation and somatic LOH of the maternal allele unmasked a recessive disorder and confirmed focal disease (see Figure 1). The deleted region on chromosome 11 contains two clusters of candidate genes. The first cluster at 11p15.5 is an imprinted domain containing maternally expressed genes *H19* and *CDKN1C* ($p57^{KIP2}$) and the paternally expressed *IGF2* gene.[23,24] The second cluster at 11p15.1 contains the *KCNJ11* (SUR1) and *ABCC8* (KIR6.2) genes that code for subunits of the β cell K_{ATP} channel. *KCNJ11* and *ABCC8* are not known imprinted genes but lie next to an imprinted domain and could be involved in epigenetic control.[3] Normal cell growth requires a balance between *H19* (tumor suppressor) and *IGF2* (autocrine growth factor). In our case, loss of *H19* expression with upregulation of *IGF2* caused β cell proliferation and the reduction to homozygosity of the *ABCC8* variant led to focal hyperinsulinism. The size of the focal lesion is dependent on the stage when LOH occurs, which most likely occurs in the late embryonic or early neonatal period.[8,25]

Focal CHI results from a constitutional *ABCC8* paternal mutation in combination with somatic loss of maternal 11p15.5-15.1 or a "second hit phenomenon".[26-28] Prior knowledge of the genetic etiology can guide surgical procedures, inform prognosis and aid genetic counseling.[29] The availability of rapid gene sequencing and ^{18}F-DOPA PET-CT imaging is critical to the management of diazoxide-unresponsive patients.[30] Recent advances in genomics have facilitated earlier diagnosis with a transition from single-gene testing by Sanger sequencing to comprehensive genomic testing by next-generation sequencing. This has improved diagnostic yield with increased detection of deletions and mosaic variants.[18]

When CHI was first diagnosed in our patient in 1997, genetic testing was not available and the parents were given a 1:4 recurrence risk. Following genetic testing in 2013, the family's recurrence risk was lowered substantially. The proband has four younger siblings and their prior risk of inheriting the paternal mutation is 1:2. When this is combined with the risk of a sporadic event in the pancreas giving rise to maternal LOH, reported as 1:600, their prior risk of inheriting CHI is 1:1200.[31] Genetic counseling is recommended for families of children with focal CHI as although, the sporadic event leading to maternal LOH is a rare event during fetal development, there is one reported case of focal CHI occurring in two siblings.[31]

CHI is a heterogenous disorder with high morbidity and limited therapeutic options. Knowledge of the underlying genetic etiology is key as potassium channel mutations are often refractory to medical management but minimum

pancreatic resection can be curative. Although treatment for CHI has improved over the last decade, the risk of adverse neurodevelopment remains high with studies quoting incidence frequencies between 26% and 48%.[20,32] Kaiser et al report early transient newborn hypoglycemia associated with poor academic performance as evidenced by lower achievement test scores at 10 years of age.[33] Consequently, reliable newborn screening programs are urgently needed for the early detection of CHI to prevent irreversible brain injury.

The strength of our case is its educational value from a patient management perspective. It highlights the molecular mechanisms underlying CHI while focusing on the merits of genomic testing and diagnostic imaging to guide surgical management, inform genetic counseling, and improve clinical outcomes. Had these modalities been available at the time of our patient's presentation, there is no doubt his medical management would have been different. He would have had a partial pancreatectomy for his focal CHI, and this would have likely mitigated against his development of iatrogenic diabetes. For older cases which have not previously been worked up from a genetic perspective, we would recommend pursing archival tissue to fully elucidate the genotype. This case highlights the need to develop clinical guidelines for retrospective investigation of older CHI cases so that future risks to offspring may be calculated.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The author(s) declare no potential conflict of interest that could be perceived as prejudicing the impartiality of this case report, authorship, and/or publication of this article.

AUTHOR CONTRIBUTIONS

CJ: drafted the case report. CJ and SMOC: worked up the genetics in this case. SMOC and PMOS: reviewed the draft manuscript. JAH: performed the Sanger sequencing and the microsatellite analysis. DOH: diagnosed diabetes in the proband. All authors: read the final manuscript.

ORCID

Caroline M. Joyce https://orcid.org/0000-0001-7231-8331

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