Vietnam National Children's Hospital

CONGENITAL HYPERINSULINEMIC HYPOGLYCEMIA IN INFANTS: GENOTYPE AND PHENOTYPE OF 102 CASES

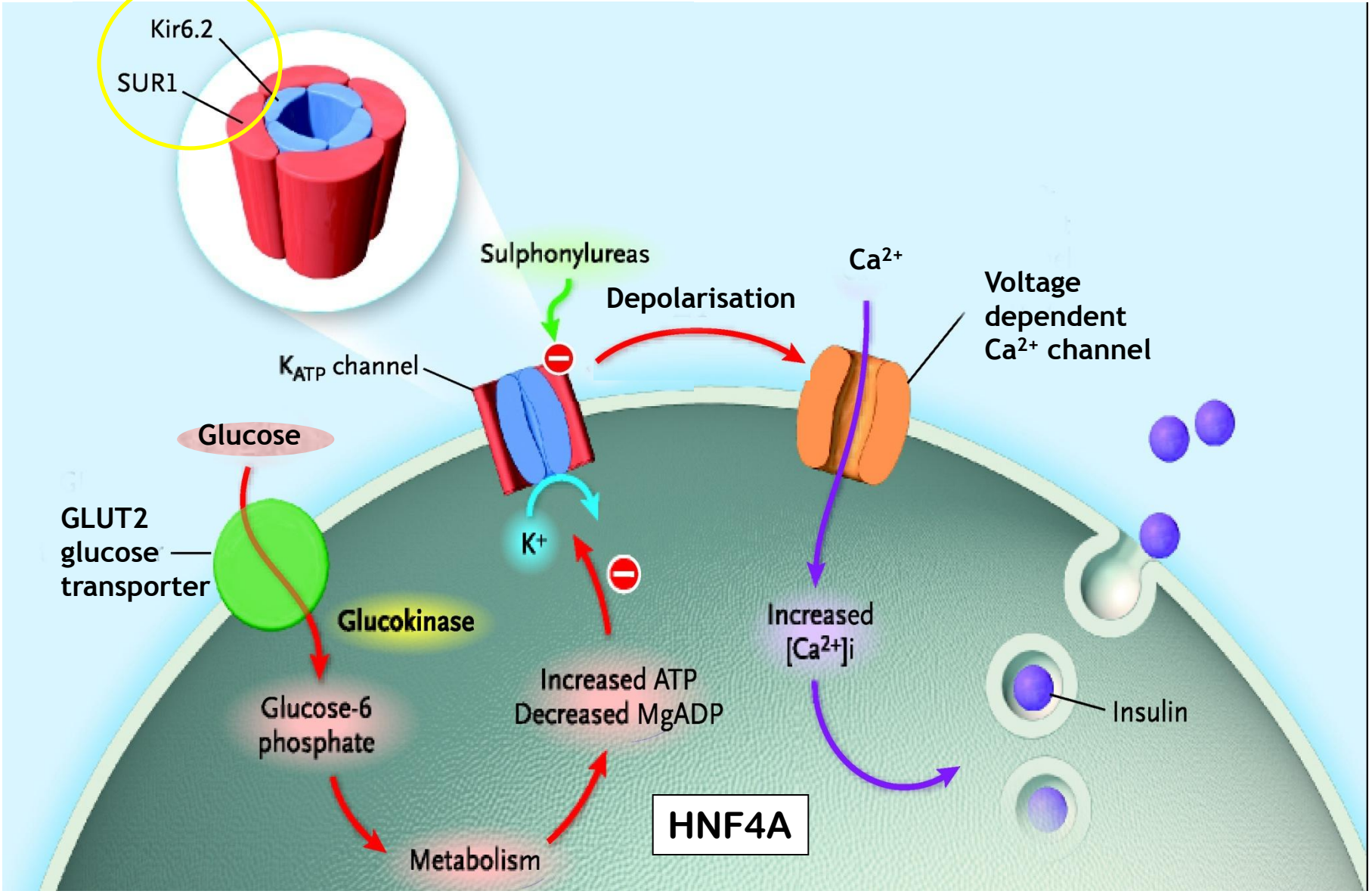
Can Thi Bich Ngoc, Vu Chi Dung et al

The National Children's Hospital . Hanoi. Vietnam

Introduction

- Congenital hyperinsulinism (CHI): inappropriate of insulin secretion despite low blood glucose levels
- Absence of treatment → irreversible brain damage
- Incidence 1/50,000 → 1/2,500 live births

Insulin secretion in the pancreatic beta-cell



BACKGROUND

Summary of genetic causes of isolated HI

	Gene	Protein	Inheritance	Diazoxide-Resp.	Histology	Comment
K_{ATP} Channel	***ABCC8***	SUR1	AR	No	F or D	
			AD	Usually	D	
	KCNJ11	Kir6.2	AR	No	F or D	
Enzymes/Transporters	***GLUD1***	GDH	AD or DN	Yes	D	HIHA syndrome
	GCK	GCK	AD or DN	Usually	D	MODY 2
	HADH	SCHAD	AR	Yes	D	
	SLC16A1	MCT1	AD	Usually	D	EIHI
	UCP2	UCP2	AD	Yes	D	
Transcription Factor	***HNF4A***	HNF4A	AD or DN	Yes	D	MODY 1

AR: autosomal recessive; **AD**: autosomal dominant; **DN**: De Novo; **F**: Focal Form; **D**: Diffuse Form; **HI/HA**: hyperammonemia/hyperinsulinism syndrome; **EIHI**: Exercise-induced hyperinsulinism; **GDH**: Glutamate Dehydrgenase; **GCK**: Glucokinase; **HADH**: Hydroxy-Acyl-CoA Dehydrogenase; **MCT1**: Monocarboxylate transporter 1; **MODY**: Maturity-onset diabetes of the young: **UCP2**: Uncoupling protein 2.

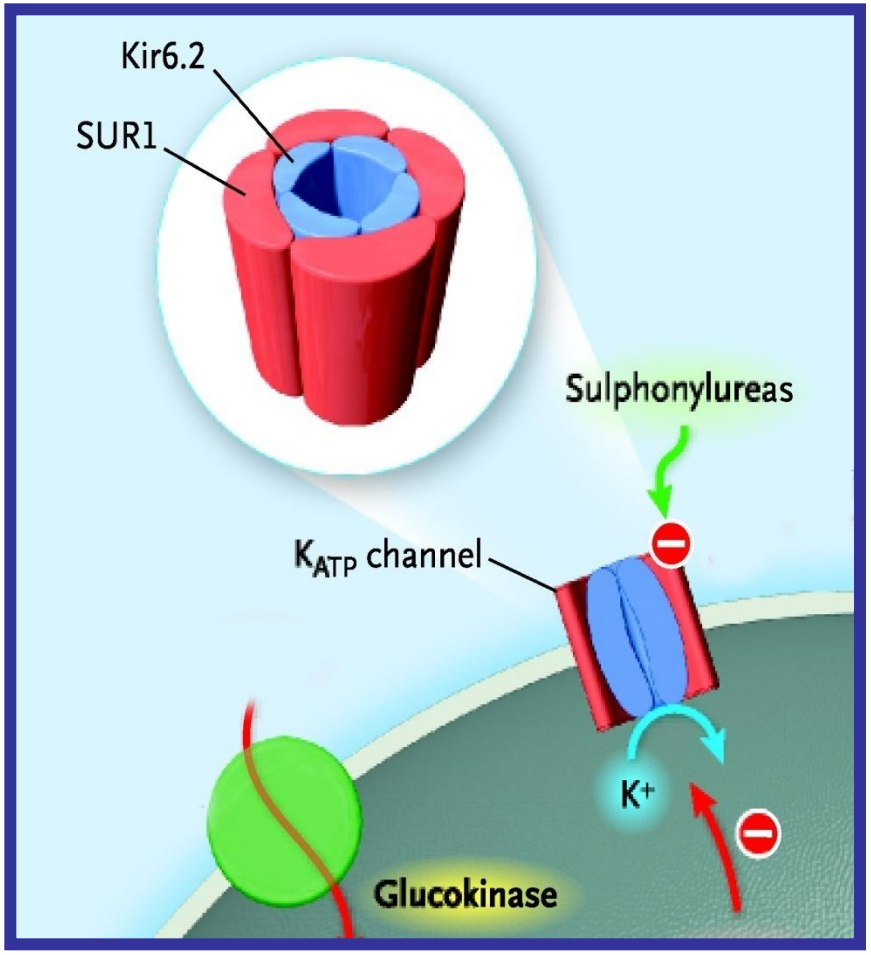
Arnoux *et al. Orphanet Journal of Rare Diseases* 2011, **6**:63

BACKGROUND

Beta-cell potassium ATP (K_{ATP}) channel genes

- *ABCC8* gene: 39 exons, 100 kb, encoding a 1582-amino acids protein (SUR1)
- *KCNJ11* gene: single exon encoding a 390-amino acid protein (Kir6.2)
- Interestingly, location of *KCNJ11* only 4.5 kb from *ABCC8* gene on 11p15.1
- *GLUD1*: 45 kb; 13 exons on 10q23.2
- *HNF4A*: ~74 kb; 10 exon on 20q13.12

Hyperinsulinism results from loss-of-function K_{ATP} channel mutations



Protein does not reach cell surface/or channels do not function

↓

Membrane Depolarised

↓

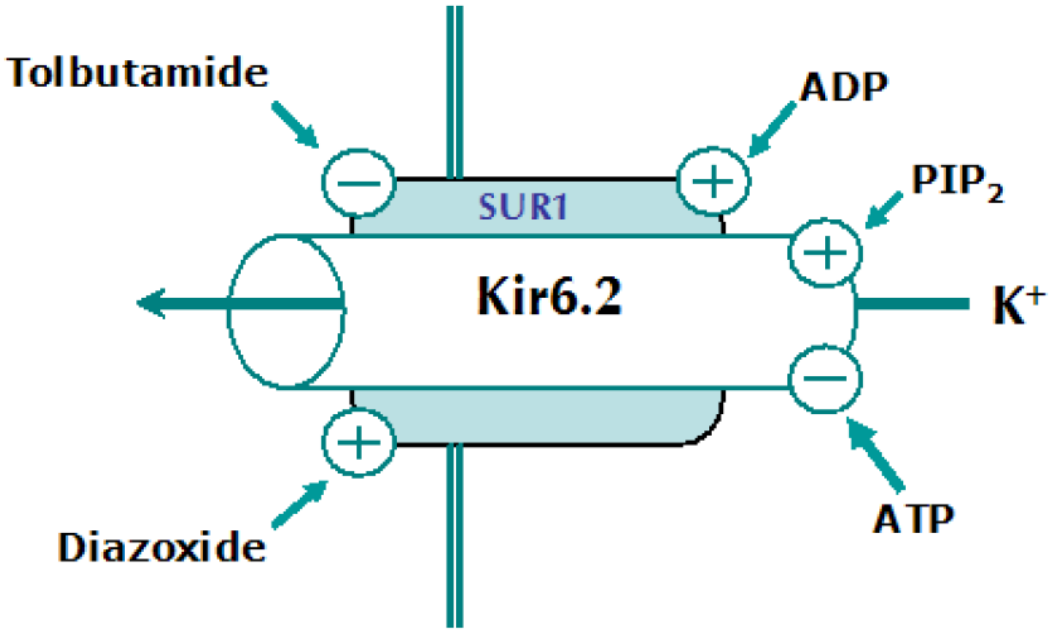
Calcium influx

↓

Unregulated insulin secretion

= Hyperinsulinism

Control Elements for the K_{ATP} Channel in Pancreatic ß-Cells, 2006



SUR=SulfonylUrea Receptor

- Diazoxide blocks insulin secretion by activating (opening) SUR1
- Sulfonylureas (tolbutamide) stimulate insulin secretion by closing SUR1

SPECIFIC AIMS

- *To identify mutations in the ABCC8 and KCNJ11, HNF4A and GLUD genes*
- *To describe genotype and phenotype correlations of Vietnamese children with congenital hyperinsulinism*

PATIENTS

- Patients

  102 cases with CHI at NHP (male: 60; female:42)

  Diagnosis age: 1 - 30 days of age
- From Jan.2010 to Dec. 2016 at the National Children’s Hospital

PATIENTS

Diagnostic criteria (Hussain K. 2008)

1. Fasting & post-prandial hypoglycemia (< 2.5–3 mmol/l) with unsuppressed insulin secretion & c-peptide levels (plasma insulin concentrations > 1 mU/l).
2. Positive response to subcutaneous or intramuscular administration of glucagon (plasma glucose concentration increase by 2 to 3 mmol/l following a 0.5 mg glucagon subcutaneous injection)
3. Negative ketone bodies in urine or blood
4. Prolonged dependence on treatment to prevent hypoglycemia throughout first months/years of life

PATIENTS

Excluded criteria

- Syndromic: e.g

✓ Beckwith-Wiedemann

✓ Trisomy 13

✓ Mosaic Turner

- Metabolic conditions
- Secondary to (usually transient)

✓ Maternal diabetes mellitus (gestational & insulin dependent)

✓ Intra-uterine growth retardation

✓ Perinatal asphyxia

METHODS

- Genomic DNA was extracted from peripheral leukocytes using standard procedures.
- Single exon of *KCNJ11*; 39 exons of *ABCC8; 10 exons of HNF4A & 13 exons of GLUD1* were amplified & sequenced.
- Sequencing reactions were analyzed on an ABI 3730 capillary sequencer & were compared to published sequences using Mutation Surveyor version 3.24.

Ellard S et al. Am J Hum Genet 2007: 81: 375-382.
Flanagan SE, et al. Diabetologia 2006: 49: 1190-1197.

Best Practice Guideline article

Congenital hyperinsulinism

Table 1
Infusion of glucose.

Peripheral catheter: glucose 10%
2 ml/kg/h (=3.3 mg/kg/min) 4 ml/kg/h (=6.7 mg/kg/min) 6 ml/kg/h (=10 mg/kg/min) 8 ml/kg/h (=13.3 mg/kg/min)
Central catheter: glucose 10%, 20%, 30% or 50%
e.g. Glucose 30%
0.5 ml/kg/h (=2.5 mg/kg/min) 1 ml/kg/h (=5 mg/kg/min) 2 ml/kg/h (=10 mg/kg/min) 3 ml/kg/h (=15 mg/kg/min)

Early Human Development 86 (2010) 287–294

A specialized team approach to diagnosis and medical versus surgical treatment of infants with congenital hyperinsulinism

Andrew A. Palladino, MD, Charles A. Stanley, MD

From the Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania.

