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Phenotype of a patient with a de novo mutation in the hepatocyte nuclear factor 1β/maturity-onset diabetes of the young type 5 gene

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Abstract

Mutations in the gene encoding the transcription factor hepatocyte nuclear factor *(HNF) 1β* cause various phenotypes including maturity-onset diabetes of the young type 5 (MODY5) and kidney disease. We provide molecular and pathophysiologic characterization of a 23-year-old male patient with clinical presentation typical for MODY5 with renal involvement. Clinical studies (including intravenous glucose tolerance test and magnetic resonance imaging) of the patient and 5 family members in comparison with unrelated control subjects and molecular analysis of the *HNF-1β* gene (direct sequencing, paternity testing, and restriction fragment length polymorphism analysis for parental mosaicism) were performed. The patient was born with low birth weight (2250 g), whereas his dizygotic twin sister was of normal weight (3500 g) and healthy. He had cystic renal dysplasia with progressive renal failure and pancreas atrophy with β-cell dysfunction and early-onset diabetes mellitus but no family history of diabetes. Intravenous glucose tolerance test showed a markedly reduced but not absent acute insulin response compared with controls (n = 6). A mutation in the *HNF-1β* gene S148L (C443T) in exon 2 within the pseudo-POU domain was identified. All other family members and the control group (n = 255) did not have the mutation, suggesting that we described a de novo mutation in *HNF-1β*. Paternity was confirmed, and no signs of mosaicism in DNA analysis of both parents could be detected. Of note, the low birth weight of the patient in contrast to his healthy twin sister provides interesting support for the fetal insulin hypothesis for reduced birth weight.

1. Introduction

Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous form of diabetes mellitus characterized by an onset usually before the age of 25 years, varying degrees of β-cell dysfunction, and an autosomal dominant mode of inheritance. It is estimated to account for 1% to 5% of all diabetes cases in industrialized countries. Five of the 6 identified MODY subtypes are associated with heterozygous mutations of different genes encoding specific transcription factors [1]. Mutations in the gene encoding the transcription factor hepatocyte nuclear factor *(HNF) 1β* cause various phenotypes including MODY type 5 (MODY5) and abnormalities in kidney, pancreas, and genital tract formation [2-15].

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2. Materials and methods

2.1. Intravenous glucose tolerance test

The patient was taken off insulin 12 hours before the study; 0.3 g/kg body weight of a 20% glucose solution was given at time 0. Blood samples for the measurement of serum insulin were obtained at −10, −5, 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, and 60 minutes. The results were compared with those previously obtained in 6 nondiabetic 24- to 30-year-old control subjects (3 women and 3 men; body mass index [BMI], 20.0-26.6 kg/m^2).

2.2. Sequencing/mutational analysis

Genomic DNA was isolated from peripheral blood leukocytes using a QIAmp DNA mini kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. To identify genetic variants, all 9 exons including intron/exon splicing sites as well as the 5′ and 3′ untranslated regions of the *HNF-1β* gene were then amplified in 25-μL

reactions containing 50 ng of genomic DNA from all 6 family members. Sequencing was performed using the Big Dye Terminator (Applied Biosystems, Foster City, CA) on an automated DNA capillary sequencer (ABI PRISM 3100 Avant, Applied Biosystems). The novel missense mutation was confirmed with an additional primer pair. Oligonucleotide sequences were as follows: forward primer 5′-AGGGAGGTGGTCGATGTCA-3′ and reverse primer 5′-GGTGCCCTTGTTGAGATGCT-3′; TaqMan probes VIC dye 5′-CTGAACCAGTCGCACCT-3′ and FAM dye 5′-CTGAACCAGTTGCACCT-3′.

2.3. Paternity testing

Paternity testing was performed by using highly polymorphic markers D7S440, D10S1653, D11S4046, D16S403, D16S497, D19S418, D20S195, D21S1256, and DXYS233. Briefly, for each marker, one of the primers was labeled at the 5′end with the fluorescence dye fluorescein (FAM). Polymerase chain reaction (PCR) was then performed in 20-μL reactions containing 50 ng of genomic DNA, 2 pmol each of labeled and unlabeled primer, and the same buffer as for the genomic DNA amplification on an MJ PTC 225 Tetrad Cycler (MJ Research, Waltham, MA). The PCRs were denatured at 94°C for 3 minutes, followed by 35 cycles at 94°C for 30 seconds and touchdown annealing with 6 cycles starting at 64°C for each primer pair. The fragments were then visualized with ABI PRISM 3100 Avant (Applied Biosystems).

