Molecular mechanisms of congenital hyperinsulinism

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Abstract

Congenital hyperinsulinism (CHI) is a complex heterogeneous condition in which insulin secretion from pancreatic β -cells is unregulated and inappropriate for the level of blood glucose. The inappropriate insulin secretion drives glucose into the insulin-sensitive tissues, such as the muscle, liver and adipose tissue, leading to severe hyperinsulinaemic hypoglycaemia (HH). At a molecular level, genetic abnormalities in nine different genes (*ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HNF4A*, *HNF1A*, *SLC16A1*, *UCP2* and *HADH*) have been identified which cause CHI. Autosomal recessive and dominant mutations in *ABCC8/KCNJ11* are the commonest cause of medically unresponsive CHI. Mutations in *GLUD1* and *HADH* lead to leucine-induced HH, and these two genes encode the key enzymes glutamate dehydrogenase and short chain 3-hydroxyacyl-CoA dehydrogenase which play a key role in amino acid and fatty acid regulation of insulin secretion respectively. Genetic abnormalities in *HNF4A* and *HNF1A* lead to a dual phenotype of HH in the newborn period and maturity onset-diabetes later in life. This state of the art review provides an update on the molecular basis of CHI.

Key Words

- ▶ glucose
- ▶ insulin
- ▶ congenital hyperinsulinism
- ▶ hypoglycaemia
- ▶ K_{ATP} channels
- ▶ genetics
- ▶ human
- ▶ development

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Introduction

Congenital hyperinsulinism (CHI) refers to a group of disorders characterised by dysregulated insulin secretion from pancreatic β -cells. As insulin is the key hormone involved in the regulation of the blood glucose level, its inappropriate release leads to persistent hyperinsulinaemic hypoglycaemia (HH). This drives glucose into the insulin sensitive tissues and simultaneously suppresses lipolysis and ketogenesis. The combined hypoketonaemic and hypoglycaemic characteristic of CHI increases the risk of brain injury in infants and children with this disorder (Aynsley-Green *et al.* 2000).

CHI typically presents in the newborn period with symptoms of hypoglycaemia, with most cases being sporadic; however, late-onset presentations of CHI can also occur. The general CHI prevalence is 1:50 000 births increasing to 1:2500 in consanguineous families (Bruining 1990).

The molecular basis of CHI involves defects in key genes that control the complex mechanisms of insulin secretion in the pancreatic β -cell. Insulin secretion is controlled to keep the fasting blood glucose level between 3.5 and 5.5 mmol/l. Genetic mechanisms that perturb this precise control lead to HH. This review discusses the current knowledge of the molecular mechanisms that lead to unregulated insulin secretion.

Normal physiological mechanisms of insulin secretion

Insulin secretion from pancreatic β -cells is primarily regulated by glucose metabolism. Post-prandial glucose enters the β -cells via the non-insulin-dependent glucose transporter type 2 (GLUT2). Glucose is then metabolised

via glycolysis and tricarboxylic acid (TCA) cycles and yields the high-energy molecule, ATP. Pancreatic β -cells control insulin release by two major pathways; by means of the ATP-sensitive potassium channels (K_{ATP}) and by K_{ATP} -channel-independent means.

The K_{ATP} -dependent control of insulin release is dependent on changes in the ratio of ATP:ADP leading to closure of potassium channels and promotes membrane depolarisation. This triggers voltage-gated calcium channels to open causing an influx of extracellular calcium, which leads to exocytosis of insulin-containing granules. On the other hand K_{ATP} -independent pathways involves the augmentation of insulin secretion by increasing cytosolic Ca^{2+} concentration and the recruitment of both β -cell protein kinases A and C to promote insulin exocytosis (Gembal *et al.* 1992, Komatsu *et al.* 1995).

In this review, we discuss the molecular mechanisms of CHI that have given valuable insights into β -cell physiology and have aided in patient care and management. We outline the genetic basis of CHI and crucial studies that have been pivotal in understanding this complex and rare disease.

Molecular genetics of CHI

So far, abnormalities in nine genes have been implicated in CHI; these are classified into two categories namely: 'channelopathies' and 'metabolopathies' (Dunne *et al.* 2004). The former is attributed to the K_{ATP} channel genes (*ABCC8* and *KCNJ11*) and latter to genes regulating different metabolic pathways (*GLUD1*, glucokinase (*GCK*), hepatocyte nuclear factor 4 homeobox A (*HNF4A*), *HNF1A*, *SLC16A1*, uncoupling protein 2 (*UCP2*) and *HADH*) (Table 1). The most common disorders are those affecting the K_{ATP} channel genes and these are predominantly recessive mutations. The other seven genes are less common causes of CHI, but are dominant mutations.

