

*Disease Reviews*  
IN ENDOCRINOLOGY

Congenital  
Hyperinsulinism  
(*ABCC8*, *KCNJ11*,  
*GLUD1*, *GCK*)

*Testing You Can Count On*



**Frequently Used Abbreviations:** **CH:** Congenital Hyperinsulinism; **GK:** glucokinase; **GDH:** glutamate dehydrogenase; **HI:** Hyperinsulinism; **KATP channel:** ATP-regulated potassium channel; **SCHAD:** short-chain 3-hydroxyacyl-CoA dehydrogenase.

## Introduction

Congenital Hyperinsulinism (CH), which is also known as familial hyperinsulinism (FHI) or persistent hyperinsulinemic hypoglycemia of infancy (PHHI), is the most frequent cause of severe, persistent hypoglycemia in newborns and children. In 25-40% of affected neonates, CH leads to lasting consequences such as developmental delay, mental retardation, or even death.<sup>1</sup> CH is due to unregulated insulin release from either the entire pancreas (diffuse CH) or confined pancreatic areas (focal CH). In most countries, CH occurs at an approximate frequency of 1/25,000 to 1/50,000 live births.

To date, mutations in any one of five different genes have been associated with CH. Depending on the underlying genetic defect, prognosis and treatment of CH may vary. While CH due to mutations in *GLUD1*, *GCK*, or *HADHSC* are generally responsive to drug therapy, CH associated with mutations in *ABCC8* or *KCNJ11* often requires variable degrees of pancreatectomy.<sup>2,3</sup> Mutations in *ABCC8* or *KCNJ11* can cause both focal CH, which can be cured by partial pancreatectomy, and diffuse CH, which may require near-total pancreatectomy, potentially leading to life-long sequelae.<sup>4</sup>

**Genetic testing for CH-associated loss-of-function mutations in *ABCC8*, *KCNJ11*, *GLUD1*, and *GCK* can diagnose about 85%<sup>5</sup> of the most severe cases of CH. In addition, genetic testing may allow distinction between diffuse and focal forms of *ABCC8*- or *KCNJ11*-associated CH in many cases and identify cases of CH that are likely to respond to drug therapy.<sup>6</sup>**

## Types and Causes of CH

Known Types of CH		Mutated Gene	Prevalence	Typical Therapy
KATP HI	Diffuse Focal	<i>ABCC8</i>	45%	Near-total pancreatectomy Partial pancreatectomy
KATP HI	Diffuse Focal	<i>KCNJ11</i>	5%	Near-total pancreatectomy Partial pancreatectomy
GDH HI	Diffuse	<i>GLUD1</i>	5%	Diazoxide, low leucine (protein) diet
GK HI	Diffuse	<i>GCK</i>	<1%	Diazoxide
SCHAD HI	Diffuse	<i>HADHSC</i>	unknown	Diazoxide

### KATP Hyperinsulinism (KATP HI)

KATP HI is due to loss-of-function mutations in either *ABCC8* or *KCNJ11*, which encode the two protein components (Sur1 and Kir6.2, respectively) of the ATP-regulated potassium channels (KATP channels) in the membrane of pancreatic  $\beta$ -cells.<sup>2</sup> Closing of the KATP channels represents a crucial step in the complex chain of events that links a rise in blood glucose

concentration to insulin release (see Normal Physiology of Insulin Release). In KATP HI, KATP channels are defective and remain closed most of the time, decoupling insulin release from the blood glucose concentration.

Of note, gain-of-function mutation in *KCNJ11* do not lead to CH, but to the “complementary” condition, neonatal diabetes mellitus.<sup>7</sup>

### **Glutamate Dehydrogenase Hyperinsulinism (GDH HI)**

GDH HI is due to gain-of-function mutations in *GLUD1*, the gene for the enzyme glutamate dehydrogenase (GDH).<sup>8</sup> GDH catalyzes the oxidative deamination of glutamate, leading to a rise in the intracellular ATP/ADP ratio. GDH activity is stimulated by leucine and inhibited by GTP. CH-associated mutations in GDH lower the enzyme's sensitivity to its inhibitor GTP, allowing increased enzymatic activity in response to leucine. In pancreatic  $\beta$ -cells, the increased GDH activity can give rise to an intracellular ATP/ADP ratio sufficient to trigger insulin release, decoupling insulin release from blood glucose concentration.

### **Glucokinase Hyperinsulinism (GK HI)**

GK HI is due to gain-of-function mutations in *GCK*, the gene for the enzyme glucokinase (GK).<sup>9</sup> GK catalyzes the first and rate-limiting step of glycolysis and thus determines the rate of ATP production in  $\beta$ -cells in response to the blood glucose concentration. If GK activity is increased due to a gain-of-function mutation, an intracellular ATP/ADP ratio sufficient to trigger insulin release is reached at lower than normal glucose concentrations.

Of note, loss-of-function mutations in *GCK* do not lead to CH, but to the "complementary" condition, diabetes mellitus.<sup>10,11</sup>

### **SCHAD Hyperinsulinism (SCHAD HI)**

SCHAD HI is due to loss-of-function mutations in *HADHSC*, the gene for the enzyme short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD).<sup>3,12</sup> SCHAD is involved in fatty acid oxidation.

### **Congenital Hyperinsulinism Due to Unknown Causes**

In about 45% of all cases, the cause of CH is not clear. It is likely that at least some of these cases are due to regulatory or intronic mutations or major deletions/ rearrangements in the five genes known to be associated with CH.<sup>13</sup> In addition, mutations in other, as yet unidentified, genes may lead to CH.

### **Clinical Presentation of CH**

**KATP HI** can occur in a diffuse and a focal form. Focal CH has been estimated to account for 40-70% of all cases of CH requiring pancreatectomy, with the remainder due to diffuse CH.<sup>6,14,15</sup> Patients with KATP HI usually have high birth weights and present with severe hypoglycemia during the first days of life. In addition, they often manifest hypotonia, poor feeding, and apnea. Exceptions to this rule are cases of diffuse KATP HI caused by rare dominant mutations in *ABCC8*; such autosomal dominant diffuse KATP HI is associated with a much milder phenotype.<sup>16,17,18</sup>

**GDH HI** is a diffuse form of CH and typically much milder than KATP HI, so that it may not be recognized until the affected infant is several months old.<sup>19</sup> GDH HI is characterized by post-prandial hypoglycemic episodes and mildly elevated, apparently asymptomatic plasma ammonia levels. GDH HI is also known as hyperinsulinism-hyperammonemia syndrome.

**GK HI** is a diffuse form of CH with a typically very mild phenotype, although a severe case of GK HI has also been reported.<sup>20</sup> Onset of presentation can range from infancy to adulthood.<sup>9,21,22</sup>

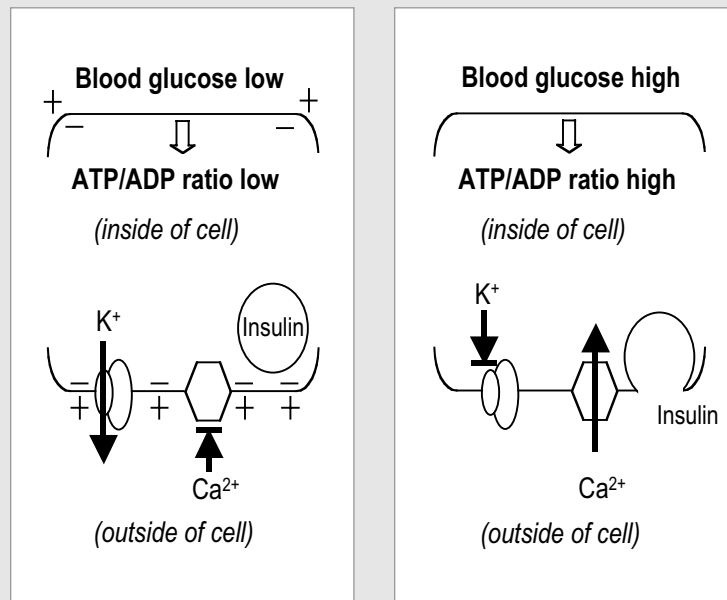
**SCHAD HI** is a diffuse form of CH that presents during infancy. The phenotype can range from mild to severe.<sup>3,12</sup>

