Novel insights into the clinical and genomic characteristics of congenital hyperinsulinism

T I Hewat

Doctor of Philosophy in Medical Studies

September 2022

Novel insights into the clinical and genomic characteristics of congenital hyperinsulinism

Submitted by Thomas Iain Hewat

to the University of Exeter as a thesis for the degree of

Doctor of Philosophy in Medical Studies

September 2022

I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

Acknowledgements

There are so many people that I would like to thank for their support, not just during the last three years but for their help in getting me to a place where I could do a PhD in the first place.

My family have been a massive support, both in the last few years and in helping me develop into the man I am. The dogs (Alfie before he passed away, Stanley, and Molly) have been fantastic, always bringing a smile to my face. My brother Sam never fails to make me laugh, and our Football Manager and Civilisation sessions were a much-needed break. My mam, Paola, is the kindest, most caring person I know, and has always believed in me even when I didn't. My dad, Andy, has always taught me to see the funny side and not take myself too seriously, and I've learned so much about the importance of hard work and graft from him. As a family, they have always been an immense support, and I don't think I could ever thank them enough for what they've done for me. I'm so grateful for all of them.

Many of my friends have supported me throughout the course of this PhD. Particular thanks go to Sian Grace, who has been a massive support, whether we were rolling our chairs back to each other's desks in the open office or through Zoom calls over the pandemic, and to Amy Haeffner, who has been my absolute rock since I was an undergraduate, and has always helped me to see the best in myself. Jason Taylor, Nicole Howcroft, and Ross McLetchie have all been a great support from a distance, while in Exeter I'm incredibly grateful for the friendship of Dr Taona Nyamapfene, Dr Hannah Mulligan, and Dr Lavin Assad.

Perhaps the strangest thanks to go into a PhD thesis goes to an online writing community based on the website NationStates, where I've written about a fictional nation and specifically its sports over the past ten years (with some large gaps when I've been busy). Having joined the community as a 14 year old with very little experience in writing, various members of the community there guided me in developing a writing style and understanding how to communicate clearly. I genuinely don't believe I'd be as good of a written communicator without the time spent on that site, and for that the whole community deserves my thanks.

A special thanks in terms of the development of my career goes to Dr Sam Washer, who took me under his wing when I spent six weeks in the Complex Disease

Epigenetics group in the summer of 2018. The time spent with him made me realise that I actually wanted to be a scientist, and that it was something I could seriously pursue. Dr Tom Laver, first as my dissertation supervisor and then as a colleague in the Hyperinsulinism Genes team, told me about the PhD project I eventually took on and encouraged me to apply, then became a great mentor. Dr Matt Johnson is someone that also deserves my thanks, for supporting me both scientifically and personally in some of the more stressful times of my PhD.

Perhaps most of all, my supervisors are a pair of incredible scientists, and I cannot thank them enough for their support and mentorship in these last three years. Dr Emma Dempster facilitated my time in the Complex Disease Epigenetics group as an undergraduate, and then became a great guide in my learning when it came to epigenetics as a PhD student. Prof. Sarah Flanagan is someone I look up to and aspire to be like one day: she combines being an incredible scientist with being incredibly supportive of colleagues in the team, and also shows that it’s possible to still be humble when you’re a world-leading scientist who has made a major difference to the lives of children with rare diseases.

Abstract

Congenital hyperinsulinism is a disorder affecting the pancreatic beta cell, where insulin is inappropriately secreted during hypoglycaemia. It often appears within the first few weeks of life, and can have a severe impact on a neonate's health and development. It is a genetically heterogeneous disorder, with 10 genes reported to cause isolated hyperinsulinism, and 28 known causes of syndromic hyperinsulinism. This thesis aimed to gain novel insights into the clinical and genetic basis of congenital hyperinsulinism, using the large cohort of individuals referred for genetic testing in Exeter.

The first section of this thesis introduces the clinical, molecular, and genetic basis of congenital hyperinsulinism, along with how genetic testing and clinical management are carried out. The following section describes how the cohorts included in this thesis were recruited.

In Chapter 1, I used clinical features commonly observed in the early stages of hyperinsulinism, including birth weight, response to treatment, and biochemical measurements, in order to identify the likelihood of individuals having a mutation in either *ABCC8* or *KCNJ11*, the two most common causes of monogenic hyperinsulinism. I identified that an increased birth weight and failure to respond to diazoxide, the first line treatment for hyperinsulinism, are highly predictive of a mutation in one of these two potassium channel genes. These features can be used to predict an individual's likelihood of carrying one of these mutations, which may have an impact on their medical management.

In Chapter 2, I identified eleven cases of coinciding Down syndrome and congenital hyperinsulinism, and performed a review of the clinical features of these individuals in order to identify the links between these two disorders. I identified that the prevalence of Down syndrome in the Exeter hyperinsulinism cohort is around four times higher than that found in the general population. From the review of clinical details, I found that non-genetic risk factors for hyperinsulinism were present in the majority of cases: these risk factors included complications of gastric surgery, chemotherapy treatment for acute lymphoblastic leukaemia, prematurity, and intra-uterine growth restriction. This indicated that the link between Down syndrome and hyperinsulinism was most likely based in these non-genetic risk factors.

In Chapter 3, I identified tandem duplications in the vicinity of the gene *KDM6A* in three patients. Point mutations and deletions in *KDM6A* are known to cause Kabuki syndrome and syndromic hyperinsulinism but there have been no reports of duplications causing disease. I used genetic and epigenetic analyses, including publically available data, in order to predict the likelihood that these duplications were leading to a loss of function, and therefore to disease. In one case, a frameshift leading to a premature stop codon was detected. In two cases, I performed analysis of DNA methylation data and compared it to known cases of Kabuki syndrome, showing that one duplication was likely to be pathogenic, while the other did not have evidence to support that conclusion.

In Chapter 4, I identified individuals with clinical features suggestive of a disorder of DNA methylation, and performed whole genome sequencing in order to identify novel causes of syndromic hyperinsulinism. By this method, I identified two individuals with a protein-truncating variant in the gene *MAGEL2*, known to cause Schaaf-Yang syndrome. I identified that previous reports of the endocrine features of this disorder have indicated a predisposition towards hypoglycaemia, though this is caused by growth hormone deficiency in some cases. Ultimately, I believe these two cases of hyperinsulinism, in addition to one previously reported in the literature, provide sufficient evidence to include *MAGEL2* in genetic testing panels for hyperinsulinism.

In summary, this thesis has resulted in a better understanding of the clinical, molecular, and genetic basis of congenital hyperinsulinism. This understanding is vital, as it can inform medical management, identify new genes involved in insulin secretion pathways, and inform on the development of new treatments for this disorder. The work presented in this thesis expands our understanding of the most common cause of congenital hyperinsulinism, as well as identifying novel causes of syndromic hyperinsulinism, and helps us to determine avenues which should be followed in further studies of this disorder. It will also inform the development of guidelines for the management of monogenic hyperinsulinism, and inform on the genes that should be included in routine sequencing panels for this disorder.

Table of Contents

Organisation of thesis

As a number of chapters in this thesis are based on work published or prepared for publication, each chapter is preceded by a statement of the work completed by myself and acknowledgements of co-authors.

Introduction

The introduction of this thesis is split into two parts. The first section discusses the molecular basis of isolated HI, along with the histology that results from mutations in *ABCC8* and *KCNJ11*. It also discusses syndromic forms of hyperinsulinism, and the medical and surgical management of this disorder. The second part is a review published in Frontiers in Endocrinology, written with Dr. Matthew Johnson and Prof. Sarah Flanagan. This review looks at the isolated and syndromic causes of congenital hyperinsulinism, along with discussing different methodologies used to investigate the genetics of patients with congenital HI.

Cohort Recruitment

This section discusses the methods by which the cohorts studied in this thesis were recruited, and the ethics under which this was conducted.

Chapter 1

Chapter 1 is an article published in the European Journal of Endocrinology titled "Birth weight and diazoxide unresponsiveness strongly predict the likelihood of congenital hyperinsulinism due to a mutation in *ABCC8* or *KCNJ11*". In this study, we used statistical methods to determine which clinical features were the strongest predictors of a mutation in *ABCC8* or *KCNJ11*, the most common causes of congenital hyperinsulinism.

Chapter 2

Chapter 2 is an article published in Pediatric Diabetes titled "Increased referrals for congenital hyperinsulinism genetic testing in children with trisomy 21 reflects the high burden of non-genetic risk factors in this group". In this study, we identified an increased prevalence of individuals with Down syndrome in a hyperinsulinism genetic testing cohort compared to the general population, and performed a retrospective study in order to determine the cause of this increase in prevalence.

Chapter 3

Chapter 3 is an article prepared for publication in Clinical Epigenetics titled “DNA methylation data can support the pathogenicity of duplications within the KDM6A gene”. In this study, we identified patients with duplications in *KDM6A*, a gene known to cause Kabuki syndrome, and confirmed the pathogenicity of two of these duplications by studying the episignature of these patients, and comparing it to that of patients with truncating and missense mutations causing Kabuki syndrome.

Chapter 4

Chapter 4 is an article prepared for publication in Pediatric Diabetes titled “Loss of function variants in the maternally imprinted gene, *MAGEL2* are a rare but important cause of congenital hyperinsulinism”. In this study, we identified the second and third cases of mutations in *MAGEL2* causing congenital hyperinsulinism.

Conclusions

This chapter discusses the key findings of the four data chapters described above, and puts them in the context of wider research into the genetics and epigenetics of congenital hyperinsulinism. It also discusses the issues faced in the course of the research that produced this thesis and how these issues were confronted, along with future avenues of research to expand on the work presented in this thesis.

Abbreviations

ABCC8 – ATP-binding cassette, subfamily C, member 8

ACMG - American College of Medical Genetics and Genomics

ADK – Adenosine kinase

AGA – Appropriate for gestational age

ALG3 - Alpha-1,3- mannosyltransferase

ASD – Atrial septal defect

ATP - Adenosine triphosphate

AUC – Area under the curve

BW – Birth weight

BWS – Beckwith-Wiedemann syndrome

CACNA1D - Calcium channel, voltage-dependent, L type, alpha 1D subunit

CDKN1C – Cyclin dependent kinase inhibitor 1C

CGH - Comparative genomic hybridisation

CHD8 – Chromodomain helicase DNA binding protein 8

CHI – Congenital hyperinsulinism

CI – Confidence interval

CNV – Copy number variant

CREBBP – Cyclic adenosine monophosphate response element binding protein binding protein

DIS3L2 - DIS3 like 3'-5' exoribonuclease 2

DNA – Deoxyribonucleic acid

DR – Diazoxide responsiveness

EHMT1 - Euchromatic histone-lysine N-methyltransferase 1

EIF2S3 - Eukaryotic translation initiation factor 2 subunit 3

EP300 - E1A binding protein p300

FAH - Fumarylacetoacetate hydrolase

FOXA2 - Forkhead box protein A2

GB – Gigabyte

GCK – Glucokinase

GI - Gastrointestinal

GLUD1 – Glutamate dehydrogenase 1

GnomAD – Genome Aggregation Database

GORD – Gastro-oesophageal reflux disease

GOSH – Great Ormond Street Hospital for Children

GPC3 – Glypican 3

HADH - Hydroxyacyl-Coenzyme A dehydrogenase

HI – Hyperinsulinism

HK1 – Hexokinase 1

HNF1A – Hepatocyte nuclear factor 1 homeobox A

HNF4A – Hepatocyte nuclear factor 4 alpha

HRAS - HRas proto-oncogene, GTPase

INSR – Insulin receptor

Indel – insertion/deletion

IUGR – Intra-uterine growth restriction

IV - Intravenous

KATP – ATP-sensitive potassium channel

KCNJ11 – Potassium channel, inwardly rectifying, subfamily J, member 11

KDM6A – Lysine demethylase 6A

Kir6.2 - Inward-rectifier potassium channel 6.2

KMT2C – Lysine-specific methyltransferase 2C

KMT2D – Lysine-specific methyltransferase 2D

LGA – Large for gestational age

MAGEL2 – Mage-like 2

MLPA - Multiplex-ligation dependent probe amplification

mmol/L – millimole per litre

MODY – Maturity-onset diabetes of the young

MPI – Mannose phosphate isomerase

MS-MLPA – Methylation-specific multiplex-ligation dependent probe amplification

NSD1 – Nuclear receptor-binding SET domain protein 1

OMIM – Online Mendelian Inheritance in Man

OR – Odds ratio

PCR – Polymerase chain reaction

PDA – Patent ductus arteriosus

PHOX2B – Paired like homeobox 2B

PMM2 - Phosphomannomutase 2

pmol/L – picomole per litre

RNA – Ribonucleic acid

ROC – Receiver operating curve

SD – Standard deviation

SGA – Small for gestational age

SLC16A1 – Solute carrier family 16 member 1

SNV – Single nucleotide variant

SUR1 – Sulfonylurea receptor 1

TRMT10A – tRNA methyltransferase 10A

UK – United Kingdom

USH1C - USH1 protein network component harmonin

VSD – Ventricular septal defect

WGS – Whole genome sequencing

YARS - Tyrosyl-tRNA synthetase, cytoplasmic

Introduction

Structure of introduction

This introduction is structured in two sections. The first part provides an overview of how congenital hyperinsulinism (HI) is managed, both in general and specifically within the UK, including its clinical diagnosis and medical and surgical management. It also includes details on the underlying biology of HI, the differences between transient and persistent forms of the condition, and how the underlying genetics impact on the age at onset.

The second part of the introduction takes the form of a review article written for ‘The Problem of Childhood Hypoglycemia’, a research topic that was published in Frontiers in Endocrinology. This review covers what is already known about the various genetic causes of HI, along with how different genomic methodologies are used in the diagnosis of this disorder, including Sanger sequencing, targeted next-generation sequencing panels, and whole genome/exome sequencing.

Introduction part 1

The underlying biology of isolated HI

Congenital hyperinsulinism (HI) results from the over-secretion of insulin at times of hypoglycaemia, and this generally results from defects in pathways of insulin secretion from the pancreatic beta cell. Many of the known genetic causes of HI, particularly those which lead to isolated disease, include genes whose protein products are critical for the normal function of these pathways. The following section describes the normal process of insulin secretion, with notes on where this pathway is disrupted in congenital hyperinsulinism.

GLUT2, encoded by the *SLC2A2* gene, is the major glucose sensor in the pancreatic beta cell (1). High glucose influx into the beta cell is maintained due to this protein having a low substrate affinity (2). After entering the beta cell, glucose is phosphorylated. Unlike most mammalian cell types, where this process is undertaken by the hexokinase 1 enzyme (encoded by the *HK1* gene), in beta cells this occurs through the hexokinase 4 enzyme (glucokinase), encoded by the *GCK* gene (3). This enzyme is the rate limiting step in glucose-stimulated insulin secretion because of two factors. Firstly, it has a lower affinity for glucose relative to other members of the hexokinase family, and secondly, it is not inhibited by the presence of glucose-6-phosphate, the product of this enzymatic reaction, which allows it to continue to function at times when glucose levels are high (3). Disruption of this process, as a result of activating mutations in the *GCK* gene, or non-coding mutations leading to inappropriate expression of the *HK1* gene, can lead to HI by introducing a glucose sensor to the beta cell with increased affinity for glucose (4, 5). This process is depicted graphically in Figure 1.

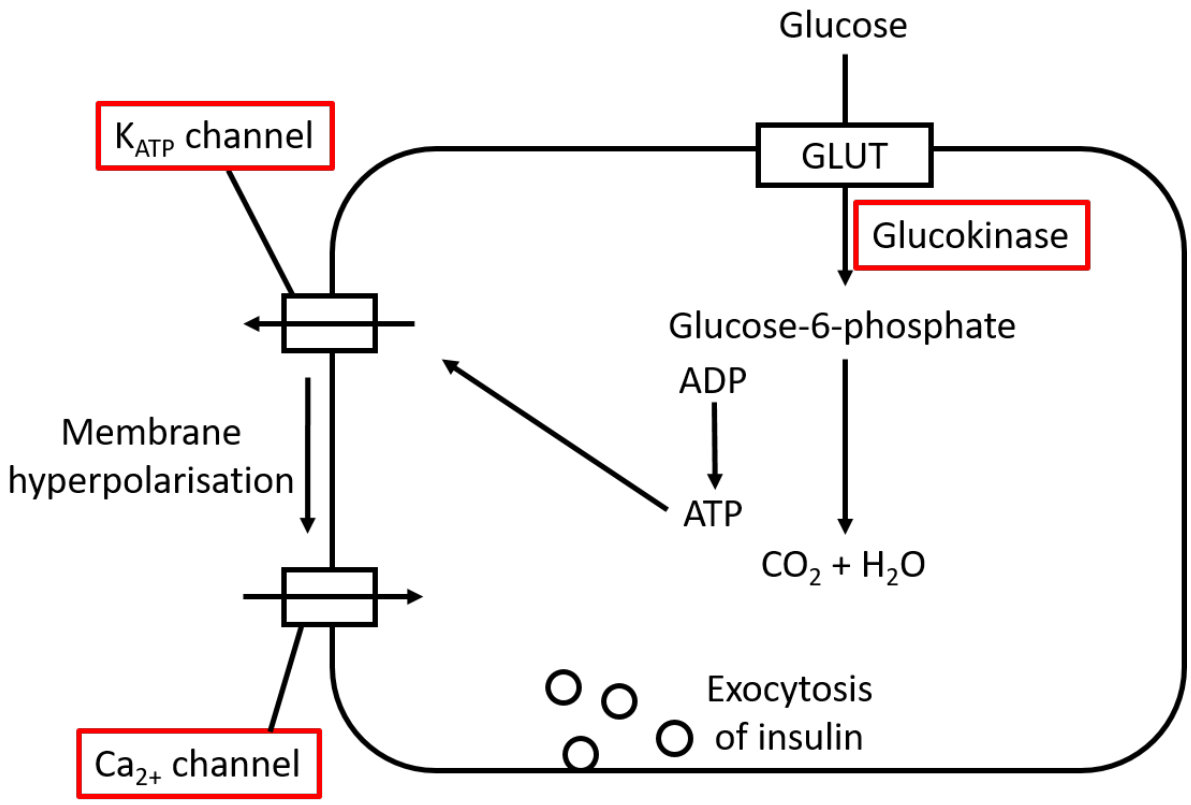

Figure 1 - A graphical depiction of the process of glucose-stimulated insulin secretion from the pancreatic beta-cell. Red boxes indicate proteins that when dysregulated can cause congenital hyperinsulinism.

The process of glycolysis leads to the production of pyruvate, which is an important substrate for the tricarboxylic acid cycle, by which the mitochondria produce ATP (6). This increased concentration of ATP leads to an increase in the cell's ATP/ADP ratio, resulting in the closure of voltage-gated potassium (KATP) channels at the cellular membrane. This leads to depolarisation of the cell plasma membrane, followed by the opening of voltage-gated calcium channels. This influx of Ca^{2+} then leads to the exocytosis of granules containing insulin. This signalling pathway shows the vital role of ion channels, and the genes that encode them in the process of glucose-stimulated insulin secretion, with genes such as *ABCC8*, *KCNJ11*, and *CACNA1D* all involved in this pathway. Disruption of all these genes can cause HI (7-9).

Substrates other than glucose can trigger the secretion of insulin, and disruption of these 'alternative' pathways can also lead to HI. For example, the presence of

glutamine and leucine, as free amino acids, can lead to an increase in insulin secretion (10). Leucine activates the glutamate dehydrogenase enzyme, encoded by the *GLUD1* gene. After glutamine is converted into glutamate in the cytosol of the cell, the glutamate dehydrogenase enzyme acts to convert it to α-ketoglutarate, which can be used in the tricarboxylic acid cycle to produce ATP, thereby triggering the closure of KATP channels and the release of insulin as described above (11). Disruption of this process as a result of activating mutations in *GLUD1*, or inactivating mutations in the *HADH* gene, leads to HI (12, 13).

Syndromic HI and its causes

While the most common causes of congenital HI lead to an isolated pancreatic phenotype, there are 28 described syndromes where HI is reported to occur. A list of the syndromes is included in Part 2 of this introduction, but some of the causes most relevant to this thesis are discussed in detail in this section.

Syndromic HI can have a wide range of genetic aetiologies: this includes chromosomal abnormalities (aneuploidies), such as Turner syndrome (14) and Patau syndrome (15), large CNVs, such as those that lead to Usher syndrome (16) and Chromosome 9p deletion syndrome (17), disorders of genomic imprinting in the case of Beckwith-Wiedemann syndrome (18), and disorders caused by single nucleotide changes. This last category includes Kabuki syndrome (19), Sotos syndrome (20), and Rubinstein-Taybi syndrome (21), all of which are known to lead to changes in DNA methylation, along with wider effects on the epigenetic machinery (22).

Kabuki syndrome is a disorder where HI has been described as a presenting feature, meaning that it is the reason for which an individual might seek medical attention leading to their eventual diagnosis (23). A consensus statement on Kabuki syndrome in 2019 identified that the disorder is characterised by distinctive facial dysmorphism, developmental delay, and infantile hypotonia, with HI presenting in infancy considered to be “supportive” of a diagnosis of Kabuki syndrome (24). There are two known genetic causes of Kabuki syndrome, with loss of function mutations in the *KMT2D* gene (25) accounting for around 90% of genetic diagnoses. The remainder are due to loss of function mutations in *KDM6A* (26). HI is reported to occur at a

higher rate in individuals with *KDM6A* mutations than those with *KMT2D* mutations (24).

Both the *KDM6A* and *KMT2D* genes play a role in the epigenetic machinery (27), with *KMT2D* methylating the H3K4 mark (28), while *KDM6A* demethylates the H3K27 mark (29). Both these changes in methylation are reported to lead to an increase in gene expression. As a result of their role in the epigenetic machinery, Kabuki syndrome can be identified by a pattern of DNA methylation, also known as an episignature (30). These episignatures have been identified for 42 disorders at the time of writing, including Sotos syndrome and Rubinstein-Taybi syndrome, and can be used to aid in the diagnosis of these disorders (22).

Hyperinsulinism in the United Kingdom

Congenital hyperinsulinism is a complex condition requiring specialised care through a multidisciplinary team led by an expert paediatric endocrinology service. The management of HI, particularly medically unresponsive HI is challenging. The nationally designated HI service in the United Kingdom is commissioned on behalf of the NHS and comprises of two specialist centres: Great Ormond Street Hospital for Children NHS Foundation Trust in London and The Northern Congenital Hyperinsulinism Service (NORCHI). NORCHI comprises of two centres, the Royal Manchester Children's Hospital (RMCH) and Alder Hey Children's Hospital (AHCH) in Liverpool. These centres offer the gold standard of care to neonates with HI, with a particular focus on ensuring normoglycaemia using IV glucose initially, and then with drugs such as diazoxide or octreotide. These centres are equipped to perform 18-F-DOPA PET scanning, which allows for the identification of focal or diffuse disease, and they work extremely closely with the NHS Genomics Laboratory in Exeter, which performs genetic testing for HI for all individuals referred within the England and Wales.

The existence of two clinical centres for HI treatment and one genetic testing laboratory allows for large studies that identify the incidence of this disorder in the UK. Through these collaborations, a 2020 study identified the minimal incidence of HI in the UK to be 1 in 28,389 (31). This is comparable to other estimates of the incidence of HI in outbred European populations, with estimates ranging from 1 in

27,000 in Ireland to 1 in 50,000 in the Netherlands (32-34) (Figure 2). There are also examples of populations with a higher incidence of HI: for example, in Central Finland the incidence increases to 1 in 3,200 as a result of two common founder mutations in the *ABCC8* gene. In Saudi Arabia the incidence was shown to be 1 in 2,675 which is likely to result from an increase in recessively inherited disease as a result of the high rates of consanguineous unions in the population (Figure 2).

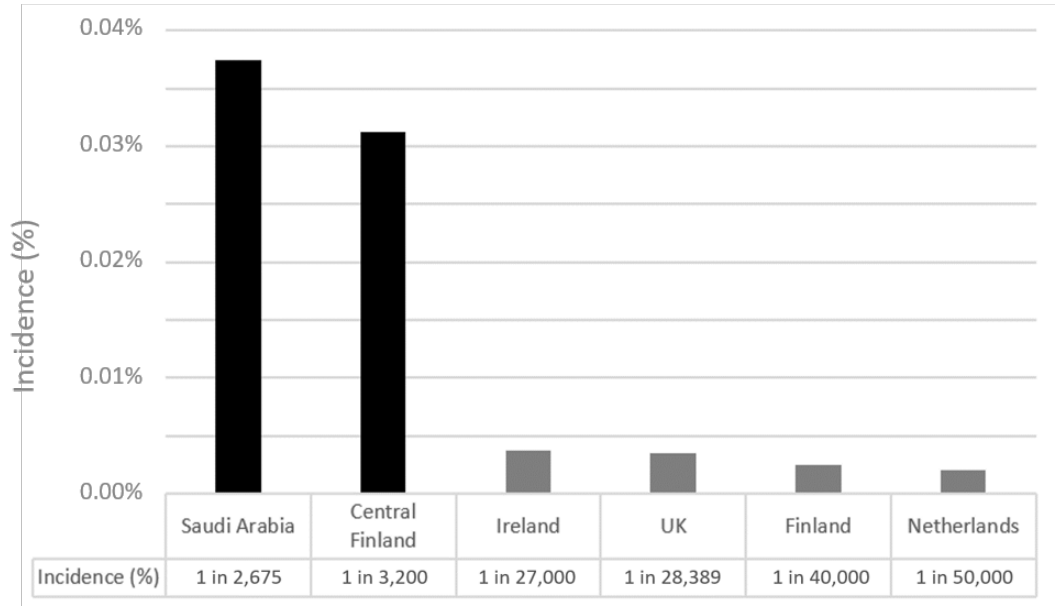

Figure 2 – The reported incidence of HI in 6 studies. Black bars indicate populations with increased rates of HI, while grey bars indicate outbred populations.

Diagnosing neonatal hypoglycaemia and HI

As hypoglycaemia often presents in the neonatal period in cases of HI, it can be challenging to obtain a diagnosis, particularly as neonates are unable to communicate their symptoms, thus rendering traditional diagnostic thresholds such as Whipple's triad difficult to utilise (35). The initial symptoms of HI are often non-specific, such as poor feeding and lethargy (36). However, if the presence of severe hypoglycaemia goes unrecognised, the consequences can include seizures, coma, and long-term neurological sequelae (37). As such, it is vital to identify neonatal hypoglycaemia, and HI, at the earliest possible stage to enable the best medical management and ensure the best outcome for the individual.

Birth weight can in some cases help to identify newborns who are at high risk of having hypoglycaemia and congenital HI. Insulin acts as a fetal growth factor *in utero*, and consequently individuals who are over secreting insulin *in utero* will have an increased birth weight. This is often observed in individuals with monogenic HI who have pathogenic variants in a gene affecting the glucose-stimulated insulin secretion pathway (e.g. *ABCC8*, *KCNJ11*, *CACNA1D*) (38-41) highlighting the importance of this pathway in foetal insulin secretion.

In 2015, the Pediatric Endocrine Society presented a suite of recommendations on the evaluation and management of individuals with persistent hypoglycaemia in the paediatric setting (35). In the initial stages, they suggest evaluation of those with a plasma glucose level measured under 3.3 mmol/L on a laboratory quality assay, and that neonates at high risk of persistent hypoglycaemia are evaluated after 48 hours following their birth to assess them after the period of transitional glucose regulation. They listed seven features considered to indicate neonates at a high risk of persistent hypoglycaemia: symptomatic hypoglycaemia, being large for gestational age, perinatal stress, premature or postmature delivery, being the infant of a diabetic mother, a family history of a genetic form of persistent hypoglycaemia, or signs of a congenital syndrome (35).

Transient and persistent HI

While persistent HI, which lasts longer than six months, is usually considered most likely to be a monogenic disease, transient HI, which remits within the first few months of life, can result from many factors. In transient HI it is important to differentiate between disease and normal transitional neonatal hypoglycaemia, a commonly observed phenomenon which occurs within the first few days of life (42). In contrast transient HI persists longer than transitional neonatal hypoglycaemia, and usually results from perinatal stress, including intrauterine growth restriction and prematurity, along with maternal factors such as pre-eclampsia and diabetes (43). This perinatal stress-linked HI is generally understood to remit spontaneously within the first three to six months of life (44). However, without treatment transient HI can have many of the same outcomes as in those individuals with persistent HI, with

around 30% of individuals with transient HI reported to have some form of abnormal neurodevelopment (45).

HI can also occur later in life as a result of many different factors unlinked to a monogenic cause of HI. One of the best recognised of these is dumping syndrome, where iatrogenic hypoglycaemia occurs as a result of gastric surgery, particularly in children (46). This results from the increased speed at which food passes into the small intestine in individuals who have undergone certain forms of gastric surgery, such as fundoplication, leading to hyperglycaemia, which is quickly met by increased insulin secretion, which leads to post-prandial hypoglycaemia occurring around 1-3 hours after a meal (47) (Figure 3). Another disorder which can be misdiagnosed as congenital HI is insulinoma. An insulinoma is defined as a tumour of insulin-secreting cells in the pancreas, and they are the most common endocrine tumour of the pancreas (48, 49). Insulinomas are not generally caused by germline changes in an individual's genetics, though in a small proportion of cases mutations can be identified in genes such as *MEN1* (50).

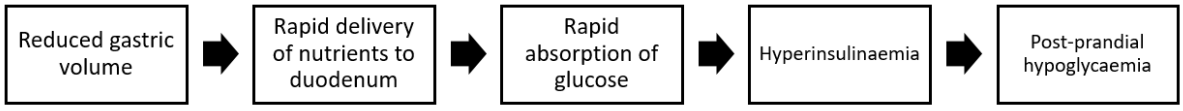

Figure 3 – The process of how reduced gastric volume resulting from gastric surgery can lead to post prandial hypoglycaemia, known as dumping syndrome.

