Observation

Identification of a new case of hepatocyte nuclear factor-1 β mutation with highly varied phenotypes

To the Editor: Mutations in the hepatocyte nuclear factor (HNF)-1 β gene cause a rare form of MODY5 [1]. The various phenotypic features found in patients are related to tissues that express HNF-1 β , including pancreas, kidney, liver and uterus. As the highest level of expression is in the kidney, a range of developmental abnormalities of the kidney, which may cause non-diabetic renal dysfunction, have been described [2, 3, 4, 5, 6]. Of these, multiple renal cysts are the commonest phenotypic feature. Recently, the apparently distinct hereditary diseases, familial hypoplastic glomerulocystic kidney disease and familial juvenile hyperuricaemic nephropathy (FJHN), were also found to be associated with HNF-1 β mutations [3, 4], although there are other established genetic loci for FJHN. Because HNF-1 β is expressed from the earliest stages in the development of Wolffian duct in the fetus, mutations may also be associated with hereditary urogenital dysplasia [5, 6]. Thus, it is important to accumulate knowledge from various cases to understand the broad spectrum of clinical features associated with HNF-1 β mutation. However, only 13 families with mutations have so far been described, regardless of the presence or absence of diabetes. Here, we present a new case of HNF-1 β mutation which has highly varied symptoms.

The affected subject is a 17-year-old woman. Four weeks after birth (full-term birthweight 2930 g) she was found to have renal insufficiency with a blood urea nitrogen level of 15.35 mmol/l and a serum creatinine level of 194.48 μ mol/l. Bilateral multiple renal cysts in normal-sized kidneys were found by ultrasonography. Peritoneal dialysis was initiated at the age of 4 months owing to rapid progress of renal dysfunction. High blood glucose was first noticed at the age of 6 years (insulinogenic index 0.20; HbA_{1c} 7–8%), which is the earliest onset reported to date, when she was admitted to hospital with bacterial meningitis. Autoimmune antibodies were all negative. At the age of 7 years, her left kidney was resected and renal transplantation from her mother was performed. Ultrasonography before the operation showed that she had small kidneys (left 6.2 cm long; right 6.0 cm long). The pathological findings from the resected kidney (weighing 76 g) have previously been reported [7]. Briefly, most of the cut surface of the kidney is occupied by the cortex; the papillae and medulla are small and atrophic. The cortex contained numerous cysts of various sizes, which consisted of a dilated Bowman's space

and collapsed glomerular tufts. Clusters of immature metanephric cells, which were often arranged in a circular pattern forming lumens, were scattered throughout the area. Since the transplanted kidney function is currently normal, renal transplantation is effective for treatment of the renal phenotypes.

After the operation, various doses of prednisolone, ciclosporin and mizoribin were used for immunosuppression. Two years after renal transplant, blood glucose levels were highly elevated and the patient was treated by intensive insulin therapy (48 units/day). Urinary C-peptide level at initiation was 1.92 nmol/l, suggesting that the diabetes became insulin dependent. There has been no obesity throughout the clinical course. There is no diabetic retinopathy or neuropathy at present. Because of early-onset diabetes and multiple renal cysts, which are characteristic of MODY5, all of the exons and flanking regions of the HNF-1 β gene were screened for mutations by direct sequencing, as described previously [1, 2], resulting in the identification of a novel heterozygous frameshift mutation, L95fsinsAGCT (insertion of AGCT at the codon 95 for Leu), which could generate a truncated 116 amino acid protein. Gene testing of both parents, who were shown to be non-diabetic by an oral glucose tolerance test, revealed no mutation in the corresponding site of the gene, indicating that this is in fact a new HNF-1 β mutation.

Based on the diagnosis of MODY5, other tissues expressing HNF-1 β were extensively examined. Magnetic resonance imaging (MRI) revealed bicornuate uterus. Vaginal aplasia was also observed. Since similar abnormalities have been observed in three other families with the mutations, genital malformations may be an important manifestation of MODY5. Liver dysfunction with increased levels of transaminase was noticed shortly after birth in the patient and continues to the present (aspartate aminotransferase 79 U/l, alanine aminotransferase 138 U/l), although the liver size was normal according to MRI. Also observed was HDL-dominant hypercholesterolaemia (total cholesterol 6.31 mmol/l, HDL cholesterol 3.15 mmol/l). However, although the liver expresses both HNF-1 β and a heterodimerising partner HNF-1 α , which was found to be essential for bile acid metabolism and cholesterol metabolism in the liver, this is the first case representing liver dysfunction and hypercholesterolaemia.

The patient also has unusual neurological symptoms of epilepsy and learning difficulty. Similar learning difficulty was previously described in another patient with a mutation in the gene [3]. While HNF-1 β is known to regulate regional specification of zebrafish brain, the significance in human brain development is unknown. The patient also presents the unusual feature of prognathism of mandibula, although HNF-1 β is not expressed in bone tissue. Interestingly, prognathism has previously been described in two families with different mutations in HNF-1 β [3], suggesting that HNF-1 β may regulate genes encoding possible secreted proteins involved in bone growth of the mandibula.

The patient presents highly varied clinical features, some of which are quite severe. To investigate the molecular mechanism by which the mutation might cause the various phenotypic

Fig. 1. Functional analysis of the identified HNF-1 β mutation. The wild-type expression construct was prepared using human HNF-1 β cDNA and vector pCMV6b. The L95fsinsAGCT mutation was introduced in the sequence by site-directed mutagenesis and cloned into pCMV6b. The reporter construct pGL3-RA was prepared by cloning the rat albumin gene promoter into the firefly luciferase reporter vector pGL3-Basic (Promega, Madison, Wis., USA). HepG2 cells were transfected with various amounts of the reporter and test plasmid constructs. The transactivation activity of the wild-type and mutant proteins was measured using the Dual-Luciferase Reporter Assay System (Promega) as described previously [8]

ic features, wild-type and mutant proteins were transiently expressed in cultured hepatic cells. Luciferase reporter assay revealed L95fsinsAGCT to be a null function mutation (Fig. 1). However, when co-expressed with wild-type, the mutation behaved as strong dominant negative regulation; an equimolar amount of the mutant reduced wild-type activity by ~85%. However, whether the dominant negative mechanism influences the diversity of phenotypes seen in this patient is unknown.

Since molecular cooperation with other diabetogenes might increase the severity of symptoms or hasten onset, other MODY genes were also screened for mutations in this patient, resulting in the identification of one missense mutation, D78H, in the BETA2/NeuroD1 (MODY6) gene; the allele frequency in the general population is 0.002 (three in 1330 alleles). Since the mutation was transmitted from the non-diabetic mother, this mutation alone should not cause diabetes. However, because HNF-1 directly regulates BETA2/NeuroD1 gene transcription, some synergistic effect on increased morbidity is possible.

This report of a solitary case owing to a new mutation expands our clinical knowledge on HNF-1 β deficiency. Accumulation of additional cases and identification of the molecular participants in the transcriptional events are required to clarify this distinct syndrome of varied phenotypes.

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N. Shihara, Y. Horikawa

Laboratory of Molecular Genetics, Department of Cell Biology, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Gunma 371-8512, Japan

T. Onishi, M. Ono, K. Kashimada

Department of Pediatrics and Developmental Biology, Graduate School of Medicine, Tokyo Medical and Dental University, Japan

J. Takeda

Department of Endocrinology, Diabetes and Rheumatology, Division of Bioregulatory Medicine, Gifu University School of Medicine, Gifu, Japan

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Y. Horikawa (✉)

Laboratory of Molecular Genetics,
Department of Cell Biology,
Institute for Molecular and Cellular Regulation,
Gunma University, 3-39-15, Showa-machi, Maebashi,
Gunma 371-8512, Japan
E-mail: yhorikaw@showa.gunma-u.ac.jp
Tel.: +81-27-2208831, Fax: +81-27-2208889

Abbreviations: HNF, hepatocyte nuclear factor · MRI, magnetic resonance imaging · FJHN, familial juvenile hyperuricaemic nephropathy