Table 1 Incidence of CHI due to known genetic mutations

Gene	Number of CHI cases due to this gene (%)	References
<i>ABCC8/KCNJ11</i>	36.3	Kapoor <i>et al.</i> (2013)
<i>GLUD1</i>	5.9	Flanagan <i>et al.</i> (2010)
<i>HADH</i>	1	Kapoor <i>et al.</i> (2013)
<i>GCK</i>	<1	Glaser <i>et al.</i> (1998)
<i>SLC16A1</i>	<1	Otonkoski <i>et al.</i> (2003)
<i>HNF4A</i>	5	Flanagan <i>et al.</i> (2010)
<i>HNF1A</i>	≪1	Snider <i>et al.</i> (2013)
<i>UCP2</i>	≪1	Gonzalez-Barroso <i>et al.</i> (2008)

Channelopathies

The *ABCC8* gene: K_{ATP} -CHI The *ABCC8* gene is found on chromosome 11p15.1, and contains 39 exons (Inagaki *et al.* 1995). This encodes the sulfonylurea receptor 1 (SUR1) protein or an alternative spliced variant. SUR1 is a member of the ATP-binding cassette transporters, which promote ATP hydrolysis to transport substrates. However, SUR1 does not directly transport substrates, instead it regulates the activity of Kir6.2 in the K_{ATP} channel complex. A single SUR1 subunit is composed of three transmembrane domains (TMD0, TMD1 and TMD2) containing 17 transmembrane helices, two cytosolic nucleotide-binding domains (NBD1 and NBD2) and a cytosolic linker (L0) (Fig. 1).

The *KCNJ11* gene: K_{ATP} -CHI Like *ABCC8*, the *KCNJ11* gene is also located on chromosome 11p15.1, containing a single exon. This encodes Kir6.2, a 390 amino acid protein (Inagaki *et al.* 1995). It is originally from a superfamily that is further divided into seven subfamilies ranging from Kir1 to Kir7. Kir6.2 is an unconventional pore-forming subunit of K_{ATP} channels which acts as a bio-diode in the cell surface. Kir proteins favour passing positive currents 'inward', even when the potassium (K^+) concentration is equal on both sides of the membrane, and hence is termed an 'inward rectifier'. Kir6.2 is a 'weak' inward rectifier unlike classic Kir channels (Doupnik *et al.* 1995). A single Kir6.2 subunit contains two putative TMDs, linked by an extracellular pore-forming region and cytoplasmic -NH₂ and -COOH terminal domains. Within the K_{ATP} channel complex, the Kir6.2 subunit forms a tetrameric complex, harbouring the inhibitory binding site (Saint-Martin *et al.* 2011).

K_{ATP} -CHI Recessive inactivating mutations in the *ABCC8* and *KCNJ11* genes are the most common cause of CHI. To date, 150 types of mutations in *ABCC8* and 24 in *KCNJ11* have been identified (Flanagan *et al.* 2009), these account for approximately 36.3% of all CHI cases (Kapoor *et al.* 2013). Mutations in *ABCC8* or *KCNJ11* can affect the functioning of K_{ATP} channels by either affecting the surface expression or impairing the ability of MgADP to stimulate channel activity (Nichols *et al.* 1996). In either case, there is continuous depolarisation of the pancreatic β -cell membrane which leads to unregulated insulin secretion.

Class 1 mutations in *ABCC8* or *KCNJ11* lead to proteins which fail to reach the plasma membrane due to the impaired synthesis or maturation of SUR1.

Figure 1

Image of K_{ATP} channel structure in the cell membrane. (A) SUR1 is the regulatory subunit composed of three TMDs (TMD0, TMD1 and TMD2), each with five to six transmembrane helices. There are two cytosolic nucleotide-binding domains (NBD1 and NBD2), and the conserved sequences include the Walker A and Walker B motifs. The linker regions are located between the Walker A and B motifs. Kir6.2 is the pore-forming subunit. It is a tetrameric complex made up of four proteins, each with two

putative membrane-spanning domains, which are linked by an extra-cellular pore-forming region and cytoplasmic -NH₂ and -COOH terminal domains. Both SUR1 and Kir6.2 contain the RKR sequence which is the endoplasmic reticulum retention signal. (B) Illustration of the predicted octameric structure of K_{ATP} channels. It is made up of four Kir6.2 proteins in the centre forming the pore, each with an associated SUR1 protein.