Persistent HI is usually defined as HI lasting longer than six months, and is considered likely to be a monogenic disease, with around 50% of individuals having an identified monogenic cause. The genetics and testing approaches for persistent HI will be discussed further in the second part of this introduction.

Associations between genetic aetiologies and age at HI onset

The age at presentation of HI in an individual is influenced by the genetic aetiology with the majority of patients with disease-causing variants in the KATP channel genes present in the first few days of life (51). This early age at diagnosis is not consistently observed in all genetic causes of HI. Pathogenic mutations in *GLUD1*

cause HI which often presents later in infancy or early childhood. One study of a Finnish HI cohort reports the diagnosis of *GLUD1* mutations occurring in a range of 4-31 weeks, while another study focussed on neurological outcomes reported diagnosis as late as 34 years (52, 53). In addition, the Finnish study also reported individuals being diagnosed with HI caused by mutations in *SLC16A1* between 1.5 and 2.5 years of life, along with an individual diagnosed with HI caused by a mutation in the *GCK* gene at 8 years of life (52). These later diagnoses may also be influenced by the underlying biology of these genetic aetiologies. *GLUD1* is activated by the presence of leucine, and this pathway of insulin secretion may become more active after birth, therefore leading to a later onset in some cases. *GCK* mutations can also lead to wide range in the age at onset of HI. In these cases the severity of the disease is likely to be associated with the effect of the mutation on the enzymatic function of the GCK protein (54). Finally, as *SLC16A1* mutations are linked to a phenotype of exercise-induced HI (with hypoglycaemia occurring around half an hour after a period of anaerobic exercise), it is possible that these mutations are not detected earlier in life as a result of babies not performing anaerobic exercise, meaning that the HI does not present until later in life (55).

Medical management of HI

The treatment of HI begins with the administration of intravenous glucose, to quickly stabilise a patient's blood glucose levels. After this there are very few treatments available that can be used to effectively manage hyperinsulinism.

Diazoxide is generally used as the first line medical treatment for hyperinsulinism. This drug binds to the SUR1 component of the KATP channel where it acts to prevent the channel from closing (51). A failure of the channel to close keeps the cellular membrane in a hyperpolarised state, which prevents opening of the calcium channels and thereby inhibiting insulin secretion. However, this drug does not have a universal response. In particular, individuals with mutations in *ABCC8* or *KCNJ11* that disrupt either the function or the presence in the membrane of the KATP channel are less likely to respond to diazoxide, as the mechanism by which this drug acts is unable to occur (56). In addition, diazoxide has serious side effects that prevent the drug from being used in some individuals. These include pulmonary

hypertension, oedema, and neutropenia (57). Furthermore, the drug is also not available in some countries. Other medical treatments can be used, such as octreotide, a somatostatin receptor analogue (58), though side effects can also occur in octreotide treatment, including elevation of liver enzymes that resolves transiently, along with gallbladder pathologies (59). Recently, some reports have suggested the use of nifedipine (60) and sirolimus (61) to treat hyperinsulinism though others have suggested that success rates with these drugs are variable (60, 62). As such, given the prevalence of diazoxide unresponsiveness in HI, it is vital that more drugs are developed with which HI can be safely treated.

The histology of HI

HI can occur in distinct histological forms, including diffuse, focal and more rarely atypical histology. These forms are difficult to differentiate in a clinical setting as shown in unpublished data presented in Table 1. Both birth weight and insulin are seen to be higher in those with diffuse disease, but this cannot be used to define discrete cut-offs in these metrics for clinical use as a result of the overlap in the ranges. A determination of whether focal or diffuse disease is present is vital for individuals with diazoxide-unresponsive hyperinsulinism, as it will inform on medical management, such as whether 18-F-DOPA PET CT scanning is needed, and whether lesionectomy, in the case of focal disease, can be performed to cure the disease. Children with diazoxide-unresponsive disease are most likely to have a KATP channel mutation as these mutations disrupt the function of the channel that diazoxide acts on: as a result, I will be focussing on KATP channel mutations in this section.

	Diffuse histology, n=129	**Focal histology, n=122**	P=
Age of diagnosis (days)	2 (1 – 7)	2 (1 – 14)	0.103
Corrected birth weight (g)	4281 (3729 – 4736)	3836 (3484 – 4273)	**0.0001**
Female sex	61 (47.3%)	44 (36.1%)	0.075
Insulin (pmol/l)	178.7 (88.2 – 347.1)	115.2 (57.3 – 253.2)	**0.0086**
Glucose (mmol/l)	1.5 (1.1 – 1.9)	1.6 (1.2 – 2.1)	0.111
Diazoxide responsive	23 (31.1%)	11 (17.7%)	0.074

Table 1 – Unpublished data showing the differences between diffuse and focal disease in HI. Bold indicates features significantly different between the two groups.

The inheritance of biallelic, recessively acting, or monoallelic, dominantly acting mutations in *ABCC8* or *KCNJ11* confirms a diagnosis of diffuse disease, where affected beta cells occur throughout the pancreas (7, 8). In contrast, the presence of a recessive-acting mutation on the paternally inherited copy of *ABCC8* or *KCNJ11* can be combined with a loss of heterozygosity of the 11p15 region within the pancreas. The loss of heterozygosity results from paternal uniparental disomy, which unmasks the affected copy of the gene. This leads to reduced expression of the maternally expressed tumour suppressors *H19* and *CDKN1C*, and increased expression of the paternally expressed growth factor *IGF2*. This disruption leads to the proliferation of beta-cells in one region of the pancreas, leading to a focal lesion. The gold standard of treatment in HI includes the use of 18-F-DOPA PET CT scanning to assess the location of a focal lesion where it is indicated genetically, as this can support surgical treatment of the focal lesion (63-65).

Surgical management of HI

Surgical treatment can be used in HI, with two main indications. In individuals with a focal pancreatic lesion, partial pancreatectomy, where the focal lesion is resected, has a 97% success rate in curing hyperinsulinism (66). Near-total pancreatectomy, where up to 95% of the pancreas is resected, may be used in some individuals

where HI persists despite medical treatment (67). However, this treatment can have variable efficacy, with up to 60% of individuals reported to have continual hypoglycaemia post-pancreatectomy (68). In addition, near-total pancreatectomy can lead to issues with the exocrine function of the pancreas (67), along with a greatly increased risk of developing diabetes (68).

Summary of introduction

Congenital HI is a complex disorder both in terms of its molecular basis and its clinical management, and this is also true of its genetics, which will be discussed in the next section. As such, it is clear that a deeper understanding of these factors could lead to better diagnosis and treatments of neonates with HI, thus improving outcomes.

References

1. Fu Z, Gilbert ER, Liu D. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Curr Diabetes Rev. 2013;9(1):25-53.
2. Thorens B. GLUT2, glucose sensing and glucose homeostasis. Diabetologia. 2015;58(2):221-32.
3. Suckale J, Solimena M. Pancreas islets in metabolic signaling--focus on the beta-cell. Front Biosci. 2008;13:7156-71.
4. Glaser B, Kesavan P, Heyman M, Davis E, Cuesta A, Buchs A, et al. Familial hyperinsulinism caused by an activating glucokinase mutation. N Engl J Med. 1998;338(4):226-30.
5. Wakeling MN, Owens NDL, Hopkinson JR, Johnson MB, Houghton JAL, Dastamani A, et al. A novel disease mechanism leading to the expression of a disallowed gene in the pancreatic beta-cell identified by non-coding, regulatory mutations controlling HK1. medRxiv. 2021:2021.12.03.21267240.
6. Martinez-Reyes I, Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. Nat Commun. 2020;11(1):102.
7. Thomas P, Ye Y, Lightner E. Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. Hum Mol Genet. 1996;5(11):1809-12.
8. Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. Science. 1995;268(5209):426-9.
9. Flanagan SE, Vairo F, Johnson MB, Caswell R, Laver TW, Lango Allen H, et al. A CACNA1D mutation in a patient with persistent hyperinsulinaemic hypoglycaemia, heart defects, and severe hypotonia. Pediatr Diabetes. 2017;18(4):320-3.
10. Dixon G, Nolan J, McClenaghan N, Flatt PR, Newsholme P. A comparative study of amino acid consumption by rat islet cells and the clonal beta-cell line BRIN-BD11 - the functional significance of L-alanine. J Endocrinol. 2003;179(3):447-54.
11. Sener A, Malaisse WJ. L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. Nature. 1980;288(5787):187-9.
12. Stanley CA, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, et al. Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. N Engl J Med. 1998;338(19):1352-7.
13. Clayton PT, Eaton S, Aynsley-Green A, Edginton M, Hussain K, Krywawych S, et al. Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of beta-oxidation in insulin secretion. J Clin Invest. 2001;108(3):457-65.
14. Alkhayyat H, Christesen HB, Steer J, Stewart H, Brusgaard K, Hussain K. Mosaic Turner syndrome and hyperinsulinaemic hypoglycaemia. J Pediatr Endocrinol Metab. 2006;19(12):1451-7.
15. Tamame T, Hori N, Homma H, Yoshida R, Inokuchi M, Kosaki K, et al. Hyperinsulinemic hypoglycemia in a newborn infant with trisomy 13. Am J Med Genet A. 2004;129A(3):321-2.
16. Bitner-Glindzicz M, Lindley KJ, Rutland P, Blaydon D, Smith VV, Milla PJ, et al. A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene. Nat Genet. 2000;26(1):56-60.
17. Banerjee I, Senniappan S, Laver TW, Caswell R, Zenker M, Mohnike K, et al. Refinement of the critical genomic region for congenital hyperinsulinism in the Chromosome 9p deletion syndrome. Wellcome Open Res. 2019;4:149.
18. Munns CF, Batch JA. Hyperinsulinism and Beckwith-Wiedemann syndrome. Arch Dis Child Fetal Neonatal Ed. 2001;84(1):F67-9.
19. White SM, Thompson EM, Kidd A, Savarirayan R, Turner A, Amor D, et al. Growth, behavior, and clinical findings in 27 patients with Kabuki (Niikawa-Kuroki) syndrome. Am J Med Genet A. 2004;127A(2):118-27.

20. Carrasco Salas P, Palma Milla C, Lezana Rosales JM, Benito C, Franco Freire S, Lopez Siles J. Hyperinsulinemic hypoglycemia in a patient with an intragenic NSD1 mutation. Am J Med Genet A. 2016;170A(2):544-6.
21. Welters A, El-Khairi R, Dastamani A, Bachmann N, Bergmann C, Gilbert C, et al. Persistent hyperinsulinaemic hypoglycaemia in children with Rubinstein-Taybi syndrome. Eur J Endocrinol. 2019;181(2):121-8.
22. Aref-Eshghi E, Kerkhof J, Pedro VP, Groupe DIF, Barat-Houari M, Ruiz-Pallares N, et al. Evaluation of DNA Methylation Episignatures for Diagnosis and Phenotype Correlations in 42 Mendelian Neurodevelopmental Disorders. Am J Hum Genet. 2020;106(3):356-70.
23. Yap KL, Johnson AEK, Fischer D, Kandikatla P, Deml J, Nelakuditi V, et al. Congenital hyperinsulinism as the presenting feature of Kabuki syndrome: clinical and molecular characterization of 9 affected individuals. Genet Med. 2019;21(1):233-42.
24. Adam MP, Banka S, Bjornsson HT, Bodamer O, Chudley AE, Harris J, et al. Kabuki syndrome: international consensus diagnostic criteria. J Med Genet. 2019;56(2):89-95.
25. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet. 2010;42(9):790-3.
26. Lederer D, Grisart B, Digilio MC, Benoit V, Crespin M, Ghariani SC, et al. Deletion of KDM6A, a histone demethylase interacting with MLL2, in three patients with Kabuki syndrome. Am J Hum Genet. 2012;90(1):119-24.
27. Fahrner JA, Bjornsson HT. Mendelian disorders of the epigenetic machinery: postnatal malleability and therapeutic prospects. Hum Mol Genet. 2019;28(R2):R254-R64.
28. Shilatifard A. The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. Annu Rev Biochem. 2012;81:65-95.
29. Chen J, Xu X, Li Y, Li F, Zhang J, Xu Q, et al. Kdm6a suppresses the alternative activation of macrophages and impairs energy expenditure in obesity. Cell Death Differ. 2021;28(5):1688-704.
30. Aref-Eshghi E, Schenkel LC, Lin H, Skinner C, Ainsworth P, Pare G, et al. The defining DNA methylation signature of Kabuki syndrome enables functional assessment of genetic variants of unknown clinical significance. Epigenetics. 2017;12(11):923-33.
31. Yau D, Laver TW, Dastamani A, Senniappan S, Houghton JAL, Shaikh G, et al. Using referral rates for genetic testing to determine the incidence of a rare disease: The minimal incidence of congenital hyperinsulinism in the UK is 1 in 28,389. PLoS One. 2020;15(2):e0228417.
32. Glaser B, Thornton P, Otonkoski T, Junien C. Genetics of neonatal hyperinsulinism. Arch Dis Child Fetal Neonatal Ed. 2000;82(2):F79-86.
33. Bruining GJ. Recent advances in hyperinsulinism and the pathogenesis of diabetes mellitus. Current Opinion in Pediatrics. 1990;2(4):758-65.
34. Otonkoski T, Ammala C, Huopio H, Cote GJ, Chapman J, Cosgrove K, et al. A point mutation inactivating the sulfonylurea receptor causes the severe form of persistent hyperinsulinemic hypoglycemia of infancy in Finland. Diabetes. 1999;48(2):408-15.
35. Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, et al. Recommendations from the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. J Pediatr. 2015;167(2):238-45.
36. Demirbilek H, Hussain K. Congenital Hyperinsulinism: Diagnosis and Treatment Update. J Clin Res Pediatr Endocrinol. 2017;9(Suppl 2):69-87.
37. Helleskov A, Melikyan M, Globa E, Shcherderkina I, Poertner F, Larsen AM, et al. Both Low Blood Glucose and Insufficient Treatment Confer Risk of Neurodevelopmental Impairment in Congenital Hyperinsulinism: A Multinational Cohort Study. Front Endocrinol (Lausanne). 2017;8:156.
38. Pedersen J. Diabetes and pregnancy; blood sugar of newborn infants during fasting and glucose administration. Ugeskr Laeger. 1952;114(21):685.
39. Falzone N, Harrington J. Clinical Predictors of Transient versus Persistent Neonatal Hyperinsulinism. Horm Res Paediatr. 2020;93(5):297-303.

40. Pinney SE, MacMullen C, Becker S, Lin YW, Hanna C, Thornton P, et al. Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant KATP channel mutations. J Clin Invest. 2008;118(8):2877-86.
41. Flanagan SE, Kapoor RR, Hussain K. Genetics of congenital hyperinsulinemic hypoglycemia. Semin Pediatr Surg. 2011;20(1):13-7.
42. Stanley CA, Rozance PJ, Thornton PS, De Leon DD, Harris D, Haymond MW, et al. Re-evaluating "transitional neonatal hypoglycemia": mechanism and implications for management. J Pediatr. 2015;166(6):1520-5 e1.
43. Lord K, De Leon DD. Hyperinsulinism in the Neonate. Clin Perinatol. 2018;45(1):61-74.
44. Hoe FM, Thornton PS, Wanner LA, Steinkrauss L, Simmons RA, Stanley CA. Clinical features and insulin regulation in infants with a syndrome of prolonged neonatal hyperinsulinism. J Pediatr. 2006;148(2):207-12.
45. Avatapalle HB, Banerjee I, Shah S, Pryce M, Nicholson J, Rigby L, et al. Abnormal Neurodevelopmental Outcomes are Common in Children with Transient Congenital Hyperinsulinism. Front Endocrinol (Lausanne). 2013;4:60.
46. Chesser H, Abdulhussein F, Huang A, Lee JY, Gitelman SE. Continuous Glucose Monitoring to Diagnose Hypoglycemia Due to Late Dumping Syndrome in Children After Gastric Surgeries. J Endocr Soc. 2021;5(3):bvaa197.
47. Calabria AC, Charles L, Givler S, De Leon DD. Postprandial Hypoglycemia in Children after Gastric Surgery: Clinical Characterization and Pathophysiology. Horm Res Paediatr. 2016;85(2):140-6.
48. Service FJ, McMahon MM, O'Brien PC, Ballard DJ. Functioning insulinoma--incidence, recurrence, and long-term survival of patients: a 60-year study. Mayo Clin Proc. 1991;66(7):711-9.
49. Lam KY, Lo CY. Pancreatic endocrine tumour: a 22-year clinico-pathological experience with morphological, immunohistochemical observation and a review of the literature. Eur J Surg Oncol. 1997;23(1):36-42.
50. Agarwal SK. The future: genetics advances in MEN1 therapeutic approaches and management strategies. Endocr Relat Cancer. 2017;24(10):T119-T34.
51. Aynsley-Green A, Hussain K, Hall J, Saudubray JM, Nihoul-Fekete C, De Lonlay-Debeney P, et al. Practical management of hyperinsulinism in infancy. Arch Dis Child Fetal Neonatal Ed. 2000;82(2):F98-F107.
52. Mannisto JME, Maria M, Raivo J, Kuulasmaa T, Otonkoski T, Huopio H, et al. Clinical and Genetic Characterization of 153 Patients with Persistent or Transient Congenital Hyperinsulinism. J Clin Endocrinol Metab. 2020;105(4).
53. Rosenfeld E, Nanga RPR, Lucas A, Revell AY, Thomas A, Thomas NH, et al. Characterizing the neurological phenotype of the hyperinsulinism hyperammonemia syndrome. Orphanet J Rare Dis. 2022;17(1):248.
54. Martinez R, Gutierrez-Nogues A, Fernandez-Ramos C, Velayos T, Vela A, Spanish Congenital Hyperinsulinism G, et al. Heterogeneity in phenotype of hyperinsulinism caused by activating glucokinase mutations: a novel mutation and its functional characterization. Clin Endocrinol (Oxf). 2017;86(6):778-83.
55. Otonkoski T, Jiao H, Kaminen-Ahola N, Tapia-Paez I, Ullah MS, Parton LE, et al. Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic beta cells. Am J Hum Genet. 2007;81(3):467-74.
56. De Franco E, Saint-Martin C, Brusgaard K, Knight Johnson AE, Aguilar-Bryan L, Bowman P, et al. Update of variants identified in the pancreatic beta-cell KATP channel genes KCNJ11 and ABCC8 in individuals with congenital hyperinsulinism and diabetes. Hum Mutat. 2020;41(5):884-905.
57. Herrera A, Vajravelu ME, Givler S, Mitteer L, Avitabile CM, Lord K, et al. Prevalence of Adverse Events in Children With Congenital Hyperinsulinism Treated With Diazoxide. J Clin Endocrinol Metab. 2018;103(12):4365-72.

58. van der Steen I, van Albada ME, Mohnike K, Christesen HT, Empting S, Salomon-Estebanez M, et al. A Multicenter Experience with Long-Acting Somatostatin Analogues in Patients with Congenital Hyperinsulinism. Horm Res Paediatr. 2018;89(2):82-9.
59. Demirbilek H, Shah P, Arya VB, Hinchey L, Flanagan SE, Ellard S, et al. Long-term follow-up of children with congenital hyperinsulinism on octreotide therapy. J Clin Endocrinol Metab. 2014;99(10):3660-7.
60. Guemes M, Shah P, Silvera S, Morgan K, Gilbert C, Hinchey L, et al. Assessment of Nifedipine Therapy in Hyperinsulinemic Hypoglycemia due to Mutations in the ABCC8 Gene. J Clin Endocrinol Metab. 2017;102(3):822-30.
61. Senniappan S, Alexandrescu S, Tatevian N, Shah P, Arya V, Flanagan S, et al. Sirolimus therapy in infants with severe hyperinsulinemic hypoglycemia. N Engl J Med. 2014;370(12):1131-7.
62. Maria G, Antonia D, Michael A, Kate M, Sian E, Sarah FE, et al. Sirolimus: Efficacy and Complications in Children With Hyperinsulinemic Hypoglycemia: A 5-Year Follow-Up Study. J Endocr Soc. 2019;3(4):699-713.
63. Ribeiro MJ, De Lonlay P, Delzescaux T, Boddaert N, Jaubert F, Bourgeois S, et al. Characterization of hyperinsulinism in infancy assessed with PET and 18F-fluoro-L-DOPA. J Nucl Med. 2005;46(4):560-6.
64. Otonkoski T, Nanto-Salonen K, Seppanen M, Veijola R, Huopio H, Hussain K, et al. Noninvasive diagnosis of focal hyperinsulinism of infancy with [18F]-DOPA positron emission tomography. Diabetes. 2006;55(1):13-8.
65. Adzick NS, Thornton PS, Stanley CA, Kaye RD, Ruchelli E. A multidisciplinary approach to the focal form of congenital hyperinsulinism leads to successful treatment by partial pancreatectomy. J Pediatr Surg. 2004;39(3):270-5.
66. Scott Adzick N. Surgical treatment of congenital hyperinsulinism. Semin Pediatr Surg. 2020;29(3):150924.
67. Banerjee I, Salomon-Estebanez M, Shah P, Nicholson J, Cosgrove KE, Dunne MJ. Therapies and outcomes of congenital hyperinsulinism-induced hypoglycaemia. Diabet Med. 2019;36(1):9-21.
68. Beltrand J, Caquard M, Arnoux JB, Laborde K, Velho G, Verkarre V, et al. Glucose metabolism in 105 children and adolescents after pancreatectomy for congenital hyperinsulinism. Diabetes Care. 2012;35(2):198-203.

Introduction Part 2

Congenital hyperinsulinism: current laboratory-based approaches to the genetic diagnosis of a heterogeneous disease

Thomas I. Hewat, Matthew B. Johnson, Sarah E. Flanagan

Institute of Biomedical and Clinical Science, University of Exeter Medical School, UK

This manuscript was published in Frontiers in Endocrinology –

Front Endocrinol (Lausanne). 2022;13.
https://doi.org/10.3389/fendo.2022.873254

Abstract

Congenital hyperinsulinism is characterised by the inappropriate release of insulin during hypoglycaemia. This potentially life-threatening disorder can occur in isolation, or present as a feature of syndromic disease. Establishing the underlying aetiology of the hyperinsulinism is critical for guiding medical management of this condition especially in children with diazoxide-unresponsive hyperinsulinism, where the underlying genetics determines whether focal or diffuse pancreatic disease is present.

Disease-causing single nucleotide variants affecting over 30 genes are known to cause persistent hyperinsulinism with mutations in the KATP channel genes (*ABCC8* and *KCNJ11*) most commonly identified in children with severe persistent disease. Defects in methylation, changes in chromosome number, and large deletions and duplications disrupting multiple genes are also well described in congenital hyperinsulinism, further highlighting the genetic heterogeneity of this condition.

Next-generation sequencing has revolutionised the approach to genetic testing for congenital hyperinsulinism with targeted gene panels, exome, and genome sequencing being highly sensitive methods for the analysis of multiple disease genes in a single reaction. It should though be recognised that limitations remain with next-generation sequencing with no single application able to detect all reported forms of genetic variation. This is an important consideration for hyperinsulinism genetic testing as comprehensive screening may require multiple investigations.

Introduction

Persistent congenital hyperinsulinism (HI) is characterised by the inappropriate secretion of insulin during hypoglycaemia which continues beyond 6 months. A prompt diagnosis of HI and effective management of glucose levels is critical to prevent adverse outcomes (1).

Persistent HI affects approximately 1 in 13,500 to 1 in 45,000 new-borns in non-consanguineous populations (2-5). In some isolated communities where founder mutations have been reported, and in populations with high rates of consanguinity, the incidence can increase to approximately 1 in 3,000 (6, 7). At least 36 different genetic causes of HI have been reported which follow recessive, dominant, X-linked, or sporadic inheritance (Table 1). The underlying genetic aetiology of disease will determine whether the HI presents as isolated pancreatic disease or occurs as part of a rare syndrome.

Many laboratories provide genetic testing for congenital HI; however, strategies vary between testing centres both in terms of the genes that are screened and the types of variation that can be detected (8-10). The different approaches to testing employed by each laboratory could help explain the differences in the percentage of mutation positive cases between cohorts which range from 45% to 79% (3, 4, 11, 12). Furthermore, the large number of genes which cause HI, the variable penetrance observed both within and between families with the same disease-causing variants, and the multiple modes of inheritance reported can hinder genetic interpretation which will also impact on the pick-up rates reported by each laboratory.

In this review, we describe the genetic causes of HI and discuss the benefits and limitations of the different methodological approaches currently used for genetic screening of this condition.

Genetic types of Congenital Hyperinsulinism

Disease-causing variants in 10 genes have been reported to cause isolated, persistent HI (Table 1). Loss-of-function variants in the *ABCC8* and *KCNJ11* genes, which encode the two subunits of the pancreatic beta-cell ATP-sensitive potassium (KATP) channel, are most common and reported in 30-66% of cases referred for

genetic testing (3, 4, 11, 12). A wide range of clinical severity is associated with KATP-HI with the functionally mildest variants causing transient disease which responds well to diazoxide treatment (the frontline drug for HI), whilst the most functionally severe variants cause diazoxide-unresponsive HI that persists throughout childhood (13-15). For individuals with diazoxide-unresponsive HI, pancreatic resection may be required to prevent life-threatening hypoglycaemia. For these infants, rapid genetic testing of the KATP channel genes is critical as it will determine the histological subtype of disease. Identifying biallelic (two disease-causing variants on opposite alleles) or a single dominant KATP channel disease-causing variant confirms diffuse pancreatic disease. In contrast finding a paternally inherited, recessive KATP channel variant, predicts focal disease with a sensitivity of 97% (12, 16). In these individuals the variant is rendered homozygous by a second somatic genetic event within the focal lesion in the pancreas (uniparental isodisomy) (17, 18). This can be genetically confirmed by testing the pancreatic tissue following a lesionectomy, which proves curative in most cases.

Clinical characteristics can help to predict some genetic forms of isolated HI. For example, high ammonia concentrations are a consistent feature of *GLUD1*-HI (19), a family history of Maturity-Onset Diabetes of the Young (MODY) can predict *HNF4A* or *HNF1A*- HI (20, 21), and exercise-induced HI suggests a role for the beta-cell disallowed gene, *SLC16A1* in disease pathogenesis (22).

Over 28 different syndromes which feature HI have been reported with the most common being Beckwith-Wiedemann syndrome (BWS) and Kabuki syndrome (23) (Table 2). The proportion of individuals with syndromic disease who present with HI varies between genetic subgroups. In some conditions HI is reported as a cardinal feature (e.g. Beckwith-Wiedemann syndrome (24)) whilst for others it is reported as a rare feature of the disease (e.g. Chromosome 9p deletions (25)). Without genetic testing it can be hard to accurately diagnose syndromic disease, especially when HI is the presenting feature and dysmorphism develops after birth, or when the clinical features are not specific to a genetic syndrome (26). For individuals with syndromic HI a genetic diagnosis is important as it will inform on prognosis and allow for the effective monitoring of new features of the disease.

Sanger sequencing

Causative genes for HI were historically screened by Sanger sequencing; an approach that allows a few hundred nucleotides (typically a single exon) to be rapidly sequenced in a single reaction. This is followed by semi-automated analysis by alignment and inspection of the DNA sequence. These constraints force laboratories to screen genes sequentially in descending order of prior probability based on clinical characteristics and how commonly disease-causing variants in the gene are identified. Whilst this phenotype-driven approach works well in many scenarios (for example in the rapid screening of KATP channel genes in individuals with diazoxide-unresponsive disease (27, 28)), the reliance of clinical features to guide testing can delay a genetic diagnosis for individuals with an atypical presentation. This is an important consideration for HI, as phenotypic variability is described within most genetic subgroups, for example the presence of normal ammonia levels in some children with *GLUD1*-HI (29, 30). Using the clinical characteristics to guide genetic testing in syndromic HI should also be applied with caution as additional features may develop after the diagnosis of HI (31).

A further major limitation of Sanger sequencing is its inability to detect heterozygous deletions and duplications that extend beyond the targeted region, changes in the number of chromosomes (aneuploidies), and defects in methylation, all of which have been reported to cause HI (Table 1).

Despite its limitations, Sanger sequencing remains a highly sensitive test for the rapid detection of single-nucleotide variants and small insertion/deletion variants (indels) in both the coding and non-coding regions of the genome. It can also detect mosaic variants (i.e. a genetic variant that is introduced during cell division that does not affect every cell within the body) that are present in the sampled tissue at a level of >8% (32). This is important, as disease-causing mosaic variants have been reported in the known HI genes including *KMT2D*, *KDM6A*, *NSD1*, and *CREBBP* (33-35).

Next-generation sequencing

Since 2005, next-generation sequencing has provided a method to allow for the simultaneous analysis of multiple genes in a single assay (36). This technology

revolutionised diagnostic testing for genetically heterogeneous disorders such as HI by allowing for the parallel screening of all known disease-causing genes/genomic regions in a single assay at a much lower cost than Sanger sequencing. This led to a paradigm shift for conditions like syndromic HI where genetic testing can precede the development of the full clinical spectrum of disease, serving to make, rather than confirm, the clinical diagnosis (26).

Targeted gene panel analysis by next-generation sequencing

A targeted gene panel typically includes all known genetic causes of a disease and DNA samples are enriched for DNA in these loci prior to next-generation sequencing. For most targeted gene panels, the average coverage achieved often reaches many hundreds of reads over each base (37). This high-depth sequencing data can be exploited to detect changes in copy number over targeted regions and allows for the accurate detection of mosaic variants occurring at a level of >1% (32). Recent studies have shown that off-target reads generated during the sequencing process can be analysed to assess read-depth across the entire genome allowing for the detection of large deletions and duplications outside of targeted regions (38). These off-target reads have been used successfully to detect disease-causing deletions on chromosome 9p in individuals with HI (25). The potential to identify large deletions and duplications from off-target reads will though depend on the methodology used for the targeted next-generation sequencing; amplicon-based approaches that sequence PCR products will not generate the off-target sequencing data.