Seminars in Pediatric Surgery (2011) 20, 32-37

Table 1 Timeline for diagnosing HI, initiating medical therapy, and referring to specialized center

Day 1	Establish diagnosis of HI (see Table 3)
	Begin 5-d trial of diazoxide If HI is severe begin at max dose (15 mg/kg/d) If HI less severe/perinatal-stress, start diazoxide at 5-10 mg/kg/d* Consider starting a diuretic with diazoxide, especially if on high GIR
Day 2-5	Determine minimum GIR required to maintain blood glucose between 70 and 100 If HI is severe or GIR is >10 mg/kg/min, send mutation analysis on HI genes for infant and parents
Day 6	Determine fasting tolerance on diazoxide Failure to fast >12 h with BS >70 mg/dL indicates diazoxide unresponsiveness Diazoxide failure suggests a K_{ATP} channel HI and potential surgical candidate Begin arrangements for transfer to a specialized HI center with ^{18}F-DOPA PET scan capability
Day 7	Discontinue diazoxide; consider octreotide, 5 μg kg^{-1} d^{-1} divided every 6-8 h Desensitization to octreotide is common after 2-3 doses If required, octreotide can be increased to maximum of 15 μg/kg/d
Day 8-14	Evaluate effectiveness of octreotide with fasting test while awaiting transfer of patient

Abbreviations: GIR, glucose infusion rate (mg/kg/min); HI, hyperinsulinism.

*See text for further discussion of tachyphylaxis.

METHODS

- Definition of diazoxide efficiency: normalization of glycemia > 3 mmol/l measured before & after each meal in patients fed normally with a physiological overnight fast, after stopping intravenous glucose & any other medications for at least five consecutive days

  Arnoux JB et al. Early Human Development 2010;86:287–294

- Non responsive with diazoxide

➢ Surgery

➢ Octreotide

RESULTS

CLINICAL SYMPTOMS

- ❖ Weight of birth: 4.1 0.9 (2.3 – 5.6) kg
- ❖ Age at presentation: < 24 hours: 47/102 (46.1%)
- ❖ Symptoms:
  - ✓ Poor feeding, lethargy: 89/102 (87.3%)
  - ✓ Seizures 14/102 (13.7%)
  - ✓ Apnea, cyanosis 9/102 (8.8%)
- ❖ Glucose infusion rate: 12 – 28 mg/kg/mn

RESULTS

Distribution of mutations in different genes

Gene	Number of patients	%
ABCC8	47	46.1
KCNJ11	5	4.9
HNF4A	1	0.9
GLUD1	0	0
Total	**53**	**51.9**

HNF4A: **c.659T>C (**p.L220P): novel mutation & mother inheritance

RESULTS

Mutations in *ABCC8*

- 25 different mutations: 13 novel; 12 reported one in *ABCC8*
- Homozygous/compound heterozygous mutations in *ABCC8*

  27/47 (57.4%)
- Hemizygous mutations in *ABCC8* from father or mother

  20/27 (42,6%)

RESULTS

Mutations in *ABCC8* and genotype

Genotype with *ABCC8* mutations	Number of families
c.3403-1G>A	13
c.3403-1G>A/c.3403-1G>A	1
c.3403-1G>A/c.2995C>T	1
c.2057T>C	2
c.2057T>C/c.2057T>C	1
c.2417G>A/c.2995C>T	1
c.4160_4162del	2
c.1467+5G>A/c.2800C>T	1
c.2041-21G>A	1

RESULTS

Mutations in *ABCC8* and genotype

Genotype with *ABCC8* mutations	Number of families
c.2041-21G>A/c.3978del	1
c.2041-21G>A/c.2041-21G>A	1
c.2056T>A/c.2057T>C	1
c.2057T>C/c.3403-1G>A	2
c.2057T>C/c.2995C>T	1
c.2995C>T	3
c.3293A>G	1
c.3403-1G>A/c.4462C>T	1
c.4415-13G>A	1