Alternatively, class 1 mutations can also lead to defective trafficking of SUR1 and as Kir6.2 expression is dependent on SUR1 surface expression, Kir6.2 is also absent from the membrane (Saint-Martin *et al.* 2011). Whereas, class 2 mutations produce K_{ATP} channel complexes which are present in the cell membrane but are non-functional. These defective K_{ATP} channels are insensitive to the changing concentrations of MgADP, hence they remain closed even when the glucose levels are low (Saint-Martin *et al.* 2011).

Recessive *ABCC8/KCNJ11* mutations The molecular mechanisms of recessive *ABCC8* or *KCNJ11* disease causing mutations have been described in the literature over the past 18 years. The molecular basis involves the following mechanisms:

Defects in biogenesis and turnover Results of pulse labelling studies have been used to identify individual SUR1 expression with a half-life of 25.5 h, whereas Kir6.2 has a shorter half-life of just 36 min. However, when the two subunits are co-expressed, Kir6.2 degradation is eliminated. The *ABCC8* F1388del mutation and the *KCNJ11* W91R mutation are two examples of mutations resulting in subunits that associate, but then dissociate freely and degrade rapidly, affecting biogenesis and turnover of K_{ATP} channels (Crane & Aguilar-Bryan 2004).

Defects in channel trafficking The correct formation of K_{ATP} channels in the endoplasmic reticulum (ER) requires the shielding of the ER retention signal (RKR) (Arg-Lys-Arg)

and enables the protein complex to exit the ER. The RKR is present in the C-terminal region of Kir6.2 and N-terminal side of NBD2 in SUR1. Hence the last 36 amino acid residues of Kir6.2 are highly significant as they contain the RKR signal peptide. Removal of these amino acids results in the surface expression of homotetrameric Kir6.2 in the absence of the SUR1 proteins (Tucker *et al.* 1997).

E282K is a *KCNJ11* mutation which prevents the K_{ATP} channel surface expression; however, unlike other trafficking mutations this one does not affect protein folding (Taneja *et al.* 2009). It has been reported that the C-terminus of the SUR1 subunit is required for K_{ATP} channel function (Giblin *et al.* 2002) whilst others have shown it to be required for membrane expression (Sharma *et al.* 1999).

Abnormalities in regulation The SUR1 protein acts as conductance regulator of Kir6.2, and both subunits are sensitive to changes in nucleotides such as ATP, ADP, GTP and GDP. ATP-induced inhibition of Kir6.2 is countered by the activation of ADP at SUR1. The T1139M and R1215Q mutations in the *ABCC8* gene cause loss of ADP-dependent gating properties of the channel and result in constitutive inhibition of K_{ATP} channels by ATP (Dunne *et al.* 2004). The R1420C mutation is located in SUR1-NBD2, and results of mutational studies have indicated that it reduces the affinity of NBD2 for ATP and ADP (Matsuo *et al.* 2000).

Dominant *ABCC8/KCNJ11* channel mutations The identification of a single-dominant *ABCC8* mutation causing CHI was identified by Huopio and colleagues.

E1506K is a dominant heterozygous mutation, which changes the amino acid at position 1506 from glutamic acid (E) to lysine (K), and caused CHI in seven related patients (Huopio *et al.* 2000). The functional consequences of the E1506K mutant were determined using a *Xenopus laevis* oocytes expression system, and studied using electrophysiological techniques. The cells were investigated under metabolic inhibition using NaAzide, which is known to stimulate K_{ATP} channels in intact cells (Trapp & Ashcroft 2000). The results indicate that when E1507K is expressed alone it is not responsive to metabolic inhibition, but with the addition of diazoxide it elicited a small current. This indicates that, unlike recessive *ABCC8* mutations, the E1506K mutant SUR1 subunit could form K_{ATP} channels with Kir6.2 which can be activated by diazoxide. The mutation is distal to the Walker B motif, which a highly conserved region known to be activated by nucleotides. Hence it is likely that the mutation prevents Mg^{2+} binding to the SUR1 Walker B motif, thereby impairing the Mg^{2+} binding interactions with SUR1 (Huopio *et al.* 2000).

