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Letters

Splice site mutation in the hepatocyte nuclear factor-1β Gene, IVS2nt + 1G > A, associated with maturity-onset diabetes of the young, renal dysplasia and bicornuate uterus

Dear Sir,

Mutations in the homeodomain-containing transcription factor hepatocyte nuclear factor (HNF)-1β cause one form of maturity-onset diabetes of the young (MODY5) [1]. This relatively uncommon form of MODY has been reported to date in only four families [1–4]. The HNF-1β variation of diabetes is associated with not only hyperglycaemia but also impaired renal function that can include defects in kidney development such as renal cysts, oligomeganephronia and abnormal nephrogenesis. In addition, two of the four female carriers of a deletion mutation (R137-K161del) in the HNF-1β gene had internal genital abnormalities (vaginal aplasia and rudimentary uterus) [3]. Since HNF-1β transcripts can be detected in mesoderm-derived cells differentiating into the polarized epithelium during organogenesis of rat kidney [5], renal tubular functions could deteriorate in HNF-1β diabetes. Renal tubular function in MODY5 patients has, however, not been reported. We studied a Japanese family whose members had HNF-1β diabetes because of an inherited novel mutation located in the splice donor site of exon 2. This mutation co-segregated with MODY and renal dysfunction with mild impairment of tubular function. In addition, one female carrier had a bicornuate uterus. These results are consistent with the view that mutations in *HNF-1β* gene affect beta-cell, renal and genital development thereby leading to a broad spectrum of clinical symptoms.

The subject studied (Table 1) had polydypsia, polyuria and weight loss (6 kg) over a 3 month period at 11 years of age. Two months later during the course of a routine school health examination, she was noted to have glucosuria. Her fasting

Table 1. Clinical and laboratory findings in subjects with IVS2nt + 1G > A mutation in HNF-1β gene

	Subject	Sister	Mother
Present age (years)	19	13	42
Age at diagnosis of diabetes (years)	11	11	27
Body mass index (kg/m^2)	19.6	16.9	17.6
Treatment – insulin (U/day)	34	27	14
HbA_{1c} (%)	5.8	6.9	6.4
Fasting C peptide at presentation (ng/ml)	0.68	2.9	ND
Urine C peptide at presentation (μg/day)	10.1	ND	ND
Islet cell antibodies at presentation	Negative	ND	ND
Retinopathy	Absent	Absent	Absent
Neuropathy	Absent	Absent	Absent
Renal function			
Blood urea nitrogen (mg/dl) (8–20 mg/dl)	9.3	20.2	ND
Serum creatinine (mg/dl) (0.5–1.2 mg/dl)	0.8	1.1	2.18
Uric acid (mg/dl) (< 7.0 mg/dl)	6.4	8.4	ND
Urinary protein	–	+ /–	ND
Urinary albumin/creatinine (mg/g) (< 18 mg/g)	13.6	253.0	ND
Urinary β2-microglobulin (μg/l) (< 250 μg/l)	101	282	ND
Urinary N-acetyl-β-D glycosaminadase (U/l) (0–10 U/l)	11.1	7.0	ND
Ultrasonography	Bilateral renal cysts Increased cortical echogenicity Poor differentiation of corticomedurally junction in both kidneys	Bilateral renal cysts Increased cortical echogenicity Poor differentiation of corticomedurally junction in both kidneys	Right renal cysts Left renal dysplasia Bicornuate uterus

Tests were carried out during a clinical examination in September 1999 unless otherwise indicated
ND = not determined

Corresponding author: N. Iwasaki, MD, FJSIM, Fellow of Japanese Society of Internal Medicine, Diabetes Center, Tokyo Women's Medical University, 8–1 Kawada-cho, Shinjuku-ku, Tokyo 162–8666, Japan

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plasma glucose and insulin concentrations were 10.3 mmol/l and 1.33 pmol/l. She was diagnosed with diabetes and insulin therapy was started. Her younger sister was also found to have glucosuria at 11 years of age during the course of a school health examination. The mother, currently 42 years of age, has been treated for diabetes since she was 27 years of age. None of the mother's three siblings is known to have diabetes but her father had been treated for diabetes. The presence of renal cysts and left renal dysplasia in the mother in addition to diabetes prompted us to screen the *HNF-1β* gene for mutations. DNA was prepared from the peripheral blood after signed informed consent was obtained from these three patients. The minimal promoter and 9 exons of the *HNF-1β* gene were screened for mutations by direct sequencing of the polymerase chain reaction products as previously described (1). We identified a G-to-A mutation of the splice donor site of exon 2 (designated IVS2nt + 1G > A) that was present in the heterozygous state in the three subjects. This mutation, which is located at the boundary between exon 2 and intron 2, changes the conserved GT-dinucleotide at the splice donor site to AT and would affect synthesis and processing of *HNF-1β* mRNA. This substitution was not found in 100 normal chromosomes.

