Early Development of Hyperparathyroidism Due to Loss of *PTH* Transcriptional Repression in Patients With HNF1β Mutations?

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Context: Heterozygous mutations or deletions of the transcription factor hepatocyte nuclear factor 1β (HNF1β) result in a heterogeneous syndrome characterized by renal cysts and diabetes, together with a variety of other extrarenal and renal manifestations. Interestingly, in several patients with HNF1β abnormalities, we observed early hyperparathyroidism and PTH levels that we judged inappropriately high compared with the degree of renal function decline.

Objective: Based on the above clinical observations, we tested the hypothesis of a direct role of HNF1β in the transcriptional regulation of the human *PTH* gene in the parathyroid gland.

Design, Setting, and Patients: Immunostaining of human parathyroid sections, RT-PCR, chromatin immunoprecipitation (ChIP), and luciferase reporter assays in human embryonic kidney cells (HEK293) were performed. We eventually report clinical data from all 11 HNF1β patients known at our institute, 9 with heterozygous *HNF1*β whole-gene deletions and 2 with heterozygous *HNF1*β mutations.

Results: PTH levels were high in 8 patients. In 2 of these patients, the hyperparathyroidism was clearly appropriate for the level of kidney function, whereas PTH might be discrepant in the others. We demonstrated HNF1β expression in PTH-positive cells of human parathyroid gland. Chromatin immunoprecipitation analysis showed that HNF1β directly binds responsive elements within the human *PTH* promoter. Cotransfection of a *PTH* promoter- luciferase construct with a wild-type HNF1β construct resulted in a maximal reduction of 30% of *PTH* promoter activity. Importantly, HNF1β mutants lacked this inhibitory property. Serial deletions in the *PTH* promoter construct revealed that the inhibitory effect of HNF1β resides between -200 and -70 bp from the transcription initiation site.

Conclusions: Our data demonstrate that HNF1β is a novel repressor of human *PTH* gene transcription, which could contribute to the development of hyperparathyroidism in patients with *HNF1*β mutations or deletions. **(*J Clin Endocrinol Metab* 98: 4089–4096, 2013)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received September 26, 2012. Accepted August 8, 2013.
First Published Online August 26, 2013

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Abbreviations: CaSR, Ca^{2+}-sensing receptor; ChIP, chromatin immunoprecipitation; 1,25-D_3, 1,25-dihydroxyvitamin D_3; DAPI, 4′,6-diamidino-2-phenylindole; FGF, fibroblast growth factor; FGFR1, FGF receptor 1; GFR, glomerular filtration rate; HNF1β, hepatocyte nuclear factor 1β; POU, *Pit-1*, *OCT1/2*, *UNC-86*; VDR, vitamin D receptor; VDRE, vitamin D-responsive element.

The parathyroid gland has a central role in calcium (Ca^{2+}) and phosphate (PO_4^{3-}) homeostasis. PTH regulates the synthesis of 1,25-dihydroxyvitamin D_3 (1,25-D_3), alters Ca^{2+} and PO_4^{3-} (re)absorption in the kidney and intestine and modulates bone metabolism (1–3). Serum PTH levels depend on direct secretion of PTH from the secretory granules in the parathyroid gland as well as on synthesis of new PTH molecules secondary to *PTH* gene transcription. PTH expression is restricted to the parathyroid glands in humans and is under the control of specific stimuli and repressors. A low serum Ca^{2+} alters the activation of the Ca^{2+}-sensing receptor (CaSR) on the surface of parathyroid glands, leads to the rapid release of PTH from the secretory granules, and stimulates *PTH* gene expression, whereas high Ca^{2+} inhibits PTH secretion (4). Contrary to Ca^{2+}, high PO_4^{3-} leads to increased PTH levels. Furthermore, *PTH* transcription is repressed by binding of a complex of 1,25-D_3, the vitamin D receptor (VDR), and retinoic acid X receptor (RXR) to vitamin D-responsive elements (VDREs) in the promoter region of the *PTH* gene (5, 6). Recently, it was shown that *PTH* gene transcription is also inhibited by fibroblast growth factor (FGF) 23, a novel phosphaturic hormone that acts through the FGF receptor 1 (FGFR1)/klotho receptor complex present in parathyroid cells (7, 8). Finally, the *PTH* promoter activity is regulated by the concerted action of tissue-specific transcription factors, such as glial cells missing B (4), and nonspecific transcription factors, like specificity protein 1, nuclear transcription factor-Y, cyclic AMP-responsive element-binding protein, and transcription factors GATA (9, 10).

The hepatocyte nuclear factor 1β (HNF1β) is a *Pit-1*, *OCT1/2*, *UNC-86* (POU) domain transcription factor that participates in organogenesis during early embryonic development (11). More specifically, it regulates tubulogenesis in the liver, pancreas, kidney, and genital tract. In the kidney and urinary tract, HNF1β is expressed in renal tubules as well as developing ureters. Heterozygous mutations or deletions in the *HNF1β* gene are responsible for a dominant syndrome characterized by highly heterogeneous renal and extrarenal phenotypes that can comprise: 1) renal malformations with or without cyst formation (glomerulocystic disease, cystic renal dysplasia, calyceal abnormalities, oligomeganephronia, or solitary kidney); 2) liver and genital tract abnormalities; and 3) defects in the exocrine and endocrine pancreatic functions including maturity-onset diabetes of the young type 5 (MODY5; OMIM 137920). Furthermore, HNF1β nephropathy is distinguished by a large variability in renal tubular transport abnormalities (12). Functional HNF1β binding sites have been identified in the promoter regions of many renal cystic genes, (13–15) as well as genes involved in tubular transport (16, 17), such as the *FXYD2* gene encoding for the γ-subunit of the Na^+/K^+-ATPase. The impaired transcription of this gene by HNF1β is suggested to be involved in the renal Mg^{2+} wasting observed in almost half of the patients (18).

After observing early hyperparathyroidism and PTH levels that were judged inappropriately high by us in several index patients with known *HNF1β* mutations and/or deletions, we hypothesized that HNF1β might regulate PTH expression in the parathyroid gland. Because HNF1β is a tissue-specific transcription factor highly expressed in the epithelia of specialized endocrine organs and tissues with secondary endocrine functions (19–22), the aim of our study was to investigate whether HNF1β could act as a transcriptional regulator of the human *PTH* gene, possibly by directly affecting *PTH* promoter activity.

Patients and Methods

Patients with *HNF1β* mutations and/or deletions

All patients were diagnosed by nephrologists and/or clinical geneticists at the Radboud University Nijmegen Medical Centre, The Netherlands. Clinical histories were collected from hospital records. Informed consent for genetic analyses was obtained from all patients. Intact PTH was measured on an Architect random access analyzer (Abbott). In a limited number of samples, intact PTH was measured by another method that gave comparable results and conform to the evaluation protocol 9 (EP9, Clinical and Laboratory Standards Institute) protocol.

DNA constructs

The 5′-promoter region of the human *PTH* gene (−1476/+25; +1 designates the transcription start site, NM_000315.2) was obtained by amplification of genomic DNA using a high-fidelity DNA polymerase (Phusion, Finnzymes; forward 5′-AAAAAAGGTACCCAGCTATAAAGTCATCCCGTCTT-3′ and reverse 5′-GGGGGGGAGCT CGCAGACCCCTTAAATGGTGA-3′). To generate a firefly luciferase reporter construct, the PCR product was cloned into a pGL3-Basic vector (Promega), using the restriction sites *Kpn*I and *Sac*I. Firefly luciferase constructs for deletion analysis were prepared with a similar cloning strategy after amplification of the promoter regions −1000/+25 (forward 5′-AAAAAAGGTACCAGTCAGACATGTGGCAGCATCATG-3′ and reverse 5′-GGGGG GGAGCTCGCAGACCCCTTAAATGGTGA-3′), −630/+25 (forward 5′-AAAAAAGGTA CCCATGCAGTTAGTGCTTATCAAATG-3′ and reverse 5′-GGGGGGGAGCTCGCAGACC CCTTAAATGGTGA-3′), −500/+25 (forward 5′-AAAAAAGGTACCCAAATTATTCTTAA CACTTCCTTTAAG-3′ and reverse 5′-GGGGGGGAGCTCGCAGACCCCTTAAATGGTGA-3′), −200/+25 (forward 5′-AAAAAAGGTACCGTCTTTGCATAAGCCCCTTGTC-3′ and reverse 5′-GGGGGGGAGCTCGCAGACCCCTTAAATGGTGA-3′), and −70/+25 (forward 5′-AAAAAAG GTACCCAGAGAATTGGGAGTGACATC-3′ and reverse 5′-GGGGGGGAGCTCGCAGA CCCCTTAAATGGTGA-3′). The pRL-CMV vector encoding Renilla luciferase was commercially available (Promega) and used to correct for transfection efficiency. HA-HNF1β wild-

type and HA-HNF1β p.His69fsdelAC, p.Lys156Glu, p.His324Ser325fsdelCA, and p.Tyr352fsinsA. pCINEO IRES GFP were cloned as previously described (23). All constructs were verified by sequence analysis.

Cell culture and transfection

Human embryonic kidney cells (HEK293) were grown in DMEM (Bio Whittaker-Europe) containing 10% (vol/vol) FBS (Thermo Fisher HyClone), 2mM L-glutamine, and 10 μg/mL ciproxin at 37°C in a humidity-controlled incubator with 5% (vol/vol) CO_2. The cells were transiently transfected with the respective constructs using polyethylenimine cationic polymer (PEI, Polysciences Inc) and assayed 48 hours after transfection. When performing dose-response analysis, an empty vector (including nonsense mock DNA) was used to keep the total amount of transfected DNA constant.

Luciferase reporter assay

In a 12-well plate, 700 ng of the *PTH* promoter-luciferase constructs and 100 ng of either empty vector (mock DNA) or HA-HNF1β wild-type, HA-HNF1β p.His69fsdelAC, p.Lys156Glu, p.His324Ser325fsdelCA, and p.Tyr352fsinsA. pCINEO IRES GFP constructs were cotransfected into HEK293 cells. To correct for transfection efficiency, 10 ng of pRL-CMV was used as a reference. Firefly and renilla luciferase activities were measured with the dual-luciferase reporter assay (Promega).

Immunohistochemistry

Staining was performed on 6-μm sections of frozen human cadaveric parathyroid samples that were fixed with 100% methanol for 10 minutes at −20°C. Subsequently, sections were washed 3 times with buffer (0.15 mol/L NaCl, 0.1 mol/L Tris adjusted to pH 7.6 with HCl) before incubation in blocking buffer for 30 minutes. Sections were stained overnight at 4°C with a rabbit antihuman HNF1β (1:20, sc-22840; Santa Cruz Biotechnology). The next day, sections were washed and incubated with a goat antirabbit secondary antibody coupled to Alexa Fluor 488 (1:300, A11008; Invitrogen) for 1 hour at room temperature. Sections were washed and incubated for 2 hours with a mouse antihuman PTH antibody (1:50, 7170–6216; AbD Serotec). After washing, a goat antimouse secondary antibody conjugated to Alexa Fluor 594 (1:300, A11005; Invitrogen) was applied for 1 hour. Subsequently, sections were washed, incubated for 30 minutes with 4′,6-diamidino-2-phenylindole (DAPI), and mounted with Mowiol. Photographs were taken using a Zeiss Axio Imager 1 microscope equipped with a HXP120 Kubler Codix fluorescence lamp and a Zeiss Axiocam MRm digital camera.

RT-PCR analysis

Total RNA from human parathyroid glands was isolated using Trizol (Ambion, Life Technologies). Total RNA (1.5 μg) was reverse transcribed and end-point PCR performed. Part of the RNA sample was not reverse transcribed before PCR and thereby served as a negative control. Sequences of the oligonucleotides used for the RT-PCR are shown in Table 1 and Supplemental Table 1 (published on The Endocrine Society's Journals Online website at http://jcem.endojournals.org).

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) analysis was performed using HEK293 cells cotransfected with 700 ng of the human *PTH* promoter luciferase constructs −1476/+25 or −70/+25 and 100 ng of the HNF1β pCINEO IRES GFP construct. Cells were harvested 48 hours after transfection, and a Magna ChIP A assay was performed according to the manufacturer's protocol (Merck Millipore). In short, cells were treated with formaldehyde to cross-link the chromatin. After cell lysis, samples were sonicated twice for 30 seconds on wet ice using a 22-μm amplitude with a Soniprep 150 (Measuring and Scientific Equipment). Samples were incubated with 5.0 μg rabbit polyclonal antihuman HNF1β antibody (Santa Cruz; sc-22840), rabbit IgG isotype antibodies as negative control, or anti-trimethylhistone H3 antibodies as positive control, which were bound to protein A magnetic beads. Subsequently, chromatin complexes were eluted, the cross-links were reversed, and the DNA was isolated. The presence of *PTH* promoter DNA was evaluated using real-time PCR targeting the human *PTH* promoter (5′-GCCTGGAGCAACACTCTAAG-3′ and 5′-CATCCTG-GCTTCATGTCATCC-3′). Subsequently, samples were loaded on a 2% agarose gel and visualized using ProXima C16 software version 3.0 (Isogen Life Science).

Data analysis

Results are based on a minimum of 3 independent experiments, with each condition performed in triplicate, unless otherwise stated. Values are expressed as means ± SEM. Statistical significance ($P < .05$) was determined using unpaired Student's *t* tests.

Results

PTH levels in a cohort of HNF1β patients

We initially reviewed the cases of several HNF1β patients, which raised the hypothesis of a link between *HNF1β* mutations or whole-gene deletions and early hyperparathyroidism. One patient underwent parathyroidectomy at the age of 23 years for primary hyperparathyroidism (patient VII, Table 2). This patient was 36 years of age before any connection between *HNF1β* mutations and his clinical symptoms was revealed, at which time he again dis-

Table 1. Oligonucleotide Sequences Used for RT-PCR Analysis

Gene	Accession	Forward (5′–3′)	Reverse (5′–3′)	Amplicon Size, bp
hPTH	NM_000315.2	CATTGTATGTGAAGATGATACCTGC	GCAGCATGTATTGTTGCCCT	424
hHNF1β	NM_000458.2	CATACTCTCACCAACGGCCA	AAACAGCAGCTGATCCTGACT	426
hGAPDH	NM_002046.4	GGAGTCAACGGATTTGGTCGTA	GGCAACAATATCCACTTTACCAGAGT	78

Table 2. Laboratory Investigations of 11 Patients With *HNF1β* Abnormalities[a]

Patient	Gender	Age, y	PTH, pmol/L	Ca^{2+}, mmol/L	PO_4^{3-}, mmol/L	Mg^{2+}, mmol/L	Cr, μmol/L	C_{Cr}, mL/min	eGFR, mL/min/1.73 m^2	Urinary Ca^{2+}/Cr, mmol/mmol	TRP, %	Abnormality	Additional Information
I.1	M	51	13.9	2.26	0.80	0.55	114		62	<0.1	71	Deletion	
I.2	F	47	14.7	2.31	0.79	0.54	141		35	<0.01	82	Deletion	
I.3	F	42	8.8	2.38	0.98	0.54	121	83		0.18	81	Deletion	
I.4	F	15	9.2	2.38	1.27	0.41	65	109		<0.01	88	Deletion	
I.5	M	14	6.6	2.52	1.01	0.51	64		>90	ND	ND	Deletion	
II	M	7	ND	2.52	1.40	0.65	41		>90	0.23	91	Deletion	
III	M	10	5.2	2.45	1.31	0.70	37		>90	0.96	94	Deletion	
IV	F	42	5.3	2.39	1.35	0.43	107	48		<0.1	87	Deletion	
V	F	39	9.5	2.34	0.88	0.79	93	87		<0.1	ND	c.883C→T p.Arg295Cys	
VI	F	1	23.1	2.52	1.53	1.21	331	ESRD		ND	ND	c.18delG p.Ser7Argfs*7	
		9	13.4	2.57	1.36	0.77	79	69		0.29	86	c.18delG p.Ser7Argfs*7	Data after kidney transplantation
VII	M	23	ND	2.96	0.52	0.47	128		64	ND	ND	Deletion	Parathyroidectomy for hyperparathyroidism at age 23
		36	16.4	2.30	0.79	0.64	150	74		<0.06	86	Deletion	Data at eventual diagnosis

Abbreviations: C_{Cr}, creatinine clearance; Cr, creatinine; eGFR, estimated GFR; ESRD, end-stage renal disease; F, female; M, male; ND, not determined; TRP, renal tubular reabsorption of phosphate.

[a] Normal levels are as follows: PTH, 1.0–6.5 pmol/L; serum Ca^{2+}, 2.20–2.65 mmol/L; serum PO_4^{3-}, for age <12 years, 1.3–1.9 mmol/L, and for age >12 years, 0.8–1.4 mmol/L; serum Mg^{2+}, 0.7–1.1 mmol/L; serum Cr, 45–110 μmol/L; C_{Cr}, 89–143 mL/min; Ca^{2+}/Cr ratio, 0.40–0.57 mmol/mmol; TRP, >85%. eGFR was calculated by Modification of Diet in Renal Disease formula (adults) or Cockcroft-Gould (children).

played hyperparathyroidism. Three patients from one family (patients I.3, I.4, and I.5), showed increased PTH levels without clearly reduced renal function based on creatinine clearance or estimated glomerular filtration rate (GFR).

Including the patients just described, we retrospectively reviewed 11 patients, 5 familial and 6 sporadic cases, with known *HNF1β* mutations or whole-gene deletions visiting the outpatient clinic of our institution (Table 2). Nine patients had an *HNF1β* whole-gene deletion, 1 patient had a frameshift mutation (c.18delG, p.Ser7Argfs*7), and 1 patient had a missense mutation (c.883C→T, p.Arg295Cys).

Serum intact PTH levels were available for 10 of 11 patients. Eight of these 10 patients had hyperparathyroidism (6.6–16.4 pmol/L, normal range 1.0–6.5 pmol/L). Eight patients had hypomagnesemia (0.41–0.65 mmol/L), whereas their plasma PO_4^{3-} and Ca^{2+} levels were within the normal range. Five of 7 patients with increased PTH levels belong to the same family (patients I.1–I.5) and showed concomitant hypomagnesemia. No hypomagnesemia was observed in a patient with a c.883C→T (p.Arg295Cys) *HNF1β* mutation and high PTH levels (patient V, Table 2). In 2 patients, the hyperparathyroidism was initially clearly appropriate for the level of kidney function (patient I.2 and VI, Table 2). After renal transplantation, the former patient with a c.18delG (p.Ser7Argfs*7) *HNF1β* mutation showed improved renal function and normal plasma Mg^{2+} levels, whereas the PTH levels remained increased. Urinary calcium to creatinine ratio revealed that 9 of 10 patients whose data were available displayed hypocalciuria. Tubular reabsorption of phosphate was at or below the lower limit of normal in all patients suggested to display hyperparathyroidism. Overall, to us, these data appeared in line with the hypothesized link between *HNF1β* mutations or whole-gene deletions and hyperparathyroidism, distinct from secondary hyperparathyroidism due to renal function decline. Therefore, we proceeded to evaluate the hypothesis that HNF1β could act as a transcriptional regulator of the *PTH* gene.

HNF1β is expressed in parathyroid cells

First, we investigated HNF1β expression in human parathyroid gland tissue. HNF1β mRNA expression in human parathyroid glands was detected by end-point PCR and compared with HNF1β mRNA expression in a control human kidney sample (Figure 1A). The specificity of the tissue was confirmed by amplification of the PTH transcript, which was clearly present in parathyroid tissue but, as expected, not in the kidney. The histology of parathyroid glands is easily recognizable by the densely packed cells. PTH was highly expressed in the cytosol of the parathyroid cells (Figure 1B). Importantly, HNF1β was localized in PTH-positive cells in a nuclear pattern, as shown by the colocalization with the nuclear marker DAPI that binds to double-stranded DNA (Figure 1B).

Wild-type HNF1β binds the human *PTH* gene promoter and inhibits its activity

HNF1β affects transcription of target genes through binding of the POU domains to a DNA consensus sequence, reported in Figure 2A. Using the Consite program (asp.ii.uib.no:8090/cgi-bin/CONSITE/consite), prediction analysis for HNF1 binding sites in the −1476 bp region upstream of the transcription initiation site of the human *PTH* promoter identified 2 relatively well-conserved sites, at −1238 and −690 (Figure 2B). Demay et al

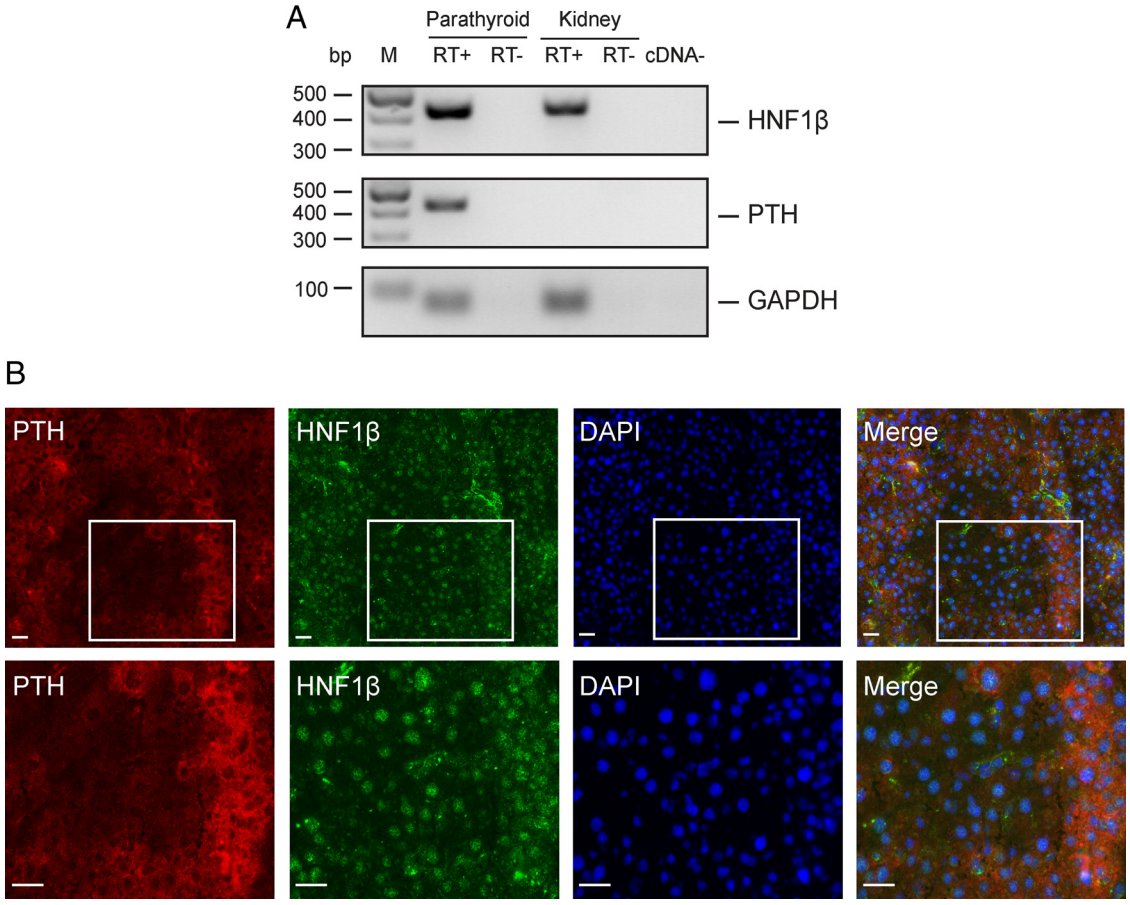

Figure 1. HNF1β expression in human parathyroid glands. A, Endogenous expression of HNF1β in human parathyroid tissue was investigated using RT-PCR. PTH was included as positive control for tissue specificity and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as housekeeping gene. RT+ indicates RT sample; RT− indicates no RT control; cDNA− indicates no cDNA control. B, Representative images of immunohistochemical analysis of HNF1β and PTH colocalization in human parathyroid tissue. DAPI is a nuclear marker. Scale bars, 20 μm.

(24) have previously suggested the presence of a poorly conserved consensus sequence of a POU transcription factor around position −101. To determine whether the *PTH* promoter is bound by HNF1β, we performed a ChIP assay using a human *PTH* −1476 promoter construct transfected in HEK293 cells, which do not endogenously express HNF1β (25). The immunoprecipitated genomic fragment bound by exogenously expressed HNF1β was quantified by real-time PCR analysis, which showed a 9-fold enrichment of the *PTH* promoter when precipitated with the anti-HNF1β antibody (Figure 2C) compared with the rabbit IgG isotype control (Figure 2C). Using a human *PTH* −70 promoter construct, no significant difference was seen between both antibodies. Immunoprecipitation with an anti-trimethyl-histone H3 antibody was performed as positive control. Two percent of the chromatin used for immunoprecipitation (input) was also included in the analysis (Figure 2C). To study the involvement of HNF1β in the transcriptional regulation of the human *PTH* gene, luciferase-reporter assays were performed. When wild-type HNF1β was transiently cotransfected with the human *PTH* −1476 promoter construct, a dose-dependent reduction of the promoter activity was observed, up to a 30% decrease compared with cells transfected with the mock plasmid (Figure 2D). This inhibitory effect was not demonstrated for the p.His69fsdelAC, p.Lys156Glu, p.His324Ser325fsdelCA, and p.Tyr352fsinsA mutants. When the latter were coexpressed with the *PTH* reporter construct, the promoter activity was comparable or higher to what was observed in mock plasmid-expressing cells (Figure 3A). To pinpoint the HNF1β-responsive region within the *PTH* promoter, serial deletions of the reporter construct were prepared (Figure 3B). HNF1β p.His324Ser325fsdelCA lacks a complete functional transactivation domain and does not downregulate *PTH* promoter activity. For the wild-type HNF1β, inhibition of *PTH* promoter activity persisted until −200 bp from the transcription initiation site. A further deletion to −70 bp no longer showed a significant difference between wild-type and mutant HNF1β (Figure 3E). These data demonstrate that 1 or more HNF1β-responsive elements reside in this proximal promoter region of the human *PTH* gene.

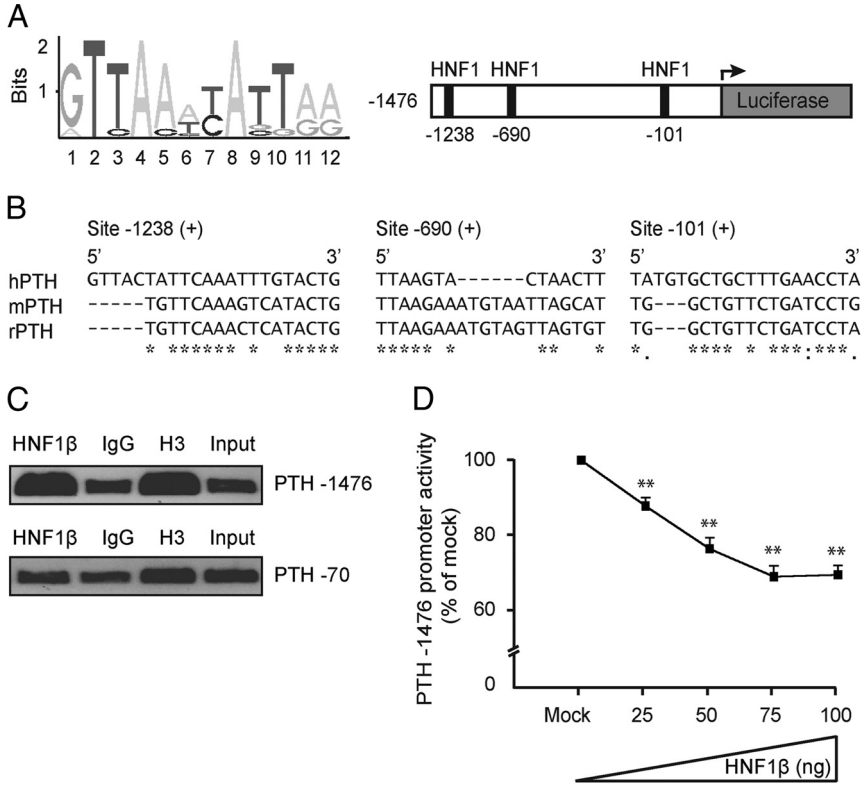

Figure 2. The human *PTH* gene as target of the HNF1β transcription factor. A, Sequence logo of the HNF1β motif from the Jaspar database (ID MA0153.1). In the human *PTH* promoter region (−1476 bp from the transcription initiation site), 3 putative HNF1 binding sites map at −1238, −690, and −101 of the forward DNA strand (+). B, Sequence alignment of the 3 putative HNF1 recognition sites in the PTH promoter region of different mammalian species. C, Binding of the *PTH* promoter by HNF1β was verified by a ChIP assay in HEK293 cells cotransfected with the human *PTH* promoter luciferase constructs, −1476/+25 or −70/+25, and wild-type HNF1β. For immunoprecipitation, an anti-HNF1β antibody or a rabbit IgG isotype control antiserum was used. Immunoprecipitation with an anti-trimethyl-histone H3 antibody was included as positive control. Two percent of the chromatin used for immunoprecipitation (input) was also included as a control. D, Dose-response curve of increasing amounts of wild-type HNF1β cotransfected in HEK293 cells with a luciferase construct carrying the human *PTH* gene promoter region −1476/+25 (n = 9). **, $P < .001$, compared with mock.

Discussion

In the present study, we demonstrated that the nuclear transcription factor HNF1β is expressed in the parathyroid gland and acts as a repressor of human *PTH* gene expression. ChIP experiments and luciferase-assays in HEK293 cells showed that HNF1β binds and inhibits the *PTH* promoter via *cis*-elements located in the proximal promoter of the *PTH* gene. This inhibition is lost when *HNF1β* is mutated or absent. Although further experiments are needed to determine which molecular pathways govern HNF1β activity in the parathyroid gland, our data demonstrated that HNF1β inhibits human *PTH* gene transcription.

Our hypothesis that HNF1β might be involved in parathyroid gland function was based on our observation that, in patients with *HNF1β* mutations or whole-gene deletions, PTH levels in several instances were relatively high compared with the degree of renal function decline, if present. This was accompanied by reduced urinary Ca^{2+} excretion and renal tubular reabsorption of PO_4^{3-} that was in the lower range of normal or reduced. Importantly, HNF1β was known to function as a transcription factor regulating gene transcription in a number of specialized endocrine organs and tissues with secondary endocrine functions (19–22). Previously, Adalat et al (18) reported plasma PTH levels in a cohort of patients with chronic kidney disease, in stages 1 through 3, with and without HNF1β-associated disease. They mainly focused on the hypomagnesemia observed in 44% of their patients with *HNF1β* defects, but they also reported plasma PTH levels were 6.3 pmol/L in patients with *HNF1β* defects versus 4.9 pmol/L in the patients with renal dysplasia in the absence of such defects ($P = .2$). Possibly due to the small series of patients, these data did not reach statistical significance. Importantly, Ca^{2+} and PO_4^{3-} levels were within normal ranges, whereas the GFR was similar in both groups.

The present data clearly show that HNF1β is expressed in a nuclear pattern in the PTH-positive cells of human parathyroid gland. Importantly, we demonstrated that HNF1β binds to the *PTH* promoter and that the presence of HNF1β inhibits *PTH* transcription, at least in part by a direct effect on the activity of the *PTH* promoter. This inhibitory effect was lost when known mutations in *HNF1β* were introduced. Taken together, our data indicated that HNF1β acts as a functional repressor of *PTH* gene transcription. Unfortunately, direct evidence that HNF1β decreases the synthesis or secretion of PTH in vitro cannot be provided due to the lack of an available PTH secretory cell line.

So far, many regulatory pathways that control PTH secretion, both transcriptionally and posttranscriptionally, have been defined, and several are currently therapeutic targets for the treatment of secondary hyperparathyroidism in the course of chronic kidney disease (2). These include active vitamin D (1,25-D_3) analogs and calcimimetics (26–29). The best-known *PTH* transcriptional repressor, 1,25-D_3, acts by way of the liganded 1,25-D_3 receptor-retinoic acid X receptor (VDR-RXR) complex

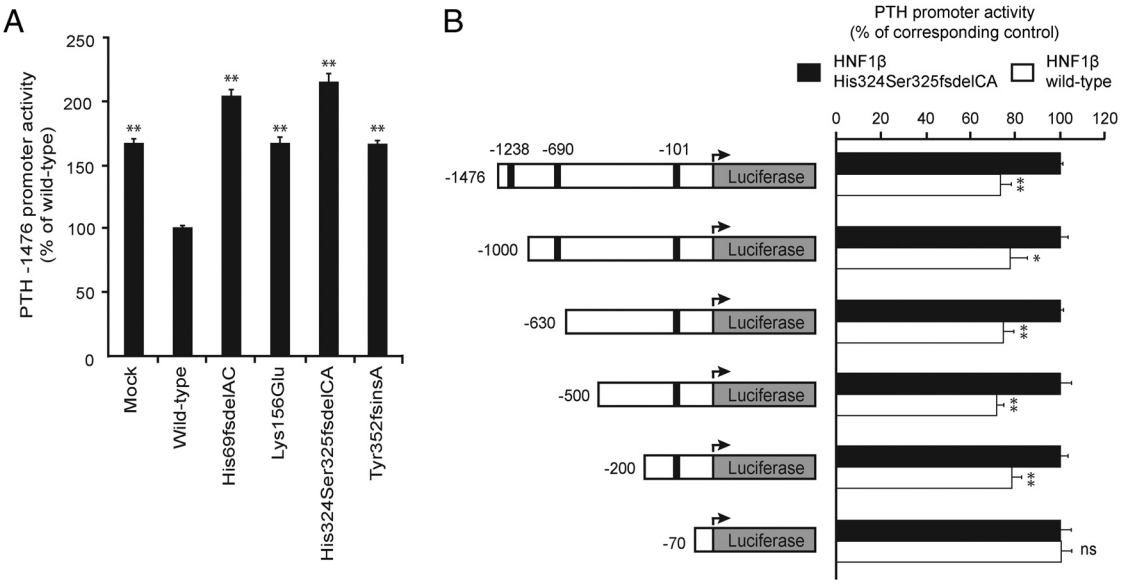

Figure 3. HNF1β mutants lack the ability to inhibit *PTH* promoter activity. A, Study of the human *PTH* promoter activity when cotransfected with wild-type HNF1β or HNF1β p.His69fsdelAC, p.Lys156Glu, p.His324Ser325fsdelCA, p.Tyr352fsinsA. mutants or the mock plasmid in HEK293 cells (n = 3). **, $P < .001$, compared with wild-type HNF1β. B, Serial deletion analysis in which the *PTH* promoter activity was tested in HEK293 cells in the presence of wild-type HNF1β (empty bars) or HNF1β p.His324Ser325fsdelCA mutant (black bars) (n = 9). **, $P = .001$, compared with HNF1β p.His324Ser325fsdelCA; *, $P = .006$, compared with HNF1β p.His324Ser325fsdelCA; ns, nonsignificant compared with HNF1β p.His324Ser325fsdelCA, $P = .9$.

binding to VDRE in the promoter region of the *PTH* gene. Interestingly, this VDRE was previously mapped to a region very close to the putative HNF1β binding site in the *PTH* promoter (24). There might be cross-talk between pathways involving 1,25-D_3 and HNF1β, but the exact signaling mechanism in which HNF1β is the final effector remains to be determined. Other possibilities include involvement in CaSR-mediated signaling or the recently identified FGF23-FGFR1/klotho axis (30, 31). MAPK pathways presumably play a role downstream of both CaSR and FGFR1/klotho activation in the parathyroid gland, and an interaction between FGF-induced/MAPK-mediated signaling and HNF1β has been previously suggested in other tissues (32–36).

Of note, in our patients with HNF1β-associated disease, a concomitant hypomagnesemia was diagnosed in most individuals. It was previously reported that in the distal part of the nephron, impaired transcriptional regulation of the *FXYD2* gene, encoding for the γ-subunit of the Na^+/K^+-ATPase, could be responsible for this hypomagnesemic effect (23). Because the Na^+/K^+-ATPase pump is essential to generate the driving force for PTH secretion (37), a hypothesis that remains to be tested is whether in the parathyroid glands the impaired transcriptional regulation of *FXYD* family members due to HNF1β abnormalities may contribute to increase PTH expression. FXYD proteins are known to associate with the Na^+/K^+-ATPase and to modulate its kinetic properties in a tissue-specific manner. However, RT-PCR experiments we performed on human parathyroid samples (Supplemental Figure 2 and Supplemental Table 1) and a human tissue microarray study failed to detect *FXYD2* expression in the parathyroid glands (38). So far, impaired renal *FXYD2* expression is the only molecular mechanism connecting the misregulation of the Na^+/K^+-ATPase activity to a tissue-specific effect due to *HNF1*β mutations.

In conclusion, we identified HNF1β as a novel transcriptional regulator of the *PTH* gene and demonstrated that it is expressed in the parathyroid gland. The exact HNF1β binding site within the *PTH* promoter needs to be identified, and the role of HNF1β in the responsiveness of parathyroid cells to extracellular Ca^{2+}, 1,25-D_3, and FGF23 has to be investigated. Loss of the HNF1β-mediated repression of the *PTH* promoter activity in the parathyroid gland could contribute to the high PTH levels observed in our cohort of patients with HNF1β-associated disease. However, it is very difficult to dissect this effect from the secondary hyperparathyroidism that is concomitantly present in some of these patients. Apart from our observations, a single reference to a patient with hyperparathyroidism in a cohort of 27 patients with HNF1β-associated disease (12), and the previously mentioned study by Adalat et al (18) there are to our knowledge no other reports of PTH levels in patients with HNF1β-related disease. To study the clinical significance of HNF1β affecting the synthesis of PTH in patients with *HNF1*β mutations, future studies will have to evaluate PTH levels in a larger cohort of patients, preferably before the onset of renal function decline. Furthermore, it would be of interest to measure an index of PTH action on the kidney, such as renal cAMP,

as a valuable approach to discriminate the tubular defects associated with the changes in PTH expression from the ones associated with *HNF1β* mutations.

Acknowledgments

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Financial support was provided by the Netherlands Organization for Scientific Research (ZonMw 9120.8026, NWO ALW 818.02.001), a Vici Grant for J. Hoenderop (NWO-ZonMw 016.130.668), and EURenOmics funding from the European Union seventh Framework Programme (FP7/2007–2013, agreement n° 305608).

Disclosure Summary: The authors have nothing to disclose.

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