The most significant difference between dominant and recessive mutations is diazoxide-responsiveness (MacMullen *et al.* 2011). Unlike patients carrying recessive mutations, patients with dominant mutations are able to maintain normoglycaemia with diazoxide and regular feeds. This is consistent with the functional analysis of dominant mutations which displays normal channel assembly and trafficking to the plasma membrane, whereby the normal allele confers partial channel response to increased ATP and diazoxide, thus eliminating the need for a pancreatectomy. This is in stark contrast to recessive CHI where a near-total pancreatectomy is the likely outcome for most patients (Shilyansky *et al.* 1997, Otonkoski *et al.* 2006).

The phenotype associated with dominant *ABCC8/KCNJ11* mutations is also quite varied, ranging from asymptomatic to persistent HH in childhood (Kapoor *et al.* 2011). For adult mutation carriers this may be a cause of dominantly inherited early-onset diabetes.

Dominant mutation carriers developing diabetes A prominent feature observed in some dominant mutation carriers is their tendency to develop diabetes mellitus (DM). However, not all cohorts have reported DM, despite family members experiencing hypoglycaemia continuing into adulthood and beyond the age of 60 (Thornton *et al.* 2003). The proposed mechanism for development of DM has been attributed to β -cell apoptosis mediated via enhanced β -cell depolarisation, resulting in increased Ca^{2+} entry into the cell (Huopio *et al.* 2000). Hence, the use of diazoxide may protect β -cells by

reducing Ca^{2+} influx by delaying onset of apoptosis or postpone development of DM. β -cell apoptosis or the development of DM has not been observed in SUR1 knock-out (KO) mice or Kir6.2 KO mice. This indicates that there are K_{ATP} channel-independent pathways involved in the regulation of insulin secretion (Pinney *et al.* 2008).

Dominant *ABCC8/KCNJ11* mutations with medically unresponsive diffuse disease Results of several studies have indicated the presence of dominant inactivation of mutations in *ABCC8* and *KCNJ11* which are medically unresponsive. In one study 12 novel missense mutations from 15 families were identified (MacMullen *et al.* 2011). Most required a pancreatectomy (15/17). The patients were large for gestational age, and the median age during presentation was closer to that of recessive patients, indicating a more severe form of CHI. The diazoxide-unresponsive mutations have been located in NBD2, an area where many of the known diazoxide-responsive mutations have been found. This indicates that the clinical effects of dominant mutations cannot be distinguished by location alone (MacMullen *et al.* 2011).

It has been largely assumed that dominant *ABCC8/KCNJ11* mutations which cause CHI are mainly diazoxide-responsive, due to the availability of 'spare channels' made entirely of WT K_{ATP} channel subunits. However the 'spare' channel theory is not sufficient to explain the mechanism by which dominant mutations cause severe medically unresponsive CHI.

Paternal mutations in *ABCC8/KCNJ11* causing diffuse disease There are two histological forms of CHI: focal CHI (FCHI) and diffuse CHI (DCHI). The focal form occurs when abnormal pancreatic β -cells are localised to a single location in the pancreas, resulting in a focal lesion. The diffuse form occurs when all the cells in the pancreas are abnormal in size, and have hypertrophied insulin cells (De Lonlay *et al.* 1997).

Patients who have a single paternally inherited recessive mutation in the *ABCC8* or *KCNJ11* gene could have a focal form of CHI, which accounts for 30–40% of all cases (De Lonlay *et al.* 1997). However, there are now clinical descriptions of heterozygous paternal mutations causing DCHI and atypical CHI (Chandran *et al.* 2013). It may be the case that paternal mutations causing DCHI act via a different mechanism from recessive mutations, which generally cause DCHI that is unresponsive to diazoxide. There is also the possibility of a second mutation in the maternal allele, which was not detected by the initial genetic analysis; alternatively there could be a mutation in a non-*ABCC8* non-*KCNJ11* gene.

The other known genetic causes of CHI have so far been identified in seven genes involved in β -cell dysregulation and inappropriate insulin release (Fig. 2).

Metabolopathies

The *GLUD1* gene: hyperinsulinism/hyperammonaemia syndrome Glutamate is a key compound involved in amino-acid-induced modulation of insulin secretion. Dietary proteins are metabolised to amino acids, which serve as alternative metabolic fuel source in the absence of glucose. Glutamate is degraded by transamination to form α -ketoglutarate (and ammonia in the liver and kidney) and enters the TCA cycle to promote ATP and insulin exocytosis.