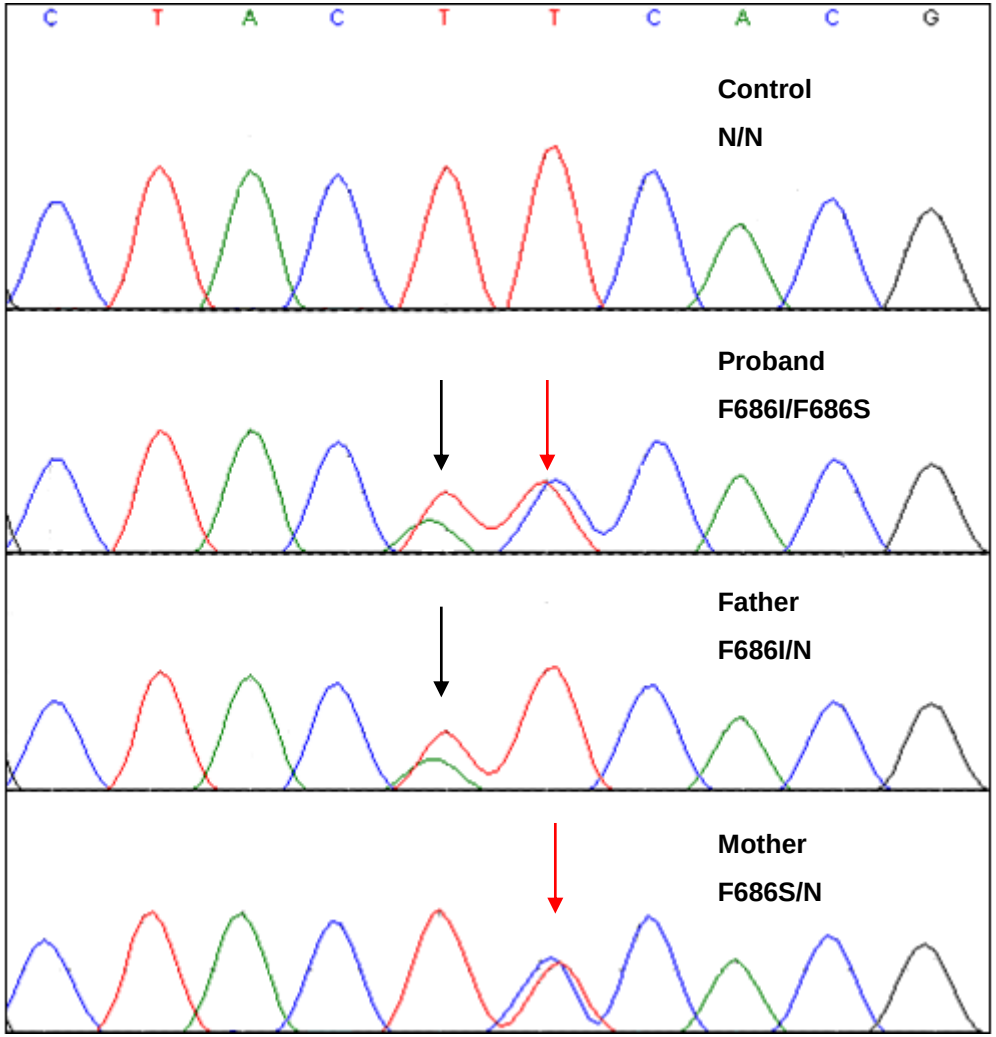
RESULTS

Mutations in *ABCC8* and genotype

Genotype with *ABCC8* mutations	Number of families
c.4610C>T	1
c.655C>A/c.892C>T	2
c.1106A>G/ c.4611G>A	1
c.1183A>T	1
c.2056T>A/c.2057T>A	1
c.3293A>G	1
c.4061A>G *	1
c.4135G>A	1
Deletion of exons 23-26	1

RESULTS

Sequencing of *ABCC8*



RESULTS

Mutations in *KCNJ11*

- 3 novel mutations from father (**c.482C>T, c.512C>A, c.820G>C)** in 2 unrelated families
- Homozygous **c.185delC** of *KCNJ11* in two sibling of 1 family.

RESULTS

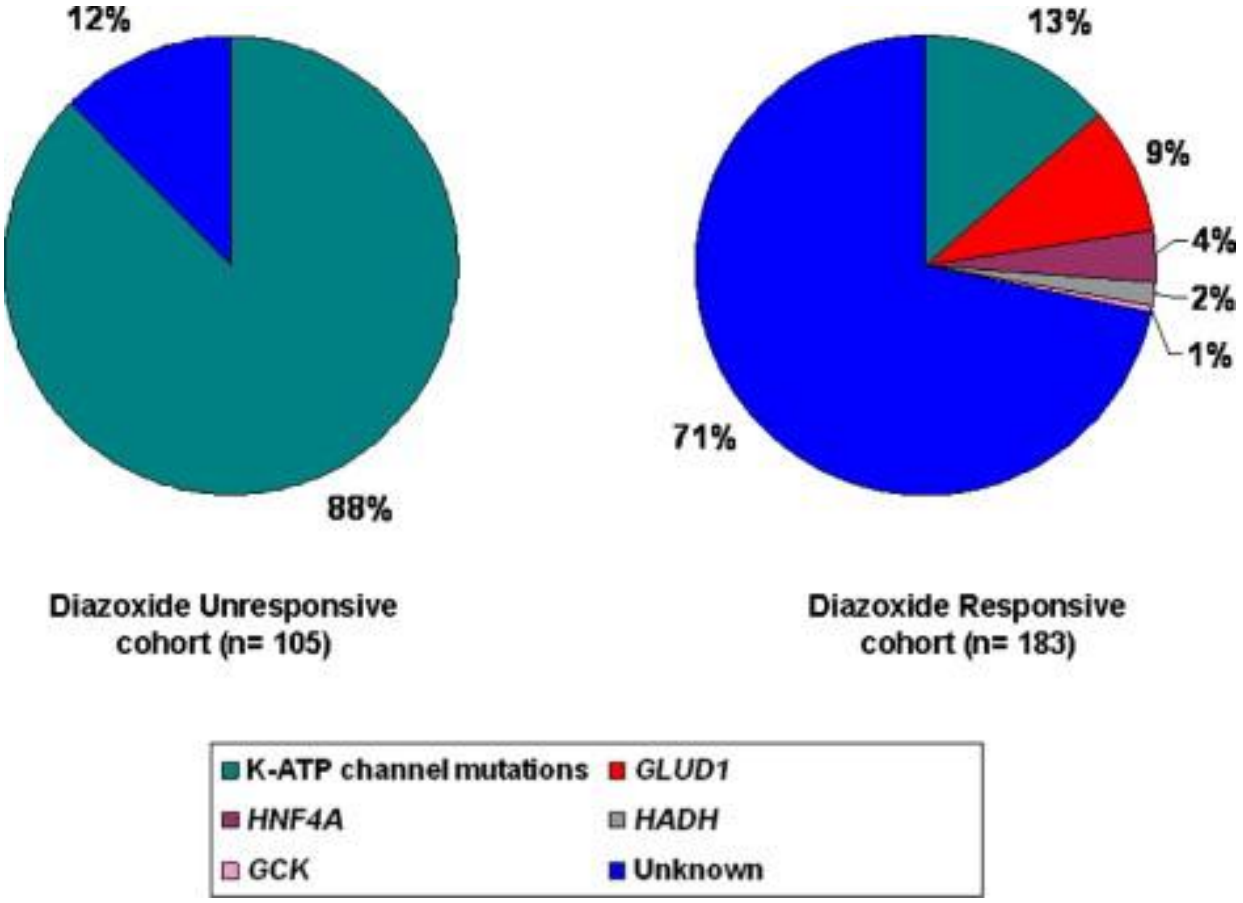
Correlation of genotype - phenotype

- Responsive with diazoxide: 52 cases:
  - 49 without mutations
  - 1 case with maternal mutation in *ABCC8*
  - 1 case with mutation in *HNF4A*
  - 1 case with mutation in *KCNJ11*

Kết quả
Correlation of genotype - phenotype

- Non responsive with diazoxide (surgery and/or octreotide): 48 cases
  - 4 cases with mutations in *KCNJ11*
  - 44 cases with homozygous/compound heterozygous or paternal mutations in ABCC8

DISCUSSION



Flanagan S et al. Semina in Pediatric Surgery 2011'20(1):13-17

DISCUSSION

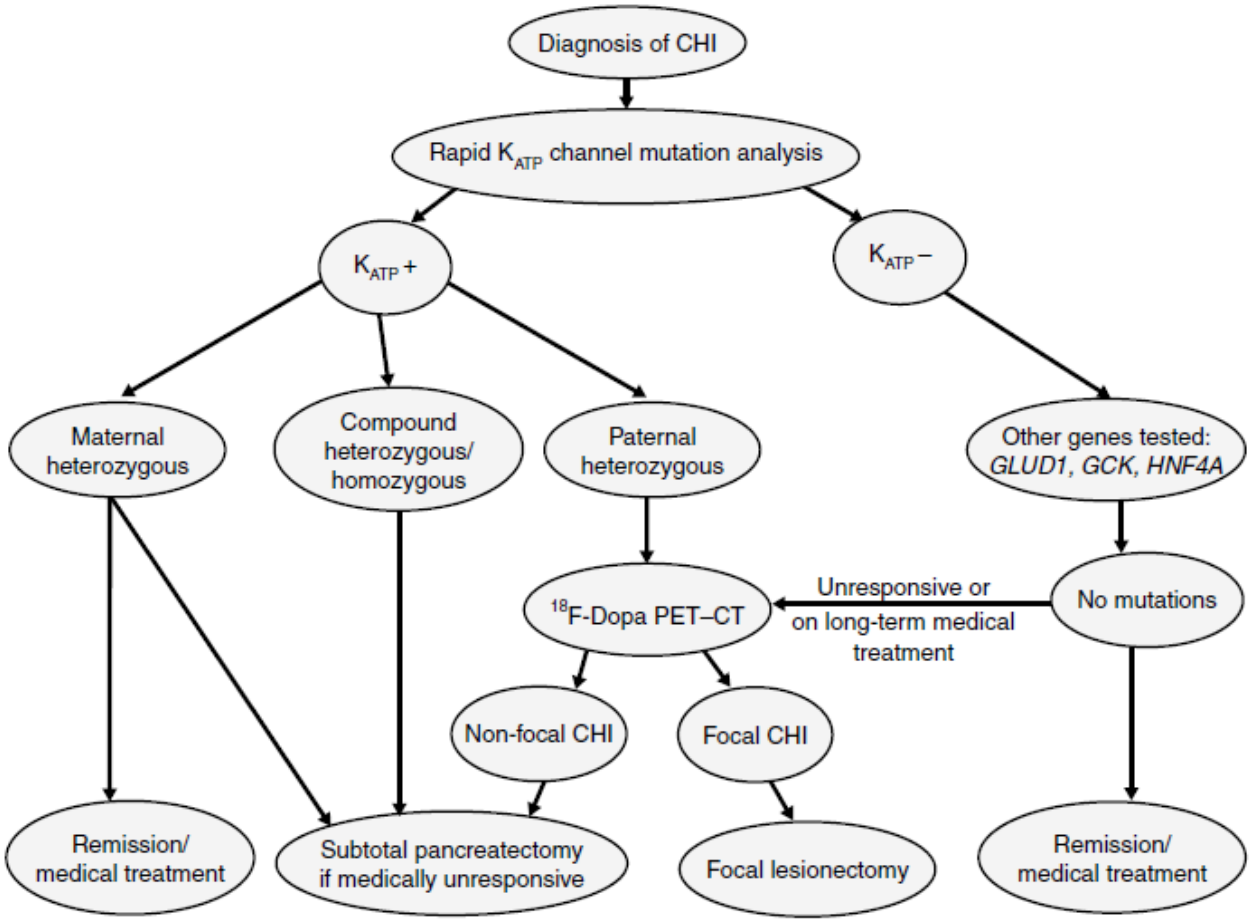
- Mutation in ABCC8 (SUR1): most common cause of CHI and were first to be described
- Approximately 45% of affected individuals have mutations in *ABCC8* [Nestorowicz et al 1998, Aguilar-Bryan & Bryan 1999, Meissner et al 1999, Fournet & Junien 2003, Tornovsky et al 2004].
- Almost 20 years after discovery of first mutation
- Over 200 mutations identified
- Distribution of mutations throughout the gene

Sarah E et al. Human Mutation 2009;30:170-180

DISCUSSION

- Diazoxide is effective in virtually all forms of CHI except in inactivating recessive mutations in *ABCC8*
- Rapid genetic analysis for mutations in *ABCC8 & KCNJ11* → identification of majority of patients with diffuse disease (homozygous or compound heterozygous mutations)

Kapoor RR et al. Arch Dis Child 2009;94:450-457



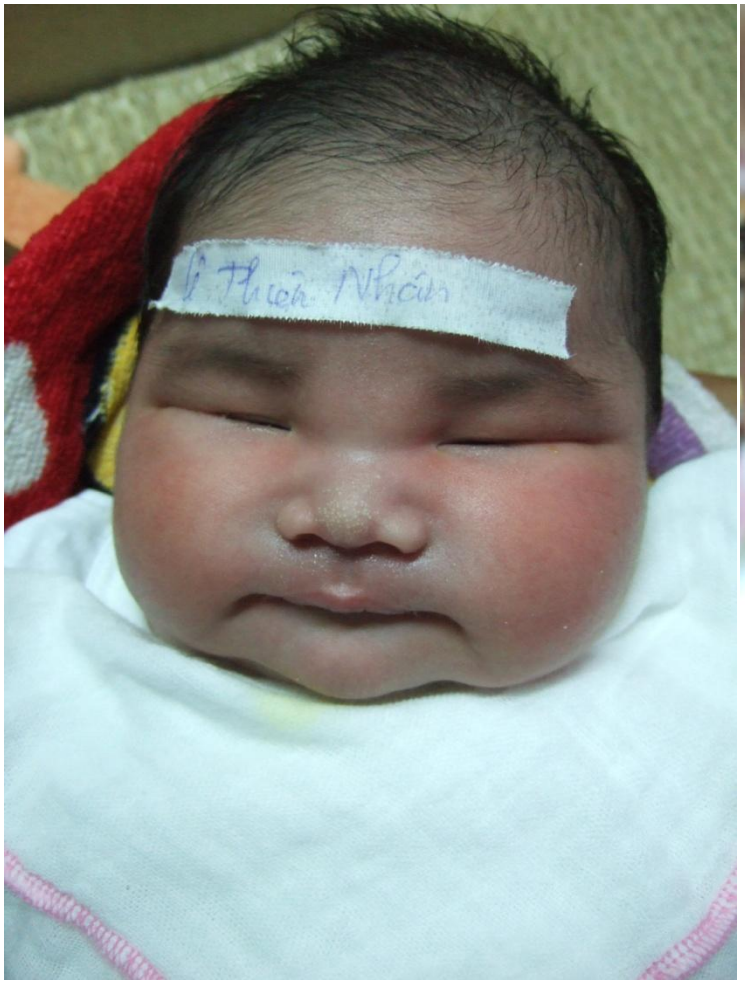
Flowchart of investigation and management of children with CHI.

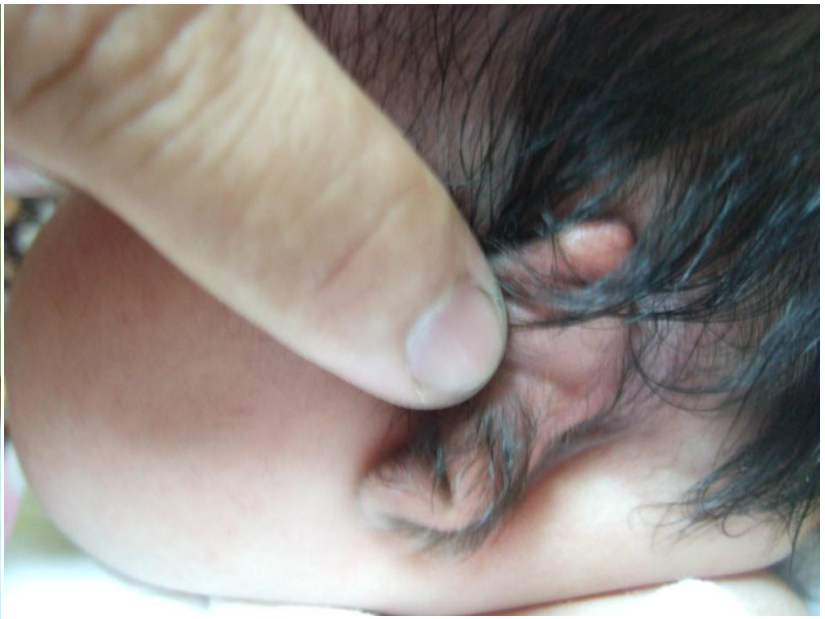
CONCLUSIONS

- Understanding genetic basis of CHI provide novel insights into β-cell physiology
- Prediction phenotype, management & genetic counseling

