GENETIC CAUSES OF CONGENITAL HYPERINSULINISM IN SLOVAKIA

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INTRODUCTION AND AIM

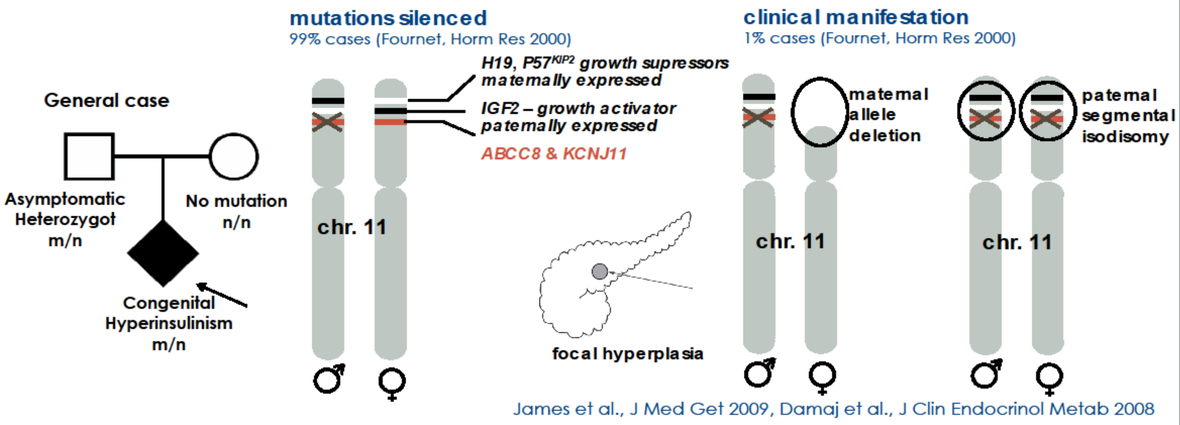
Congenital hyperinsulinism (CHI) is the most common cause of persistent hypoglycemia in neonates and infants with incidence of 1:50 000 live births (Bruining, 1990) due to insulin hypersecretion.

Genetically, CHI is a heterogeneous condition with mutation in several key genes involved to the insulin secretion. The most common are mutations in *KCNJ11* and *ABCC8* genes (coding potassium channel subunits), much rarer are mutations in *GCK*, *HNF4A*, *HNF1A*, *HADH*, *GLUD1*, *UCP* and *SLC16A1* genes. However, the etiology of more than 50% CHI patients is unknown. The type of B-cell hyperplasia: diffuse forms can be inherited in both recessive or dominant manner; focal forms are sporadic and can arise either from maternal allele deletion or paternal segmental isodisomy (Fig. 1).

Treatment of choice for CHI is diazoxide, however *ABCC8* and *KCNJ11* recessive and focal forms are diazoxide resistant and require other medicaments or surgery.

This study **aimed** to evaluate genetic cause of severe hypoglycemia and recommend an appropriate therapeutic approach in particular cases.

Figure 1: Focal form – loss of heterozygozity:



James et al., J Med Get 2009, Damaj et al., J Clin Endocrinol Metab 2008

- Focal hyperplasia involves specific loss of the maternal 11p15 region and constitutional mutation of a paternally inherited allele of the genes *ABCC8/KCNJ11*
- Imbalanced expression of imprinted genes (increased insulin-like growth factor II IGF2, diminished HI19 and $p57^{kip2}$) leads to activation of growth factors and increase proliferation of β-cells with insulin overproduction

METHODS

For genetic testing, 16 unrelated probands with CHI were referred throughout Slovakia over years 2004 – 2014. **Inclusion criteria:** insulin levels >2μU/ml by plasma glucose < 3.0mmol/l. DNA analysis: direct sequencing of ***ABCC8, KCNJ11, HNF4A, GCK*** *and* ***HNF1A*** genes. One patient (HI-48) had whole exome sequencing (BGI, Hong Kong).

RESULTS

- **Incidence** of CHI in Slovakia is **1:34 375** live births
- **Mutation** was identified in 6/16 (**37.5%**) patients
- 3/16 (**19%**) were diazoxide resistant with more severe hypoglycemia
- Genotype & diazoxide sensitivity determined the therapy choice

Table 1: Genotype, phenotype and therapy of patients with mutations associated with CHI.

ID	Gene	Region	Nucleotide position	Protein position	Inheritance	Mode of inheritance	Functional analysis	Reference	Age of onset	Diazoxide responsive	Birth weight (gest. week)	Management	Form
HI-26	ABCC8	exon 1	c.50T>C	V17A	Paternal	N/A	No	Mohnike et al., 2014	6 months	Yes	3 400g (40)	Glucose, diazoxide	N/A
HI-07	ABCC8	exon 8	c.1332G>T	Q444H	Paternal	Recessive	No	Hardy et al., 2007	1st day	No	3920g (36)	Surgrical	Focal
P586	ABCC8	intron 22	c.2694+1G>C	-	Paternal	Recessive	No	Novel, G>A Snider et al., 2013	1st day	No	3270g (39)	Surgical	Focal
HI-36	KCNJ11	exon 1	c.154C>T/ c.901C>G	Q52*/R301G	Paternal/Maternal	Recesisve/Recessive	No/Yes	Novel/ Lin et al., 2008	1st day	No	4150g (40)	Glucose, octreotide, frequent feeding	Predicted Diffuse
HI-50	KCNJ11	exon 1	c.539C>A	T180N	Paternal	Recessive	No	Novel	1st day	Yes	4800 (40)	Diazoxide	Unknown
HI-48	HNF4A	intron 4	c.427-1G>A	-	Maternal	Dominant	No	Novel	1st day	Yes	3900g (39)	Diazoxide	Unknown

All sequence information is based on GenBank reference nucleotide and protein sequences: NM_000352.4 → NP_000343.2, NM_000525.3 → NP_000516.3, NM_175914.4 → NP_787110.2 for *ABCC8, KCNJ11* and *HNF4A* genes respectively. Nucleotide numbering reflects cDNA position, with +1 corresponding to the A of the major start codon of exon 1.

Diazoxide resistant forms: 3/16 patients (19%)

Case 1 (ID P586):

At presentation

- lowest glycaemia: 0.9mmol/l
- insulin in hypoglycaemia 1.9mmol/l: 15.7μU/ml
- therapy: i.v. glucose (18mg/kg/min), glucagon, corticoids, diazoxide

DNA diagnosis

- Paternally inherited ABCC8 mutation **intron 22 (c.2694+1G>C) (novel) – focal form**

Clinical management

- **recommendation for the therapy** discontinue diazoxide add octreotide PET-CT + surgery (**Prof. O. Blankenstein**, Charité, Berlin, Germany)
- **surgery – partial pancreatectomy** in 6 months of life, hypoglycaemia cured

Before surgery

After surgery

Current age 5.5 years normal psychomotor development

Case 2 (ID HI-07):

At presentation

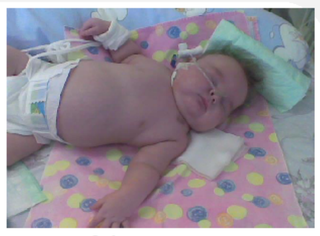
- lowest glycaemia: 0.1mmol/l
- insulin in hypoglycaemia: 90.8μU/ml
- therapy: i.v. glucose (24mg/kg/min), glucagon, corticoids, diazoxide

DNA diagnosis

- **Paternally** inherited *ABCC8* mutation in exon 8 **(Q444H) – focal form**

Clinical management

- **recommendation for the therapy** discontinue diazoxide add octreotide PET-CT + surgery (**Prof. O. Blankenstein**, Charité, Berlin, Germany)
- **surgery – partial pancreatectomy** In age 2 months, persisting hypoglycaemia
- **resurgery** + octreotide lanreotide, percutanneous gastrostomy



Before surgery

After surgery

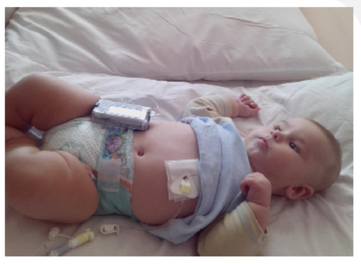
Current age 5.5 years PM delay, epilepsy

Case 3 (ID HI-36):

At presentation

- lowest glycaemia: 0.8mmol/l
- insulin in hypoglycaemia 0.9mmol/l: 10.0μU/ml
- therapy: p.o. maltodextrin, diazoxide

DNA diagnosis



Compound heterozygot for *KCNJ11* gene **(Q52*/R301G), both pathogenic – diffuse form**

Clinical management

- **recommendation for the therapy**

severe form → i.v. glucose
discontinue diazoxide
add octreotide
frequent feeding

Current age 2.5 years

normoglyceamia on octreotide s.c. insulin pump, decreasing p.o. intake, percutaneous gastrostomy

Diazoxide sensitive forms: 13/16 patients (81%)

Case 4 (ID HI-48):

At presentation - lowest glycaemia: 0.8mmol/l, insulin in hypoglycaemia 2.8mmol/l: 18.0μU/ml

- triglycerides 3.5mmol/l, lactate 4.03mmol/l, glycogen in erythrocytes: positive
- therapy: p.o. sacharides (15g/kg/24h)

Working Diagnosis Glygogenosis III (IV)

WES – glycogenosis genes – no mutation, Maternally inherited *HNF4A splicing* mutation **(c.427-1G>A)**

Clinical management - Normoglycaemia on diazoxide 3mg/kg

Follow up in maturity age for hyperglycaemia screening due to HNF4A-MODY

CONCLUSIONS

Incidence of CHI in Slovakia is 1:35 000 live births. We have resolved etiology in 37.5% (6/16) CHI cases, with ABCC8 mutations as the most common.
The knowledge of the genetic etiology of CHI helped us to choose the most appropriate therapeutic approach.

REFERENCES

1.Bruining GJ: Recent advances in hyperinsulinism and the patho- genesis of diabetes mellitus. Current Opinion in Pediatrics, 1990, 2 758–765.
2.James C, Kapoor RR, Ismail D and Hussain K: The genetic basis of congenital hyperinsulinism. Journal in Medical Genetics, 2009, 46, 289-299.
3.Fournet JC1 et al.: Loss of imprinted genes and paternal SUR1 mutations lead to focal form of congenital hyperinsulinism., Horm Res. 2000;53 Suppl 1:2-6.
4.Damaj L et al.: Chromosome 11p15 Paternal Isodisomy in Focal Forms of Neonatal Hyperinsulinism, JCEM 10/2008; 93(12):4941-7. DOI: 10.1210/jc.2008-0673

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