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Germline hepatocyte nuclear factor 1α and 1β mutations in renal cell carcinomas

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Mutations in one copy of the hepatocyte nuclear factors (HNF) 1α and 1β homeodomain containing transcription factors predispose the carrier to maturity-onset diabetes of the young (MODY) types 3 and 5, respectively. Moreover, previous identification of biallelic inactivation of *HNF1α* in hepatocellular adenoma identified its tumor suppressor function in hepatocarcinogenesis. The seminal observation of an ovarian carcinoma in a MODY5 patient who subsequently developed a chromophobe renal cell carcinoma, prompted us to screen for HNF1β and HNF1α inactivation in a series of 20 ovarian and 35 renal neoplasms. Biallelic *HNF1β* inactivation was found in two of 12 chromophobe renal carcinomas by association of a germline mutation and a somatic gene deletion. In these cases, the expression of *PKHD1* (polycystic kidney and hepatic disease 1) and *UMOD* (Uromodulin), two genes regulated by HNF1β, was turned off. Interestingly, in two of 13 clear cell renal carcinomas, we found a monoallelic germline mutation of *HNF1α* with no associated suppression of target mRNA expression. In normal and tumor renal tissues, we showed the existence of a network of transcription factors differentially regulated in tumor subtypes. We identified two related clusters of co-regulated genes associating *HNF1β*, *PKHD1* and *UMOD* in the first group and *HNF1α*, *HNF4α*, *FABP1* and *UGT2B7* in the second group. Finally, these results suggest that germline mutations of *HNF1β* and *HNF1α* may predispose to renal tumors. Furthermore, we suggest that HNF1β functions as a tumor suppressor gene in chromophobe renal cell carcinogenesis through a PKHD1 expression control.

INTRODUCTION

Hepatocyte nuclear factors HNF1α, HNF1β and HNF4α were initially described as liver-enriched transcription factors. They are also expressed in other tissues and participate in a network of transcription factors that control gene expression during embryonic development and in adult tissue, particularly in liver, kidney and pancreas (1). HNF1α and HNF1β are two closely related homeodomain-containing transcription factors that bind DNA as homodimers or heterodimers and activate transcription of targeted genes (2–5). HNF4α is a transcription factor of the nuclear hormone receptor family essential for hepatocyte differentiation during mammalian liver development (6,7). In humans, heterozygous germline mutations of hepatocyte nuclear factors have been previously described to predispose to maturity-onset diabetes of the young (MODY). MODY are monogenic forms of non-ketotic diabetes mellitus characterized by an early age at onset (usually before 25 years) and autosomal dominant inheritance (reviewed in 8). MODY3 and MODY5 are defined by inactivation of the HNF1α and 1β encoded by *TCF1* and *TCF2* (Transcription factor 1 and 2) genes, respectively ((9,10), OMIM nos 600496 and 604284). Rare mutation of HNF4α leads to MODY1 (11) (OMIM no. 125850).

Mutations in HNF1 are also associated with various phenotypes in human. In patients with MODY5, diabetes is always

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associated with renal involvement including dysplastic kidneys, antenatally detected renal cysts, familial glomerulocystic kidney disease and oligomeganephronia (10,12,13). *HNF1α* gene inactivation was observed in half of the hepatocellular adenomas and in rare cases of well-differentiated hepatocellular carcinomas developed in the absence of liver cirrhosis (14). In 90% of the *HNF1α*-mutated adenomas, both mutations are of somatic origin, whereas in the remaining cases, corresponding to MODY3 patients, one mutation is germline and the second allele inactivation is a somatic event observed in the tumor (14–16), (OMIM no. 142330). In liver tumors, inactivation of both *HNF1α* alleles is required for tumor development, meeting the genetic criteria for a tumor suppressor gene. On the other hand, monoallelic somatic *HNF1α* mutations are observed in 23% of colon cancers that are associated with microsatellite instability phenotype (MSI-H) (17). These results suggest that HNF1α alteration might participate in colorectal carcinogenesis through a haplo-insufficiency mechanism.