2.4. PCR–restriction fragment length polymorphism analysis

For detection of mosaicism associated with the S148L mutation, we used the restriction fragment length polymorphism–based method to detect low-level mosaicism. Exon 2 of the *HNF-1β* was amplified as for the mutation analysis described above. Twenty microliters of the PCR product was digested with 1 U of *HpyCH4V* in a 30-μL reaction at 37°C for 1 hour and then electrophoresed through a 12% polyacrylamide gel, which was then stained with ethidium bromide.

2.5. Genotyping of the S148L variant

Genotyping of the S148L variant was done using the TaqMan allelic discrimination assay (Custom TaqMan Single Nucleotide Polymorphism [SNP] Genotyping Assay, Applied Biosystems). Oligonucleotide sequences are available upon request. The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 9600 (95°C for 10 minutes, 95°C for 15 seconds, and 62°C for 1 minute, for 38 cycles), and fluorescence was detected on an ABI PRISM 7500 sequence detector (Applied Biosystems). To assess genotyping reproducibility, a random ~10% selection of the sample was regenotyped in both SNPs; all genotypes matched the initial designated genotypes.

3. Results

We report a 23-year-old patient who presented with advanced-stage chronic kidney disease (glomerular filtration rate, 15 mL/min) with tertiary hyperparathyroidism and insulin-treated diabetes mellitus. He was born as a hypotrophic dizygotic twin with low birth weight (2250 g; gestational age, 39 weeks). His healthy twin sister weighed 3500 g. An older brother, a half sister, and his parents are healthy (Fig. 1). Immediately after birth, serum creatinine concentrations were already elevated (130-165 μmol/L); and overt proteinuria (250 mg/d), anemia, and metabolic acidosis were present. Urinary sediment was normal, and urine cultures were negative. Kidney morphology by ultrasound showed organ degeneration, and renal biopsy performed 3 months after birth revealed small cystic renal dysplasia. By this time, creatinine concentration had further increased (147-205 μmol/L); but proteinuria was stable. Apart from this, physical and mental development during early life was normal. Because of developing renal osteopathy, replacement therapy with calcium and vitamin D was started in the first 3 months. At age 13 years, diabetes mellitus was detected by a routine physical examination; and 3 years later, insulin therapy had to be started. At that time, a large cyst in the head of the pancreas was detected by ultrasound.

On present clinical examination, the patient was lean (BMI, 22.4 kg/m^2); and his insulin requirements were approximately 33 IU/d (equivalent to 0.48 IU/kg, 18 IU long-acting insulin, 15 IU short-acting insulin). Hemoglobin A_{1c} was 6.2%, and basal C-peptide plasma concentration (0.44 nmol/L) was in the lower reference range. Islet-cell antibodies and glutamic acid decarboxylase autoantibodies were negative. Values from liver function tests were not

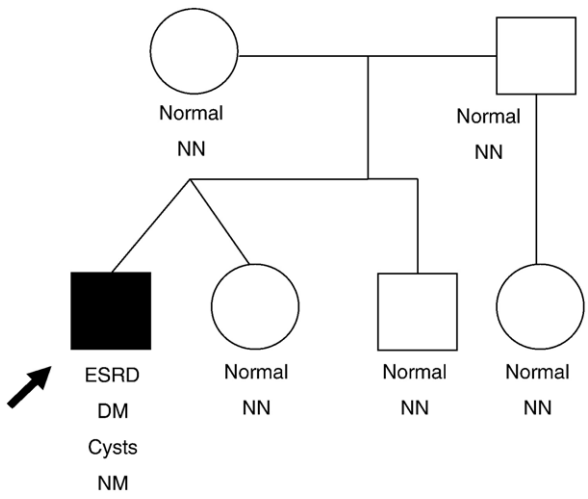

Fig. 1. Pedigree of the patient's family. The arrow indicates the patient. Subjects affected are indicated by a filled symbol; nonaffected subjects are indicated by an open symbol. Genotype: N, normal allele; M, mutation allele S148L (C443T). ESRD indicates end-stage renal disease; DM, diabetes mellitus.

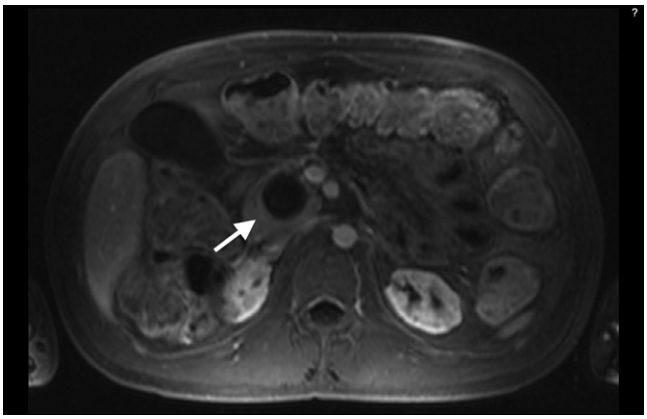

Fig. 2. Magnetic resonance tomography performed at age 23 years showing pancreas atrophy and kidney malformation. The head of the pancreas was presented with a large cyst surrounded by a thin ring of residual pancreatic tissue (white arrow). The body and tail of the pancreas were not detectable. Both kidneys were markedly reduced in size with bilateral small cystic renal dysplasia.

elevated. There were no clinical signs of exocrine pancreas dysfunction, and measurement of fecal elastase concentration was normal. No genital tract abnormalities were found in ultrasound and physical examination. Abdominal magnetic resonance imaging showed a hypotrophic pancreas with only a small tissue residue surrounding a large (33 × 28 mm) cyst. The size of both kidneys was markedly reduced, with bilateral small cystic renal dysplasia corresponding to end-stage renal disease (Fig. 2). Because of progressive decline in glomerular filtration rate, hemodialysis was started.

To characterize insulin secretion, we performed an intravenous glucose tolerance test (IVGTT) and compared the results with that of a control group (n = 6) matched for age and BMI. As shown in Fig. 3A, 2 phases of insulin secretion were discernible in the patient but were clearly reduced compared with the controls. It appears that the reduction in first phase was more pronounced than that in second phase. In addition, Fig. 3B demonstrates reduced C-peptide levels in the patient compared with the control group in the IVGTT.

Because phenotypic characterization of the patient was consistent with diagnosis of MODY5, molecular analysis of blood samples obtained from all family members was performed. Direct sequencing of the *HNF-1β* gene in the patient revealed a novel missense mutation S148L (C443T) in exon 2 within the pseudo-POU domain for which the patient was heterozygous. The sequence was confirmed by sequencing with a different primer pair as well as by genotyping using the TaqMan technique. All of the other family members including the twin sister carried the wild-type genotype at this position. Furthermore, we found some SNPs within the 3′ untranslated region (rs2689, rs2688, rs10962, T2221A) in the index patient and his twin sister.

This suggested that the S148L variant was a de novo mutation, and further tests were performed to support this hypothesis. (*a*) By analyzing 9 highly polymorphic markers, we confirmed paternity. (*b*) Because the S148L mutation generated a novel *HpyCH4V* restriction site, we used PCR–restriction fragment length polymorphism analysis to examine potential mosaicism in both parents. We did not detect any signs of low-level mosaicism in the DNA of the mother or father because only the PCR fragment of the patient's DNA showed additional bands resulting from the cut by *HpyCH4V* at the newly occurring restriction site. To confirm the mutation status of the S148L variant, we genotyped 255 additional nonrelated white subjects from the same geographical area. None of these subjects carried the mutation.

4. Discussion

Renal dysfunction and early-onset diabetes mellitus are typical characteristics of subjects affected with MODY5 because *HNF-1β* plays a critical role in normal kidney development and pancreatic *β*-cell function [16]. In this context, renal involvement seems to be a primary feature, with symptoms evident often at early months of age and possibly at birth [5]. The renal pathologies typically associated with MODY5 are not related to diabetes and

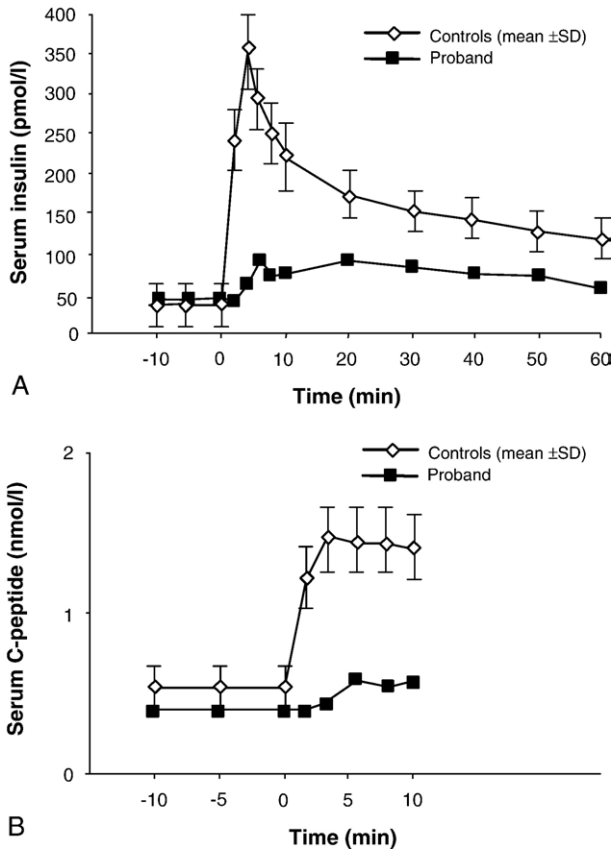

Fig. 3. Serum insulin (A) and C-peptide (B) concentrations during an IVGTT (0.3 g/kg body weight) in the patient and a matched control group (n = 6).

Table 1
Cases of S148 *HNF-1β* mutations in literature

Mutation	Mode	Characteristics	Literature
S148W (2 siblings)	Maternal germ line mosaicism	Female: neonatal diabetes, normal kidney function Male: cystic renal disease, renal failure, kidney transplantation at age 2 y	[22]
S148L	De novo	Renal dysplasia at birth, no renal failure, diabetes at age 13 y	[24]
S148L	De novo	Neonatal diabetes, pancreas atrophy, renal dysplasia at birth, no renal failure	[25]
S148L	De novo	Renal dysplasia at birth, renal failure, dialysis at age 23 y, diabetes at age 13 y, pancreas atrophy, healthy dizygotic twin sister	This case

cover a wide clinical spectrum of various renal abnormalities including multicystic dysplastic kidneys, noncystic renal parenchymal disease, oligomeganephronia, familial glomerulocystic kidney disease, or atypical familial juvenile hyperuricemic nephropathy [5,16-19]. In our patient, severe cystic renal dysplasia was already confirmed by an open renal biopsy in early childhood. Genital tract malformations were absent.

Although islet antibodies were negative clinically, he had been classified as type 1. Plasma C-peptide concentrations were in the lower reference range despite only a thin residue of pancreatic tissue around a large abdominal cyst. His daily insulin requirements were moderate like in a lean subject with type 1 diabetes mellitus. β-Cell dysfunction and reduced insulin sensitivity of endogenous glucose production are described in patients with *HNF-1β* mutations [20,21]. The IVGTT of the patient clearly suggests that some residual β-cell function is present, albeit inadequate to produce a first phase and control blood glucose.

The mutation we detected (S148L [C443T]) was heterozygous and located in exon 2 within the pseudo-POU domain of the gene that determines the target sequence specificity of the *HNF-1β* molecule. The mutation was not present in both parents (confirmed paternity) or in any other family member studied. We also excluded genetic mosaicism in both parents. We therefore conclude that the S148L mutation in our patient represents a de novo mutation.

A different mutation at the same codon, S148W (C443G), was previously found in 2 Japanese siblings with phenotypically very discordant affection, one with permanent neonatal diabetes mellitus and the other with neonatal polycystic, dysplastic kidneys due to maternal germ line mosaicism. Divergent influences of additional genetic or environmental factors on the phenotypic expression of mutations were discussed in this report, and further molecular analysis showed that the S148W variant resulted in a loss of functional protein [22]. The S148 residue is crucial for successful DNA sequences targeting, and 4 cases of S148 mutations with highly different phenotypes have been reported (Table 1). It thus appears that this specific region may present a genetic hot spot for mutations in *HNF-1β*.

Finally, the low birth weight of the patient in contrast to the normal birth weight of his healthy dizygotic twin sister is of note. The recently described observation of reduced birth weight in *HNF-1β* mutation carriers born to an unaffected mother is consistent with reduced secretion of insulin in utero that is an important component of fetal growth. In addition to its involvement in kidney development, there is increasing evidence of a critical role of *HNF-1β* in pancreatic development [23-25]. In our case, despite the same maternal intrauterine environment for both twins, reduced fetal insulin secretion in the carrier of the *HNF-1β* mutation resulted in intrauterine growth retardation and reduced birth weight.

In conclusion, we identified a de novo mutation in the *HNF-1β* gene resulting in a phenotype of severe renal involvement manifested at birth and early-onset diabetes with β-cell dysfunction. The prevalence of MODY5 is probably underestimated because the extent and severity of organ dysfunction related to *HNF-1β* mutations are highly variable and the typical mode of inheritance is not present in all cases. Descriptions of MODY5 cases with restricted phenotypes may help define clinical criteria warranting *HNF-1β* gene testing.

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