The major limitation of targeted next-generation sequencing is that it only allows screening of a predetermined list of genomic regions, and this list often differs between laboratories. For genetically heterogeneous conditions such as HI, it is therefore important that clinicians who order panel testing are aware of which genes are included on the targeted panels and whether copy number analysis has been performed as this requires a separate bioinformatic analysis.

Exome and genome sequencing

The introduction of next-generation sequencing has enabled the rapid sequencing of the coding regions of all genes (the exome) or the entire human genome (coding and

non-coding regions) at much lower cost than previous methods. The approach to the interpretation of exome and genome sequencing data will differ between centres with some analysing variants called within a pre-defined set of known disease-causing genes whilst other laboratories will perform a gene-agnostic analysis. The latter approach has the advantage of being able to identify new genes for HI, with recent successes including the discovery of the syndromic HI genes *CACNA1D*, *PMM2*, *FOXA2*, *TRMT10A*, *EIF2S3*, *YARS*, and *KMT2D* by exome sequencing and more recently the finding of regulatory variants deep within intron 2 of the beta-cell disallowed gene, *HK1,* by genome sequencing in individuals with isolated hyperinsulinism (39-46). The ability of a laboratory to utilise next-generation sequencing data for genetic discovery will largely depend on their ability to perform robust genetic and functional studies to assess novel variation.

Exome sequencing targets the ~2% of the genome which codes for protein, making it a cheaper alternative to genome sequencing. This, together with the knowledge that 85% of known disease-causing mutations reside within coding regions, has led to exome sequencing being widely adopted within the clinical setting (47). For example, in the UK, rapid exome sequencing for acutely unwell neonates is available through the country's National Health Service with 38% of patients tested receiving a rapid diagnosis (48). Unlike targeted next-generation sequencing, which screens a predetermined list of genes, exome sequencing provides an extremely effective method to comprehensively analyse the coding regions and intron/exon boundaries of all known HI genes and to assess copy number status. The major limitation of the approach is that it will not detect non-coding mutations such as the deep intronic mutations reported in *ABCC8*, *HADH* and *HK1* or promoter variants in genes such as *HNF4A*, *PMM2*, and *SLC16A1 (22, 39, 40, 49, 50)*.

Genome sequencing represents the gold standard approach to genetic testing given its ability to detect the largest range of genetic variation. As well as providing data on coding and non-coding regions, genome sequencing can be used to search for structural changes, copy number variants (large deletions, duplications, and aneuploidies) and mosaic variants although the lower read depth achieved makes this a less sensitive approach for detecting low-level mosaic variants compared to targeted next-generation sequencing.

The costs associated with sequencing the entire genome and the large amount of data produced (approximately 200GB of processed data per sample versus 11GB per sample for exome sequencing) had prohibited the adoption of routine genome sequencing. Until recently it had been largely reserved for genetic screening when a disease-causing variant had not been detected by targeted next-generation sequencing or exome sequencing. This approach successfully resulted in an increase in diagnostic yield for many rare genetic diseases (51, 52).

Improvements in sequencing capabilities leading to reduced costs are now leading to the emergence of genome sequencing as a first line diagnostic test in specific healthcare settings, for example in the screening of some rare developmental disorders in the UK National Health Service (53). While genome sequencing is not the current approach for investigating the genetic cause of HI in many centres, it seems likely that this will become the first line test in the coming years.

Non sequencing based methods to detect copy number variants and methylation defects

Aneuploidies and large deletions and duplications (copy number variants) are a rare but important cause of HI (Tables 1-2). Unlike Sanger sequencing, next-generation sequencing can detect these forms of genetic variation, but many laboratories will not routinely screen for them as a separate analysis pipeline is required. This is an important consideration when disease-causing variants are not detected in children with HI and particularly for those where there are additional syndromic features (Table 2).

Multiplex-ligation dependent probe amplification (MLPA) can detect disease-causing deletions and duplications in individuals with HI. This approach is commonly used to screen for deletions in the *ABCC8* gene and can detect mosaicism (54). The usefulness of MLPA is limited by its ability to analyse a maximum of 60 different small genomic regions (generally single exons) in a single assay thus preventing the simultaneous analysis of all HI genes in which copy number changes have been reported.

Microarray-based comparative genomic hybridization (array CGH) is a well-established method that is used to detect large deletions/duplications and aneuploidies in individuals with HI. Unlike MLPA, array CGH is not able to detect low level mosaicism (<30% mosaicism for deletions and duplications and <10% for aneuploidies). The approach does however allow for the analysis of copy number variation across a greater percentage of the genome although the targeted region will vary across arrays and will not always target the regions known to cause HI with enough precision.

Current diagnostic sequencing approaches are also unable to detect changes in DNA methylation. Individuals with clinical suspicion of an imprinting disorder such as Beckwith-Wiedemann syndrome may therefore require additional methylation studies, such as methylation-specific MLPA (MS-MLPA)(55) or Infinium Methylation EPIC array analysis (56). Emerging technologies, such as Oxford Nanopore sequencing, may allow for the simultaneous detection of sequence variation and DNA methylation status but have not been widely used clinically. This technology does offer the hope of a single comprehensive test for genetically heterogeneous disorders like HI although to date it has mainly been used for genes that are hard to sequence by other methodologies (57-60).

Further considerations and concluding remarks

Diagnostic testing for HI is routinely performed on DNA extracted from peripheral blood leukocytes, saliva, or buccal samples. For conditions such as HI it is important to consider the source of DNA being screened, given that somatic mutations which are only present in the pancreatic tissue have been reported (12, 61). Therefore, when a mutation is not identified in the blood, and a pancreatectomy has been performed, re-testing the known HI genes to search for a variant present only within the pancreatic DNA should be considered.

In conclusion, several different genetic approaches exist for routine diagnostic screening in HI with genome sequencing representing the gold standard approach to testing. For healthcare professionals managing this genetically heterogeneous disorder it is important that the limitations of each approach including genome sequencing, are recognised as no single test can detect all known types of genetic

variation reported in HI. This is particularly important when managing syndromic disease, where copy number variants or defects in methylation are common. Despite there being a broad range of genetic screening approaches that are available for HI, in reality the testing strategy is most likely to be influenced by the capabilities of the local genetic diagnostic laboratory, affordability and importantly how quickly the tests can be performed and results reported back. This is especially critical for children with diazoxide-unresponsive disease as identifying a paternally inherited KATP disease-causing variant suggests focal pancreatic disease which can be cured by lesionectomy.

Acknowledgements

S.E.F. is a Wellcome Trust Senior Research Fellow (Grant Number 223187/Z/21/Z). T.I.H is the recipient of a PhD studentship and M.B.J has an Independent Fellowship from the Exeter Diabetes Centre of Excellence funded by Research England's Expanding Excellence in England (E3) fund. The authors thank Dr Thomas Laver and Dr Matthew Wakeling for their contributions to this review.

Gene	Zygosity	Mutation type	Sanger Sequencing[1]	Next Generation Sequencing			Ref
				Targeted Panel	Exome	Genome	
ABCC8	Dominant or recessive	SNVs/indels	✓	✓	✓[2]	✓	(13, 54, 62)
		Large CNVs	X	✓	✓	✓	
GCK	Dominant	SNVs/indels	✓	✓	✓	✓	(63)
GLUD1	Dominant	SNVs/indels	✓	✓	✓	✓	(19)
HADH	Recessive	SNVs/indels	✓	✓	✓[2]	✓	(64)
		Large CNVs	X	✓	✓	✓	(65)
HK1	Dominant	SNVs/indels	✓	✓	X	✓	(39)
		Large CNVs	X	✓	X	✓	
HNF1A	Dominant	SNVs/indels	✓	✓	✓	✓	(21)
HNF4A	Dominant	SNVs/indels	✓	✓	✓[2]	✓	(20)
		Large CNVs	X	✓	✓	✓	(66)
INSR	Dominant	SNVs/indels	✓	✓	✓	✓	(67)
KCNJ11	Dominant or recessive	SNVs/indels	✓	✓	✓	✓	(68)
SLC16A1	Dominant	SNVs/indels	✓	✓	X	✓	(22)

Table 1: **Known genetic causes of isolated congenital hyperinsulinism and current approaches to genetic testing for this condition.** A tick (✓) or cross (X) denote whether the form of genetic variation can be detected by the screening approach. None of the variants listed will be detected by methylation studies or array-CGH analysis. SNVs are single nucleotide variants, Indels are insertion/deletion variants and CNVs are copy number variants (deletions and duplications).

Footnotes:

[1]Sanger sequencing will not detect heterozygous deletions of duplications that extend beyond the targeted region. Homozygous deletions that encompass a primer binding site may be detected by a failure to amplify the sequence, but this will require verification by an independent method.

[2] Exome sequencing will not detect the deep intronic mutations or promoter mutations reported in these genes (49).

Gene	Zygosity	Syndrome	Mutation type	Sanger Sequencing[1]	Next Generation Sequencing			Array-CGH	Methylation studies	Ref
					Targeted Panel	Exome	Genome			
ABCC8	Recessive	Usher Syndrome	Large CNVs[2]	X	✓	✓	✓	X	X	(69)
ADK	Recessive	*ADK* deficiency	SNVs/indels	✓	✓	✓	✓	X	X	(70)
ALG3	Recessive	Congenital disorder of glycosylation	SNVs/indels	✓	✓	✓	✓	X	X	(71)
CACNA1D	Dominant	Primary aldosteronism, seizures & neurological abnormalities	SNVs/indels	✓	✓	✓	✓	X	X	(41)
CDKN1C	Dominant	Beckwith-Wiedemann	SNVs/indels	✓	✓	✓	✓	X	X	(72)
Chr5q35 deletion	Dominant	Sotos	Large CNVs	X	✓	✓	✓	✓	X	(73)
Chr9p deletion	Dominant	Chr9p deletion	Large CNVs	X	✓	✓	✓	✓	X	(25)
Chr11p15.5 loss of methylation	Dominant	Beckwith-Wiedemann	Imprinting abnormality	X	X[3]	X	X	X[3]	✓	(74)
CREBBP	Dominant	Rubinstein-Taybi	SNVs/indels	✓	✓	✓	✓	X	X	(75)
			Large CNVs	X	✓	✓	✓	X	X	
DIS3L2	Recessive	Perlman	SNVs/indels	✓	✓	✓	✓	X	X	(76, 77)
			Large CNVs	X	✓	✓	✓	X	X	
EIF2S3	X-linked recessive	MEHMO	SNVs/indels	✓	✓	✓	✓	X	X	(43)
EP300	Dominant	Rubinstein-Taybi	SNVs/indels	✓	✓	✓	✓	X	X	(75)
			Large CNVs	X	✓	✓	✓	X	X	
FAH	Recessive	Tyrosinaemia type I	SNVs/indels	✓	✓	✓	✓	X	X	(78)

FOXA2	Dominant	Syndromic	SNVs/indels	✓	✓	✓	✓	X	X	(42)
GPC3	X-linked recessive	Simpson-Golabi-Behmel	SNVs/indels	✓	✓	✓	✓	X	X	(79)
			Large CNVs	X	✓	✓	✓	X	X	
HNF4A	Dominant	Fanconi renotubular syndrome 4	SNV	✓	✓	✓	✓	X	X	(21)
HRAS	Dominant	Costello	SNVs/indels	✓	✓	✓	✓	X	X	(80)
KDM6A	X-linked dominant	Kabuki	SNVs/indels	✓	✓	✓	✓	X	X	(81)
			Large CNVs	X	✓	✓	✓	X	X	
KMT2D	Dominant	Kabuki	SNVs/indels	✓	✓	✓	✓	X	X	(45, 82)
			Large CNVs	X	✓	✓	✓	X	X	
MAGEL2	Dominant[4]	Schaaf-Yang	SNVs/indels	✓	✓	✓	✓	X	X	(83)
MPI	Recessive	Congenital disorder of glycosylation	SNVs/indels	✓	✓	✓	✓	X	X	(84)
NSD1	Dominant	Sotos	SNVs/indels	✓	✓	✓[5]	✓	X	X	(85-87)
			Large CNVs	X	✓	✓[5]	✓	X	X	
PHOX2B	Dominant	Congenital central hypoventilation	SNVs/indels	✓	✓	✓	✓	X	X	(88)
PMM2	Recessive	Polycystic Kidney Disease with HI	SNVs/indels	✓	✓	X	✓	X	X	(40)
		Congenital disorder of glycosylation	SNVs/indels	✓	✓	✓	✓	X	X	(89)
Trisomy 13	Dominant	Patau	Aneuploidy (Trisomy)	X	✓	✓	✓	✓	X	(90)
TRMT10A	Recessive	Syndromic	SNVs/indels	✓	✓	✓	✓	X	X	(46)
YARS	Recessive	Syndromic	SNVs/indels	✓	✓	✓	✓	X	X	(44)
45,X	Dominant	Turner	Aneuploidy (Monosomy)	X	✓	✓	✓	✓	X	(91)

Table 2: **Known genetic causes of syndromic disease in which congenital hyperinsulinism can be a rare or common feature and the current approaches to genetic testing for this condition.** A tick (✓) or cross (X) denote whether the form of genetic variation can be detected by the screening approach. Methylation studies refer to methodologies that can detect changes in DNA methylation patterns (e.g. Epic array analysis, Methylation-specific MLPA). SNVs are single nucleotide variants, Indels are insertion/deletion variants and CNVs are copy number variants (deletions and duplications).

Footnotes:

[1] Sanger sequencing will not detect heterozygous deletions of duplications that extend beyond the targeted region. Homozygous deletions that encompass a primer binding site may be detected by a failure to amplify the sequence, but this will require verification by an independent method.

[2] Congenital hyperinsulinism, profound congenital sensorineural deafness, enteropathy and renal tubular dysfunction is causes by a contiguous deletion extending over *ABCC8* and *USH1C*.

[3] Rare deletions and duplications of the Chr11p15.5 imprinted region(s) can cause Beckwith-Wiedemann syndrome (92). Their size and location will determine whether they can be detected by next-generation sequencing or microarray analysis.

[4] *MAGEL2* is an imprinted gene, loss-of-function mutations only cause disease when present on the paternal allele.

[5] Intergenic mutations affecting *NSD1* have been reported; these would not be detected by exome sequencing (85).

References

1. Helleskov A, Melikyan M, Globa E, Shcherderkina I, Poertner F, Larsen AM, et al. Both Low Blood Glucose and Insufficient Treatment Confer Risk of Neurodevelopmental Impairment in Congenital Hyperinsulinism: A Multinational Cohort Study. Front Endocrinol (Lausanne). 2017;8:156.
2. Yau D, Laver TW, Dastamani A, Senniappan S, Houghton JAL, Shaikh G, et al. Using referral rates for genetic testing to determine the incidence of a rare disease: The minimal incidence of congenital hyperinsulinism in the UK is 1 in 28,389. PLoS One. 2020;15(2):e0228417.
3. Rozenkova K, Malikova J, Nessa A, Dusatkova L, Bjorkhaug L, Obermannova B, et al. High Incidence of Heterozygous ABCC8 and HNF1A Mutations in Czech Patients With Congenital Hyperinsulinism. J Clin Endocrinol Metab. 2015;100(12):E1540-9.
4. Mannisto JME, Jaaskelainen J, Otonkoski T, Huopio H. Long-Term Outcome and Treatment in Persistent and Transient Congenital Hyperinsulinism: A Finnish Population-Based Study. J Clin Endocrinol Metab. 2021;106(4):e1542-e51.
5. Kawakita R, Sugimine H, Nagai S, Kawai M, Kusuda S, Yorifuji T. Clinical Characteristics of Congenital Hyperinsulinemic Hypoglycemia in Infant: A Nationwide Epidemiological Survey in Japan. Nihon Shonika Gakkai Zasshi. 2011;115:563-9.
6. Otonkoski T, Ammala C, Huopio H, Cote GJ, Chapman J, Cosgrove K, et al. A point mutation inactivating the sulfonylurea receptor causes the severe form of persistent hyperinsulinemic hypoglycemia of infancy in Finland. Diabetes. 1999;48(2):408-15.
7. Mathew PM, Young JM, Abu-Osba YK, Mulhern BD, Hammoudi S, Hamdan JA, et al. Persistent neonatal hyperinsulinism. Clin Pediatr (Phila). 1988;27(3):148-51.
8. Novoa-Medina Y, Dominguez Garcia A, Quinteiro Gonzalez S, Garcia Cruz LM, Santana Rodriguez A. Congenital hyperinsulinism in Gran Canaria, Canary Isles. An Pediatr (Engl Ed). 2021;95(2):93-100.
9. Casertano A, Rossi A, Fecarotta S, Rosanio FM, Moracas C, Di Candia F, et al. An Overview of Hypoglycemia in Children Including a Comprehensive Practical Diagnostic Flowchart for Clinical Use. Front Endocrinol (Lausanne). 2021;12:684011.

10. Razzaghy-Azar M, Saeedi S, Dayani SB, Enayati S, Abbasi F, Hashemian S, et al. Investigating Genetic Mutations in a Large Cohort of Iranian Patients with Congenital Hyperinsulinism. J Clin Res Pediatr Endocrinol. 2022;14(1):87-95.
11. Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S, Hussain K. Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism. Eur J Endocrinol. 2013;168(4):557-64.
12. Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, et al. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. J Clin Endocrinol Metab. 2013;98(2):E355-63.
13. Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. Science. 1995;268(5209):426-9.
14. Taschenberger G, Mougey A, Shen S, Lester LB, LaFranchi S, Shyng SL. Identification of a familial hyperinsulinism-causing mutation in the sulfonylurea receptor 1 that prevents normal trafficking and function of KATP channels. J Biol Chem. 2002;277(19):17139-46.
15. Kumaran A, Kapoor RR, Flanagan SE, Ellard S, Hussain K. Congenital hyperinsulinism due to a compound heterozygous ABCC8 mutation with spontaneous resolution at eight weeks. Horm Res Paediatr. 2010;73(4):287-92.
16. Mohnike K, Wieland I, Barthlen W, Vogelgesang S, Empting S, Mohnike W, et al. Clinical and genetic evaluation of patients with KATP channel mutations from the German registry for congenital hyperinsulinism. Horm Res Paediatr. 2014;81(3):156-68.
17. de Lonlay P, Fournet JC, Rahier J, Gross-Morand MS, Poggi-Travert F, Foussier V, et al. Somatic deletion of the imprinted 11p15 region in sporadic persistent hyperinsulinemic hypoglycemia of infancy is specific of focal adenomatous hyperplasia and endorses partial pancreatectomy. J Clin Invest. 1997;100(4):802-7.
18. Damaj L, le Lorch M, Verkarre V, Werl C, Hubert L, Nihoul-Fekete C, et al. Chromosome 11p15 paternal isodisomy in focal forms of neonatal hyperinsulinism. J Clin Endocrinol Metab. 2008;93(12):4941-7.
19. Stanley CA, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, et al. Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. N Engl J Med. 1998;338(19):1352-7.

20. Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, et al. Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. PLoS Med. 2007;4(4):e118.
21. Stanescu DE, Hughes N, Kaplan B, Stanley CA, De Leon DD. Novel presentations of congenital hyperinsulinism due to mutations in the MODY genes: HNF1A and HNF4A. J Clin Endocrinol Metab. 2012;97(10):E2026-30.
22. Otonkoski T, Jiao H, Kaminen-Ahola N, Tapia-Paez I, Ullah MS, Parton LE, et al. Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic beta cells. Am J Hum Genet. 2007;81(3):467-74.
23. Kostopoulou E, Dastamani A, Guemes M, Clement E, Caiulo S, Shanmugananda P, et al. Syndromic Forms of Hyperinsulinaemic Hypoglycaemia-A 15-year follow-up Study. Clin Endocrinol (Oxf). 2021;94(3):399-412.
24. Brioude F, Kalish JM, Mussa A, Foster AC, Bliek J, Ferrero GB, et al. Expert consensus document: Clinical and molecular diagnosis, screening and management of Beckwith-Wiedemann syndrome: an international consensus statement. Nat Rev Endocrinol. 2018;14(4):229-49.
25. Banerjee I, Senniappan S, Laver TW, Caswell R, Zenker M, Mohnike K, et al. Refinement of the critical genomic region for congenital hyperinsulinism in the Chromosome 9p deletion syndrome. Wellcome Open Res. 2019;4:149.
26. Hoermann H, El-Rifai O, Schebek M, Lodefalk M, Brusgaard K, Bachmann N, et al. Comparative meta-analysis of Kabuki syndrome with and without hyperinsulinaemic hypoglycaemia. Clin Endocrinol (Oxf). 2020;93(3):346-54.
27. Hewat TI, Yau D, Jerome JCS, Laver TW, Houghton JAL, Shields BM, et al. Birth weight and diazoxide unresponsiveness strongly predict the likelihood of congenital hyperinsulinism due to a mutation in ABCC8 or KCNJ11. Eur J Endocrinol. 2021;185(6):813-8.
28. Banerjee I, Skae M, Flanagan SE, Rigby L, Patel L, Didi M, et al. The contribution of rapid KATP channel gene mutation analysis to the clinical management of children with congenital hyperinsulinism. Eur J Endocrinol. 2011;164(5):733-40.
29. Brandt A, Agarwal N, Giri D, Yung Z, Didi M, Senniappan S. Hyperinsulinism hyperammonaemia (HI/HA) syndrome due to GLUD1 mutation: phenotypic variations

ranging from late presentation to spontaneous resolution. J Pediatr Endocrinol Metab. 2020;33(5):675-9.
30. Kapoor RR, Flanagan SE, Fulton P, Chakrapani A, Chadefaux B, Ben-Omran T, et al. Hyperinsulinism-hyperammonaemia syndrome: novel mutations in the GLUD1 gene and genotype-phenotype correlations. Eur J Endocrinol. 2009;161(5):731-5.
31. Yap KL, Johnson AEK, Fischer D, Kandikatla P, Deml J, Nelakuditi V, et al. Congenital hyperinsulinism as the presenting feature of Kabuki syndrome: clinical and molecular characterization of 9 affected individuals. Genet Med. 2019;21(1):233-42.
32. Brewer CJ, Gillespie M, Fierro J, Scaringe WA, Li JM, Lee CY, et al. The Value of Parental Testing by Next-Generation Sequencing Includes the Detection of Germline Mosaicism. J Mol Diagn. 2020;22(5):670-8.
33. Murakami H, Tsurusaki Y, Enomoto K, Kuroda Y, Yokoi T, Furuya N, et al. Update of the genotype and phenotype of KMT2D and KDM6A by genetic screening of 100 patients with clinically suspected Kabuki syndrome. Am J Med Genet A. 2020;182(10):2333-44.
34. Castronovo C, Rusconi D, Crippa M, Giardino D, Gervasini C, Milani D, et al. A novel mosaic NSD1 intragenic deletion in a patient with an atypical phenotype. Am J Med Genet A. 2013;161A(3):611-8.
35. Lin S, He Z, Huang L, Liu J, Lei T, Wu J, et al. Case Report: Low-Level Maternal Mosaicism of a Novel CREBBP Variant Causes Recurrent Rubinstein-Taybi Syndrome in Two Siblings of a Chinese Family. Front Genet. 2021;12:640992.
36. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, et al. Genome sequencing in microfabricated high-density picolitre reactors. Nature. 2005;437(7057):376-80.
37. Cleveland MH, Zook JM, Salit M, Vallone PM. Determining Performance Metrics for Targeted Next-Generation Sequencing Panels Using Reference Materials. J Mol Diagn. 2018;20(5):583-90.
38. Laver TW, De Franco E, Johnson MB, Patel KA, Ellard S, Weedon MN, et al. SavvyCNV: Genome-wide CNV calling from off-target reads. PLoS Comput Biol. 2022;18(3):e1009940.
39. Wakeling MN, Owens NDL, Hopkinson JR, Johnson MB, Houghton JAL, Dastamani A, et al. A novel disease mechanism leading to the expression of a

disallowed gene in the pancreatic beta-cell identified by non-coding, regulatory mutations controlling HK1. medRxiv. 2021:2021.12.03.21267240.
40. Cabezas OR, Flanagan SE, Stanescu H, Garcia-Martinez E, Caswell R, Lango-Allen H, et al. Polycystic Kidney Disease with Hyperinsulinemic Hypoglycemia Caused by a Promoter Mutation in Phosphomannomutase 2. J Am Soc Nephrol. 2017;28(8):2529-39.
41. Flanagan SE, Vairo F, Johnson MB, Caswell R, Laver TW, Lango Allen H, et al. A CACNA1D mutation in a patient with persistent hyperinsulinaemic hypoglycaemia, heart defects, and severe hypotonia. Pediatr Diabetes. 2017;18(4):320-3.
42. Giri D, Vignola ML, Gualtieri A, Scagliotti V, McNamara P, Peak M, et al. Novel FOXA2 mutation causes Hyperinsulinism, Hypopituitarism with Craniofacial and Endoderm-derived organ abnormalities. Hum Mol Genet. 2017;26(22):4315-26.
43. Gregory LC, Ferreira CB, Young-Baird SK, Williams HJ, Harakalova M, van Haaften G, et al. Impaired EIF2S3 function associated with a novel phenotype of X-linked hypopituitarism with glucose dysregulation. EBioMedicine. 2019;42:470-80.
44. Zeiad R, Ferren EC, Young DD, De Lancy SJ, Dedousis D, Schillaci LA, et al. A Novel Homozygous Missense Mutation in the YARS Gene: Expanding the Phenotype of YARS Multisystem Disease. J Endocr Soc. 2021;5(2):bvaa196.
45. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet. 2010;42(9):790-3.
46. Gillis D, Krishnamohan A, Yaacov B, Shaag A, Jackman JE, Elpeleg O. TRMT10A dysfunction is associated with abnormalities in glucose homeostasis, short stature and microcephaly. J Med Genet. 2014;51(9):581-6.
47. Scacheri CA, Scacheri PC. Mutations in the noncoding genome. Curr Opin Pediatr. 2015;27(6):659-64.
48. McDermott H, Baple E, Ellard S, Naik S. 1435 Rapid exome sequencing in acutely unwell children – providing new diagnostic options in intensive care settings2021. A368-A9 p.
49. Flanagan SE, Xie W, Caswell R, Damhuis A, Vianey-Saban C, Akcay T, et al. Next-generation sequencing reveals deep intronic cryptic ABCC8 and HADH splicing founder mutations causing hyperinsulinism by pseudoexon activation. Am J Hum Genet. 2013;92(1):131-6.

50. Colclough K, Bellanne-Chantelot C, Saint-Martin C, Flanagan SE, Ellard S. Mutations in the genes encoding the transcription factors hepatocyte nuclear factor 1 alpha and 4 alpha in maturity-onset diabetes of the young and hyperinsulinemic hypoglycemia. Hum Mutat. 2013;34(5):669-85.
51. Mattick JS, Dinger M, Schonrock N, Cowley M. Whole genome sequencing provides better diagnostic yield and future value than whole exome sequencing. Med J Aust. 2018;209(5):197-9.
52. Investigators GPP, Smedley D, Smith KR, Martin A, Thomas EA, McDonagh EM, et al. 100,000 Genomes Pilot on Rare-Disease Diagnosis in Health Care - Preliminary Report. N Engl J Med. 2021;385(20):1868-80.
53. Caulfield M, Davies J, Dennys M, Elbahy L, Fowler T, Hill S, et al. The National Genomics Research and Healthcare Knowledgebase[Internet]2020.
54. Flanagan S, Damhuis A, Banerjee I, Rokicki D, Jefferies C, Kapoor R, et al. Partial ABCC8 gene deletion mutations causing diazoxide-unresponsive hyperinsulinaemic hypoglycaemia. Pediatr Diabetes. 2012;13(3):285-9.
55. Scott RH, Douglas J, Baskcomb L, Nygren AO, Birch JM, Cole TR, et al. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) robustly detects and distinguishes 11p15 abnormalities associated with overgrowth and growth retardation. J Med Genet. 2008;45(2):106-13.
56. Aref-Eshghi E, Kerkhof J, Pedro VP, France GD, Barat-Houari M, Ruiz-Pallares N, et al. Evaluation of DNA Methylation Episignatures for Diagnosis and Phenotype Correlations in 42 Mendelian Neurodevelopmental Disorders. Am J Hum Genet. 2021;108(6):1161-3.
57. Nowak A, Murik O, Mann T, Zeevi DA, Altarescu G. Detection of single nucleotide and copy number variants in the Fabry disease-associated GLA gene using nanopore sequencing. Sci Rep. 2021;11(1):22372.
58. Leija-Salazar M, Sedlazeck FJ, Toffoli M, Mullin S, Mokretar K, Athanasopoulou M, et al. Evaluation of the detection of GBA missense mutations and other variants using the Oxford Nanopore MinION. Mol Genet Genomic Med. 2019;7(3):e564.
59. Mahaweni NM, Olieslagers TI, Rivas IO, Molenbroeck SJJ, Groeneweg M, Bos GMJ, et al. A comprehensive overview of FCGR3A gene variability by full-length gene sequencing including the identification of V158F polymorphism. Sci Rep. 2018;8(1):15983.