### Normal Physiology of Insulin Release

When blood glucose levels are low, glycolysis in  $\beta$ -cells is limited by the sparse influx of glucose, and the intracellular ATP/ADP ratio is low. Under these conditions, KATP channels in the membrane of  $\beta$ -cells remain open, allowing  $K^+$ , but not  $Na^+$  ions, to freely pass through. The cell membrane of  $\beta$ -cells also contains energy-dependent ion pumps that pump  $Na^+$  ions out while pumping  $K^+$  ions in, thus creating opposing  $Na^+$  and  $K^+$  concentration gradients across the cellular membrane. Through the KATP channels,  $K^+$  ions can flow out of the cell along their concentration gradient, leaving behind an excess of negative charges carried by intracellular proteins. This excess of negative charges creates a negative membrane potential similar to the resting potential seen in nerve cells. The negative

membrane potential causes voltage-dependent  $Ca^{2+}$  channels in the  $\beta$ -cell membrane to remain shut and, consequently, the intracellular  $Ca^{2+}$  concentration to remain low. Under these conditions, no insulin is released from  $\beta$ -cells.

When blood glucose levels are high, more glucose is taken up by the  $\beta$ -cells, glycolysis is increased, and the intracellular ATP/ADP ratio rises. The increased intracellular ATP/ADP ratio triggers closing of the KATP channels in the cell membrane of the  $\beta$ -cells. Since  $K^+$  ions can no longer flow out of the cells, the membrane potential becomes more positive, causing the opening of voltage-gated  $Ca^{2+}$  channels in the membrane of the  $\beta$ -cells.  $Ca^{2+}$  ions flow in and stimulate the release of insulin from secretory vesicles stored in the cells.



## Diagnosis of CH

CH is suspected in infants and young children with episodes of spontaneous severe hypoglycemia lasting longer than 48 hours. Since insulin levels in patients with CH may fluctuate widely over time, diagnosis cannot always be achieved by demonstrating an elevated blood insulin concentration at the time of hypoglycemia. Other signs of unregulated insulin action are hypoglycemia during glucose infusion, low blood levels of free fatty acids and ketones at the time of hypoglycemia, and a rise in blood glucose after glucagon administration at the time of hypoglycemia. A fasting study may be required to provoke hypoglycemia and confirm the diagnosis of CH. Due to the genetics of CH, a family history of CH is not always apparent.

It is important to distinguish between KATP HI, GDH HI, and GK HI, because treatment options may vary drastically. In addition, focal and diffuse KATP HI should be differentiated, since focal KATP HI can potentially be cured by partial pancreatectomy.

KATP HI can often, but not always, be identified through acute insulin response studies (for details, see call-out).<sup>23</sup> GDH HI is characterized

by elevated plasma ammonia levels and leucine-sensitive hypoglycemia; however, leucine-sensitivity has also been described in the case of KATP HI.<sup>18</sup> Presence of 3-hydroxyglutaric acid in urine and raised plasma levels of 3-hydroxybutyryl-carnitine are indicative of SCHAD HI.<sup>3</sup>

Differentiation between the diffuse and the focal form of KATP HI is difficult. Acute insulin response studies cannot reliably distinguish between these two forms of KATP HI,<sup>5,24</sup> and even highly involved procedures such as pancreatic arterial stimulation venous sampling or transhepatic portal venous sampling are only 70-80% accurate in differentiating diffuse and focal CH.<sup>15</sup> Preliminary reports indicate that <sup>18</sup>F-DOPA PET scans may be useful for localizing focal lesions.

**Genetic testing** allows differential diagnosis of GK HI, GDH HI, and KATP HI without lengthy, complicated, and potentially ambiguous diagnostic studies. In addition, genetic testing can help to distinguish between the diffuse and the focal form of KATP HI. Arterial stimulation venous sampling or transhepatic portal venous sampling can then be used to localize the focal lesion within the pancreas.

### Acute Insulin Response Studies

Many, but not all patients with KATP HI show a characteristic acute insulin response to an injection of  $\text{Ca}^{2+}$ , since the voltage-gated  $\text{Ca}^{2+}$  channels in the membrane of affected  $\beta$ -cells are open most of the time.<sup>23</sup> In theory, acute insulin response studies should also be able to differentiate between diffuse and focal KATP HI: In patients with diffuse KATP HI, administration of tolbutamide (a sulfonylurea compound that binds to the *ABCC8*-encoded subunit of KATP channels, causing the channels to close and insulin to be released)

should not lead to an acute insulin response, since the KATP channels in  $\beta$ -cell membranes are defective and should thus no longer be sensitive to tolbutamide action. In focal KATP HI, the remaining intact KATP channels outside of the focal lesions should give rise to an acute insulin response to tolbutamide. Contrary to these expectations, however, KATP channels affected by KATP HI-associated mutations show residual sensitivity to tolbutamide action, so that the focal and diffuse forms of KATP HI often cannot be distinguished by acute insulin response studies.<sup>15,24</sup>

### **Arterial Stimulation Venous Sampling (ASVS)**

ASVS involves sequential injection of calcium into each of the three arteries that supply blood to the head, body, and tail regions of the pancreas, using a catheter inserted into a leg artery. Through an intravenous catheter placed into the child's neck, blood samples are taken to measure the insulin secretion after each calcium injection. An increase in insulin secretion after injection of calcium into one of the three arteries suggests location of a focal lesion in the part of pancreas supplied by that artery.

### **Transhepatic Portal Venous Sampling (THPVS)**

For THPVS, a catheter is inserted through the skin and the liver into the veins of the pancreas. Blood samples are obtained throughout the various regions of the pancreas and tested for insulin concentrations. The pancreatic venous system with the highest insulin concentrations is likely to be the site of a focal lesion.

## **Treatment of CH**

GK HI, GDH HI, SCHAD HI, and autosomal dominant diffuse KATP HI due to rare, specific autosomal dominant mutations in *ABCC8*<sup>16,17,18</sup> are usually responsive to diazoxide, which is believed to inhibit insulin secretion through opening KATP channels. In contrast, most cases of diffuse and focal KATP HI cannot be adequately managed with diazoxide, since KATP channels are non-functional. Instead, octreotide (a somatostatin analogue) or continuous dextrose is used for therapy. However, in most cases of KATP HI, drug therapy fails, and pancreatectomy is required.<sup>4</sup>

In patients suffering from diffuse KATP HI, typically over 95% of the pancreas has to be removed to avoid recurrent hypoglycemia. This near-total pancreatectomy can lead to life-long diabetes. In focal KATP HI, by contrast, only the part of the pancreas containing  $\beta$ -cells with defective KATP channels has to be removed. Such partial pancreatectomy can offer a cure for focal KATP HI.

## **Genetics of CH**

**Diffuse KATP HI** is generally inherited in an autosomal recessive manner. In rare cases, specific autosomal dominant mutations in *ABCC8* can lead to a mild form of diffuse KATP HI.

**Focal KATP HI** is inherited in an autosomal dominant manner, due to somatic loss of heterozygosity.<sup>14</sup> In this form of KATP HI, only the paternally inherited allele of *ABCC8* or *KCNJ11* contains a disease-linked mutation, while the maternal copy is normal. The CH phenotype is only expressed in those  $\beta$ -cells and their clonal descendants where a somatic event has led to loss of the maternal allele; these cells create the focal lesion in the pancreas. The obligate paternal inheritance of the mutation leading to focal KATP HI may be associated with genomic imprinting.

**GDH HI** and **GK HI** are both inherited in an autosomal dominant manner. Most GHD HI cases identified to date are due to sporadic *de novo* mutations.<sup>19</sup>

**SCHAD HI** is inherited in an autosomal recessive manner.

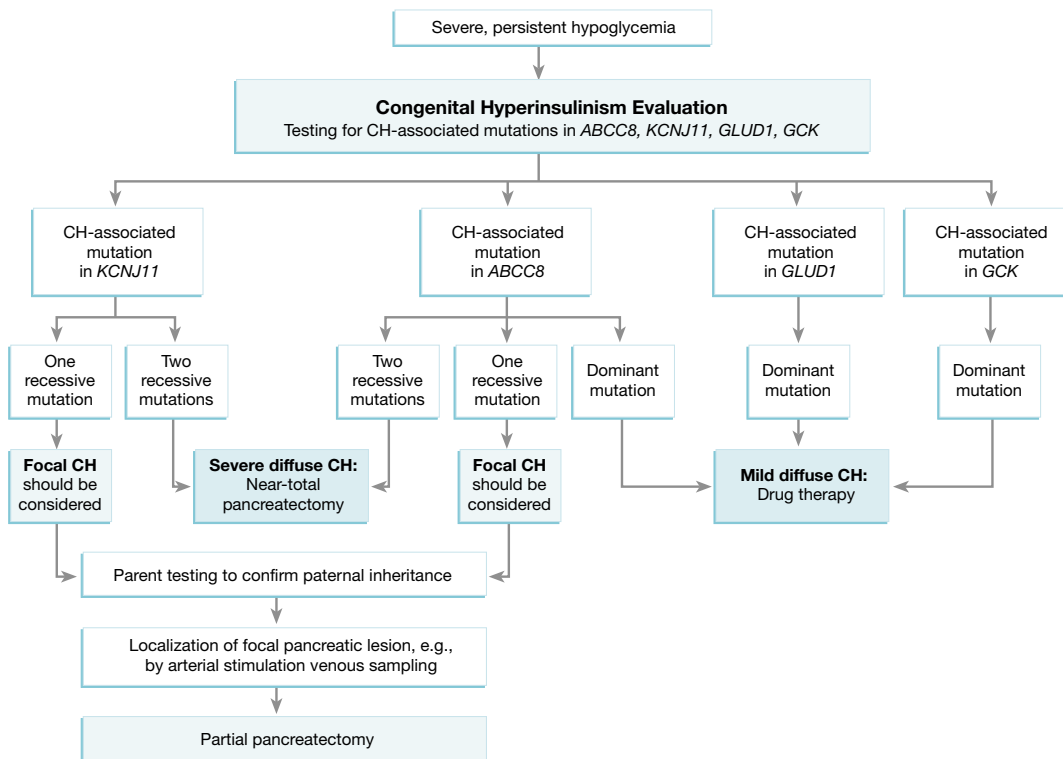
## Genomic Imprinting

In most cases, autosomal genes are expressed from both chromosomes. For some autosomal genes, however, the gene allele on one of the chromosomes is permanently silenced. This phenomenon is known as “genomic imprinting.” Genes subject to imprinting are methylated

differently in sperm and egg, and these methylation patterns later determine whether the gene is expressed or not.

The *ABCC8* and *KCNJ11* genes are located close to a chromosomal region known to contain both maternally and paternally imprinted genes.<sup>14</sup>

## An approach to CH testing and related therapeutic decision-making



## Genetic Testing for CH

The **Congenital Hyperinsulinism Evaluation** detects CH-associated mutations in *ABCC8*, *KCNJ11*, *GLUD1*, and *GCK*. It can diagnose most cases of CH requiring pancreatectomy, distinguish between different types of CH, and help to differentiate between the diffuse and the focal form of KATP

HI, thus aiding in the selection of the most appropriate treatment. Compared to current diagnostic methods, genetic testing for CH is non-invasive, accurate, and at least as fast.

For the current number of CH-associated variants in *ABCC8*, *KCNJ11*, *GLUD1*, and *GCK*, please visit: <http://www.correlagen.com/endocrinetests/>.

## How Is Genetic Testing for CH Performed?

DNA for sequencing is obtained from leukocytes present in a small blood sample. The coding sequences of *ABCC8*, *KCNJ11*, *GLUD1*, and *GCK* are amplified in a highly specific manner through a polymerase chain reaction (PCR), and all PCR products are fully sequenced.

Sequencing results are interpreted, and a detailed result report is sent to the patient's physician.

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