➢ Genetic analysis for mutation in CHI can help in genetic diagnosis → treatment

➢ Prenatal diagnosis of CHI → immediate medical management at the time of birth

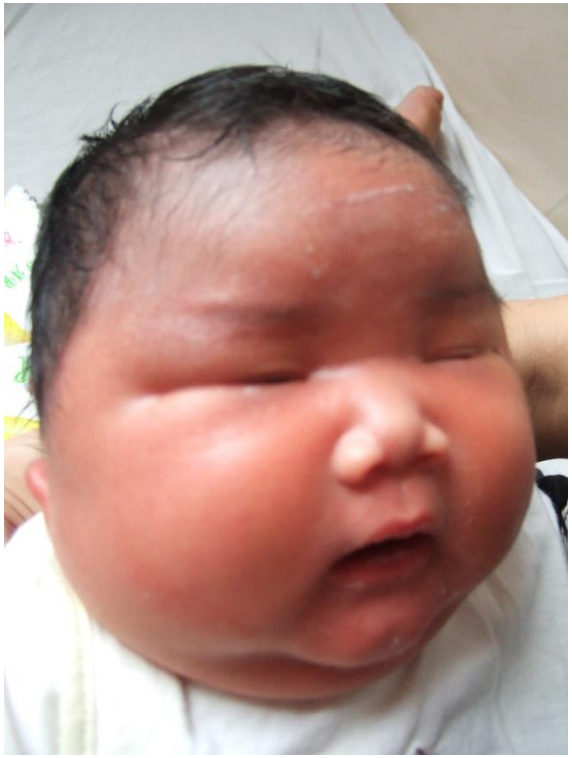
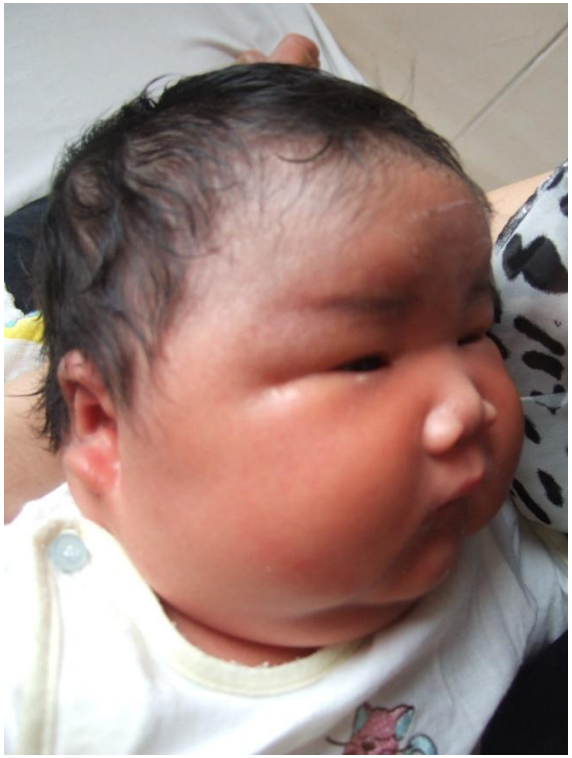
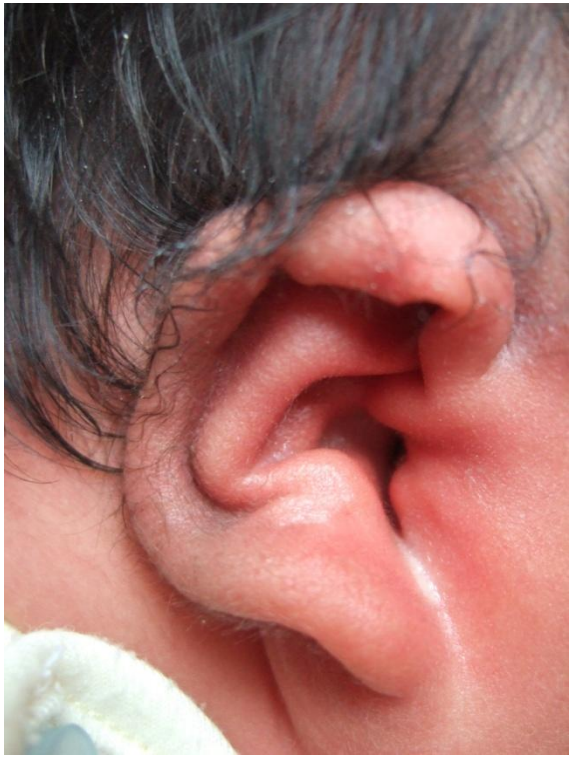




Lê Thiện N, WOB 5 kg (39 weeks)

responsive with medical treatment

Nguyen Thi Diem H. Responsive with medical treatment. WOB 3.5 kg (37 weeks). Two sibling died at Provincial Hospital at 3 days of age (cyanosis)

Vũ Hải Y. WOB 5.4 kg, responsive with medical treatment

Vuong Ha M; WOB 3.8 kg

Unresponsive with medical treatment

Mutation of ABCC8: (F686I/F686S)

Cao Bao N. WOB 5 kg;

Unresponsive with medical treatment

Mutation of ABCC8 F686S/IVS27-1G>A

Thank you very much!