**Background.** Neonatal hyperinsulinemic hypoglycemia (HH) has recently been recognized as a consequence of mutations in HNF1A, which also cause diabetes later in life.

**Aims.** To report phenotypic and genetic investigations of two patients with functional characterisation of identified mutations in HNF1A.

**Case reports.** Two unrelated patients presented with hyperinsulinemic hypoglycemia requiring intravenous glucose administration during the neonatal period. Patient 1 repeatedly developed hyperglycaemia during acute respiratory infections in infancy. Macrosomia was observed in all affected family members (Fig.1). Patient 2 was recently observed for glycosuria and fasting glycemia 7 mmol/L at the age of 18 months (Fig.2). Positive family history of diabetes was reported in both families.

Age: 57 y
- Macrosomia (BW 4000 g)
- DM since 18 years of age
- HbA1c 54 mmol/mol

Age: 34 y
- Macrosomia (BW 4070 g)
- GDM – every pregnancy
- HbA1c 39 mmol/mol

Age: 31 y
- Macrosomia (BW 4700 g)
- HbA1c 36 mmol/mol

Age: 2 y
- Macrosomia (BW 4110 g)
- Neonatal hyperinsulinemic hypoglycemia
- Transient hyperglycaemia, HbA1c 29 mmol/mol

Age: 4 y
- Macrosomia (BW 4100 g)
- Neonatal hyperinsulinemic hypoglycemia
- HbA1c 35 mmol/mol

Age: 48 y
- DM since young age
- Sulphonylurea treatment

Age: 22 y
- Since 13 years of age hyperglycaemia
- Without treatment
- Sulphonylurea treatment since 17 years of age
- HbA1c 56 mmol/mol

Age: 1.5 y
- Neonatal hypoglycemia. Recently observed for glycosuria
- HbA1c 34 mmol/mol

**Fig.1** Family pedigree of patient 1. Affected members of family were presented with macrosomia, HH and diabetes.

**Fig.2** Family pedigree of patient 2. Diabetes and HH appeared in affected members of family.

**Methods.** DNA of two patients and their family members were analysed by directed sequencing (Fig.3). The pathogenic effect of the novel HNF1A mutations on normal HNF1A function was assessed by transcriptional activation assay in transffected HeLa cells (Fig.4), and DNA binding studies using in vitro expressed (TrT) proteins and analysed by Electrophoretic Mobility Shift Analysis (Fig.5).

![Figure 3: Diagram showing the mutations Leu254Gln (upper one) and Asn62LysFs93 (lower one) were detected by directed Sanger sequencing. All affected family members and patients carried heterozygous form of mutations.](image)

**Fig.3** The mutations Leu254Gln (upper one) and Asn62LysFs93 (lower one) were detected by directed Sanger sequencing. All affected family members and patients carried heterozygous form of mutations.

**Fig.4** The cells were transiently transfected with WT or mutant HNF1A plasmids and rat albumin reporter construct. Results are expressed as percentage of WT HNF1A activity. Independent experiments were performed in triplicates at least 3 times. Error bars represent the mean ± SEM. ***p < 0.001

![Figure 5: Xpress-epitope-tagged WT and HNF1A mutant proteins, expressed in an in vitro transcription/translation system, were incubated with a radiolabeled oligonucleotide containing the HNF1A-binding site in the rat albumin promoter.](image)

**Fig.5** Xpress-epitope-tagged WT and HNF1A mutant proteins, expressed in an in vitro transcription/translation system, were incubated with a radiolabeled oligonucleotide containing the HNF1A-binding site in the rat albumin promoter. Mutant p.Pro112Leu by reduced DNA-binding was included as control. Addition of the anti-Xpress antibody induced a supershift of the DNA-protein complex, confirming the identity of HNF1A within the complex.

**Results.** Two novel mutations in the HNF1A gene were detected: Patient 1 carried p.Leu254Gln and Patient 2 p.Asn62LysFs93. Both mutations segregated with β-cell defect within the families. Functional investigation of the p.Leu254Gln and p.Asn62LysFs93 mutation demonstrated severely reduced transcriptional activity (~20% and ~0% activity) compared to wild-type HNF1A (100% activity), respectively. Both of the in vitro expressed mutant proteins failed to bind to an HNF1A site in the rat albumin promoter.

**Conclusion.** Complex characterisation of two patients suggests that the capacity of β-cells to respond to high demands on insulin secretion may be impaired due to mutations in HNF1A at an early age. Our clinical and functional analyses confirm the role of HNF1A in pathogenesis of HH and emphasize the importance of molecular genetic testing of the HNF1A gene in patients presented with hyperinsulinemic hypoglycemia.

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