The glutamate dehydrogenase (GDH) enzyme regulates the entrance of glutamate into the TCA cycle and catalyses the glutaminolysis reaction. Leucine is thought to bind to the GDH catalytic site and promote enzyme action. Two additional sites on GDH also exist: the allosteric inhibitory site which binds GTP and ATP and an ADP-binding activator domain. These sites are thought to regulate the rate of glutaminolysis (Li *et al.* 2003). The *GLUD1* gene encodes GDH, and *GLUD1*-activating mutations are the next most genetically

prevalent cause of CHI after *ABCC8/KCNJ11* (Li *et al.* 2003). Mutations in the *GLUD1* gene appear to affect GDH's allosteric binding site. These mutations are found in exons 11 and 12 and have been reported to account for more than half of the cases in a cohort of 48 unrelated GDH–CHI patients (Stanley *et al.* 2000). However, mutations have also been identified in exons 6 and 7 of the catalytic domains, causing normal GDH activity but diminished GTP-sensitivity (Santer *et al.* 2001).

The majority of *GLUD1* mutations occur *de novo*, but some are due to autosomal inheritance in a dominant fashion (Weinzimer *et al.* 1997, Stanley *et al.* 1998). GDH–CHI is also referred to as the hyperinsulinism/hyperammonaemia (HI/HA) syndrome due to fasting or recurrent postprandial HH coupled with elevated serum ammonia. The HH link to leucine-sensitivity was initially established by Cochrane *et al.* (1956) who reported on three related patients experiencing hypoglycaemia on leucine ingestion.

Moreover, elevated levels of plasma glutamine are associated with other hyperammonaemic conditions such as Reye's syndrome, but in the case of GDH (HI/HA)–CHI, this is absent (Weinzimer *et al.* 1997). All other amino acids in these patients appear within the normal ranges. Initially, the HA was proposed to arise from the liver, but

Figure 2

Known genetic mechanisms of CHI.

was later discovered to be due to renal ammoniogenesis (Treberg *et al.* 2010). HI/HA–CHI patients are diazoxide-responsive but also require a diet low in protein.

The *SCHAD* gene: protein-sensitive CHI The *SCHAD* (or *HADH*) gene encodes the mitochondrial enzyme, short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD). This enzyme is crucial for β -oxidation of fatty acids which act as the alternative source of fuel during fasting. The process of β -oxidation produces acetyl-CoA, but in the absence of carbohydrates, the acetyl-CoA cannot be recycled via the TCA cycle and its accumulation activates ketogenesis. In the penultimate step of β -oxidation, SCHAD catalyses the oxidation of 3-hydroxyacyl-CoA to 3-ketoacyl-CoA. The transcription factor *FOXA2* regulates SCHAD and is essential for β -cell function and differentiation. Results of studies of β -cell-specific *Foxa2* mice null have revealed a threefold reduction in *Schad* expression that also appears to exhibit HH (Sund *et al.* 2001).

SCHAD–CHI is caused by inactivating mutations in the *SCHAD* gene and is inherited in an autosomal recessive manner. Until recently, the role of SCHAD in CHI was unknown. Results of phenotyping of *Hadh*-null mice led to the identification of the close relationship between the SCHAD and the GDH enzyme (Li *et al.* 2010, Heslegrave *et al.* 2012). SCHAD appears to be a negative regulator of insulin secretion and in SCHAD–CHI, SCHAD loses its inhibitory action on GDH (Li *et al.* 2010, Heslegrave *et al.* 2012). Most patients affected are from consanguineous families and are diazoxide-responsive.

To date, ten patients have been identified as having SCHAD–CHI, presenting with varying symptoms from mild late-onset hypoglycaemia to severe neonatal hypoglycaemia. Though HI/HA and SCHAD–CHI patients have similar leucine-sensitive features (Heslegrave *et al.* 2012), SCHAD–CHI do not present with HA.

The *GCK* gene: GCK–CHI The *GCK* gene is conserved amongst different species. Two tissue-specific promoter regions of exon 1 exist, with one specific for the liver and the other for the pancreatic β -cells. Though these two promoters give rise to different molecules, the two isoforms remain functionally similar.