As noted in Table 1, the mother of the subject had right renal cysts and dysplasia of the left kidney. Bilateral renal cysts 10 mm in diameter were observed in the subject and her younger sister. In addition, we observed increased cortical echogenicity and poor differentiation of corticomedullary junction in both sisters indicating some renal developmental defect. The results of various renal function tests, including renal tubular function in the mutation carriers, are summarized in Table 1: some were in the normal range whereas others were higher. The presence of renal dysfunction with no evidence of diabetic retinopathy or neuropathy suggests that the observed abnormalities are not a consequence of hyperglycaemia itself.

The subject's mother also had a bicornuate uterus. The subject and her sister were not examined for internal genital malformations. This is the second family in which a female carrier of a mutation in *HNF-1β* gene has been reported to have an abnormality related to the development of Müllerian system which gives rise to the uterus and upper vagina. The combination of anomalies of the Müllerian duct with developmental errors of the urinary tract are features of the condition termed hereditary urogenital dysplasia (OMIM 191 830). The variable expression of mutations in the *HNF-1β* gene could result in a diverse clinical phenotype.

Sincerely yours,
N. Iwasaki, I. Okabe, M. Y. Momoi, H. Ohashi, M. Ogata, Y. Iwamoto

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C Peptide and insulin do not influence plasma semicarbazide-sensitive amine oxidase activity

Dear Sir,
In diabetes mellitus plasma activities of semicarbazide-sensitive amine oxidase (SSAO) have been reported to be raised in childhood Type I (insulin-dependent) diabetes mellitus at first clinical diagnosis [1]. SSAO belongs to a group of enzymes which convert primary amines into aldehydes, hydrogen peroxide and ammonia; increased plasma SSAO concentrations have been shown to have deleterious effects on vascular endothelium [2]. The precise physiological role of SSAO is not fully understood but it plays a part in glucose transportation into adipocytes through the GLUT4 transporter [3]. The origin of plasma SSAO and the cause of the rise in activity in pathological conditions like diabetes mellitus and congestive heart failure are not known. It has been suggested that naturally occurring inhibitors or activators or both are responsible for such changes [4, 5]. In newly onset Type I diabetes a drastic fall in both C peptide and insulin occurs, while Type II (non-insulin-dependent) diabetes mellitus usually has increased insulin and C-peptide concentrations. We wondered whether C peptide and insulin might have inhibitory or activating effects on SSAO, and that the rise in SSAO could be explained by the changing concentrations of these compounds.

SSAO activity was determined in plasma by a functional assay measuring the amount of benzaldehyde generated by SSAO from the substrate benzylamine during a 1-h incubation period [6]. Activities were measured in plasma as such, and in the same plasma to which C peptide (Eli Lilly) or insulin (Actrapid) or both had been added, each in 6 different concentrations ranging from 0.5 nmol/l to 50 μmol/l, so that $3 \times 7 = 21$ SSAO activities were determined for each plasma sample. Experiments were done with 6 different plasma samples: 2 control pools (with normal and high SSAO activity, 388 and 720 mU/l respectively), 2 from patients with diabetes mellitus (336 and 1132 mU/l) and 2 from patients with chronic heart failure (332 and 1089 mU/l). No effects of C peptide, insulin or a combination of both were seen. The 21 measurements in each plasma sample averaged 394 ± 28, 717 ± 41, 339 ± 25, 1099 ± 67, 339 ± 14 and 1074 ± 49 mU/l, respectively. The average variation coefficient was 5.9 % (range 4.3–7.5 %), not dif-

Corresponding author: F. Boomsma, Department of Internal Medicine, Erasmus university Medical Center, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands