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HYPERGLYCEMIA PRECEDED BY NEONATAL HYPERINSULINEMIC HYPOGLYCEMIA IN INFANTS WITH NOVEL HNF1A MUTATIONS

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The authors have no relevant conflicts of interest.

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 hyperglycemia 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.

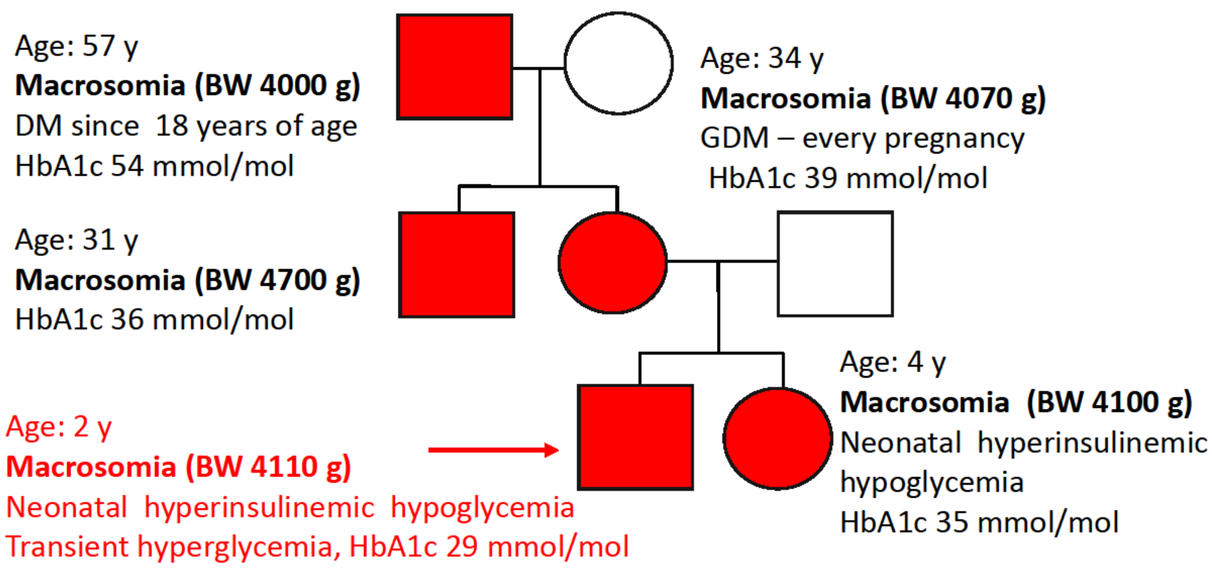

Fig.1 Family pedigree of patient 1. Affected members of family were presented with macrosomia, HH and diabetes.

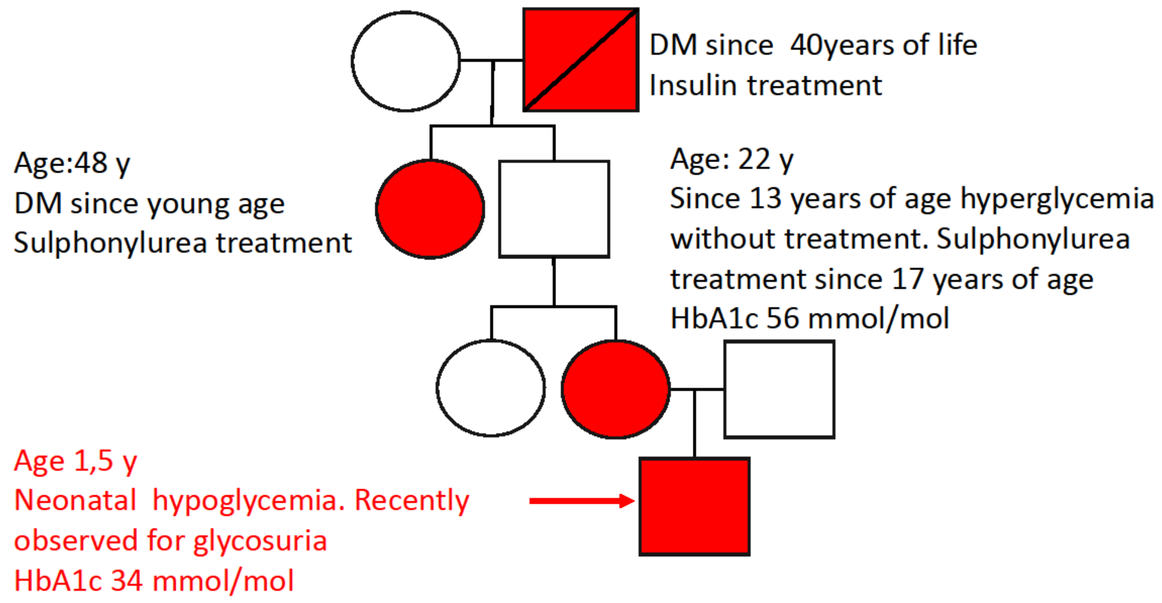

Fig.2 Family pedigree of patient 2. Diabetes and HH appeared in affected members of family.

Methods. DNA of two patients and their family members were analysed by directed sequencing (Fig3). the pathogenic effect of the novel *HNF1A* mutations on normal HNF1A function was assessed by transcriptional activation assay in transfected HeLa cells (Fig.4),and DNA binding studies using in vitro expressed (TnT) proteins and analysed by Electrophoretic Mobility Shift Analysis (Fig.5).