GCK, which encodes the GCK enzyme, is particularly central to the glycolytic pathway and is referred to as the ‘glucose sensor’. Its key role is to catalyse the phosphorylation of carbon-6 on glucose giving rise to glucose-6-phosphate (G6P). This in turn is metabolised further and activates insulin release via the regulation by K_{ATP} and calcium channels. The rate of phosphorylation of glucose

in the β -cells is dependent on the physiological glucose concentration and thus regulates insulin secretion accordingly. These features include a low affinity for glucose molecules and the absence of inhibition by G6P.

Currently, heterozygous gain-of-function *GCK* mutations (which are inherited in an autosomal-dominant manner) are known to cause CHI. However, to date there is no support for an increase in *GCK* expression causing CHI. So far 15 mutations of GCK–CHI have been identified in various sites of the enzyme, including the allosteric domain (A456V, V455M, T65I, G68V, V91L, E422K, V389L, T103S, W99R, W99L, S64Y, V452L, ins454A, M197I and Y214C; Matschinsky 2002). GCK–CHI patients have a higher affinity for glucose and lower threshold for glucose-stimulated insulin secretion (GSIS). CHI caused by *GCK* mutations accounts for around 7% of medically responsive HH and is mainly diazoxide-responsive, although unresponsive forms have also been described. Clinical presentation of GCK–CHI can vary from postprandial hypoglycaemia to mild fasting hypoglycaemia or even seizures.

The *SLC16A1* gene: exercise-induced-CHI During glycolytic metabolism, glucose gives rise to pyruvate which is either converted to lactate (during anaerobic exercise) or enters the TCA cycle to produce reducing equivalents for ATP synthesis. The pancreatic β -cells carefully control the metabolic demand to ensure that blood glucose levels remain within a tight and narrow range. Hence, insulin release requires effective stimulation when glucose concentrations are high and is required switch off just as effectively during states of hypoglycaemia.

These glucose-mediated features which promote insulin release include expression of the glucose-sensing genes such as *GLUT2* and *GCK*, which together enable degradation of glucose as blood sugar levels rise. In addition, by directing glycolysis-derived pyruvate to the mitochondrial TCA cycle, the new pathway diverts insulin secretion by glucose-independent means (Sekine *et al.* 1994). This speculation was confirmed by the finding of very low lactate dehydrogenase A expression in pancreatic β -cells as well as slow transport of lactate into the cells (Sekine *et al.* 1994).

The *SLC16A1* gene encodes the monocarboxylate transporter subtype 1 (MCT1). Generally, MCT1 expression is either low or undetectable in β -cells. *SLC16A1* genetic defects are gain-of-function mutations and promote increased β -cell MCT1 expression which is otherwise transcriptionally silenced. The transporter is therefore thought to allow circulating pyruvate to enter

the β -cells and fuel the TCA cycle, and thus inappropriately promote insulin secretion. However, the genotype–phenotype link between MCT1 and CHI was confirmed only after mice overexpressing *Mct1* in β -cells were generated (Pullen *et al.* 2012).

SLC16A1 mutations are also referred to as exercise-induced-CHI and characteristically induce HH after strenuous exercise or during a pyruvate load (Otonkoski *et al.* 2003). HH is inherited in an autosomal-dominant manner, and mutations have been identified in the regulatory regions of *SLC16A1* (Otonkoski *et al.* 2003). Patients are diazoxide-responsive and are advised to avoid strenuous exercise.

The HNFs Transcription factors are formed by a DNA-binding domain specific for a short sequence of DNA and a domain that influences the transcription of genes by activators or repressors. HNFs are expressed in the pancreas and are involved in pancreatic development, β -cell mass and insulin release.

The *HNF4A* gene: nuclear transcription factor 1; CHI to maturity-onset diabetes of youth 1 switch *HNF4A* encodes the HNF4a protein. The gene has two promoter regions, P1 and P2, which have tissue-specific expression. The P1 promoter gives rise to hepatic-tissue-derived *HNF4A* isoforms, whereas the P2 transcription is most abundant in the pancreatic β -cells and important in the regulation of GSIS (Miura *et al.* 2006).

Heterozygous dominant inactivating mutations of *HNF4A* cause CHI and then eventually maturity-onset diabetes of youth (MODY1: an autosomal dominant disorder; Pearson *et al.* 2007). Mice lacking β -cell *Hnf4a* have been reported to have a reduction in the expression of *Kcfn11* (Gupta *et al.* 2005). However, this finding has not been reproduced in other *Hnf4a* mice models (Miura *et al.* 2006), hence the mechanism of HNF4a action is still unknown.

Further speculation regarding the switch from hypoglycaemia to hyperglycaemia includes the notion that hypersecretion of insulin causes exertion on the β -cells (Glaser 2007). Other possible mechanisms of HNF4a–CHI may be the loss of interactions between HNF4a and other nuclear factors such as peroxisome proliferator-activated receptor α (PPAR α).

Exposure to low fatty acid concentrations has been shown to promote a loss of GSIS in islets, which is reversible on fatty acid replacement (Stein *et al.* 1996). Long-term exposure to fatty acids causes a rise in the level of transcription factors such as PPAR- α . It has been suggested that PPAR- α directs fatty acids to the

β -oxidation pathway and promotes an elevation of insulin secretion during hypoglycaemia (Sugden & Holness 2004). This hypothesis is supported by the reduction in PPAR- α expression in β -cell *Hnf4a*-null mice (Gupta *et al.* 2005). Moreover, *Ppara*-null mice have also been reported to develop fasted HH (Gremlich *et al.* 2005).

In HNF4a–CHI patients, severity of fasting hypoglycaemia ranges from transient to persistent, requiring medical therapy. However, the majority of cases appear to be diazoxide-responsive and HH characteristically improves with age. However, the mechanism for this mutation still remains unknown.

The *HNF1A* gene: nuclear transcription factor 2; CHI to MODY3 switch *HNF1A* is a gene that encodes the protein HNF1- α . It is found highly expressed in the liver as well as the pancreatic acinar- and β -cells. HNF1- α is thought to be involved in the expression of several tissue-specific genes. Loss-of-function of HNF1- α has been identified in macro-somic births presenting with diazoxide-responsive CHI. However, in later life, patients appear to switch from hypoglycaemia to MODY3. Though the loss of HNF1- α function does inevitably cause insulin dysregulation as shown using *Hnf1a*-null mice (Pontoglio *et al.* 1998), the exact mechanism is unknown.

Moreover, the pancreatic *HNF4A* P2 promoter appears to regulate HNF1- α because binding sites for this exist on its P2 region. Furthermore, deletion of a HNF4- α binding site on the *HNF1A* promoter abolishes the promoters activity (Kuo *et al.* 1992). The results of these studies have highlighted the importance of these interlinked transcription factors and the involvement they have in causing this dual phenotype of hypoglycaemia to hyperglycaemia during development. However, further work is still required to elucidate the exact mechanism of the dysregulation that causes HH and then MODY.

The *UCP2* gene UCP2 is a mitochondrial carrier and is encoded by the *UCP2* gene found in the pancreatic α - and β -cells. UCP2 contains six TMDs and one nucleotide binding site, which dimerises and forms a proton channel. UCP2 is thought to produce proton leaks across the inner mitochondrial membrane, which uncouples oxidative phosphorylation from ATP synthesis (Zhang *et al.* 2001).

Rats that fasted for 24 h had an 82% increase in islet *Ucp2* expression (Gremlich *et al.* 2005). However, UCP2 has been implicated as a negative regulator of insulin secretion because it reduces the formation of ATP from glucose. PPAR- α is also known to upregulate islet UCP2 expression in hypoglycaemic states, such as fasting.

Under normal conditions, UCP2 reduces ATP synthesis and thus suppresses GSIS even in the presence of enhanced fatty acid oxidation (Tordjman *et al.* 2002).

Ucp2-null mice have been shown to display HH with an increase in islet ATP production (Zhang *et al.* 2001). This led to the discovery of an inactivating *UCP2* mutation in a group of CHI patients who were all negative for the other known genetic causes of HH (Gonzalez-Barroso *et al.* 2008). However, the results of this study indicated that *UCP2* indirectly inhibited ATP production. It has also been proposed that *UCP2* acts to reduce the formation of mitochondrial reactive oxygen species and thus attenuate GSIS (Emre *et al.* 2007). In contrast, findings of studies using *Ucp2* transgenic mice as well as *in vitro* studies using β -cell lines did not support the hypothesis that a rise in *UCP2* *per se* decreases insulin secretion. Hence, a debate still exists as to whether *UCP2* mutations promote hypoglycaemia.

UCP2 loss-of-function appears to be inherited in an autosomal-dominant fashion and patients described so far are reportedly diazoxide-responsive. The mutation was originally identified in two unrelated patients with no known genetic cause of CHI (Gonzalez-Barroso *et al.* 2008). However, the mutation may cause transient or mild fasting CHI since the patients described are less than 6 years old.

