Somatic paternal UPD on chromosome 11p15 in focal form of congenital hyperinsulinism (CHI) causes monoallelic expression of mutant *ABCC8* and *KCNJ11*

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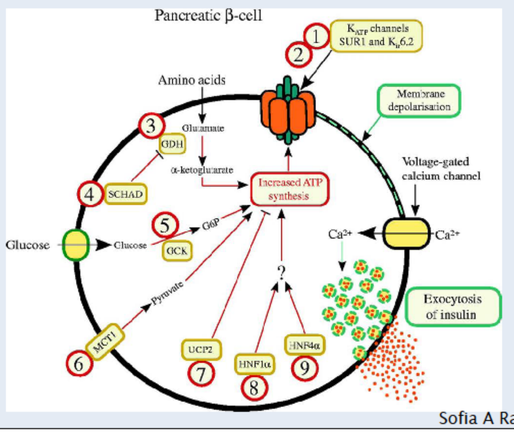
Background

Congenital hyperinsulinism (CHI) is a disorder characterized by dysregulation of insulin secretion that leads to severe hypoglycemia in neonates and infants. There are two major forms of CHI. The diffuse form affects all β-cells in the pancreas and is caused by autosomal recessive or dominant inherited mutations. The focal form of CHI is caused by an autosomal recessive mutation in the genes *ABCC8* and *KCNJ11* inherited from the father and a second somatic event in the affected islet of Langerhans.

Mechanisms of CHI



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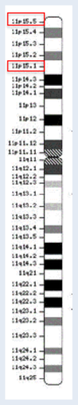
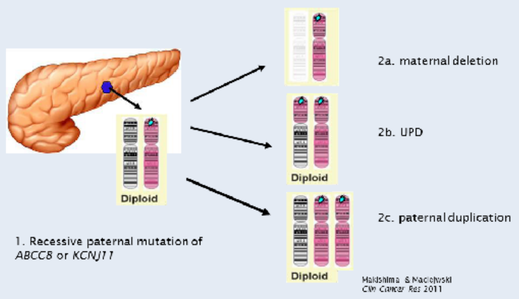
Most commonly, mutations occur in *ABCC8* (45%) and *KCNJ11* (5%).
Both genes are located on chromosome 11p15.1. The BWS imprinted region is located in proximity to these genes on 11p15.5.



Objective

Several possible mechanisms exist which may cause the second genetic event in focal CHI: maternal deletion, paternal duplication or paternal UPD 11p15. We report molecular genetic examination of focal pancreatic lesions of patients receiving therapeutic surgery to discover the genetic mechanisms in focal form of CHI.

Possible mechanisms of focal islet hyperplasia



Material and Methods

Patients with proven *ABCC8* or *KCNJ11* mutations and treated by surgical therapy were selected from the German Registry for Congenital Hyperinsulinism. Genomic DNA and RNA were extracted from pancreatic tissue. Loss of heterozygosity (LOH) and gene expression levels were analysed by PCR, RT-PCR and Sanger sequencing in 11 patients with focal form of CHI. Deletions, duplications and uniparental isodisomy (UPD) were tested by methylation-specific MLPA (MS-MLPA).

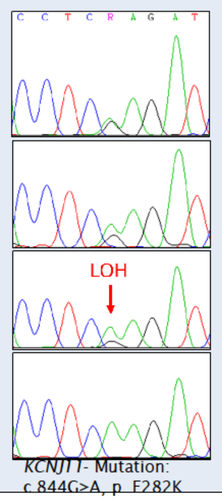
Results

Genetic and expression analysis in pancreatic lesions from focal CHI

Patient. No.	Mutation	LOH	UPD 11p15	mRNA- Expression
ABCC8				
1	c.4162_4164delTCT	++	++	mutant allele
2	c.1530G>T	++	++	mutant allele
3	c.50T>C	++	++	mutant allele
4	c.4241C>T	++	++	mutant allele
5	c.1792C>T	++	++	Ø (NMD)
6	c.4259C>T	+	+	mutant allele
7	c.2560-?_2697+?del	ROH	n.a.	mutant allele (del Exon 21)
KCNJ11				
8	c.612C>A	++	++	mutant allele
9	c.844G>A	++	++	mutant allele
10	c.286G>A	+	+	mutant/wildtyp allele 75%/25%
11	c.901C>G	(+)	+	mutant allele

LOH, loss of heterozygosity; ++, >80-100% loss of the maternal allele; +, >50-80% loss of the maternal allele; (+), >20-50% loss of the maternal allele; ROH, retention of heterozygosity; UPD 11p15, uniparental isodisomy including paternal imprint; ++, pUPD; +, UPD; n.k.,not known; n.d., not determined

Results



KCNJ11- Mutation:
c.844G>A, p. E282K

DNA pancreas Normal tissue

cDNA pancreas Normal tissue

DNA pancreas Focal lesion

cDNA pancreas Focal lesion

MS- MLPA shows no deletion or duplication of the BWS-Region 11p15.5, but an imbalance of methylation.

Conclusions

Molecular genetic analysis of *ABCC8* and *KCNJ11* in pancreatic lesions revealed expression of the paternally transmitted mutant allele and loss of the maternal allele. This supports somatic mosaicism specific in pancreatic endocrine cells. Both, *ABCC8* and *KCNJ11*, are located in proximity to the Beckwith-Wiedemann imprinting region on chromosome 11p15 that is also known for UPD. Paternal UPD is responsible for LOH and leads to a growth advantage of β-cells in focal lesions. This growth advantage results from increased expression of genes, which promote proliferation *(IGF2)* and decreased expression of genes which inhibit proliferation *(CDKN1C, H19).*

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