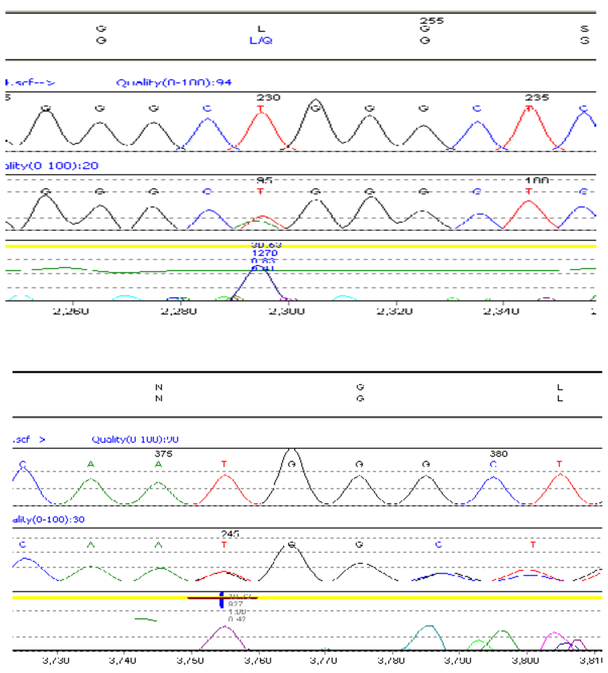

Fig.3. The mutations Leu254Gln (upper one) and Asn62LysFs93 (lower one) were detected by directed Sanger sequencing. All affected family members and patients carried heterozygous form of mutations.

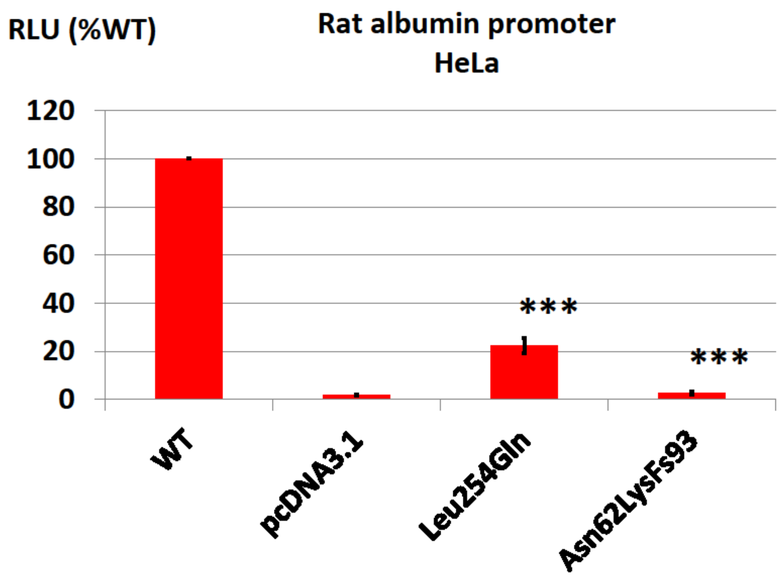

Fig.4. The cells were transiently transfected with WT or mutant HNF1A plasmids and rat albumin reporter construct. Results are expressed as percentage of WT HNF1A activity. Independent experiments were performed in triplicates at least 3 times. Error bars represent the mean ± SEM. *** $p<0.001$

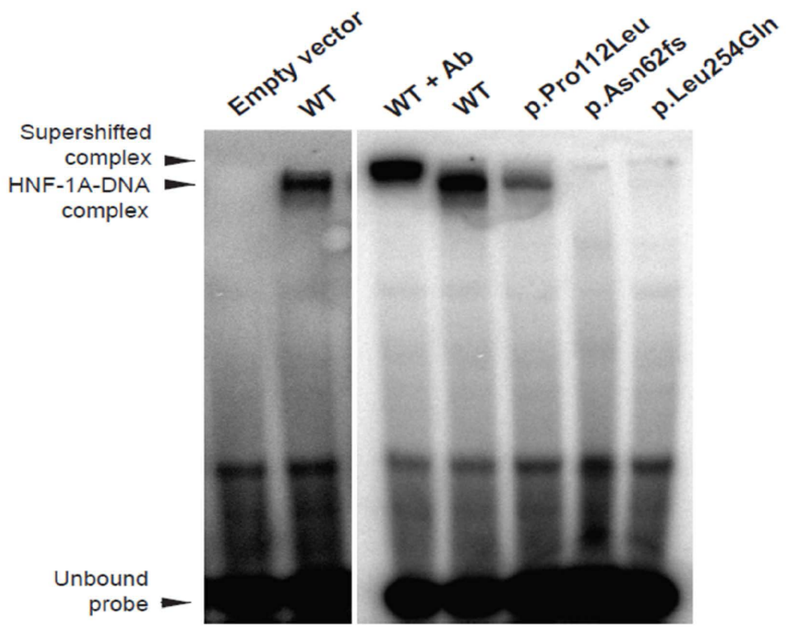

Fig.5. Xpress-epitope-tagged WT and HNF1A mutant proteins, expressed in an in vitro transcription/translation system, were incubated with a radiolabeled oligonucleotide containing the HNF1A-binding site in the rat albumin promoter. Mutant p.Pro112Leu by reduced DNA-binding was included as control. Addition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