Histological characterisation of CHI

Currently, there are three histological forms of CHI: FCHI, DCHI and atypical. FCHI occurs when the abnormal pancreatic β -cells are localised to a single specific location in the pancreas. The DCHI form occurs when all the islet cells in the pancreas are abnormal (Goossens *et al.* 1989, Sempoux *et al.* 2004). The clinical presentation of FCHI and DCHI appear to be similar, although their molecular mechanisms are quite different. In most cases DCHI is inherited in an autosomal recessive manner, whereas FCHI is sporadic (Huopio *et al.* 2000, Henquin *et al.* 2011). However, where the cases are difficult to histologically categorise these are classified as atypical forms. Generally, atypical forms are thought to be due to morphological mosaicism in a portion of the pancreas.

Molecular basis of FCHI

Focal lesions are the result of two unique events, first the inheritance of a paternally inherited *ABCC8* or *KCNJ11* mutation at 11p15.1, and secondly the loss of the corresponding maternal allele within the focal lesion.

This causes an imbalance in the expression of the imprinted genes such as the maternally expressed tumour suppressor gene *H19* and *CDKN1C*, and the paternally expressed growth factor *IGF2* at 11p15.5 (Sempoux *et al.* 2011) leading to β -cell hyperplasia. This loss of heterozygosity for the maternal 11p region causes a disruption in the expression of genes in a single pancreatic β -cell clone, which leads to deregulated insulin secretion from the resulting focal islet cell adenomatous hyperplasia with abnormally large nuclei.

A duplication of the paternal allele located on chromosome 11 has also been found in cases of FCHI (Damaj *et al.* 2008). Outside the lesion, normal endocrine tissue with small nuclei exists. These cells appear to have less cytoplasm and diminished proinsulin production (Rahier *et al.* 1984, 2000, Goossens *et al.* 1989, Sempoux *et al.* 1998).

Patients who have a heterozygous paternally inherited mutation in the *ABCC8* or *KCNJ11* gene could have FCHI, which accounts for 30–40% of all CHI cases (Rahier *et al.* 1998). FCHI is confirmed by a fluorine-18 dihydroxyphenylalanine-positron emission tomography scan, which can show the presence of a focal lesion and determine its location with diagnostic accuracy (Otonkoski *et al.* 2006). Surgery is needed to remove the focal lesion(s), which usually cure the patients of the hypoglycaemia.

Molecular basis of DCHI

Patients with a homozygous recessive or a compound heterozygote mutation in their *ABCC8* or *KCNJ11* genes present with DCHI. These patients are usually medically unresponsive, and this histological form accounts for 60–70% of all CHI cases. In the DCHI state all the pancreatic β -cells are affected (Rahier *et al.* 2000). Most islets throughout the entire pancreas are affected with the presence of large hyperchromatic nuclei (Rahier *et al.* 1984, 2000, Goossens *et al.* 1989, Sempoux *et al.* 1998, 2004). The vast majority of patients in this category undergo a 'near total pancreatectomy', which means more than 95% of the pancreas is removed through surgery, causing diabetes and exocrine insufficiency.

Molecular basis of atypical CHI

Histologically, atypical forms of CHI are categorised when the pancreatic morphology does not fit into the FCHI or DCHI types and are a mosaic pattern of the two (Hussain *et al.* 2008, Sempoux *et al.* 2011). The islets can be either enlarged or shrunken. Some cases have been cured with

a lesionectomy, whilst others also required medical management. However, to date only one patient has been described with a *ABCC8* nonsense mutation (Q54X) causing this histological form of CHI (Hussain *et al.* 2008). Results of studies have indicated that the heterogeneous expression of β -cells hexokinase 1 in the atypical CHI may be causing the abnormal insulin release (Henquin *et al.* 2013, Shi *et al.* 2015).

Conclusion

CHI is a complex heterogeneous condition which leads to unregulated insulin secretion causing HH. The molecular basis of CHI involves perturbations in the key genes involved in controlling insulin secretion from pancreatic β -cells. Interestingly the molecular basis of CHI is only known in about 50% of patients so far, indicating that there are as yet unidentified mechanisms involved in the regulation of insulin secretion. Understanding these novel mechanisms will provide fundamental new insights into the function of the pancreatic β -cell.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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