60. Minervini CF, Cumbo C, Orsini P, Brunetti C, Anelli L, Zagaria A, et al. TP53 gene mutation analysis in chronic lymphocytic leukemia by nanopore MinION sequencing. Diagn Pathol. 2016;11(1):96.
61. Houghton JA, Banerjee I, Shaikh G, Jabbar S, Laver TW, Cheesman E, et al. Unravelling the genetic causes of mosaic islet morphology in congenital hyperinsulinism. J Pathol Clin Res. 2020;6(1):12-6.
62. De Franco E, Saint-Martin C, Brusgaard K, Knight Johnson AE, Aguilar-Bryan L, Bowman P, et al. Update of variants identified in the pancreatic beta-cell KATP channel genes KCNJ11 and ABCC8 in individuals with congenital hyperinsulinism and diabetes. Hum Mutat. 2020;41(5):884-905.
63. Glaser B, Kesavan P, Heyman M, Davis E, Cuesta A, Buchs A, et al. Familial hyperinsulinism caused by an activating glucokinase mutation. N Engl J Med. 1998;338(4):226-30.
64. Clayton PT, Eaton S, Aynsley-Green A, Edginton M, Hussain K, Krywawych S, et al. Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of beta-oxidation in insulin secretion. J Clin Invest. 2001;108(3):457-65.
65. Flanagan SE, Patch AM, Locke JM, Akcay T, Simsek E, Alaei M, et al. Genome-wide homozygosity analysis reveals HADH mutations as a common cause of diazoxide-responsive hyperinsulinemic-hypoglycemia in consanguineous pedigrees. J Clin Endocrinol Metab. 2011;96(3):E498-502.
66. Tung JY, Boodhansingh K, Stanley CA, De Leon DD. Clinical heterogeneity of hyperinsulinism due to HNF1A and HNF4A mutations. Pediatr Diabetes. 2018;19(5):910-6.
67. Hojlund K, Hansen T, Lajer M, Henriksen JE, Levin K, Lindholm J, et al. A novel syndrome of autosomal-dominant hyperinsulinemic hypoglycemia linked to a mutation in the human insulin receptor gene. Diabetes. 2004;53(6):1592-8.
68. Thomas P, Ye Y, Lightner E. Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. Hum Mol Genet. 1996;5(11):1809-12.
69. Bitner-Glindzicz M, Lindley KJ, Rutland P, Blaydon D, Smith VV, Milla PJ, et al. A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene. Nat Genet. 2000;26(1):56-60.

70. Staufner C, Lindner M, Dionisi-Vici C, Freisinger P, Dobbelaere D, Douillard C, et al. Adenosine kinase deficiency: expanding the clinical spectrum and evaluating therapeutic options. J Inherit Metab Dis. 2016;39(2):273-83.
71. Sun L, Eklund EA, Chung WK, Wang C, Cohen J, Freeze HH. Congenital disorder of glycosylation id presenting with hyperinsulinemic hypoglycemia and islet cell hyperplasia. J Clin Endocrinol Metab. 2005;90(7):4371-5.
72. Hatada I, Ohashi H, Fukushima Y, Kaneko Y, Inoue M, Komoto Y, et al. An imprinted gene p57KIP2 is mutated in Beckwith-Wiedemann syndrome. Nat Genet. 1996;14(2):171-3.
73. Matsuo T, Ihara K, Ochiai M, Kinjo T, Yoshikawa Y, Kojima-Ishii K, et al. Hyperinsulinemic hypoglycemia of infancy in Sotos syndrome. Am J Med Genet A. 2013;161A(1):34-7.
74. Munns CF, Batch JA. Hyperinsulinism and Beckwith-Wiedemann syndrome. Arch Dis Child Fetal Neonatal Ed. 2001;84(1):F67-9.
75. Welters A, El-Khairi R, Dastamani A, Bachmann N, Bergmann C, Gilbert C, et al. Persistent hyperinsulinaemic hypoglycaemia in children with Rubinstein-Taybi syndrome. Eur J Endocrinol. 2019;181(2):121-8.
76. 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(5):439-46.
77. Astuti D, Morris MR, Cooper WN, Staals RH, Wake NC, Fews GA, et al. Germline mutations in DIS3L2 cause the Perlman syndrome of overgrowth and Wilms tumor susceptibility. Nat Genet. 2012;44(3):277-84.
78. Baumann U, Preece MA, Green A, Kelly DA, McKiernan PJ. Hyperinsulinism in tyrosinaemia type I. J Inherit Metab Dis. 2005;28(2):131-5.
79. Terespolsky D, Farrell SA, Siegel-Bartelt J, Weksberg R. Infantile lethal variant of Simpson-Golabi-Behmel syndrome associated with hydrops fetalis. Am J Med Genet. 1995;59(3):329-33.
80. Alexander S, Ramadan D, Alkhayyat H, Al-Sharkawi I, Backer KC, El-Sabban F, et al. Costello syndrome and hyperinsulinemic hypoglycemia. Am J Med Genet A. 2005;139(3):227-30.
81. Gole H, Chuk R, Coman D. Persistent Hyperinsulinism in Kabuki Syndrome 2: Case Report and Literature Review. Clin Pract. 2016;6(3):848.

82. White SM, Thompson EM, Kidd A, Savarirayan R, Turner A, Amor D, et al. Growth, behavior, and clinical findings in 27 patients with Kabuki (Niikawa-Kuroki) syndrome. Am J Med Genet A. 2004;127A(2):118-27.
83. Soden SE, Saunders CJ, Willig LK, Farrow EG, Smith LD, Petrikin JE, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. Sci Transl Med. 2014;6(265):265ra168.
84. Deeb A, Al Amoodi A. A novel homozygous mutation in the mannose phosphate isomerase gene causing congenital disorder of glycation and hyperinsulinemic hypoglycemia in an infant. Clin Case Rep. 2018;6(3):479-83.
85. Carrasco Salas P, Palma Milla C, Lezana Rosales JM, Benito C, Franco Freire S, Lopez Siles J. Hyperinsulinemic hypoglycemia in a patient with an intragenic NSD1 mutation. Am J Med Genet A. 2016;170A(2):544-6.
86. Grand K, Gonzalez-Gandolfi C, Ackermann AM, Aljeaid D, Bedoukian E, Bird LM, et al. Hyperinsulinemic hypoglycemia in seven patients with de novo NSD1 mutations. Am J Med Genet A. 2019;179(4):542-51.
87. Douglas J, Tatton-Brown K, Coleman K, Guerrero S, Berg J, Cole TR, et al. Partial NSD1 deletions cause 5% of Sotos syndrome and are readily identifiable by multiplex ligation dependent probe amplification. J Med Genet. 2005;42(9):e56.
88. Hennewig U, Hadzik B, Vogel M, Meissner T, Goecke T, Peters H, et al. Congenital central hypoventilation syndrome with hyperinsulinism in a preterm infant. J Hum Genet. 2008;53(6):573-7.
89. Bohles H, Sewell AA, Gebhardt B, Reinecke-Luthge A, Kloppel G, Marquardt T. Hyperinsulinaemic hypoglycaemia--leading symptom in a patient with congenital disorder of glycosylation Ia (phosphomannomutase deficiency). J Inherit Metab Dis. 2001;24(8):858-62.
90. Tamame T, Hori N, Homma H, Yoshida R, Inokuchi M, Kosaki K, et al. Hyperinsulinemic hypoglycemia in a newborn infant with trisomy 13. Am J Med Genet A. 2004;129A(3):321-2.
91. Alkhayyat H, Christesen HB, Steer J, Stewart H, Brusgaard K, Hussain K. Mosaic Turner syndrome and hyperinsulinaemic hypoglycaemia. J Pediatr Endocrinol Metab. 2006;19(12):1451-7.
92. Brioude F, Lacoste A, Netchine I, Vazquez MP, Auber F, Audry G, et al. Beckwith-Wiedemann syndrome: growth pattern and tumor risk according to

molecular mechanism, and guidelines for tumor surveillance. Horm Res Paediatr. 2013;80(6):457-65.

Cohort recruitment

Individuals were recruited for the studies included in this thesis in tandem with their referral for genetic testing for HI in Exeter. This process was conducted jointly by Professor Sarah Flanagan, of the University of Exeter Medical School, and Dr Jayne Houghton, of the Molecular Genetics Laboratory at the Royal Devon & Exeter Hospital (both Exeter, UK).

Individuals with HI were recruited by their local clinician, who obtained consent for both routine genetic testing and for research into novel causes of HI where appropriate. All individuals gave informed consent, which was held in Exeter as part of the Exeter Beta Cell Research Bank (http://www.diabetesgenes.org/content/genetic-beta-cell-research-bank).

A standardised clinical request form accompanied samples and was used to collate known clinical information on cases of HI referred to Exeter for genetic testing and research. This request form can be found in Appendix 1. The form was designed in order to allow for the collation of standardised data that allowed for the appropriateness of genetic testing to be determined, help with the interpretation of genetic variation, and inform on research studies. Clinical data requested included birth weight, gestational age, and biochemical measures relevant to the diagnosis of treatment of HI (which included insulin, glucose, and in some cases C-peptide). In addition, free text boxes were included in order to collate information on extra-pancreatic features that may indicate the presence of syndromic disease, along with any family history of either hypoglycaemia or diabetes.

Clinical and genetic information was stored in a password-protected database held on the servers of the Royal Devon & Exeter NHS Foundation Trust, only accessible to scientists of the Molecular Genetics Laboratory, and researchers from the Hyperinsulinism Genes and Diabetes Genes teams at the University of Exeter Medical School. Correspondence with clinicians regarding genetic testing results and further clinical questions was conducted via email.

Blood samples sent to Exeter for genetic testing and research were stored securely at the Royal Devon & Exeter Hospital's Wonford site by the Molecular Genetics Laboratory, with access available for researchers on an agreed basis.

Chapter 1

Birth weight and diazoxide unresponsiveness strongly predict the likelihood of congenital hyperinsulinism due to a mutation in *ABCC8* or *KCNJ11*

Thomas I. Hewat [1], Daphne Yau [2], Joseph C. S. Jerome [1], Thomas W. Laver [1],Jayne A. L. Houghton [3], Beverley M. Shields [4], Sarah E. Flanagan [1*], Kashyap A. Patel [1,3*]

1. Institute of Biomedical and Clinical Science, University of Exeter Medical School, UK
2. Department of Paediatric Endocrinology, Royal Manchester Children's Hospital, UK
3. Royal Devon and Exeter Foundation Hospital, Exeter, UK
4. National Institute for Health Research Exeter Clinical Research Facility, University of Exeter Medical School, UK

**These authors contributed equally to this work*

This manuscript has been published in the European Journal of Endocrinology: Eur J Endocrinol. 2021 Oct 30;185(6):813-818. doi: 10.1530/EJE-21-0476.

My contribution to the chapter

I selected the cohort for this study based on data cleaning to ensure all patients included had a biochemical diagnosis of congenital hyperinsulinism and all data necessary to conduct the study. I performed statistical analyses to determine the clinical features that differed between the two groups. I designed and ran a regression model to determine the predictive power of those clinical features. I drafted the manuscript, revised the manuscript according to comments from co-authors, submitted the manuscript, and revised the manuscript in response to review.

Acknowledgements

TIH, DY, SEF, and KAP designed the study. JALH and SEF recruited patients for the study. TIH, DY, JCSJ, BMS, and KAP performed statistical analyses. TIH, TWL, SEF, and KAP drafted the manuscript.

ABSTRACT

Objective

Mutations in the KATP channel genes, *ABCC8* and *KCNJ11,* are the most common cause of congenital hyperinsulinism. The diagnosis of KATP hyperinsulinism is important for the clinical management of the condition. We aimed to determine the clinical features that help to identify KATP hyperinsulinism at diagnosis.

Design

We studied 761 individuals with KATP hyperinsulinism and 862 probands with hyperinsulinism of unknown aetiology diagnosed before 6 months of age. All were referred as part of routine clinical care.

Methods

We compared the clinical features of KATP hyperinsulinism and unknown hyperinsulinism cases. We performed logistic regression and ROC analysis to identify the features that predict KATP hyperinsulinism.

Results

Higher birth weight, diazoxide unresponsiveness and diagnosis in the first week of life were independently associated with KATP hyperinsulinism (adjusted Odds Ratio 4.5 (95% CI, 3.4-5.9), 0.09 (0.06-0.13) and 3.3 (2.0- 5.0) respectively). Birth weight and diazoxide unresponsiveness were additive and highly discriminatory for identifying KATP hyperinsulinism (ROC area under the curve for birth weight 0.80, diazoxide responsiveness 0.77, and together 0.88, 95% CI 0.85-0.90). 86% born large for gestation and 78% born appropriate for gestation who did not respond to diazoxide treatment had KATP hyperinsulinism. In contrast, of those individuals born small for gestation, none who were diazoxide responsive and only 4% of those who were diazoxide unresponsive had KATP hyperinsulinism.

Conclusions

Individuals with hyperinsulinism born appropriate or large for gestation and unresponsive to diazoxide treatment are most likely to have an *ABCC8* or *KCNJ11* mutation. These patients should be prioritised for genetic testing for KATP channel genes.

Introduction

Congenital hyperinsulinism (CHI) is a potentially life-threatening disorder characterised by inappropriately high levels of insulin at the time of hypoglycaemia. The incidence of CHI is estimated at 1 in 28,000 to 1 in 50,000 live births in European populations but rises to approximately 1 in 2,500 in countries with high rates of consanguineous unions [1-3].

Mutations in approximately 15 genes have been reported to cause isolated CHI or a multi-system syndromic disease where CHI is a rare feature [4]. Screening these genes identifies a mutation in 36-69% of cases [5, 6]. Inactivating mutations in the *ABCC8* and *KCNJ11* genes, encoding the SUR1 and Kir6.2 subunits of the pancreatic ATP-sensitive potassium (KATP) channel are responsible for 80-84% of confirmed monogenic cases of CHI [5,6].

Rapid screening of the KATP channel genes is critical for informing surgical and medical management of CHI [7]. Identifying a dominantly-acting or bi-allelic *ABCC8* or *KCNJ11* mutation confirms a diagnosis of diffuse pancreatic disease which is preferentially managed with medical treatment whereas a paternally-inherited recessively-acting *ABCC8* or *KCNJ11* mutation suggests a focal pancreatic lesion which can be cured by lesionectomy [8].

In this study, we undertook genetic testing of the KATP channel genes in a large cohort of children with CHI presenting before the age of 6 months with the aim to assess whether clinical features at presentation could predict which individuals were most likely to have monogenic CHI due to a KATP channel mutation.

METHODS

Study population and genetic analysis

We studied 761 probands with a pathogenic or likely pathogenic mutation in *ABCC8* (N=665) or *KCNJ11* (N=96) identified at the Exeter Genomics Laboratory between 2002 and 2018. We also reviewed 862 patients with CHI of unknown genetic aetiology who were referred to our laboratory within the same period. In all of these patients, mutations in the *ABCC8* and *KCNJ11* genes had been excluded by Sanger sequencing or gene panel testing.

Analysis of the coding regions and intron-exon boundaries of *ABCC8* and *KCNJ11* was performed using previously described methods [9, 10]. When available, parental samples were tested to confirm the inheritance of *ABCC8*/*KCNJ11* variants identified in the proband. Pathogenicity of variants was assessed according to ACMG guidelines [11].

Clinical information was provided at referral for genetic testing using a standardised request form and included sex, ethnicity, birth weight, gestational age at birth, age at diagnosis of CHI, biochemical measurements at diagnosis (including insulin and glucose), current treatment, response to diazoxide treatment if attempted and the presence of additional features. Although a precise, consensus definition of diazoxide responsiveness is lacking, diazoxide unresponsiveness in this study can be broadly defined as persistent hypoglycaemia despite treatment with maximal dose diazoxide indicating the need for additional therapies to achieve euglycaemia. Consanguinity was defined as the parents of the proband being related as second cousins or closer or if the proband was referred from a country with high reported rates of consanguinity [12].

Informed consent was obtained from the parents or guardians of all probands. This study was approved by the North Wales Research Ethics Committee (517/WA/0327).

Statistical analysis

Birth weight Z score and corrected birth weight for sex and gestation were generated using WHO standards accessed through the Zanthro package in Stata [13]. Small for gestational age (SGA) was defined as a birth weight lower than the 10th centile, and large for gestational age (LGA) was defined as a birth weight greater than the 90th centile. Age at diagnosis followed a skewed distribution, so was analysed categorically between those diagnosed in the first week of life and those diagnosed after the first week. As insulin (pmol/l) level followed a right skewed distribution, values were log transformed for the statistical analysis.

Individuals with an *ABCC8* or *KCNJ11* mutation were combined (referred to as KATP channel mutations hereafter) as no difference in clinical features was observed between the two genetic subgroups (Supplementary Table 1). Statistical analyses were performed to determine differences in the clinical features of patients with an

unknown aetiology compared to patients with a KATP channel mutation. Two-tailed P values were calculated to determine statistical significance using Pearson's chi-squared test for categorical variables, and Student's T test for continuous variables.

Univariate and multivariable logistic regression were used to assess which clinical features were independently predictive of having a KATP channel mutation. The area under the curve (AUC) of the receiver operator characteristic (ROC) was used to assess the discriminatory ability of the clinical features to identify individuals with a KATP channel mutation from those without a mutation. Stata/SE 16.0 (Stata Corp, College Station, TX, USA) was used to perform statistical analyses.

RESULTS

Seven of the clinical features studied were different between individuals with KATP CHI and those with CHI of unknown aetiology

Individuals with KATP channel mutations (n=761) had higher birthweights (mean 4333g vs 3512g, $P=6 \times 10^{-94}$, mean difference 821g, 95% CI 748g-894g), were more likely to be diagnosed in the first week of life (85% vs 72%, $P=1 \times 10^{-9}$), had a higher insulin level at diagnosis (mean 162.2pmol/l vs 115.4pmol/l, $P=1 \times 10^{-8}$) and were less likely to respond to diazoxide, the mainstay treatment for CHI (32% vs 88%, $P=2 \times 10^{-84}$) compared to individuals with CHI of unknown genetic aetiology (n=862) (Table 1). They were more likely to be female (46% vs 36%, $P=5 \times 10^{-5}$), have consanguineous parents (52% vs 34%, $P=2 \times 10^{-12}$) and were less likely to be Caucasian (30% vs 52%, $P=3 \times 10^{-18}$) compared to CHI of unknown genetic aetiology. Glucose levels at presentation and the number of individuals with extra-pancreatic features at referral were similar between the two groups.

Birth weight and diazoxide unresponsiveness are the most discriminative, independent, and additive for identifying children with KATP CHI

To identify the independent features that can help in discriminating KATP CHI from unknown cases, we performed a multivariable logistic regression analysis. We showed that higher birth weight, diazoxide unresponsiveness, and a diagnosis within the first 7 days of life were independent predictors of KATP CHI after adjustment for all variables (Table 2). We next performed ROC AUC analysis to assess the discriminatory ability of these variables. The ROC AUC for birth weight was 0.80

(95% CI 0.77-0.82), 0.77 for diazoxide responsiveness (95% CI 0.74-0.80) and 0.59 for age at diagnosis (95% CI 0.57-0.62). Combining birth weight with diazoxide unresponsiveness increased the ROC AUC to 0.88 (95% CI 0.85-0.90). The addition of other factors (age at diagnosis, sex, ethnicity, consanguinity, insulin level) only marginally increased ROC AUC (0.89, 95% CI 0.87-0.91) over the combination of birth weight and diazoxide unresponsiveness (Table 2, Figure 1). To establish if our results were consistent across institutions that may have different standards in measuring these clinical features, we performed a sensitivity analysis with the two institutions that referred the most patients, along with the two countries with the most referred patients. We observed similar results in this analysis, suggesting the presence of homogeneity in clinical features despite different patient populations and clinical practice (Supplementary Table 2).

Individuals born appropriate or large for gestation who did not respond to diazoxide had the highest likelihood of KATP CHI

Of the 220 individuals born large for gestational age who were unresponsive to diazoxide, 86% (95% CI 81%-91%) had KATP CHI, whilst no mutations were detected in those born small for gestational age who responded to diazoxide (Figure 2). Of the 179 individuals born appropriate for gestational age who were unresponsive to diazoxide, 78% (95% CI 71%-84%) had KATP CHI, whilst of the 389 individuals born appropriate for gestational age who were responsive to diazoxide, only 18% (95% CI 14%-22%) had KATP CHI.

DISCUSSION

In this large study, we showed that simple clinical features such as birth weight and diazoxide responsiveness were independent and highly predictive for identifying individuals with KATP CHI from those with CHI of unknown aetiology.

We show that patients with KATP CHI were ~830 g heavier compared to CHI due to unknown cause. Higher birth weight has been reported previously in patients with KATP CHI [14, 15] and with persistent CHI [16]. However, previous studies generally did not include control cases without KATP mutations, were smaller in size, and were limited to single centres impacting on the ability to statistically assess the importance of birth weight for identifying KATP CHI. We also show that birthweight and other clinical features were comparable between *ABCC8* and *KCNJ11* CHI thus this

finding is applicable to both KATP CHI subtypes (Supplementary Table 1). The higher birth weight in the KATP CHI patients is in keeping with the onset of hyperinsulinism with KATP CHI *in utero*, as insulin acts as a growth factor in pregnancy [17]. This is supported by the observation that most of the children with CHI are diagnosed in the first week of life.

Diazoxide is the most common medical treatment for CHI which suppresses insulin secretion by binding to and opening the KATP channel [18]. 78% of patients in our cohort who were unresponsive to diazoxide treatment had a KATP channel mutation, in keeping with the mutations leading an absence of channels or channels with disrupted function. Previous studies reported similar levels of diazoxide-unresponsiveness in patients with KATP CHI (5,6). Therefore, our finding is not unexpected, but due to large control cases, we were able to quantify the importance of lack of diazoxide response in identifying KATP CHI. Additionally, there was no difference in diazoxide responsiveness between patients with mutations in *ABCC8* and *KCNJ11* thus our findings are applicable to both genetic subtypes (Supplementary Table 1). Given that diazoxide is not universally available it will be important to perform further studies to assess whether responsiveness to other treatments (including somatostatin analogues) can help to predict KATP channel hyperinsulinism.

Our study has important clinical and research implications. Using the largest cohort of CHI cases referred from routine clinical practice, we robustly show that birth weight and response to diazoxide treatment can be used to help guide genetic testing in patients with CHI. Our cohort also included patients from multiple centres, of different ethnicities, and with different modes of inheritance for mutations in the KATP channel genes suggesting that our findings are applicable to patients world-wide. More than 80% of cases born LGA or AGA who were unresponsive to diazoxide had KATP channel mutations and therefore every effort should be made to prioritise genetic testing in these individuals as finding a KATP mutation can guide clinical management (7). Current Pediatric Endocrine Society guidelines on hypoglycaemia lack recommendation on criteria that can be used to prioritise patients for genetic testing for CHI (19). We believe our study provides important evidence in this regard which is especially relevant for resource-poor countries where access to genetic testing is limited [19]. Furthermore, the findings from this

study will help to prioritise patients for genetic discovery studies for patients with an unknown aetiology. Patients born large for gestational age who do not respond to diazoxide have an 86% chance of harbouring a mutation in a KATP channel gene (Figure 1). Therefore, patients in this category without a monogenic cause are good candidates to screen for pancreas-specific mosaic mutations and regulatory mutations affecting the KATP channel genes [20].

We acknowledge that our study has some limitations. We used clinician reported assessment of diazoxide responsiveness thus it is likely to vary by referring clinician. However, the rate of diazoxide unresponsiveness in KATP CHI is similar to studies that used a single definition for this feature [5, 6]. Furthermore, the lack of consistent definition will have reduced the discriminatory ability rather than inflated the estimate suggesting that a robust definition will provide improved or similar results to our study. Our study did not include comprehensive data on whether hyperinsulinism was transient or persistent in each patient as we wanted to identify features at diagnosis which could help predict a KATP channel mutation. However, enrichment of male and SGA patients in the unknown aetiology group suggests the presence of transient HI as reported by a recent study [21]. We used clinician reporting and country of referral to infer consanguinity. Although this approach has been used previously [22], we recognise that this has likely increased the proportion of consanguinity reported in our cohort. Despite this, consanguinity was significantly enriched in patients with KATP hyperinsulinism. This suggests that a more accurate definition will further increase its utility in identifying KATP hyperinsulinism. Finally, our study finding is not applicable to cases of non-KATP monogenic CHI. We chose to focus on KATP CHI as they are responsible for more than 80% monogenic CHI and finding these mutations is of great clinical importance.

In conclusion, this study shows that birth weight, diazoxide responsiveness and presenting with CHI in the first week of life are independent and highly predictive for KATP channel mutations in patients with congenital hyperinsulinism. Patients born normal or large for gestational age who are unresponsive to diazoxide are most likely to have KATP channel mutations and should be referred for genetic testing. Our study provides robust results that will help to shape future guidelines on genetic testing for CHI and is applicable to patients worldwide.

Funding Acknowledgements

TWL is supported by the UKRI Expanding Excellence in England award. SEF has a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (Grant Number: 105636/Z/14/Z). KAP has a Career Development fellowship funded by the Wellcome Trust (219606/Z/19/Z). This study represents independent research supported by the National Institute of Health Research Exeter Clinical Research Facility. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, Wellcome Trust, or the Department of Health and Social Care.

REFERENCES

1. Yau D, Laver TW, Dastamani A, Senniappan S, Houghton JAL, Shaikh G, Cheetham T, Mushtaq T, Kapoor RR, Randell T, Ellard S, Shah P, Banerjee I & Flanagan SE. Using referral rates for genetic testing to determine the incidence of a rare disease: The minimal incidence of congenital hyperinsulinism in the UK is 1 in 28,389. *PLoS One* 2020 **15** e0228417.

2. Bruining GJ. Recent advances in hyperinsulinism and the pathogenesis of diabetes mellitus. *Current Opinion in Pediatrics* 1990 **2** 758-765.

3. Mathew PM, Young JM, Abu-Osba YK, Mulhern BD, Hammoudi S, Hamdan JA & Sa'di AR. Persistent neonatal hyperinsulinism. *Clin Pediatr (Phila)* 1988 **27** 148-151.

4. Güemes M, Rahman SA, Kapoor RR, Flanagan S, Houghton JAL, Misra S, Oliver N, Dattani MT & Shah P. Hyperinsulinemic hypoglycemia in children and adolescents: Recent advances in understanding of pathophysiology and management. *Rev Endocr Metab Disord* 2020 **21** 577-597.

5. Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S & Hussain K. Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism. *Eur J Endocrinol* 2013 **168** 557-564.

6. Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, Ganapathy K, Bhatti T, Stanley CA & Ganguly A. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. *J Clin Endocrinol Metab* 2013 **98** E355-363.

7. Banerjee I, Skae M, Flanagan SE, Rigby L, Patel L, Didi M, Blair J, Ehtisham S, Ellard S, Cosgrove KE, Dunne MJ & Clayton PE. The contribution of rapid KATP channel gene mutation analysis to the clinical management of children with congenital hyperinsulinism. *Eur J Endocrinol* 2011 **164** 733-740.

8. Adzick NS, De Leon DD, States LJ, Lord K, Bhatti TR, Becker SA & Stanley CA. Surgical treatment of congenital hyperinsulinism: Results from 500 pancreatectomies in neonates and children. *J Pediatr Surg* 2019 **54** 27-32.

9. Ellard S, Lango Allen H, De Franco E, Flanagan SE, Hysenaj G, Colclough K, Houghton JA, Shepherd M, Hattersley AT, Weedon MN & Caswell R. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. *Diabetologia* 2013 **56** 1958-1963.

10. Flanagan SE, Dung VC, Houghton JAL, De Franco E, Ngoc CTB, Damhuis A, Ashcroft FM, Harries LW & Ellard S. An ABCC8 Nonsense Mutation Causing Neonatal Diabetes Through Altered Transcript Expression. *J Clin Res Pediatr Endocrinol* 2017 **9** 260-264.

11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL & Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015 **17** 405-424.

12. Bittles AH. A community genetics perspective on consanguineous marriage. *Community Genet* 2008 **11** 324-330.

13. Vidmar S, Carlin J, Hesketh K & Cole T. Standardizing Anthropometric Measures in Children and Adolescents with New Functions for Egen. *Stata J Promot Commun Stat Stata* 2004 **4** 50-55.

14. Pinney SE, MacMullen C, Becker S, Lin YW, Hanna C, Thornton P, Ganguly A, Shyng SL & Stanley CA. Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant KATP channel mutations. *J Clin Invest* 2008 **118** 2877-2886.

15. Flanagan SE, Kapoor RR & Hussain K. Genetics of congenital hyperinsulinemic hypoglycemia. *Semin Pediatr Surg* 2011 **20** 13-17.

16. Falzone N & Harrington J. Clinical Predictors of Transient versus Persistent Neonatal Hyperinsulinism. *Horm Res Paediatr* 2020 **93** 297-303.

17. Pedersen J. Diabetes and pregnancy; blood sugar of newborn infants during fasting and glucose administration. *Ugeskr Laeger* 1952 **114** 685.

18. Ashcroft FM. ATP-sensitive potassium channelopathies: focus on insulin secretion. *J Clin Invest* 2005 **115** 2047-2058.

19. Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, Levitsky LL, Murad MH, Rozance PJ, Simmons RA, Sperling MA, Weinstein DA, White NH, Wolfsdorf JI & Pediatric Endocrine S. Recommendations from the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. *J Pediatr* 2015 **167** 238-245.

20. Houghton JA, Banerjee I, Shaikh G, Jabbar S, Laver TW, Cheesman E, Chinnoy A, Yau D, Salomon-Estebanez M, Dunne MJ & Flanagan SE. Unravelling the genetic causes of mosaic islet morphology in congenital hyperinsulinism. *J Pathol Clin Res* 2020 **6** 12-16.

21. Hoermann H, Roeper M, Salimi Dafsari R, Koestner F, Reinauer C, Mayatepek E, Meissner T & Kummer S. Challenges in management of transient hyperinsulinism - a retrospective analysis of 36 severely affected children. *J Pediatr Endocrinol Metab* 2021.

22. De Franco E, Flanagan SE, Houghton JA, Lango Allen H, Mackay DJ, Temple IK, Ellard S & Hattersley AT. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. *Lancet* 2015 **386** 957-963.

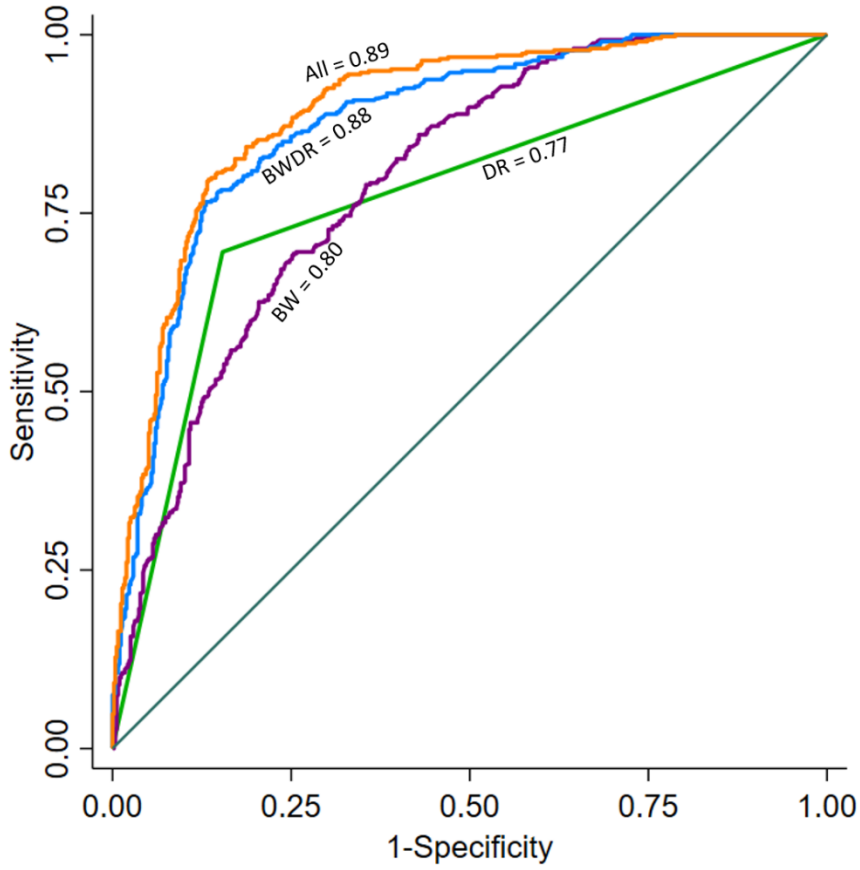

Figure 1 – Receiver operating curves analysis showing the discriminating ability of clinical features to identify individuals with KATP CHI from those with unknown aetiology. DR denotes diazoxide responsiveness. BW denotes birth weight. All includes birth weight, diazoxide responsiveness, age at diagnosis, sex, insulin level at diagnosis, ethnicity, and consanguinity.

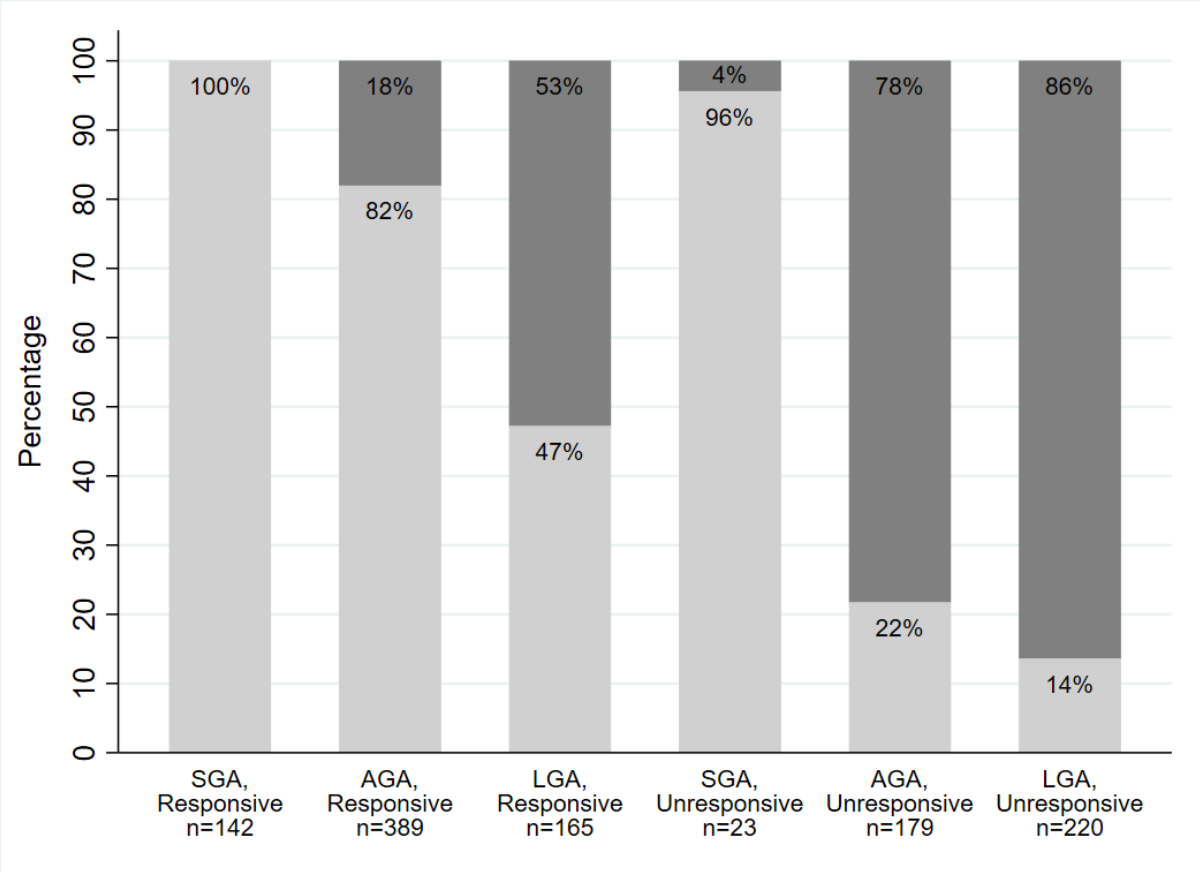

Figure 2 – Proportion of KATP Congenital Hyperinsulinism by diazoxide responsiveness and birth weight categories. Light grey bars represent the percentage number of individuals without a KATP channel mutation, dark grey bars represent the percentage number of cases with a KATP channel mutation. SGA = small for gestational age, AGA = appropriate for gestational age, LGA = large for gestational age.

Characteristics	CHI with confirmed KATP channel mutations	CHI of unknown aetiology	P value
N	761	862	-
Age at diagnosis	-	-	1×10^{-9}
≤7 days	644 (85%)	622 (72%)	-
>7 days	117 (15%)	240 (28%)	-
Female sex	352 (46%)	313 (36%)	$5x\ 10^{-5}$
Corrected birth weight (g) [n]	4333 (718) [746]	3512 (762) [843]	6×10^{-94}
Birth weight Z score [n]	1.64 (1.50) [746]	-0.13 (1.66) [843]	1×10^{-95}
Birth weight categories	-	-	8×10^{-93}
LGA	415 (56%) [746]	151 (18%) [843]	-
AGA	329 (44%) [746]	478 (57%) [843]	-
SGA	2 (0.3%) [746]	214 (25%) [843]	-
Additional features	102 (13%)	152 (18%)	0.02*
White ethnicity [n]	224 (30%) [736]	438 (52%) [841]	3×10^{-18}
Consanguineous parents	394 (52%)	296 (34%)	2×10^{-12}
Glucose (mmol/L) [n]	1.6 (0.7) [653]	1.7 (0.8) [740]	0.02*
Insulin (pmol/L) [n]	162.2 (3.0) [666]	115.4 (2.9) [715]	1×10^{-8}
Diazoxide responsive [n]	160 (32%) [495]	521 (88%) [591]	2×10^{-84}

Table 1 - Characteristics of individuals with congenital hyperinsulinism with a confirmed KATP channel mutation and those with an unknown aetiology. Categorical data are showed as n (%) whereas continuous variables are shown as mean (SD). *P value are above the Bonferroni corrected threshold for multiple comparison (0.05/8 = 0.006). Number of patients is shown in square brackets where it differs from the total number in the cohort. Patients with a confirmed KATP channel mutation included individuals with a mutation in either KCNJ11 (N=96) or ABCC8 (N=665).

Characteristics	Unadjusted Odds ratio (95% CI)	P value	Adjusted Odds ratio (95% CI)	P value
Diagnosed in first week of life	2 (1.6 -2.5)	3×10^{-10}	3.3 (2.0- 5.0)	2×10^{-6}
Female sex	1.5 (1.2 – 1.8)	5×10^{-5}	0.9 (0.6 – 1.3)	0.6
Corrected birth weight	4.4 (3.7 – 5.2)	3×10^{-68}	4.5 (3.4 – 5.9)	2×10^{-26}
Caucasian ethnicity	2.4 (2.0 – 3.0)	9×10^{-18}	1.5 (0.9 – 2.6)	0.1
Consanguinity	2.0 (1.6 – 2.4)	7×10^{-12}	1.9 (1.1 – 3.3)	0.02
Insulin at diagnosis	1.9 (1.5 – 2.4)	9×10^{-9}	0.7 (0.5 – 1.0)	0.08
Diazoxide responsive	0.08 (0.06 – 0.11)	2×10^{-65}	0.09 (0.06 – 0.13)	2×10^{-36}

Table 2 – Odds ratios for clinical characteristics from logistic regression model for identifying individuals with KATP CHI from CHI of unknown aetiology. Table shows odds ratio (95% confidence interval) for univariate and multivariate analysis after adjusting for all variables.

Characteristics	CHI with confirmed *ABCC8* mutation	CHI with confirmed *KCNJ11* mutation	P value
N	665	96	-
Age at diagnosis	-	-	0.88
≤7 days	562 (85%)	82 (85%)	-
>7 days	103 (15%)	14 (15%)	-
Female sex	313 (47%)	39 (41%)	0.27
Corrected birth weight (g) [n]	4331 (706) [650]	4341 (797)	0.90
Birth weight Z score [n]	1.64 (1.47) [650]	1.65 (1.66)	0.93
Birth weight categories	-	-	0.19
LGA	365 (56%) [650]	50 (52%)	-
AGA	284 (44%) [650]	45 (47%)	-
SGA	1 (0.2%) [650]	1 (1%)	-
Additional features	84 (13%)	18 (19%)	0.11
White ethnicity [n]	195 (30%) [642]	29 (31%) [94]	0.91
Consanguineous parents	343 (52%)	51 (53%)	0.83
Glucose (mmol/L) [n]	1.6 (0.7) [577]	1.6 (0.6) [76]	0.90
Insulin (pmol/L) [n]	161.2 (3.0) [588]	170.0 (2.9) [78]	0.69
Diazoxide responsive [n]	133 (31%) [423]	27 (38%) [72]	0.34

Supplementary Table 1 – Characteristics of individuals with congenital hyperinsulinism caused by mutations in ABCC8 and KCNJ11. Categorical data are shown as n (%) whereas continuous data are shown as mean (SD). *P values are above the Bonferroni corrected threshold for multiple comparison (0.05/8 = 0.006). Number of patients [n] is shown in square brackets where it differs from the total number in the cohort.

	No with KATP HI/total HI	ROC AUC for corrected birth weight alone (95% CI)	ROC AUC for diazoxide responsiveness alone (95% CI)	ROC AUC for corrected birth weight and diazoxide responsiveness (95% CI)	ROC AUC for corrected birth weight, diazoxide responsiveness, sex, insulin, ethnicity, consanguinity (95% CI)
Whole cohort	761/1685	0.80 (0.77 – 0.82)	0.77 (0.74 – 0.80)	0.88 (0.85 – 0.90)	0.89 (0.87 – 0.91)
Patients from the UK	173/531	0.85 (0.80 – 0.89)	0.84 (0.79 – 0.88)	0.94 (0.91 – 0.96)	0.93 (0.91 – 0.96)
Patients from Turkey	102/208	0.86 (0.80 – 0.92)	0.75 (0.68 – 0.82)	0.89 (0.84 – 0.95)	0.90 (0.85 – 0.96)
Patients referred from GOSH	116/283	0.81 (0.74 – 0.87)	0.84 (0.79 – 0.90)	0.91 (0.87 – 0.96)	0.93 (0.88 – 0.97)
Patients referred from Manchester	35/90	0.89 (0.81 – 0.98)	0.80 (0.69 – 0.91)	0.94 (0.87 – 1.00)	0.93 (0.86 – 0.99)

Supplementary Table 2 - Sensitivity analysis showing the utility of clinical features to discriminate between KATP CHI and CHI of an unknown aetiology in the two countries and institutes with the most referrals. ROC AUC = Receiver Operating Characteristic Area under the Curve

Chapter 2

Increased referrals for congenital hyperinsulinism genetic testing in children with trisomy 21 reflects the high burden of non-genetic risk factors in this group

Thomas I. Hewat[1], Thomas W. Laver[1], Jayne A. L. Houghton[2], Jonna M. E. Männistö [3], Sabah Alvi [4], Stephen P. Brearey[5], Declan Cody [6], Antonia Dastamani[7], Miguel De los Santos La Torre [8], Nuala Murphy[9], Birgit Rami-Merhar [10], Birgit Wefers [11], Hanna Huopio[12], Indraneel Banerjee[13], Matthew B. Johnson [1], Sarah E. Flanagan [1]

1. Institute of Biomedical and Clinical Science, University of Exeter Medical School, UK
2. Exeter Genomics Laboratory, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
3. Department of Pediatrics, University of Eastern Finland and Kuopio University Hospital, 70210 Kuopio, Finland
4. Leeds Children's Hospital, Leeds, UK
5. Countess of Chester Hospital, Chester, UK
6. Children's Health Ireland at Crumlin, Dublin, Ireland
7. Endocrinology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
8. Department of Endocrinology and Metabolism of the Instituto Nacional de Salud del Niño, Lima, Peru
9. Children's University Hospital, Temple Street, Dublin, Ireland
10. Medical University of Vienna, Dept. of Pediatric and Adolescent Medicine, Vienna, Austria
11. Ninewells Hospital, Dundee, UK
12. Department of Pediatrics, Kuopio University Hospital, 70210 Kuopio, Finland
13. Department of Paediatric Endocrinology, Royal Manchester Children's Hospital, Manchester, UK

This manuscript was published in Pediatric Diabetes –
Pediatr Diabetes. 2022 Jun;23(4):457-461. doi: 10.1111/pedi.13333.

My contribution to the chapter

I performed statistical analyses to confirm the increased prevalence of Down syndrome in the Exeter hyperinsulinism cohort. With colleagues, I analysed the clinical data on the Exeter cohort, and I performed literature searches to support our conclusion that non-genetic risk factors were leading to hyperinsulinism in multiple cases in this cohort. I drafted the manuscript, adapted the manuscript in accordance with co-author's comments, submitted the manuscript, and addressed reviewer's comments.

Acknowledgements

TIH, TWL, MBJ, and SEF designed the study. JALH, SA, SPB, DC, AD, MDSLT, NM, BR-M, BW, and SEF recruited patients to the study. SA, SPB, DC, AD, MDSLT, NM, BR-M, and BW provided clinical data on patients. TIH, IB, and SEF analysed clinical data. JMEM and HH provided data on a separate cohort to support findings. TIH, TWL, MBJ, and SEF drafted the manuscript.

ABSTRACT

Hyperinsulinism results from inappropriate insulin secretion during hypoglycaemia. Down syndrome is causally linked to a number of endocrine disorders including Type 1 diabetes and neonatal diabetes. We noted a high number of individuals with Down syndrome referred for hyperinsulinism genetic testing, and therefore aimed to investigate whether the prevalence of Down syndrome was increased in our hyperinsulinism cohort compared to the population.

Methods

We identified individuals with Down syndrome referred for hyperinsulinism genetic testing to the Exeter Genomics Laboratory between 2008 and 2020. We sequenced the known hyperinsulinism genes in all individuals and investigated their clinical features.

Results

We identified 11 individuals with Down syndrome in a cohort of 2011 patients referred for genetic testing for hyperinsulinism. This represents an increased prevalence compared to the population (2.5/2011 expected vs. 11/2011 observed, P=6.8 x 10^{-5}). A pathogenic *ABCC8* mutation was identified in 1 of the 11 individuals. Of the remaining 10 individuals, 5 had non-genetic risk factors for hyperinsulinism resulting from the Down syndrome phenotype: intrauterine growth restriction, prematurity, gastric/oesophageal surgery, and asparaginase treatment for leukaemia. For 5 individuals no risk factors for hypoglycaemia were reported although 2 of these individuals had transient hyperinsulinism and 1 was lost to follow-up.

Conclusions

Down syndrome is more common in patients with hyperinsulinism than in the population. This is likely due to an increased burden of non-genetic risk factors resulting from the Down syndrome phenotype. Down syndrome should not preclude genetic testing as coincidental monogenic hyperinsulinism and Down syndrome is possible.

Introduction

Hyperinsulinism (HI) is a disorder of the pancreatic beta-cell where inappropriately high levels of insulin are secreted leading to hypoglycaemia. Prolonged neonatal HI can be transient, often remitting within 6 months, with risk factors including male sex, low birth weight, and perinatal stress (1). In contrast, persistent HI is likely to be genetic with disease-causing mutations in single genes identified in 50-70% of cases (2, 3). HI has also been reported as a rare feature in patients with aneuploidies. For example, HI can present in females with Turner syndrome resulting from a complete or partial monosomy of the X chromosome and in children with Patau syndrome resulting from mosaic trisomy 13 (4, 5).

The most common aneuploidy is trisomy 21, causing Down syndrome, which affects 1 in 794 live births in the USA (6). Down syndrome is characterised by intellectual disability, microcephaly, congenital heart defects, gastrointestinal disorders, and endocrine disorders which include Type 1 diabetes or neonatal diabetes (7-9). Whilst HI has not been reported as a feature of Down syndrome, we noted a high number of individuals with the co-existence of these two conditions being referred to our laboratory for genetic testing. Our aim was to assess whether the prevalence of children with HI and Down syndrome was higher than expected in our cohort and if so to determine the reason(s) for this.

METHODS

We studied 2011 individuals referred for HI genetic testing to the Exeter Genomics Laboratory between 2008 and 2020. Clinical information was provided at referral using a standardised request form. Follow-up data by case note review were requested for all individuals with HI and Down syndrome.

We performed targeted next-generation sequencing of 13 known HI genes including *ABCC8*, *CACNA1D*, *CDKN1C*, *GCK*, *GLUD1*, *HADH*, *HNF1A*, *HNF4A*, *INSR*, *KCNJ11*, *PMM2*, *SLC16A1*, and *TRMT10A* in all individuals with HI and Down syndrome using previously described methods (10). We used Stata/SE v16.0 to perform a one-sample binomial test to assess if the prevalence of Down syndrome in our cohort was significantly higher than the population prevalence (Stata Corp, College Station, TX, USA).

Informed consent was obtained from the parents or guardians of all probands. This study was approved by the North Wales Research Ethics Committee (517/WA/0327).

RESULTS

Within our international cohort of 2011 individuals, we identified 11 cases with Down syndrome (n=11/2011 [0.55%]). This represents a minimal prevalence as we do not routinely screen for aneuploidies, and some clinicians may not have provided this information on the genetic request form. The number of children with Down syndrome was significantly higher than expected by chance given the population prevalence of Down syndrome of 12.6/10,000 (6) (2.5/2011 expected vs. 11/2011 observed, $P=6.8 \times 10^{-5}$).

We identified a mutation in a known HI gene in 1/11 (9%) patients. This individual had a pathogenic paternally inherited *ABCC8* mutation (11). Of the 10 individuals without a mutation in a known gene, 2 were born with intrauterine growth retardation (IUGR) (birth weight Z-score <-2). The median age at diagnosis of HI of the 10 individuals was 101 days (IQR 1d-581d) with insulin detected at the time of hypoglycaemia (plasma glucose < 2.8mmol/L) in all cases. Persistent HI (defined here as requiring treatment for >6 months) was confirmed in 4 of the 10 genetically unsolved individuals. In the remaining 6 individuals the HI was transient (n=5) or follow-up information was not available (n=1). One individual with persistent HI demonstrated side-effects to diazoxide and did not respond to octreotide, necessitating a near-total pancreatectomy (12). Consanguinity was reported in this individual.

Seven individuals, including the child with an *ABCC8* mutation, had undergone gastric or oesophageal surgery for duodenal atresia, duodenal stenosis, tracheomalacia, or gastro-oesophageal reflux disease (GORD). In two cases surgery had been performed prior to the onset of HI. One of these cases had also undergone surgery to repair a portosystemic shunt (13). A further individual had been diagnosed with acute lymphoblastic leukaemia and had received L-asparaginase treatment prior to the onset of HI. An overview of the clinical features of the cohort are provided in Table 1.

DISCUSSION

We identified 11 individuals with HI and Down syndrome. Given that Down syndrome has an approximate incidence of 1 in 794 live births we would have expected 2 or 3 individuals with Down syndrome in our cohort of 2011 individuals (6). The statistically significant enrichment and higher prevalence therefore suggest that the two conditions are related.

The prevalence of mutations in the known genes was low in the Down syndrome and HI cohort (n=1/11, 9%) although this increased to 20% in those with confirmed persistent HI (n=1/5). This pick-up rate is lower than anticipated given previous studies have reported mutations in the known genes in 50-70% of HI cases (2, 3). While this may reflect the small sample size, it is also possible that the Down syndrome is increasing the risk of the child developing HI.

We identified risk factors for developing HI in 5 of the 10 individuals without a mutation in a known gene. Two children had surgery to correct a gastrointestinal (GI) disorder prior to the onset of HI (Table 1). GI disorders are common in individuals with Down syndrome and surgical management of this can lead to iatrogenic hypoglycaemia as a result of dumping syndrome (14, 15). Furthermore, one of these individuals also had confirmed post-prandial hypoglycaemia following surgery lending further support to this diagnosis (16). This patient also had a portosystemic shunt, with surgical closure resulting in a resolution of the hypoglycaemia (13). In 4 further cases gastric surgery was performed but this occurred after the onset of HI in 3 cases suggesting that the HI was unlikely to be due to gastric surgery induced post-prandial hypoglycaemia. The age at gastric surgery in the remaining patient was unknown.

IUGR or biochemical evidence of perinatal and postnatal stress associated with prematurity, was reported in two individuals. These are well-recognised risk factors for prolonged neonatal hypoglycaemia (1). IUGR was reported in a second individual however the HI was ongoing at the age of 13 years suggesting it was not causative of the hypoglycaemia (17).

One individual had been diagnosed with acute lymphoblastic leukaemia that had been treated with an L-asparaginase based chemotherapy prior to the onset of HI at 4 years. Children with Down syndrome are at increased risk of developing acute

lymphoblastic leukaemia and previous studies have shown that treatment with L-asparaginase can cause hypoglycaemia in younger patients (18, 19). This could explain the transitory hypoglycaemia observed in this child.

Of the 5 individuals without an identifiable risk factor for HI, two had persistent HI, two had transient HI and one case was lost to follow-up which might suggest that the HI was transient and not severe. It is also possible that in this patient risk factors for HI were present but not reported at referral for genetic testing. The finding of two individuals with Down syndrome and persistent HI within our cohort is expected based on the population prevalence of Down syndrome. Interestingly, consanguinity was reported in one of these individuals, supporting the possibility of a recessively inherited monogenic aetiology.

Recently, a study of HI in Finland identified 5 cases with Down syndrome in a cohort of 238 individuals. The authors noted that this was a statistically significant increase compared to the population prevalence of Down syndrome (20). In keeping with our findings, screening of the known genes identified an *ABCC8* mutation in a single individual whilst the 4 mutation negative individuals had non-genetic risk factors for HI which could be attributed to the Down syndrome phenotype: extreme prematurity and cardiac insufficiency, IUGR, gastric surgery/fundoplication, and stress due to congenital heart defects (personal communication Huopio and Männistö). In two individuals the HI remitted before the age of 4 months.

Genetic testing identified an *ABCC8* mutation in one individual with Down syndrome in our cohort and this, together with the finding of an *ABCC8* mutation in an individual within the Finnish cohort, highlights the need to perform genetic testing in all individuals with persistent HI (20). Whilst a diagnosis of Down syndrome does not preclude co-incidental monogenic HI, our study suggests HI in Down syndrome is most likely to be due to non-genetic risk factors.

In conclusion, we have identified an increased referral rate for HI genetic testing for individuals with Down syndrome. Our findings suggest that HI is not a feature of Trisomy 21 but a consequence of the high burden of non-genetic risk factors resulting from the Down syndrome phenotype.

REFERENCES

1. Hoe FM, Thornton PS, Wanner LA, Steinkrauss L, Simmons RA, Stanley CA. Clinical features and insulin regulation in infants with a syndrome of prolonged neonatal hyperinsulinism. J Pediatr. 2006;148(2):207-12.
2. Güemes M, Rahman SA, Kapoor RR, Flanagan S, Houghton JAL, Misra S, et al. Hyperinsulinemic hypoglycemia in children and adolescents: Recent advances in understanding of pathophysiology and management. Rev Endocr Metab Disord. 2020;21(4):577-97.
3. Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, et al. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. The Journal of clinical endocrinology and metabolism. 2013;98(2):E355-63.
4. Gibson CE, Boodhansingh KE, Li C, Conlin L, Chen P, Becker SA, et al. Congenital Hyperinsulinism in Infants with Turner Syndrome: Possible Association with Monosomy X and KDM6A Haploinsufficiency. Hormone research in paediatrics. 2018;89(6):413-22.
5. Tamame T, Hori N, Homma H, Yoshida R, Inokuchi M, Kosaki K, et al. Hyperinsulinemic hypoglycemia in a newborn infant with trisomy 13. Am J Med Genet A. 2004;129A(3):321-2.
6. de Graaf G, Buckley F, Skotko BG. Estimates of the live births, natural losses, and elective terminations with Down syndrome in the United States. Am J Med Genet A. 2015;167A(4):756-67.
7. Startin CM, D'Souza H, Ball G, Hamburg S, Hithersay R, Hughes KMO, et al. Health comorbidities and cognitive abilities across the lifespan in Down syndrome. J Neurodev Disord. 2020;12(1):4.
8. Aitken RJ, Mehers KL, Williams AJ, Brown J, Bingley PJ, Holl RW, et al. Early-onset, coexisting autoimmunity and decreased HLA-mediated susceptibility are the characteristics of diabetes in Down syndrome. Diabetes Care. 2013;36(5):1181-5.
9. Johnson MB, De Franco E, Greeley SAW, Letourneau LR, Gillespie KM, International DSPC, et al. Trisomy 21 Is a Cause of Permanent Neonatal Diabetes That Is Autoimmune but Not HLA Associated. Diabetes. 2019;68(7):1528-35.

10. Ellard S, Lango Allen H, De Franco E, Flanagan SE, Hysenaj G, Colclough K, et al. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. Diabetologia. 2013;56(9):1958-63.
11. De Franco E, Saint-Martin C, Brusgaard K, Knight Johnson AE, Aguilar-Bryan L, Bowman P, et al. Update of variants identified in the pancreatic beta-cell KATP channel genes KCNJ11 and ABCC8 in individuals with congenital hyperinsulinism and diabetes. Human mutation. 2020;41(5):884-905.
12. Shi Y, Avatapalle HB, Skae MS, Padidela R, Newbould M, Rigby L, et al. Increased plasma incretin concentrations identifies a subset of patients with persistent congenital hyperinsulinism without KATP channel gene defects. The Journal of pediatrics. 2015;166(1):191-4.
13. Senniappan S, Pitt K, Shah P, Arya V, Jaiswal S, Haddad M, et al. Postprandial hyperinsulinaemic hypoglycaemia secondary to a congenital portosystemic shunt. Horm Res Paediatr. 2015;83(3):217-20.
14. Chicoine B, Rivelli A, Fitzpatrick V, Chicoine L, Jia G, Rzhetsky A. Prevalence of Common Disease Conditions in a Large Cohort of Individuals With Down Syndrome in the United States. J Patient Cent Res Rev. 2021;8(2):86-97.
15. Chesser H, Abdulhussein F, Huang A, Lee JY, Gitelman SE. Continuous Glucose Monitoring to Diagnose Hypoglycemia Due to Late Dumping Syndrome in Children After Gastric Surgeries. J Endocr Soc. 2021;5(3):bvaa197.
16. Calabria AC, Charles L, Givler S, De Leon DD. Postprandial Hypoglycemia in Children after Gastric Surgery: Clinical Characterization and Pathophysiology. Horm Res Paediatr. 2016;85(2):140-6.
17. Fafoula O, Alkhayyat H, Hussain K. Prolonged hyperinsulinaemic hypoglycaemia in newborns with intrauterine growth retardation. Arch Dis Child Fetal Neonatal Ed. 2006;91(6):F467.
18. Panigrahi M, Swain TR, Jena RK, Panigrahi A. L-asparaginase-induced abnormality in plasma glucose level in patients of acute lymphoblastic leukemia admitted to a tertiary care hospital of Odisha. Indian J Pharmacol. 2016;48(5):595-8.
19. Brown AL, de Smith AJ, Gant VU, Yang W, Scheurer ME, Walsh KM, et al. Inherited genetic susceptibility to acute lymphoblastic leukemia in Down syndrome. Blood. 2019;134(15):1227-37.

20. Mannisto JME, Jaaskelainen J, Otonkoski T, Huopio H. Long-Term Outcome and Treatment in Persistent and Transient Congenital Hyperinsulinism: A Finnish Population-Based Study. J Clin Endocrinol Metab. 2021;106(4):e1542-e51.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11
Genetic results	No mutation detected	No mutation detected	No mutation detected	No mutation detected	No mutation detected	No mutation detected	No mutation detected	No mutation detected	No mutation detected	No mutation detected	*ABCC8* p.G25fs/N**
Sex	Female	Female	Male	Male	Female	Female	Male	Female	Male	Female	Male
Birth weight SDS	-2.55	Not available	1.35	0.25	-0.77	0.22	-1.07	0.89	-3.74	1.25	0.65
Age at HI diagnosis (weeks)	36	20	120	0.14	83	0.14	0.14	0.14	9	208	0.43
Glucose (mmol/L) (Insulin (pmol/L)) at diagnosis	2 (108)	1.5 (60.5)	1 (18.3)	0.4 (26)	1.6 (88)	1.9 (12.8)	2.0 (347)	1.8 (12.5)	2.8 (4.5)	2.4 (141)	1.1 (56.1)
Post-prandial hypoglycaemia	Not noted	Not noted	No	No	Yes	Not noted	Not noted	Not noted	Not noted	Not noted	Not noted
Transient/persistent HI	**Persistent** Diazoxide treatment ongoing at 13 years (3 mg/kg/day)	**Transient** No treatment required	**Persistent** Pancreatectomy due to side effects of diazoxide and no response to octreotide	**Transient** Treated transiently with I.V. glucose and increased feeds	**Persistent** Diazoxide unresponsive, managed with continuous feeds until remission at 3 years following VSD correction	**Transient** Treated with diazoxide until 4 months	**Transient** No treatment required	**Unknown** Lost to follow-up (10 mg/kg/day diazoxide at referral)	**Persistent** Treated with diazoxide, until 12 months	**Transient** Treated transiently with I.V. glucose	**Persistent** Octreotide (20ug/kg/day) ongoing at 5 years
Gastric or oesophageal surgery (age)	No	Yes, prior to HI diagnosis (16 weeks)	Yes, following HI diagnosis	No	Yes, prior to HI diagnosis (1st, 4 weeks, 2nd 1 yr)	Yes, following HI diagnosis	No	Yes (age unknown)	Yes, following HI diagnosis (26 weeks)	No	Yes, following HI diagnosis

			(3 yrs)			(>0.14 weeks)					(0.86 weeks)
Additional features	ASD	GORD, VSD	West syndrome, GORD, asthma	Mild hypoventilation, ASD	Tracheo-oesophageal fistula, GORD, VSD, jaundice, portosystemic shunt	Duodenal atresia, ASD, PDA	Prematurity (31/40), perinatal compromise (poor CTG, reduced movements, at birth: raised lactate, biochemical evidence of liver & renal compromise) Cerebral palsy with right hemiplegia 2nd to left Periventricular Leukomalacia, Cataracts, Hearing loss 2nd auditory neuropathy	Duodenal stenosis, haematuria	Tracheomalacia	Acute lymphoblastic leukaemia treated with L-asparaginase at 3.8 years	Duodenal atresia

Table 1 – Clinical features of patients diagnosed with Down syndrome and Hyperinsulinism. Grey-filled boxes represent risk factors for Hyperinsulinism (HI). ASD = Atrial Septal Defect. VSD = Ventricular Septal Defect. PDA = Patent Ductus Arteriosus. GORD = Gastro-Oesophageal Reflux Disease. I.V. Intravenous. For the purposes of this study persistent disease is defined as HI requiring treatment for >6 months and transient disease is defined as HI requiring treatment for <6 months. Patient 3 previously reported in (12). Patient 5 previously reported in (13). ** indicates that mutation was previously reported in (11).

Chapter 3

Partial duplications of the *KDM6A* gene are a novel cause of congenital hyperinsulinism

Thomas I Hewat [1], Matthew B Johnson [1], Thomas W Laver [1], Joseph Burrage [1], Luke Weymouth [1], Jayne AL Houghton [2], Matthew N Wakeling [1], Emma L Dempster [1], Sarah E Flanagan [1]

1 – Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK

2 – Exeter Genomics Laboratory, Royal Devon University Healthcare NHS Foundation Trust, Exeter, UK

The content of this chapter is prepared for submission to Clinical Epigenetics.

Corresponding Author:
Prof Sarah Flanagan
University of Exeter Medical School,
Exeter, UK EX2 5DW
S.Flanagan@exeter.ac.uk

My contributions to the chapter

With colleagues, I identified the three individuals with duplications in *KDM6A* from targeted sequencing and whole genome sequencing data. I prepared the samples for EPIC array work, including selecting control samples from unaffected siblings of probands affected with Hyperinsulinism. I performed quality control on the DNA methylation data, and with colleagues analysed the data. I drafted the chapter, with feedback from colleagues.

Acknowledgements

TIH, ELD, and SEF designed the study. JALH and SEF recruited patients to the cohort, and TIH and SEF identified patients in the cohort appropriate for inclusion in this study. MNW designed bioinformatics pipelines to analyse whole genome sequencing data, and TIH, TWL, and MNW performed bioinformatic analyses for this study. JB and LW performed lab work. TIH and ELD performed quality control steps and analysed DNA methylation data. TIH drafted the manuscript with support from TWL, ELD, and SEF.

Abstract

Background

Loss of function mutations in the gene *KDM6A* are known to cause Kabuki syndrome, a syndromic cause of congenital hyperinsulinism.

Aim

We aimed to study three individuals in whom partial duplications of the *KDM6A* gene were identified from targeted sequencing or whole genome sequencing data.

Methods

Genetic testing was performed to identify tandem partial duplications of the *KDM6A* gene in three individuals referred for genetic testing for hyperinsulinism. Bioinformatic and DNA methylation analyses were performed in order to determine whether these duplications were pathogenic.

Results

Two of the duplications were predicted to be pathogenic based on either the introduction of a frameshift and premature stop codon resulting from the duplication, or by the presence of an episignature similar to that of individuals with Kabuki syndrome. In one individual, there was not sufficient evidence to support pathogenicity of the duplication, which extended outside of the *KDM6A* gene and is predicted to result in one full copy of the gene being present.

Conclusions

Tandem partial duplications in *KDM6A* are a rare cause of congenital hyperinsulinism and a Kabuki syndrome-like phenotype.

Introduction

Kabuki syndrome is a rare developmental disorder, with an estimated prevalence of around 1 in 32,000 individuals (1, 2). The condition is characterised by well-defined and distinct dysmorphism, particularly of the face, developmental delay, and infantile hypotonia (3). It can also present with a wide range of manifestations, including endocrine, neurological, and cardiac phenotypes (2, 4-7). In addition, Kabuki syndrome is also a cause of syndromic hyperinsulinism (HI), which can act as the presenting feature for this disorder (4), and as a result it is regularly included in clinical panels for HI.

Kabuki syndrome is a genetically heterogeneous disorder, with dominantly acting pathogenic variants identified in around 70% of cases with clinically diagnosed Kabuki syndrome (3). Loss of function variants in the *KMT2D* and *KDM6A* genes have been identified as causing Kabuki syndrome, with pathogenic variants in *KMT2D* reported in just under 90% of genetically confirmed cases (3, 8-10).

KMT2D and *KDM6A* are important in controlling gene expression and cell differentiation as a result of their roles as a histone lysine methyltransferase and histone demethylase respectively (11, 12). However, the precise mechanism by which mutations in these genes lead to HI is not known. These roles as mediators of histone methylation also result in direct changes in DNA methylation, which allows for identification of a Kabuki syndrome-specific pattern of DNA methylation, or an ’episignature’, that can be used to aid the diagnosis of this condition (13). In recent years, methylation profiles generated from individuals with a range of disorders of the epigenetic machinery have shown that episignatures can be used to differentiate around 42 Mendelian disorders, and thereby aid diagnosis (14).

Disruption of the *KDM6A* gene was first identified as a cause of Kabuki syndrome in 2012, with the identification of partial or complete *de novo* deletions of the *KDM6A* gene in three individuals with the Kabuki syndrome phenotype(8). More recently, a study of 80 individuals with mutations in *KDM6A* showed that the large majority of individuals with *KDM6A*-Kabuki syndrome have a point mutation rather than a large deletion, with the majority of variants identified being protein-truncating, as opposed to protein-altering variants (15). No cases of duplications in the *KDM6A* gene have been described.

In this study we studied three novel KDM6A partial gene duplications identified in three individuals presenting with congenital hyperinsulinism to assess whether duplication of this gene could represent a novel mechanism of disease.

Methods

Recruitment and ethics

Individuals identified in this study were recruited for routine genetic testing for HI. Consent was obtained for research to be performed to identify a novel cause for an individual's HI where routine testing did not identify a cause. Clinical features of these individuals were collected from referral forms submitted at the time of referral for genetic testing. Informed consent was obtained for genetic testing from the parents or guardians of all probands. This study was approved by the North Wales Research Ethics Committee (517/WA/0327).

DNA sequencing

Targeted next generation sequencing was performed on DNA extracted from peripheral blood leukocytes from three individuals as part of routine genetic testing for HI (16, 17). The targeted next generation sequencing panel captured 13 of the known disease genes for congenital hyperinsulinism including *KMT2D* and *KDM6A*. This analysis also allows for off target copy number variations (CNVs) to be called using SavvyCNV (18).

Whole genome sequencing (WGS) was performed to confirm the presence of tandem duplications and their breakpoints on DNA extracted from peripheral blood leukocytes from three individuals and their parents using Illumina HiSeq, Illumina TruSeq, or BGISeq-500 technology. Sequence data was aligned with BWA MEM 0.7.15 and processed using a pipeline based on the GATK best practices (Picard version 2.7.1, GATK version 3.7). Variants were annotated using Alamut batch standalone version 1.11 (SOPHiA genetics, Lausanne, Switzerland).

Methylation array

DNA extracted from peripheral blood was used to profile DNA methylation using the Illumina EPIC DNA methylation array on two of the patients and 8 control samples (n=3 leukocyte DNA samples from individuals with known pathogenic single nucleotide variants in *KDM6A* and HI, and n=5 unaffected controls). Quality control

was performed and methylation data was normalised using methods previously published (19).

An epigenome-wide association study (EWAS) was performed by comparing the individuals with confirmed Kabuki syndrome to unaffected controls, using a linear regression which controlled for age and sex. The process of quality control lead to the removal of 12,093 methylation probes from this study, with a final number included of 802,051. Previous studies have identified probes from the Illumina 450k array which can identify individuals with Kabuki syndrome (13). While some probes from the 450k array were not included in the EPIC array, previous studies have shown that data from these two arrays can be comnbined due to their similarities (20). As a result, we filtered the probes identified to detect Kabuki syndrome in a previous study (13) on whether or not they were significant in our EWAS, and used those probes in an exploratory analysis to identify similarities in episignature between our duplication patients, confirmed Kabuki syndrome cases, and controls.

As an additional exploratory analysis, we utilised the database of individuals with methylation disorders from the Hospital for Sick Children (Toronto, CA) (21). Normalised DNA methylation study was uploaded to the online EpigenCentral portal, and we compared methylation data from our study to that of individuals with *KMT2D* mutations identified in their cohort of individuals with methylation disorders using three models: a logistic regression with regularisation penalty, a random forest model, and a support vector machine.

Results

KDM6A partial gene duplications were identified in 3 individuals

We identified three unrelated individuals with partial *de novo* duplications of the *KDM6A* gene (Figure 1). Preliminary analysis detected no mutations in known HI genes in these individuals, except for the duplications taking in the *KDM6A* gene. In two of these individuals the duplications were identified using off-target CNVs from targeted sequencing data, with breakpoints confirmed by whole genome sequencing. In one individual, the duplication was identified initially by whole genome sequencing

data, as this individual underwent targeted sequencing before the development of the SavvyCNV pipeline (18).

All identified duplications were tandem (meaning the duplicated segments were included serially into the original gene copy), and two were contained entirely within the *KDM6A* gene (Figure 1). The duplication identified in Patient 1 was inserted after exon 26, and consisted of exons 3 through 26. Exon 3 has a 5' overhang, and as such a frameshift was introduced, leading to a premature stop codon in the second copy of exon 3. The duplication identified in Patient 2 was smaller, with an additional copy of exons 3 through 6, which was introduced after exon 6. This duplication did not lead to a frameshift or the introduction of a premature stop codon. The final duplication, identified in Patient 3, included exons 2 through 29 of the *KDM6A* gene, along with exons 6 and 5 of a nearby open reading frame, CXorf36. As such, a full copy of *KDM6A* is expected to be expressed in this individual, with the duplicated sequence introduced within CXorf36.

Methylation profiling was consistent with KDM6A-Kabuki syndrome in one individual

EPIC array analysis was performed on two patients, with 65 probes previously identified (13) also found to be significantly different in our EWAS. An analysis of DNA methylation of these demonstrated that the methylation pattern in Patient 2 clustered with that of individuals with genetically confirmed Kabuki syndrome (Figure 2). However, the methylation pattern of Patient 3 was not found to be similar to either Patient 2, or to any of the Kabuki syndrome cases identified in this study (Figure 2).

The analysis performed in the EpigenCentral database (21) found variable evidence of similarity to the individuals with *KMT2D* mutations in that database. Logistic regression with a regularisation penalty predicted Patient 2 to have Kabuki syndrome, along with one of the three cases in our cohort, while a support vector machine model predicted that same case as Kabuki syndrome, and marking Patient 2 as "uncertain" (Table 1).

Clinical phenotype supported a diagnosis of Kabuki syndrome in 2 cases

The clinical features of the three individuals are described in Table 2. Additional features were reported in 2 individuals, with Patient 1 having learning and behavioural difficulties, while Patient 3 was referred with a clinical suspicion of

Kabuki syndrome, along with hypoplastic right heart syndrome. All three individuals were diagnosed with hyperinsulinism within the first month of life.

Discussion

We identified three separate tandem duplications within the *KDM6A* gene and used DNA methylation data that supports the pathogenicity of two of these duplications.

The two of the duplications identified in this study are predicted to lead to a loss of function of *KDM6A*, and are therefore the cause of HI in these two individuals. In Patient 1, the presence of a frameshift introduced in the second copy of Exon 3 suggests that a full copy of the *KDM6A* protein would not be produced. In Patient 2, while the duplication of four exons does not introduce a frameshift, the presence of a similar pattern of DNA methylation to that seen in confirmed cases of Kabuki syndrome suggests that this duplication is pathogenic in this individual. This similarity is seen both in an analysis based on the previously established episignature of Kabuki syndrome (13), and on comparison to cases in the EpigenCentral database (21). Patient 3 is predicted to express a full, unaffected copy of the *KDM6A* gene (Figure 1), and their methylation pattern is not similar to that seen in Kabuki syndrome, suggesting that this is not the cause of this individual's HI. Further studies will be required to establish the cause of Patient 3's hyperinsulinism.

None of the patients identified in this study presented in a way that would have seen them diagnosed with Kabuki syndrome in a clinical setting according to international consensus diagnostic criteria (3). However, HI has been identified as a possible presenting feature in cases of Kabuki syndrome by previous studies (4). In addition, intellectual disability, as identified in Patient 1, and congenital heart defects, as identified in Patient 3, are cardinal features of Kabuki syndrome. Despite this, the limited clinical information available to us at the time of this study does not suggest a clinical diagnosis of Kabuki syndrome in these individuals. Further clinical analysis may reveal additional features that were not reported or not presenting in these individuals at the time of referral for genetic testing. This emphasises the importance of testing syndromic causes of HI in all cases where possible, as it may allow for better identification of individuals with Kabuki syndrome and other syndromic causes.

Kabuki syndrome was one of the first disorders in which an episignature was identified (13). Episignatures, which can help in determining pathogenicity of

variants, have now been identified for at least 65 genetic syndromes (22), with many of those being disorders that, like Kabuki syndrome, lead to disruption of the epigenetic machinery. These episignatures have allowed for improvements in the genetic diagnosis of these disorders, by helping to resolve unknown cases or by helping to classify variants of unknown significance (22). This study shows another example of where analysis of DNA methylation helps in the classification of variants in *KDM6A*, particularly where a mechanism of disease may not be immediately apparent, such as duplications in a disorder generally caused by loss of function mutations.

In the study within which the episignature of Kabuki syndrome was identified for the first time (13), all 20 affected individuals studied scored high on the model, while 417 healthy control samples scored low on the model. This suggests that the episignature identified in this study is highly sensitive and specific for Kabuki syndrome, which helps to strengthen our study. However, the original study only included one individual with Kabuki syndrome caused by a mutation in *KDM6A*. Despite this, a later study based on the same model identified that the episignature of *KMT2D* Kabuki syndrome and *KDM6A* Kabuki syndrome are highly similar (14). As such, we believe that the epigenetic analysis conducted in our study strengthens the argument that the individuals identified have a Kabuki-like phenotype.

This study was limited as we were unable to perform assays to determine whether the *KDM6A* protein is expressed in the pancreatic beta cells in these individuals, and if so in what form. It is possible that RNA sequencing of patient cells, such as fibroblasts may allow for identification of whether *KDM6A* is widely expressed in these individuals. In addition, as a referral centre for HI, rather than Kabuki syndrome, our available sample size of Kabuki syndrome cases is relatively small. However, we believe the use of data from previous studies on which probes are most suggestive of Kabuki syndrome strengthens this study (13), and although the analysis in EpigenCentral was a comparison to individuals with mutations in *KMT2D*, the similarity of Patient 2 to these individuals is further evidence of pathogenicity. Finally, we were unable to perform EPIC array analysis in Patient 1, as a result of there being insufficient DNA to perform the array. However, we believe that the presence of a frameshift introduced by the duplication is strong evidence that a full copy of the *KDM6A* gene is not carried by this individual on one allele.

In conclusion, we have identified two tandem duplications within the *KDM6A* gene that cause HI and a Kabuki syndrome-like phenotype. We have also identified a further duplication that extends outside the *KDM6A* gene that from our studies is not predicted to be pathogenic. This shows that duplications within this gene, normally affected by loss of function variants, can lead to disease if contained entirely within the gene, and therefore disrupting it.

Acknowledgments

SEF is the recipient of a Wellcome Trust Senior Research Fellowship. TIH is the recipient of a PhD studentship, TWL is the recipients of a lectureship, and MBJ and MNW are the recipients of an independent research fellowships from the UKRI's Expanding Excellence in England fund.

References

1. Li Y, Bogershausen N, Alanay Y, Simsek Kiper PO, Plume N, Keupp K, et al. A mutation screen in patients with Kabuki syndrome. Hum Genet. 2011;130(6):715-24.
2. Cheon CK, Ko JM. Kabuki syndrome: clinical and molecular characteristics. Korean J Pediatr. 2015;58(9):317-24.
3. Adam MP, Banka S, Bjornsson HT, Bodamer O, Chudley AE, Harris J, et al. Kabuki syndrome: international consensus diagnostic criteria. J Med Genet. 2019;56(2):89-95.
4. Yap KL, Johnson AEK, Fischer D, Kandikatla P, Deml J, Nelakuditi V, et al. Congenital hyperinsulinism as the presenting feature of Kabuki syndrome: clinical and molecular characterization of 9 affected individuals. Genet Med. 2019;21(1):233-42.
5. Upton S, Stadter CS, Landis P, Wulfsberg EA. Speech characteristics in the Kabuki syndrome. Am J Med Genet A. 2003;116A(4):338-41.
6. Caciolo C, Alfieri P, Piccini G, Digilio MC, Lepri FR, Tartaglia M, et al. Neurobehavioral features in individuals with Kabuki syndrome. Mol Genet Genomic Med. 2018;6(3):322-31.
7. Digilio MC, Gnazzo M, Lepri F, Dentici ML, Pisaneschi E, Baban A, et al. Congenital heart defects in molecularly proven Kabuki syndrome patients. Am J Med Genet A. 2017;173(11):2912-22.
8. Lederer D, Grisart B, Digilio MC, Benoit V, Crespin M, Ghariani SC, et al. Deletion of KDM6A, a histone demethylase interacting with MLL2, in three patients with Kabuki syndrome. Am J Hum Genet. 2012;90(1):119-24.
9. Miyake N, Mizuno S, Okamoto N, Ohashi H, Shiina M, Ogata K, et al. KDM6A point mutations cause Kabuki syndrome. Hum Mutat. 2013;34(1):108-10.
10. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet. 2010;42(9):790-3.
11. Daniel JA, Santos MA, Wang Z, Zang C, Schwab KR, Jankovic M, et al. PTIP promotes chromatin changes critical for immunoglobulin class switch recombination. Science. 2010;329(5994):917-23.
12. Lan F, Bayliss PE, Rinn JL, Whetstine JR, Wang JK, Chen S, et al. A histone H3 lysine 27 demethylase regulates animal posterior development. Nature. 2007;449(7163):689-94.
13. Aref-Eshghi E, Schenkel LC, Lin H, Skinner C, Ainsworth P, Pare G, et al. The defining DNA methylation signature of Kabuki syndrome enables functional assessment of genetic variants of unknown clinical significance. Epigenetics. 2017;12(11):923-33.
14. Aref-Eshghi E, Kerkhof J, Pedro VP, Groupe DIF, Barat-Houari M, Ruiz-Pallares N, et al. Evaluation of DNA Methylation Episignatures for Diagnosis and Phenotype Correlations in 42 Mendelian Neurodevelopmental Disorders. Am J Hum Genet. 2020;106(3):356-70.
15. Faundes V, Goh S, Akilapa R, Bezuidenhout H, Bjornsson HT, Bradley L, et al. Clinical delineation, sex differences, and genotype-phenotype correlation in pathogenic KDM6A variants causing X-linked Kabuki syndrome type 2. Genet Med. 2021;23(7):1202-10.
16. Ellard S, Lango Allen H, De Franco E, Flanagan SE, Hysenaj G, Colclough K, et al. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. Diabetologia. 2013;56(9):1958-63.
17. Flanagan SE, Dung VC, Houghton JAL, De Franco E, Ngoc CTB, Damhuis A, et al. An ABCC8 Nonsense Mutation Causing Neonatal Diabetes Through Altered Transcript Expression. J Clin Res Pediatr Endocrinol. 2017;9(3):260-4.
18. Laver TW, De Franco E, Johnson MB, Patel KA, Ellard S, Weedon MN, et al. SavvyCNV: Genome-wide CNV calling from off-target reads. PLoS Comput Biol. 2022;18(3):e1009940.
19. Hannon E, Dempster EL, Mansell G, Burrage J, Bass N, Bohlken MM, et al. DNA methylation meta-analysis reveals cellular alterations in psychosis and markers of treatment-resistant schizophrenia. Elife. 2021;10.

20. Fernandez-Jimenez N, Allard C, Bouchard L, Perron P, Bustamante M, Bilbao JR, et al. Comparison of Illumina 450K and EPIC arrays in placental DNA methylation. Epigenetics. 2019;14(12):1177-82.
21. Turinsky AL, Choufani S, Lu K, Liu D, Mashouri P, Min D, et al. EpigenCentral: Portal for DNA methylation data analysis and classification in rare diseases. Hum Mutat. 2020;41(10):1722-33.
22. Levy MA, McConkey H, Kerkhof J, Barat-Houari M, Bargiacchi S, Biamino E, et al. Novel diagnostic DNA methylation episignatures expand and refine the epigenetic landscapes of Mendelian disorders. HGG Adv. 2022;3(1):100075.

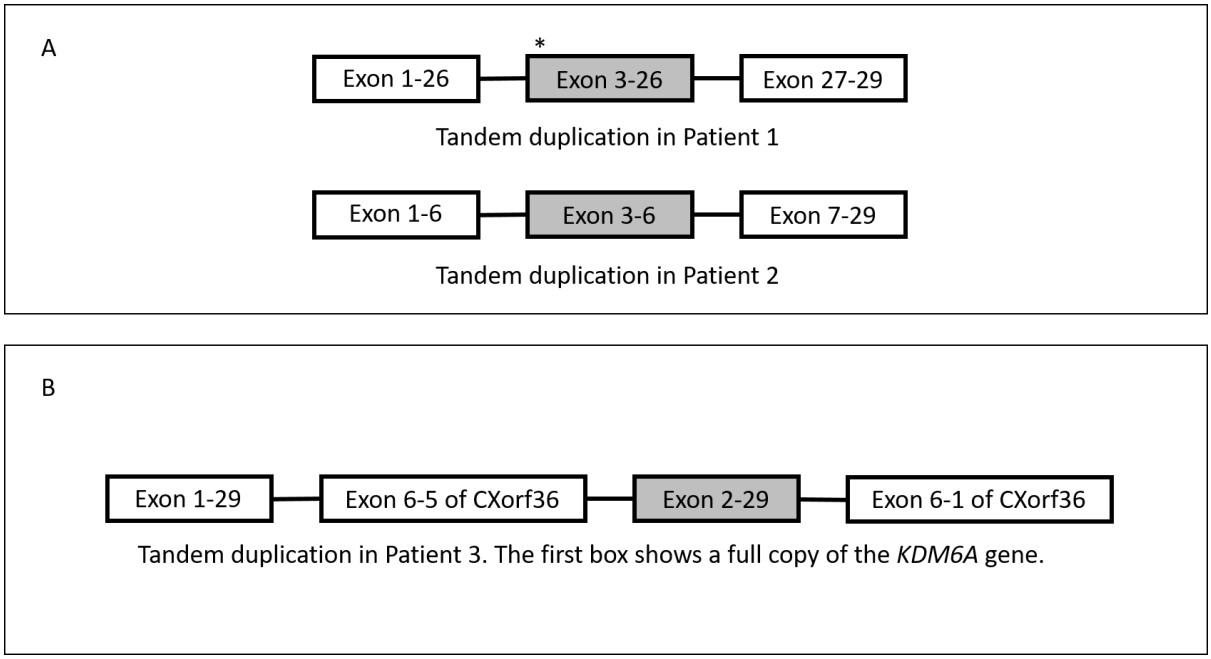

Figure 1 – Schematic representations of the partial duplications identified in the *KDM6A* gene. Panel A shows the duplications predicted to be pathogenic. * indicates the approximate position of an introduced frameshift. Panel B shows the duplication not predicted to be pathogenic.

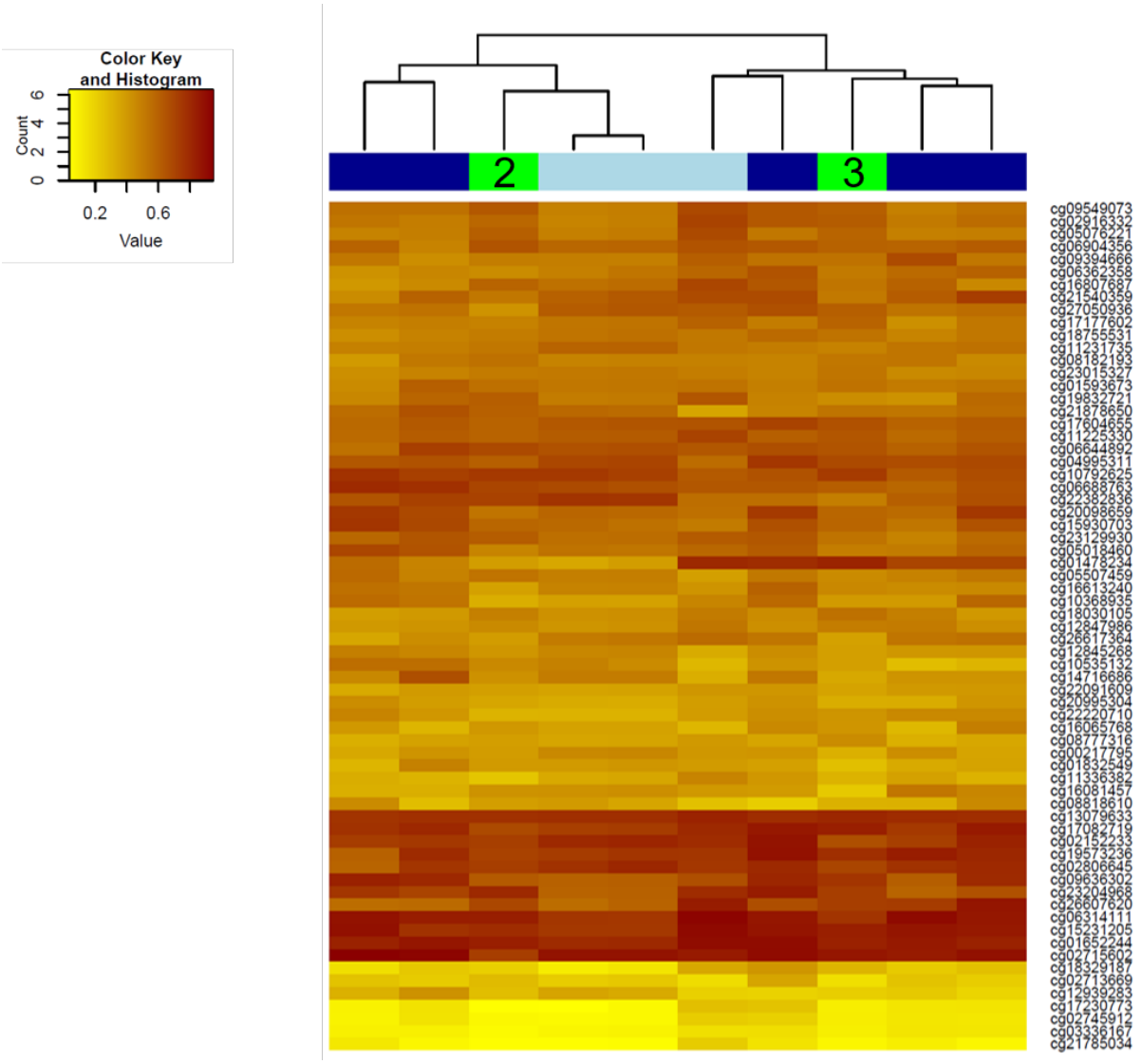

Figure 2 – Heatmap showing the methylation levels in probes previously identified to indicate Kabuki syndrome (13) and significant in an EWAS performed in the samples studied here, with a tree showing similarity between the samples. Green indicates individuals with duplications (with patient numbers for this study), light blue indicates confirmed Kabuki syndrome cases, dark blue indicates unaffected controls. The colour in the heatmap indicates level of methylation, with red indicating methylated and yellow unmethylated. Each row refers to a specific probe included in the analysis, indicated by the cg number.

	Logistic regression	**Support vector machine**
Patient 2	Case	Uncertain
Patient 3	Control	Control
Kabuki 1	Case	Case
Kabuki 2	Control	Control
Kabuki 3	Control	Control

Table 1 – Predictions of two models used by EpigenCentral (21) to detect Kabuki syndrome from normalised methylation data. Case indicates those individuals predicted as having Kabuki syndrome by the listed model.

	Patient 1	Patient 2	Patient 3
KDM6A sequencing result	X:44,787,682-44,959,415dup (Duplication of KDM6A exons 3-26)	X:44,776,422-44,893,995dup (Duplication of KDM6A exons 3-6)	X:44,799,178-45,014,969dup (Duplication of KDM6A exons 2-29)
Predicted effect on protein	Frameshift	In-frame	Unknown
EPIC Array result prediction	Not performed	Kabuki syndrome	Inconclusive
Classification of variant	Pathogenic	Pathogenic	VUS
Sex	Male	Female	Female
Ethnicity	Caucasian	Caucasian	Arab
Birth weight (g)	3760	3225	2600
Gestational age (wks)	40	39	40
Birth weight SDS	0.69	-0.23	-2.36
Age at HI diagnosis	1 day	1 day	3 weeks
Glucose at presentation (mmol/l)	Unknown	1.2	Unknown
Insulin during hypoglycaemia (pmol/l)	340	104	Unknown
Current age (yrs)	8	1	6
Treatment for hyperinsulinism (current dose/date remitted, responsiveness)	Diazoxide 5mg/kg (responsive)	Diazoxide 4.7mg/kg/day	Unknown
Additional clinical features	Learning and behavioural difficulties, umbilical hernia	None reported	Hypoplastic right heart syndrome, clinical suspicion of Kabuki syndrome

Table 2 – Clinical and genomic features of three individuals with partial duplications of the *KDM6A* gene.

Chapter 4

Loss-of-function variants in the maternally imprinted gene *MAGEL2* are a rare but important cause of congenital hyperinsulinism

Thomas I Hewat [1], Eleanor Self [1], Matthew B Johnson [1], Thomas W Laver [1], Jayne AL Houghton [2], Matthew N Wakeling [1], Emma L Dempster [1], Sarah E Flanagan [1]

1 – Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK

2 – Exeter Genomics Laboratory, Royal Devon University Healthcare NHS Foundation Trust, Exeter, UK

The content of this chapter is prepared for submission to Pediatric Diabetes.

Corresponding Author:
Prof Sarah Flanagan
University of Exeter Medical School,
Exeter, UK EX2 5DW
S.Flanagan@exeter.ac.uk

My contributions to the chapter

I identified the features common to methylation disorders that cause hyperinsulinism and performed a review of clinical features in our cohort to identify the 19 patients for this study. I used in-house previously developed pipelines to interrogate genome sequencing data to identify variants in shared genes in the 19 individuals. Through this analysis I identified mutations in the *MAGEL2* gene as a cause of hyperinsulinism. I performed literature searches to establish the previous reports of hypoglycaemia and hyperinsulinism in individuals with *MAGEL2* mutations and wrote the first draft of this manuscript.

Acknowledgements

TIH, ELD, and SEF designed the study. JALH and SEF recruited patients to the cohort, and TIH and SEF identified patients in the cohort appropriate for inclusion in this study. MNW designed bioinformatics pipelines to analyse whole genome sequencing data, and TIH, TWL, and MNW performed bioinformatic analyses for this study. ES performed co-segregation studies. TIH, TWL, and SEF helped draft the manuscript.

Abstract

Objective

Hyperinsulinism can feature as a part of six syndromes linked to DNA methylation abnormalities. Around 50% of individuals with hyperinsulinism do not have a genetic diagnosis, and some of these individuals have clinical features consistent with a methylation abnormality, but do not have one of the six known syndromes. We aimed to identify new causes of syndromic hyperinsulinism linked to aberrant DNA methylation.

Methods

We identified clinical features common to the six methylation disorders that are known to cause hyperinsulinism. We then selected individuals for whole genome sequencing with syndromic hyperinsulinism of unknown genetic cause using these clinical features to find novel disease genes that cause disease through aberrant DNA methylation.

Results

We identified 10 clinical features that occurred in two or more of the methylation disorders which feature hyperinsulinism. Within our cohort we identified 19 individuals with hyperinsulinism who had two or more of these features. Whole genome sequencing identified five genes which contained variants in more than one patient. This identified *MAGEL2* as the best biological candidate gene, as the features reported in the two individuals with protein truncating variants fit the clinical synopsis of the disorder.

Conclusions

We identified two cases with congenital hyperinsulinism caused by pathogenic variants in *MAGEL2*. This highlights the role of aberrant DNA methylation in the aetiology of hyperinsulinism. We recommend that *MAGEL2* is included on targeted next-generation sequencing panels for hyperinsulinism to ensure the best clinical management of individuals with syndromic disease.

Introduction

Congenital hyperinsulinism (HI) is a life-threatening disorder of the pancreatic beta-cell whereby insulin is inappropriately secreted despite hypoglycaemia. Persistent HI is a genetically heterogeneous condition with disease causing variants identified in 36 genes which either cause isolated HI (n=10) or where HI is a feature of syndromic disease (n=26) (1).

Abnormalities in DNA methylation are reported in six of the 26 genetic syndromes that feature HI. The most common is Beckwith-Wiedemann syndrome, a paediatric overgrowth disorder which results from the disruption of differentially-methylated genes at the 11p15.5 imprinted region (2). Syndromic forms of HI can also result from dominantly-acting loss-of-function variants in genes that regulate DNA methylation. This includes Kabuki syndrome, caused by pathogenic variants in either *KMT2D* or *KDM6A* (3-5), Sotos syndrome, caused by pathogenic variants leading to a loss of function of *NSD1* (6), and Rubinstein-Taybi syndrome, caused by pathogenic variants in *CREBBP* or *EP300* (7). While Beckwith-Wiedemann syndrome is caused by changes in DNA methylation over a single genomic region, disruption of the genes which cause Kabuki, Sotos, and Rubinstein-Taybi syndrome result in global defects in DNA methylation (2, 8-10). The methylation pattern of particular genes can also be affected by genomic imprinting, where a gene is expressed based on whether that copy of the gene is inherited from the mother or father, controlled by DNA methylation.

Global defects in methylation lead to the dysregulation of multiple genes as a large number of tissues are commonly affected in individuals with methylation disorders. The clinical features overlap between genetic subgroups of disease, with intellectual disability, growth defects, and immune dysfunction reported across a wide number of methylation disorders (11). In syndromic HI, conditions such as facial dysmorphia, heart defects, and hypotonia can help to identify individuals with HI who are most likely to have a methylation disorder, while features such as seizures and intellectual disability are not pathognomonic for a methylation defect as they can result from hypoglycaemic insult to the brain (12).

In this study, we leveraged clinical features to identify individuals with genetically undiagnosed HI who were likely to have a methylation disorder. We then performed

whole genome sequencing to search for novel causes of HI resulting from aberrant DNA methylation.

Methods

Cohort

We used OMIM's clinical synopses to define clinical features shared across the 6 methylation disorders known to feature HI: Beckwith-Wiedemann syndrome, Kabuki syndrome (*KMT2D* and *KDM6A*), Sotos syndrome, and Rubinstein-Taybi syndrome (*EP300* and *CREBBP*) (13). We next searched for individuals in our cohort of 2361 patients with HI of unknown genetic cause who had two or more clinical features suggesting an underlying defect in methylation. Informed consent was obtained for genetic testing from the parents or guardians of all probands. This study was approved by the North Wales Research Ethics Committee (517/WA/0327).

Whole genome sequencing

Whole genome sequencing (WGS) was performed on DNA extracted from peripheral blood leukocytes of all 19 probands and unaffected parents when available (n=12 sets of parental samples, trios) using Illumina HiSeq, Illumina TruSeq, or BGISeq-500 technology. Sequence data was aligned with BWA MEM 0.7.15 and processed using a pipeline based on the GATK best practices (Picard version 2.7.1, GATK version 3.7). Variants were annotated using Alamut batch standalone version 1.11 (SOPHiA genetics, Lausanne, Switzerland). An initial analysis excluded disease-causing variants in the 36 known HI genes in all individuals (1).

Variant filtering and analysis

As the parents of all 19 individuals were reported to be clinically unaffected, and all 6 known methylation disorders that can feature HI result from monoallelic mutations, we focussed our search on high-quality heterozygous variants. We searched for non-synonymous variants and changes that affected the canonical splice sites of protein coding genes, excluding those with low mapping quality or skewed read balance. We further excluded variants if they were identified in WGS data from unaffected parents (n=24), in 463 in-house controls (including individuals with genetically-solved HI, neonatal diabetes or maturity onset diabetes of the young (MODY)), or when they were listed within the gnomAD database (14). The remaining heterozygous variants

were prioritised for follow-up if they occurred in a gene which harboured variants in two or more individuals.

Results

Identification of clinical features common to methylation disorders featuring HI

We identified 10 clinical features that occurred in at least two of the six methylation disorders that feature HI (Table 1). A search of our genetically unsolved HI cohort identified 19 probands with a minimum of two of these features (Table 2). The most common were facial dysmorphia, occurring in 10/19 (52.6%) patients, heart defects, occurring in 8/19 (42.1%), and hypotonia, occurring in 7/19 (36.8%). An overview of the features of HI in the WGS cohort can be found in Supplementary Table 2.

Genetic analysis and prioritisation

A search of WGS data for novel variants within genes shared between two of more individuals identified five genes: *CDC25A*, *CHD8*, *KMT2C*, *MAGEL2*, and *PTPRCAP* (Table 3). Protein truncating variants were identified in *MAGEL2*, whereas in all other genes, the variants identified were missense.

Three of the five genes are reported to cause dominantly inherited Mendelian disease: *CHD8* causes intellectual developmental disorder with autism and macrocephaly (15), *KMT2C* causes a form of Kleefstra syndrome (16), and *MAGEL2* causes Schaaf-Yang syndrome (17). Both Kleefstra syndrome and Schaaf-Yang syndrome are linked to changes in epigenetic patterns (8, 18). *KMT2C* acts as a regulator of histone methylation and *MAGEL2* is an imprinted gene, where DNA methylation occurs on the maternally inherited copy leading to expression from the paternally inherited copy only (17). There is some evidence of wider patterns of aberrant DNA methylation in those with protein truncating *MAGEL2* mutations, though this is based on studies of a single patient with a deletion in this gene (18).

We prioritised the variants detected in *MAGEL2* as these mutations were nonsense mutations occurring in a gene where protein-truncating variants are known to cause disease. The other variants were identified from singleton WGS data and parental samples were not available in all cases. Furthermore, pathogenic variants in *MAGEL2* have previously been linked to hypoglycaemia including one case where HI was confirmed (19-21). Finally, given the presence of a protein-truncating variant in

MAGEL2 in Patient 15 (Table 2), that is more likely to be causative for this patient's HI than the missense variants in *CHD8*, *KMT2C*, and *PTPRCAP*.

Clinical features of two individuals with MAGEL2 variants

Patient 15 is a female individual of Arab-Israeli origin, born to healthy consanguineous parents. She had a birth weight of 2955g at full term (-1.30 SDS), and were diagnosed with HI at age one week, with elevated insulin on multiple samples taken at the time of hypoglycaemia (insulin 16.9 and 129 pmol/L, glucose 1.7 and 1.7 mmol/L, respectively). These samples also showed low growth hormone and elevated cortisol, though the exact value of these tests was not reported on referral for genetic testing, and it is not clear if this individual was diagnosed clinically with growth hormone deficiency. Treatment of hypoglycaemia with diazoxide (15mg/kg/day) and growth hormone (20mcg/kg/day) was not sufficient to maintain normoglycaemia, and the patient experienced seizures secondary to hypoglycaemia. Treatment with octreotide (dose unknown) was reported to lead to an improvement in glycaemic levels. The patient was diagnosed with dysmorphic features, hydronephrosis, and vesicourethral reflux within the first week of life. Later examinations revealed joint contractures, transient diabetes insipidus, respiratory difficulties, global developmental delay, and reduced stature.

Patient 19 was a male referred from Turkey. At birth the patient was considered large for gestational age (birth weight 3970g, gestation 38 weeks, 1.95 SDS), and was also diagnosed with dysmorphic features, macrocephaly, and flexion contracture. He was diagnosed with HI on the third day of life, with an insulin measurement of 12.4 pmol/L and glucose of 2.1 mmol/L. Treatment with diazoxide (dose unknown) maintained normoglycaemia. Through the course of treatment, partial diabetes insipidus, gastro-oesophageal reflux, and central hypoventilation were also identified.

An overview of the clinical features of both individuals are provided in Table 4.

Discussion

We have identified and described *MAGEL2* mutations in two cases with HI, taking the total reported in the literature to three (19).

We identified four other genes with variants in more than one individual: *CDC25A*, *CHD8*, *KMT2C*, and *PTPRCAP*. The variants identified within these genes were all missense variants. Due to lack of parental samples, we were unable to confirm if these missense variants occurred *de novo.* As such, it is possible that these genes could cause syndromic HI, but further studies are required to confirm this.

Schaaf-Yang syndrome is a genetic disorder caused by mutations on the paternal allele of the imprinted gene *MAGEL2* (17). Since the first report, protein-truncating variants in *MAGEL2* leading to disease have been identified in over 78 individuals with multi-system disease (22). The individuals identified in this study have features consistent with Schaaf-Yang syndrome, including developmental delay, hypotonia, feeding difficulties, and joint contractures.

Intellectual disability or developmental delay, autism spectrum disorder, neonatal hypotonia, infantile feeding difficulties, and joint contractures are the most common features of Schaaf-Yang syndrome (22). Endocrine manifestations in individuals with Schaaf-Yang syndrome have also been described, including growth hormone deficiency, diabetes insipidus, hypothyroidism, adrenal insufficiency, hypogonadotrophic hypogonadism, and hyperprolactinaemia (19, 20, 23-25).

Hypoglycaemia has previously been described in 11 cases of Schaaf-Yang syndrome (Figure 1). In six patients with Schaaf-Yang syndrome described in the literature, hypoglycaemia has been described as secondary to growth hormone deficiency, where low growth hormone was detected at the time of hypoglycaemia, and hormone replacement therapy helped in the maintenance of normal glycaemic levels (20, 21). HI has previously been described in one family with Schaaf-Yang syndrome, with two siblings who were affected (19). The eldest sibling required treatment for HI until the age of six, while the HI was transient in the younger sibling. In the individuals identified in this study, HI was confirmed with both patients undergoing treatment at 3 and 2 years respectively, suggesting persistent HI.

The mechanism for the HI in MAGEL2 is unknown. *MAGEL2* is expressed in human pancreatic tissue, but it is unclear if or how it plays a role in insulin secretion and glucose regulation. Mice that do not express *Magel2* are unable to mount a satisfactory counter-regulatory response to hypoglycaemia, which may result from impaired neuropeptide production in the hypothalamus, where MAGEL2 has a role in

the regulation of secretory granule biogenesis (26-28). The fact that *MAGEL2* mutations can result in HI could lead to improved understanding of the normal function of this gene in regulating insulin secretion.

The two patients with Schaaf-Yang syndrome identified in this study presented with features common to the disorder, with diabetes insipidus and joint contractures occurring in both cases, and both individuals also presenting with other features linked to Schaaf-Yang syndrome (Table 4). HI presented early in the clinical course in both of our cases: as untreated hypoglycaemia can have severe impacts, it is vital that this is identified and treated early to prevent complications (29). In addition, the early diagnosis with HI, along with other features in these cases, does not preclude additional features of this disorder occurring later in life.

It is also possible that cases of Schaaf-Yang syndrome are being missed in HI cohorts, as *MAGEL2* is not included on diagnostic panels for the disease (30-32). In addition, HI may occur as the presenting feature in some individuals, which would prevent us from selecting these individuals for testing based on syndromic features. While whole exome and genome sequencing are increasingly being used in gene discovery studies, it is rarer that these methodologies are used in clinical scenarios. As such, the gene's inclusion on targeted next generation sequencing panels is important, as it will allow prediction and monitoring of additional features of Schaaf-Yang syndrome, improving patient prognosis and management.

This study was limited by the fact that parental samples were unavailable for 6 of the 19 cases and we were therefore unable to establish inheritance or whether mutations had occurred *de novo*. In addition, the approach taken in this study would have been unable to identify dominant variants with variable penetrance or bi-allelic mutations. We were unable to perform allele-specific PCR in these cases, so could not confirm which allele these mutations occurred on. As *MAGEL2* is a maternally imprinted gene, an allele-specific PCR would allow us to confirm that these variants are occurring on the expressed paternal allele. However, given that the phenotype in these patients is consistent with that of Schaaf-Yang syndrome, we consider it likely that these variants are pathogenic.

In conclusion, we have identified two cases of *MAGEL2* mutations leading to HI, taking the total number of patients reported in the literature to three. This finding

establishes *MAGEL2* as a causative gene for HI. We recommend that this gene is included on targeted panels for HI, as early identification of these cases will allow for better medical management of these patients.

Acknowledgements

SEF is the recipient of a Wellcome Trust Senior Research Fellowship. TIH is the recipient of a PhD studentship, TWL is the recipients of a lectureship, and MBJ and MNW are the recipients of an independent research fellowships from the UKRI's Expanding Excellence in England fund.

REFERENCES

1. Hewat TI, Johnson MB, Flanagan SE. Congenital Hyperinsulinism: Current Laboratory-Based Approaches to the Genetic Diagnosis of a Heterogeneous Disease. Frontiers in Endocrinology. 2022;13.
2. Munns CF, Batch JA. Hyperinsulinism and Beckwith-Wiedemann syndrome. Arch Dis Child Fetal Neonatal Ed. 2001;84(1):F67-9.
3. White SM, Thompson EM, Kidd A, Savarirayan R, Turner A, Amor D, et al. Growth, behavior, and clinical findings in 27 patients with Kabuki (Niikawa-Kuroki) syndrome. Am J Med Genet A. 2004;127A(2):118-27.
4. Gole H, Chuk R, Coman D. Persistent Hyperinsulinism in Kabuki Syndrome 2: Case Report and Literature Review. Clin Pract. 2016;6(3):848.
5. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet. 2010;42(9):790-3.
6. Grand K, Gonzalez-Gandolfi C, Ackermann AM, Aljeaid D, Bedoukian E, Bird LM, et al. Hyperinsulinemic hypoglycemia in seven patients with de novo NSD1 mutations. Am J Med Genet A. 2019;179(4):542-51.
7. Welters A, El-Khairi R, Dastamani A, Bachmann N, Bergmann C, Gilbert C, et al. Persistent hyperinsulinaemic hypoglycaemia in children with Rubinstein-Taybi syndrome. Eur J Endocrinol. 2019;181(2):121-8.
8. Aref-Eshghi E, Kerkhof J, Pedro VP, Groupe DIF, Barat-Houari M, Ruiz-Pallares N, et al. Evaluation of DNA Methylation Episignatures for Diagnosis and Phenotype Correlations in 42 Mendelian Neurodevelopmental Disorders. Am J Hum Genet. 2020;106(3):356-70.
9. Aref-Eshghi E, Schenkel LC, Lin H, Skinner C, Ainsworth P, Pare G, et al. The defining DNA methylation signature of Kabuki syndrome enables functional assessment of genetic variants of unknown clinical significance. Epigenetics. 2017;12(11):923-33.
10. Choufani S, Cytrynbaum C, Chung BH, Turinsky AL, Grafodatskaya D, Chen YA, et al. NSD1 mutations generate a genome-wide DNA methylation signature. Nat Commun. 2015;6:10207.
11. Fahrner JA, Bjornsson HT. Mendelian disorders of the epigenetic machinery: postnatal malleability and therapeutic prospects. Hum Mol Genet. 2019;28(R2):R254-R64.
12. Mannisto JME, Jaaskelainen J, Otonkoski T, Huopio H. Long-Term Outcome and Treatment in Persistent and Transient Congenital Hyperinsulinism: A Finnish Population-Based Study. J Clin Endocrinol Metab. 2021;106(4):e1542-e51.
13. McKusick VA. Mendelian Inheritance in Man and its online version, OMIM. Am J Hum Genet. 2007;80(4):588-604.
14. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581(7809):434-43.
15. Douzgou S, Liang HW, Metcalfe K, Somarathi S, Tischkowitz M, Mohamed W, et al. The clinical presentation caused by truncating CHD8 variants. Clin Genet. 2019;96(1):72-84.
16. Kleefstra T, Kramer JM, Neveling K, Willemsen MH, Koemans TS, Vissers LE, et al. Disruption of an EHMT1-associated chromatin-modification module causes intellectual disability. Am J Hum Genet. 2012;91(1):73-82.
17. Schaaf CP, Gonzalez-Garay ML, Xia F, Potocki L, Gripp KW, Zhang B, et al. Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. Nat Genet. 2013;45(11):1405-8.
18. Salles J, Eddiry S, Lacassagne E, Laurier V, Molinas C, Bieth E, et al. Patients with PWS and related syndromes display differentially methylated regions involved in neurodevelopmental and nutritional trajectory. Clin Epigenetics. 2021;13(1):159.
19. Soden SE, Saunders CJ, Willig LK, Farrow EG, Smith LD, Petrikin JE, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. Sci Transl Med. 2014;6(265):265ra168.

20. Jobling R, Stavropoulos DJ, Marshall CR, Cytrynbaum C, Axford MM, Londero V, et al. Chitayat-Hall and Schaaf-Yang syndromes:a common aetiology: expanding the phenotype of MAGEL2-related disorders. J Med Genet. 2018;55(5):316-21.
21. Patak J, Gilfert J, Byler M, Neerukonda V, Thiffault I, Cross L, et al. MAGEL2-related disorders: A study and case series. Clin Genet. 2019;96(6):493-505.
22. McCarthy J, Lupo PJ, Kovar E, Rech M, Bostwick B, Scott D, et al. Schaaf-Yang syndrome overview: Report of 78 individuals. Am J Med Genet A. 2018;176(12):2564-74.
23. McCarthy JM, McCann-Crosby BM, Rech ME, Yin J, Chen CA, Ali MA, et al. Hormonal, metabolic and skeletal phenotype of Schaaf-Yang syndrome: a comparison to Prader-Willi syndrome. J Med Genet. 2018;55(5):307-15.
24. A DH-S, Del Carmen DeMingo-Alemany M, Moreno-Macian F, Rosello M, Orellana C, Martinez F, et al. A Novel Mutation of MAGEL2 in a Patient with Schaaf-Yang Syndrome and Hypopituitarism. Int J Endocrinol Metab. 2018;16(3):e67329.
25. Gregory LC, Shah P, Sanner JRF, Arancibia M, Hurst J, Jones WD, et al. Mutations in MAGEL2 and L1CAM Are Associated With Congenital Hypopituitarism and Arthrogryposis. J Clin Endocrinol Metab. 2019;104(12):5737-50.
26. Tennese AA, Wevrick R. Impaired hypothalamic regulation of endocrine function and delayed counterregulatory response to hypoglycemia in Magel2-null mice. Endocrinology. 2011;152(3):967-78.
27. Maillard J, Park S, Croizier S, Vanacker C, Cook JH, Prevot V, et al. Loss of Magel2 impairs the development of hypothalamic Anorexigenic circuits. Hum Mol Genet. 2016;25(15):3208-15.
28. Chen H, Victor AK, Klein J, Tacer KF, Tai DJ, de Esch C, et al. Loss of MAGEL2 in Prader-Willi syndrome leads to decreased secretory granule and neuropeptide production. JCI Insight. 2020;5(17).
29. Helleskov A, Melikyan M, Globa E, Shcherderkina I, Poertner F, Larsen AM, et al. Both Low Blood Glucose and Insufficient Treatment Confer Risk of Neurodevelopmental Impairment in Congenital Hyperinsulinism: A Multinational Cohort Study. Front Endocrinol (Lausanne). 2017;8:156.
30. Novoa-Medina Y, Dominguez Garcia A, Quinteiro Gonzalez S, Garcia Cruz LM, Santana Rodriguez A. Congenital hyperinsulinism in Gran Canaria, Canary Isles. An Pediatr (Engl Ed). 2021;95(2):93-100.
31. Casertano A, Rossi A, Fecarotta S, Rosanio FM, Moracas C, Di Candia F, et al. An Overview of Hypoglycemia in Children Including a Comprehensive Practical Diagnostic Flowchart for Clinical Use. Front Endocrinol (Lausanne). 2021;12:684011.
32. Razzaghy-Azar M, Saeedi S, Dayani SB, Enayati S, Abbasi F, Hashemian S, et al. Investigating Genetic Mutations in a Large Cohort of Iranian Patients with Congenital Hyperinsulinism. J Clin Res Pediatr Endocrinol. 2022;14(1):87-95.
33. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-91.

	Kabuki Syndrome 1 (*KMT2D*, OMIM 147920)	**Kabuki Syndrome 2 (*KDM6A*, OMIM 300867)**	**Sotos Syndrome (*NSD1*, OMIM 117550)**	**Rubinstein-Taybi Syndrome 1 (*CREBBP*, OMIM 180849)**	**Rubinstein-Taybi Syndrome 2 (*EP300*, OMIM 613684)**	**Beckwith-Wiedemann Syndrome (Chr11p15.5, OMIM 130650)**
Hyperinsulinism	Y	Y	Y	Y	Y	Y
Dysmorphic features	Y	Y	Y	Y	Y	Y
Microcephaly	Y	Y		Y	Y	
Heart defects	Y	Y	Y	Y		Y
Scoliosis	Y		Y	Y		
Renal defects	Y		Y	Y		Y
Visual impairment		Y	Y	Y		
Joint hypermobility	Y			Y		
Hypospadias	Y			Y		
Hypotonia	Y	Y	Y	Y	Y	

Table 1: The clinical features identified in 6 syndromes known to cause hyperinsulinism and aberrant DNA methylation. Features are taken from the OMIM clinical synopses for each condition (ref OMIM). The gene symbols and OMIM references numbers are listed for each syndrome.

Patient	Hyperinsulinism	Dysmorphic features	Microcephaly	Heart defects	Scoliosis	Macroglossia	Renal abnormalities	Visual impairment	Joint hypermobility	Hypospadias	Hypotonia
1	Y	-	-	-	Y	-	Y	-	-	-	-
2	Y	Y	-	Y	-	-	-	-	-	-	-
3	Y	Y	-	-	-	-	-	-	-	-	Y
4	Y	-	-	-	-	-	-	-	Y	-	Y
5	Y	-	Y	Y	-	-	-	-	-	-	-
6	Y	-	-	-	-	-	-	Y	-	-	Y
7	Y	-	Y	-	-	-	-	Y	-	-	-
8	Y	Y	-	Y	-	-	-	-	-	-	-
9	Y	-	-	-	-	-	Y	-	-	Y	-
10	Y	-	-	Y	-	-	-	-	-	-	Y
11	Y	Y	-	-	-	Y	-	-	-	-	-
12	Y	-	-	Y	-	-	-	-	-	-	Y
13	Y	-	Y	-	-	-	Y	-	-	-	-
14	Y	Y	-	-	-	Y	-	-	-	-	-
15	Y	Y	-	-	-	-	Y	-	-	-	Y
16	Y	Y	-	Y	-	-	-	-	-	-	-
17	Y	Y	-	Y	-	-	-	-	-	-	-
18	Y	Y	-	Y	-	-	-	-	-	Y	-
19	Y	Y	-	-	-	-	-	-	Y	-	Y

Table 2: Clinical features of 19 individuals studied. Y = feature reported, - = feature not reported.

Gene	Number of Variants	Genomic coordinates and protein effect	Trio?	Patient ID	pLI score	Hypoglycaemia reported?
CDC25A	2	g.48215945G>T p.Asp253Glu	No	6	0.84	No
		g.48209391G>T p.Thr325Asn	No	8		
CHD8	2	g.21862265G>A p.Arg1897Trp	No	6	1	No
		g.21861363T>C p.Thr2124Ala	No	15		
KMT2C	2	g.151860814C>G p.Ser3283Thr	No	9	1	No
		g.151836791G>C p.Ala4810Gly	No	15		
MAGEL2	2	g.23890978G>A p.Gln638*	No	15	0.98	Yes
		g.23890978G>A p.Gln638*	Yes	19		
PTPRCAP	2	g.67203727G>A p.Ser33Phe	No	9	0.54	No
		g.67205088C>T p.? (start loss)	No	15		

Table 3: Genes where heterozygous variants were detected in multiple individuals in the whole genome sequencing cohort. pLI scores measure the tolerance of a gene to loss of function variants, based on the number of protein-truncating variants identified in population databases (33). Literature searches were performed to ascertain if hypoglycaemia or hyperinsulinism had been previously reported as a feature in cases where variants in these genes cause disease.

	Patient 15	**Patient 19**
Sex	Female	Male
Ethnicity	Arab	Turkish
Birth weight (g)	2955	3970
Gestational age (wks)	40	38
Birth weight SDS	-1.30	1.95
Age at HI diagnosis	1 week	3 days
Glucose at presentation (mmol/l)	1.7	2.1
Insulin during hypoglycaemia (pmol/l)	16.9	12.4
Current age (yrs)	3	2
Treatment for hyperinsulinism (current dose/date remitted, responsiveness)	Octreotide (dose unknown, responsive)	Diazoxide (dose unknown, responsive)
Extra-pancreatic features	**Hypotonia**, hydronephrosis, **respiratory compromise**, **feeding difficulties**, **diabetes insipidus**, **developmental delay**, **joint contractures**, **impaired growth**	Macrocephaly, **facial dysmorphia**, **joint contractures**, **diabetes insipidus**, **gastro-oesophageal reflux**, central hypoventilation

Table 4: Clinical features of the two individuals with *MAGEL2* mutations identified in this study. Bolded entries in the “Extra-pancreatic features” column identifies those features which are included in the OMIM clinical synopsis for Schaaf-Yang syndrome (13).

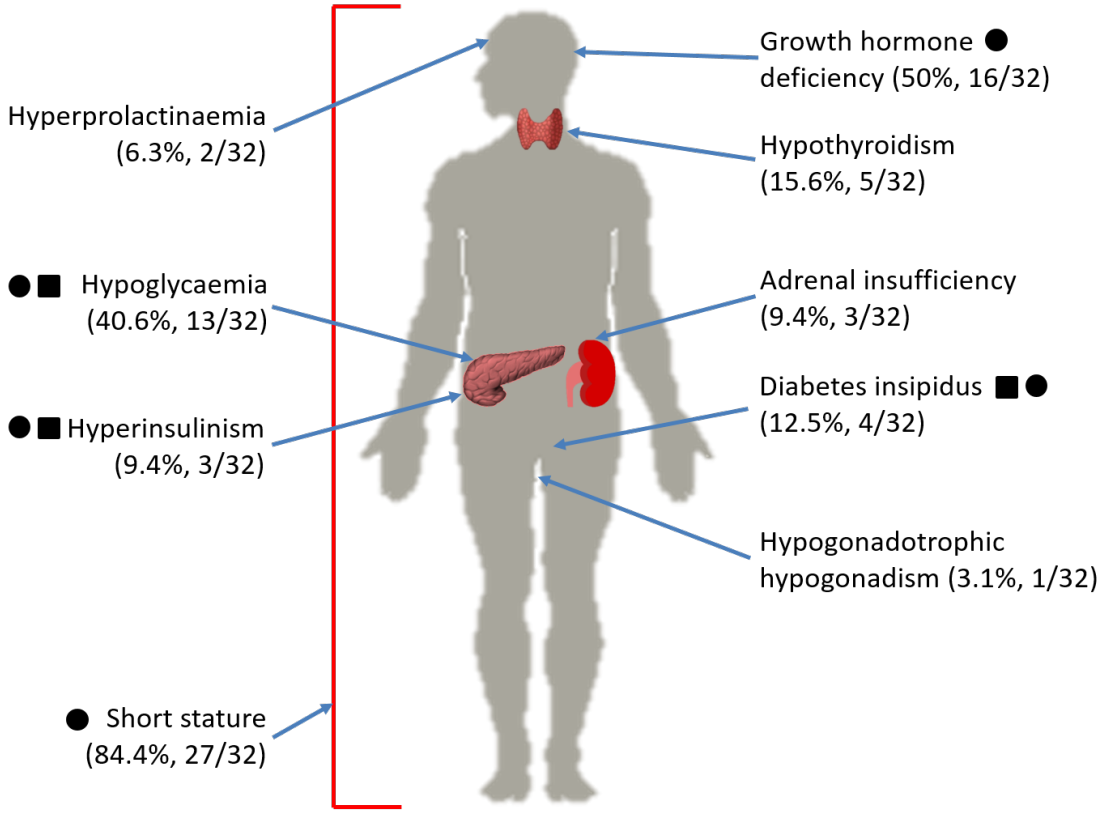

Figure 1: The endocrine features of individuals with Schaaf-Yang syndrome reported in the literature. Circles indicate features reported in Patient 15, squares indicate features reported in Patient 19 (table 4).

	Cohort
Male sex	42%
White ethnicity	47%
Birth weight (g)	2924 (695)
Gestational age (wks)	38 (3)
Birth weight SDS	-0.71 (1.75)
Age at HI diagnosis (days)	81 (138)
Glucose at presentation (mmol/l)	1.7 (0.7)
Insulin during hypoglycaemia (pmol/l)	103.4 (120)
Diazoxide responsive	88%

Supplementary Table 1: A summary of the clinical features of the cohort selected for WGS in this study. Discrete variables are shown as percentages, continuous variables are shown as mean (SD).

CONCLUSIONS

Congenital hyperinsulinism is a complex disorder, both in terms of genetic aetiologies and medical management. This thesis, along with previous literature on this disorder show the importance of understanding the molecular basis of known causes of HI, and of discovering novel aetiologies. These discoveries can lead to the identification of new genes involved in insulin secretion and glucose regulation, and also allow for the adaptation of treatment for some individuals.

This section of the thesis will summarise the findings of each data chapter, discuss the strengths and limitations of each project, and consider the impact of these studies, along with future research that may give further insights.

Chapter 1 - Birth weight and diazoxide unresponsiveness strongly predict the likelihood of congenital hyperinsulinism due to a mutation in *ABCC8* or *KCNJ11*

Main conclusions

In this chapter, I studied 761 individuals with KATP channel HI resulting from an *ABCC8* or *KCNJ11* mutation and 862 individuals with HI of unknown genetic cause to determine if clinical features could predict the likelihood of KATP channel HI. I identified that birth weight and responsiveness to diazoxide treatment are the most predictive clinical features for identifying individuals with a mutation in *ABCC8* or *KCNJ11* in HI cohorts.

Using a large cohort of individuals diagnosed with congenital HI, I confirmed that individuals with mutations in a KATP channel gene are usually born heavier than those with HI of an unknown aetiology, with an average of around 830g increase in birth weight. I also showed that response to diazoxide treatment can help to determine the likelihood of a KATP channel mutation, with 68% of those in our cohort with a KATP channel mutation reported as being unresponsive to diazoxide treatment compared to 12% of those with HI of an unknown aetiology. Linear regression models based on these features showed that birth weight (ROC AUC = 0.80) and diazoxide responsiveness (ROC AUC = 0.77) are strongly predictive alone for KATP channel HI, but that a combination of the two features produces an even stronger predictive model (ROC AUC = 0.88). I showed that these features could be combined to categorise our cohort, with 86% of individuals born large for gestational age and unresponsive to diazoxide treatment identified as having KATP channel mutations, while none of those born small for gestational age who responded to diazoxide treatment had a KATP channel mutation.

Strengths and limitations

The study population consisted of a large, ethnically diverse international cohort which makes our findings applicable across populations. It also reports the largest cohort studied to date with KATP channel HI.

The study relied on clinical information supplied on a request form completed by a clinician at the time of referral for genetic testing. Whilst measurements such as birth

weight, gestation, and age at diagnosis of HI will be robust, information such as response to diazoxide may be less reliable as responsiveness to treatment can be difficult to establish in an acutely unwell infant and may change with time. We are however not overly concerned by this as we would predict that any errors in data collection would affect both groups equally and therefore should not have a significant impact on the results.

This study was also limited by its focus on KATP channel HI. We focussed on KATP channel HI as this is the most common cause of the disease (1-4) which meant that we had a large cohort to study. Furthermore, understanding which patients are likely to have an *ABCC8* and *KCNJ11* mutation is critical for informing testing strategies for newly diagnosed patients (5).

Systematic, comprehensive screening of all the known HI genes had not been performed in all individuals with HI of unknown genetic cause and it is therefore likely that some of these individuals will have an unidentified monogenic aetiology. As a result of our study design we also do not know whether birth weight and response to diazoxide would remain as being highly predictive of KATP channel HI if we had used individuals with other genetically confirmed causes of HI as controls (e.g. *GLUD1*, *GCK*, *HADH*).

Impact of findings

The findings of this study will allow for testing strategies for congenital HI to evolve. For instance, rapid Sanger sequencing of *ABCC8* and *KCNJ11* is still used in all cases in some laboratories to identify which individuals are most likely to have a focal lesion, which can be treated surgically with overwhelmingly positive results (6). The data from this study allows us to prioritise those with an increased birth weight and a lack of response to diazoxide for rapid testing of the KATP channel genes whilst those with low birth weight and a good response to treatment could undergo targeted next generation sequencing of all known HI genes as a first line test. The ability to better stratify genetic testing is important, especially for individuals from resource poor countries where the costs associated with multiple rounds of genetic testing can be prohibitive.

The identification of routinely measured clinical features that predict the likelihood of a KATP channel mutation also allows clinicians to better counsel families in the early

stages of a HI diagnosis. A diagnosis of HI, particularly in the neonatal period, can cause a great deal of stress to family members of the affected child. The insights gained from this study allow clinicians to counsel families as to the likelihood of a KATP channel mutation while genetic testing is ongoing, as well as preparing them for the possibility that their child may require either lesionectomy for a focal pancreatic lesion, or possibly a battery of medical treatment and even sub-total pancreatectomies.

Future directions

I would like to use the insights gained into the clinical features of KATP HI to further knowledge of the wider genetic aetiology of HI. One example of this would be to sequence the non-coding regions that surround *ABCC8* and *KCNJ11* to look for regulatory or splicing mutations in children with high birthweights and unresponsiveness to diazoxide.

In addition, the results of this study can be used to inform on novel non-KATP gene discovery studies in cases of HI of an unknown aetiology. With a large proportion of those born small or appropriate for gestational age and responsive to diazoxide not having KATP channel mutations, it seems prudent to investigate using genome sequencing other possible genetic causes of disease in these individuals.

Finally, the methods used in this study should be utilised to investigate other known genetic causes of congenital HI. This would allow us to build a wider picture of the clinical features common to different causes of HI. With other known genetic causes of HI affect different pathways of insulin secretion (e.g. leucine-stimulated insulin-secretion) it would be interesting to see how these mutations impact on birth weight and other clinical features of HI. This will be important as it will provide novel insights into insulin secretion *in utero*. Importantly, the identification of clinical features that can predict mutations in other HI genes, such as *GLUD1*, *HADH*, and *HK1*, will allow for better counselling of families, and prioritisation of genetic testing.

Chapter 2 - Increased referrals for congenital hyperinsulinism genetic testing in children with trisomy 21 reflects the high burden of non-genetic risk factors in this group

Main conclusions

In this study, I identified individuals in the Exeter hyperinsulinism cohort who had concurrent hyperinsulinism and Down syndrome and investigated their clinical features to determine why these individuals were presenting with hyperinsulinism. I identified that the minimum prevalence of Down syndrome in the Exeter hyperinsulinism cohort was 0.55%, around four times higher than the prevalence of Down syndrome in the population. Once the 11 individuals in the cohort with hyperinsulinism and Down syndrome were identified, I contacted referring clinicians and reviewed referral forms to gain an insight into the mechanism of hyperinsulinism in these individuals. Through the targeted panel testing undertaken by the laboratory in Exeter, one individual was identified as having a pathogenic mutation in *ABCC8*, with no mutations identified in the other ten individuals. Six of those ten individuals had undergone some form of gastric surgery for a gastrointestinal condition that was precipitated by their Down syndrome, with this being confirmed to occur prior to the onset of hyperinsulinism in two cases. In addition, one individual underwent asparaginase treatment for acute lymphoblastic leukaemia, again prior to the onset of hyperinsulinism. Prematurity and intrauterine growth restriction were identified in one individual each. Literature searches revealed that all these features can act as risk factors for hyperinsulinism, leading me to the conclusion that a high burden of non-genetic risk factors was leading to hyperinsulinism in this cohort of individuals.

Strengths and limitations

This study was possible because of the large cohort of hyperinsulinism patient samples recruited to Exeter, which allowed us to observe this association. This study was limited as we were only able to establish a minimal prevalence for Down syndrome in hyperinsulinism cohorts, as we could only identify Down syndrome where it had been reported to us by referring clinicians. Finally, our reliance on clinical information from referral forms and the patients being lost to follow up means that some clinical features that would explain the hyperinsulinism in these individuals may have been missed as a result.

Impact of findings

This study has, for the first time, identified the increased rate of referrals to a hyperinsulinism genetic testing centre of individuals with Down syndrome, and has begun to untangle the reasons behind these referrals. Given the complex medical needs of some individuals with Down syndrome, and the possible severity of a hyperinsulinism diagnosis, these results give needed clarity to clinicians and patients over the most likely aetiology of hyperinsulinism in these individuals. As a result, clinicians may opt for more regular glucose monitoring in individuals with Down syndrome who are at risk of developing hypoglycaemia, and families can be reassured that there is likely to be a medical explanation for hyperinsulinism in these individuals, and that it can either resolve on its own, or be managed using medical treatments.

This study also highlights the important detail that monogenic hyperinsulinism and Down syndrome can occur coincidentally. This emphasises that individuals with Down syndrome and hyperinsulinism should still undergo genetic testing, particularly in cases where the individual's hyperinsulinism presents in the first weeks of life. I believe that a holistic view should be taken in diagnosing these individuals, where genetic testing is performed alongside an analysis of clinical features that identifies any possible risk factors leading to hyperinsulinism.

Future direction

I would like to take the learnings of this work to help refine the cohort for gene discovery in Exeter, by identifying the individuals in the wider cohort with similar features to those identified in our Down syndrome cohort. While there is an increased risk of many of these clinical presentations in those with Down syndrome, they do exist in the general population as well. As such, I believe it would be useful to identify those individuals with these risk factors in our wider cohort, as this may allow us to explain the hyperinsulinism occurring in these patients and remove them from costly, time consuming gene discovery studies.

As this study provides a minimum prevalence for coincidental hyperinsulinism and Down syndrome, it will be important to identify how commonly hyperinsulinism occurs in individuals with Down syndrome. The best way to do this would be to perform a larger study of individuals with Down syndrome to identify what

percentage of those individuals have experienced hyperinsulinism, either currently or at some point in the past. This could also coincide with genetic testing in these individuals, in order to ascertain how often monogenic hyperinsulinism and Down syndrome coincide.

Finally, I would like to perform a similar study in individuals with Trisomy 13, or Patau syndrome which has also been reported to cause hyperinsulinism (7). By studying the clinical features of these patients I would investigate whether there is a similarly high burden of non-genetic risk factors for hyperinsulinism, or if there is a genetic link between Patau syndrome and hyperinsulinism. The difficulty with this study is that Patau syndrome is extremely rare (8) compared to Down syndrome, however the large Exeter cohort will provide the best resource for this study.

Chapter 3 - Partial duplications of the KDM6A gene are a novel cause of congenital hyperinsulinism

Main conclusions

In this study, I identified three individuals with tandem duplications in the *KDM6A* gene, one of the two causes of Kabuki syndrome. As this syndrome is caused by loss of function mutations in this gene, it was initially unclear how these duplications may lead to disease in these individuals. Whole genome sequencing was performed to establish precise breakpoints for the duplications, along with confirming that all three duplications occurred in tandem, where the duplications were identified from off-target reads of tNGS. Bioinformatic analyses were performed in order to identify if the duplications introduced frameshifts due to split exons: this was the case in Patient 1, where a frameshift occurred, introducing a premature stop codon.

Where samples were available, DNA methylation patterns were investigated using the Illumina EPIC array. A linear regression was conducted, identifying probes that differed between those with confirmed pathogenic *KDM6A* mutations, those with duplications, and a group of unaffected controls of similar ages. These data, along with previously published data on the episignature of individuals with Kabuki syndrome, were used to identify how similar those with duplications were to those with confirmed mutations. This analysis indicated that Patient 2 had a similar DNA methylation pattern to those with confirmed Kabuki syndrome. This was not the case in Patient 3, which may be explained by the larger duplication in this individual, which extends outside of *KDM6A* and is predicted to still lead to a complete copy of *KDM6A*.

Strengths and limitations

This study utilised the large cohort of individuals referred to Exeter for genetic testing for HI with an unidentified genetic aetiology, along with expertise at the University of Exeter Medical School in how to carry out and analyse DNA methylation arrays. While the mechanism of disease was unclear on first identifying these duplications, the identification of a premature stop codon introduced by a frameshift in one individual, and the use of DNA methylation data in another, offered support to the hypothesis that in two of the three individuals, the duplication would lead to disruption of the *KDM6A* gene. This study was however limited by only a small

number of individuals with confirmed mutations in *KDM6A* having been identified in Exeter and having samples available for DNA methylation analysis. Combined with the paucity of public data available on individuals with *KDM6A* mutations leading to Kabuki syndrome, this resulted in small sample size for the DNA methylation analyses performed in this study. In addition, further blood samples were not available for Patient 1, which meant that we were unable to perform DNA methylation analyses in this individual. The presence of a premature stop codon in *KDM6A* leads me to conclude that this duplication is leading to Kabuki syndrome and HI in Patient 1.

Impact of findings

This study has identified, for the first time, tandem duplications within the *KDM6A* gene that are predicted to lead to a loss of function, and therefore to disease. Kabuki syndrome is a complex disorder with a wide range of clinical features, and HI has been previously shown to appear as the first feature with which individuals with this disorder present to clinicians (9). As such, the analysis of this gene, along with others that cause syndromic HI, in the early stages of genetic testing, is vital in order to give clinicians information with which to manage an individual through their clinical course.

This study has shown the utility of DNA methylation analyses in supporting, or disproving, the pathogenicity of variants in rare diseases affecting the epigenetic machinery. Analysis of the episignature of a variety of diseases has previously identified around 42 disorders with a distinctive signature of DNA methylation, with clinical applications being introduced in recent years (10). This analyses, which can be performed from peripheral blood samples, may have an increasing clinical utility in supporting and confirming the pathogenicity of variants of unknown significance, and in genetic diagnoses of unknown syndromic disease, such as differential diagnoses like Sotos syndrome and Rubinstein-Taybi syndrome.

Future direction

I would like to perform further studies with patient samples from this study to identify how the *KDM6A* gene, and its expression, are affected in these individuals. For example, RNA sequencing may identify if *KDM6A* is expressed in patient cells, such as fibroblasts, or if these duplications lead to nonsense-mediated decay or a

disruption of gene regulation in the region of *KDM6A*. This could lend further support to the hypothesis that the duplications identified in this study lead to disease.

Additionally, I would like to perform further studies to identify the mechanism of disease that leads to HI in some individuals with Kabuki syndrome, but not others. HI presents in three known disorders of the epigenetic machinery: Kabuki syndrome, Sotos syndrome, and Rubinstein-Taybi syndrome. Recently published work has shown that it is possible to use shared disease manifestations in different disorders of the epigenetic machinery to discover differences in expression that occur across multiple disorders (11). This methodology could therefore be used to help identify if changes in expression of particular genes in the pancreatic beta cell occur across these three disorders, and therefore give a greater insight into mechanism of disease.

Chapter 4 - Loss-of-function variants in the maternally imprinted gene, *MAGEL2*, are a rare but important cause of congenital hyperinsulinism

Main conclusions

In this study, I identified clinical features that appear in at least two of the six disorders of DNA methylation that feature hyperinsulinism. I used this list of clinical features to select individuals with hyperinsulinism of unknown genetic cause for whole genome sequencing, to identify new causes of syndromic hyperinsulinism linked to DNA methylation.

I analysed the whole genome sequencing data from these 19 individuals, 12 of whom were sequenced with parental samples. I performed stringent variant filtering to identify novel heterozygous variants in shared genes across two or more individuals. This identified five genes. Of these, three genes were reported to cause monogenic disease (*CHD8*, *KMT2C*, and *MAGEL2*). Interestingly, *MAGEL2* has been linked to aberrant DNA methylation, as this is a maternally imprinted gene. I identified that hypoglycaemia had been reported in cases of Schaaf-Yang syndrome, caused by mutations on the paternal copy of *MAGEL2*, and that one of these cases had been linked to hyperinsulinism. This allowed me to conclude that I had identified two additional cases of mutations in *MAGEL2* causing congenital hyperinsulinism.

Strengths and limitations

This study greatly benefitted from a large cohort of individuals with hyperinsulinism who had DNA samples available and were consented for genetic testing to ascertain the cause of their hyperinsulinism. In many cases, clinical information on extra-pancreatic features at the time of referral was available, and this formed the backbone of this project, as it allowed me to identify a cohort of individuals suitable for study. It seems likely that there will be more individuals that have syndromic features consistent with a disorder of DNA methylation in our cohort. These may remain undetected, either because clinical features had not become apparent at the time of referral for genetic testing, or because the reporting clinician had considered the additional features to not be relevant to the diagnosis of hyperinsulinism.

Increasing the amount of individuals in the cohort with comprehensive reporting of additional clinical features will increase the power of gene discovery studies such as

this one. Due to time constraints, I was unable to further investigate the variants in *CHD8* and *KMT2C*. As Patient 15 in our cohort is one of the individuals with a *MAGEL2* mutation, along with variants in *CHD8* and *KMT2C*, these genes would only have a single unsolved case identified in our cohort, which gives me confidence that these mutations are not causative in these cases. However, I believe it would still be prudent to investigate these variants completely.

Impact of findings

This study identified the second and third known cases of mutations in the maternally imprinted gene *MAGEL2* causing hyperinsulinism. This allows geneticists to say with confidence that *MAGEL2* is a cause of syndromic hyperinsulinism, and that the gene should be included on targeted panels for the disease. More directly, this study has identified a diagnosis for two patients who previously had a complex disorder of unknown aetiology. This study can help clinicians of individuals with a *MAGEL2* mutation and hyperinsulinism if there are any future screening programs that these children would benefit from. In addition, it provides certainty for their families, who can now continue onto the next step of the "diagnostic odyssey" with more confidence, knowing the cause of their child's health issues and the risk of recurrence in any future offspring (12).

Future direction

I would like to investigate the variants in *CHD8* and *KMT2C* identified in this study, to confirm that they are not linked to disease. These variants were identified in singleton whole genome sequencing data, where it is impossible to identify *de novo* variants, and so I believe the best course of action would be to collect parental samples from these families to confirm whether these mutations have occurred *de novo*. If they are identified as occurring *de novo*, then I would investigate whether these variants were causing disease, by investigating if the proteins have a role in insulin secretion or glucose homeostasis.

I would also seek to improve the amount of clinical data that we have on the Exeter cohort of patients with hyperinsulinism. To do this, I would, with ethical approval, contact families and referring clinicians of individuals in the Exeter cohort to request follow-up information on extra-pancreatic features that were not reported to us at the time of referral for genetic testing, either because of their presumed irrelevance to

the hyperinsulinism diagnosis or because they have emerged since the time of referral. This would allow me to identify additional individuals who may be suitable for gene discovery studies, which would serve to further our knowledge of causes of syndromic hyperinsulinism, and in particular those causes linked to aberrant DNA methylation.

I would also like to investigate if any of the other known causes of disorders of the epigenetic machinery feature hyperinsulinism. There are a large number of genes reported that are known to be involved in the epigenetic machinery (11). Comprehensive sequencing of these genes in individuals with syndromic hyperinsulinism may allow us to identify more disorders where hyperinsulinism can be a feature, and we may be able to leverage this to understand the mechanism by which these disorders lead to hyperinsulinism.

Final Remarks

This thesis intended to study the large cohort of individuals referred for genetic testing for congenital hyperinsulinism in Exeter in order to gain a greater understanding of the clinical, molecular, and genetic basis of this complex disorder. This was undertaken with a particular focus on syndromic forms of the disorder, where medical management is necessarily a more complicated venture. To this aim, it has identified new genetic causes of syndromic HI, including tandem duplications occurring in the Kabuki syndrome gene *KDM6A* (Chapter 3), and *de novo* monoallelic mutations in the imprinted gene *MAGEL2* (Chapter 4). It has also unveiled a greater understanding of the mechanism by which Down syndrome may lead to HI, and that this is more influenced by non-genetic factors (Chapter 2). This thesis also depicts work that used the clinical features of individuals with the most common cause of congenital HI, KATP channel mutations, to determine the likelihood of an individual carrying a mutation in one of these genes (Chapter 1).

The work conducted in this thesis was carried out in the face of the global COVID-19 pandemic, which presented great challenges for both the process of research, and its dissemination. Initial plans for this PhD thesis included more laboratory work to investigate the mechanism by which some forms of syndromic HI might lead to a pancreatic phenotype when others do not. Instead, much of the work necessarily became more computational, with the analysis of clinical data and a focus on statistics, as a result of the mandate to work from home in the UK. In addition, the pandemic and resulting working from home led to personal challenges, including the fact that, casual conversations with fellow researchers that would have led to fruitful scientific endeavours were far less common. However, I believe that the work conducted in this thesis is a testament to how scientific research can still take place in those most challenging of times.

The practical achievements of this thesis are laid out in Appendix 2. In summary, the work included in this thesis has led to three publications thus far, with Chapters 1 and 2 published as journal articles, along with a section of the introduction being published as a review. Work from this thesis has also been presented at international conferences, including both online and in-person events, and including an event organised by the charity Congenital Hyperinsulinism International (Glen Ridge, New

Jersey, USA) aimed to engage individuals with hyperinsulinism and their families with research into the condition being conducted around the world. I have also presented at departmental seminars, along with two occasions at which I presented my research to clinicians who are focussed on the diagnosis and treatment of monogenic diabetes.

One challenge in this research is that, despite syndromic causes of HI being included on the panel for targeted sequencing in the Exeter laboratory, the understanding of HI's place in syndromic diseases is less common. Clinicians often do not list extra-pancreatic features when referring individuals with HI for routine genetic testing, only disclosing them on a follow-up. While there are understandable reasons for this, including the lack of awareness that these features may be relevant to their diagnosis of HI, I believe that as we discover more causes of syndromic hyperinsulinism, it will become clearer that it is important to include a full clinical picture when referring an individual for genetic testing. Having information on these additional clinical features will allow for better interpretation of variants occurring in genes that lead to syndromic disease, and may also allow for research into when these features develop in a variety of syndromic disease, particularly as HI can occur as the presenting feature as a result of its early diagnosis.

While the challenges described above resulting from a global pandemic have led to focus being shifted away from the variability of HI occurrence in some syndromic disorders, I believe there is still work to be done to this end. In particular, recent work has shown that clinical features of individuals with disorders of the epigenetic machinery can be used to elucidate functional variation in the epigenome. While HI may not be a perfect model for this, given the difficulty of obtaining pancreatic samples, I believe that developments in gene editing technologies and stem cell differentiation could allow for research to be conducted into this. Mutations identified in individuals with these disorders could be edited into a cell line, or tissue samples from these individuals could be modified into induced pluripotent stem cells, which may allow for further study of the mechanism of disease in these disorders.

Given that HI occurs in Kabuki syndrome, Sotos syndrome, and Rubinstein-Taybi syndrome, it seems possible that other disorders of the epigenetic machinery may manifest with HI. While that has not been identified in this thesis, a larger cohort of

individuals with syndromic HI may allow for a greater screening of the genes that lead to disorders of the epigenetic machinery, and therefore further elucidate the role of HI in the clinical course of these disorders. This may also feed into further studies designed to understand the phenotypic variability observed in some of these disorders.

References

1. Rozenkova K, Malikova J, Nessa A, Dusatkova L, Bjorkhaug L, Obermannova B, et al. High Incidence of Heterozygous ABCC8 and HNF1A Mutations in Czech Patients With Congenital Hyperinsulinism. J Clin Endocrinol Metab. 2015;100(12):E1540-9.
2. Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S, Hussain K. Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism. Eur J Endocrinol. 2013;168(4):557-64.
3. Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, et al. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. J Clin Endocrinol Metab. 2013;98(2):E355-63.
4. Mannisto JME, Jaaskelainen J, Otonkoski T, Huopio H. Long-Term Outcome and Treatment in Persistent and Transient Congenital Hyperinsulinism: A Finnish Population-Based Study. J Clin Endocrinol Metab. 2021;106(4):e1542-e51.
5. Banerjee I, Skae M, Flanagan SE, Rigby L, Patel L, Didi M, et al. The contribution of rapid KATP channel gene mutation analysis to the clinical management of children with congenital hyperinsulinism. Eur J Endocrinol. 2011;164(5):733-40.
6. Adzick NS, De Leon DD, States LJ, Lord K, Bhatti TR, Becker SA, et al. Surgical treatment of congenital hyperinsulinism: Results from 500 pancreatectomies in neonates and children. J Pediatr Surg. 2019;54(1):27-32.
7. Tamame T, Hori N, Homma H, Yoshida R, Inokuchi M, Kosaki K, et al. Hyperinsulinemic hypoglycemia in a newborn infant with trisomy 13. Am J Med Genet A. 2004;129A(3):321-2.
8. Goel N, Morris JK, Tucker D, de Walle HEK, Bakker MK, Kancherla V, et al. Trisomy 13 and 18-Prevalence and mortality-A multi-registry population based analysis. Am J Med Genet A. 2019;179(12):2382-92.
9. Yap KL, Johnson AEK, Fischer D, Kandikatla P, Deml J, Nelakuditi V, et al. Congenital hyperinsulinism as the presenting feature of Kabuki syndrome: clinical and molecular characterization of 9 affected individuals. Genet Med. 2019;21(1):233-42.
10. Aref-Eshghi E, Kerkhof J, Pedro VP, Groupe DIF, Barat-Houari M, Ruiz-Pallares N, et al. Evaluation of DNA Methylation Episignatures for Diagnosis and Phenotype Correlations in 42 Mendelian Neurodevelopmental Disorders. Am J Hum Genet. 2020;106(3):356-70.
11. Luperchio TR, Boukas L, Zhang L, Pilarowski G, Jiang J, Kalinousky A, et al. Leveraging the Mendelian disorders of the epigenetic machinery to systematically map functional epigenetic variation. Elife. 2021;10.
12. Wu AC, McMahon P, Lu C. Ending the Diagnostic Odyssey-Is Whole-Genome Sequencing the Answer? JAMA Pediatr. 2020;174(9):821-2.