Recently, we described a female patient with MODY5 who developed two cancers around age 50, an ovarian carcinoma and a chromophobe renal cell carcinoma (RCC) (13). Taking into account the tumor suppressor role of HNF1α in hepatocytes, this seminal finding suggested that HNF1β may also be involved in ovarian and/or chromophobe RCC. To test this hypothesis, we first searched for *HNF1β* gene inactivation in randomly selected human ovarian and renal primary tumors. As renal neoplasms may derive from different cell lineages, we tested our hypothesis in three groups of renal tumors: clear cell carcinoma, chromophobe and oncocytoma. In the second step, we analyzed in renal tissues the level of RNA expression of *PKHD1* (polycystic kidney and hepatic disease 1), *UMOD* (uromodulin) and *PKD2* (polycystic kidney disease 2), genes known to be regulated by HNF1β in mouse kidney. Finally, we tested for the alteration of HNF1α network of transcription in renal tumors.

RESULTS

Screening for HNF1β alterations

To evaluate the occurrence of mutations in renal and ovarian tumors, the entire coding region of *HNF1β* was screened in 20 ovarian tumors and 35 renal tumors including 12 chromophobe RCC (11 primary tumors and one relapse), 13 clear cell renal carcinomas and 10 oncocytomas. The main clinical and pathological characteristics of the renal neoplasms are summarized in Table 1. Among these patients, diabetes was found in one out of 11 individuals presenting chromophobe RCC (index case previously diagnosed with a MODY5, patient 12). Among the 13 patients presenting clear cell renal carcinomas, diabetes was found in three patients without previous genetic test for MODY. We found a biallelic inactivation of *HNF1β* in two chromophobe carcinoma samples from two unrelated patients (Fig. 1 and Table 2). In the index case, the known germline 494G>A leading to R165H mutation was found in the tumor together with a deletion of the remaining allele leading to a biallelic *HNF1β* inactivation (Fig. 1A). Furthermore, by sequencing RT–PCR product, we found the expression of only the mutated allele. In another case (patient 2), a germline mutation, 46delC, leading to a premature frameshift, L16fsX17, was found. This mutation was not previously described in patient 2 or in other MODY5 patients. The second *HNF1β* allele was also inactivated in the tumor through a gene deletion (Fig. 1B). These genetic alterations were found in the primary tumor and similarly in the relapse developed 4 years later. Patient 2 was a 33-year-old female in whom a renal solid mass was detected at ultrasound screening following her third pregnancy, because bilateral hyperechoeic kidneys were found antenatally in her fetus. CT-scan showed bilateral small-sized cortical renal cysts and a 6 cm sized solid mass within the left kidney. A partial nephrectomy was carried out and a diagnosis of chromophobe RCC was made (Fig. 2). After 4 years, local recurrence of five renal tumors required radical nephrectomy. Despite the lack of hair follicle tumors, a genetic test for Birt–Hogg–Dubé was performed because of her young age and multifocal tumors, but no such mutation was found in the *BHD* gene. In contrast, in the recurrent tumor specimens, the genetic alterations in *HNF1β* were identical to those in the primary tumor. When evaluated for MODY5, the patient had no liver test abnormality or diabetes, whereas reappraisal of CT-scan demonstrated the absence of the body and the tail of the pancreas. Interestingly, her first child was diagnosed at birth with an abnormal pelviureteric junction that subsequently resolved spontaneously. Renal cysts that were observed antenatally in her third child resolved by year 2. No other relative exhibited findings suggestive of MODY5 in her kindred.

In the remaining renal tumors, two different *HNF1β* germline variants were identified without somatic inactivation of the second allele in three patients. A nucleotide substitution in the 5′-untranslated region of *HNF1β* at position −67 before the first translated codon was found in two patients. Patient 6 presented two lesions: a chromophobe carcinoma (case 6) and a clear cell carcinoma (case 7, Table 1). Patient 28 was a 61-year-old female, presenting a unique oncocytoma lesion of 60 mm. Although these genetic variants were not previously found in a screen of more than 300 chromosomes of patients with type 2 diabetes, we explored a putative effect of these genetic variants on HNF1β function. In tumor RNAs presenting −67C > T, a *HNF1β*, RT–PCR amplification was performed followed by sequence analysis of the 5′-untranslated region. This analysis showed similar expression of variant and normal alleles, indicating that the −67C>T variation does not disturb the level of *HNF1β* transcription and functional consequences of this variant remained unclear.

The second variant, 1414G>A, leading to a V472I amino acid substitution was found in a patient presenting with oncocytoma (patient 36). This patient was a 58-year-old male, who did not present with diabetes, nephropathy or urogenital abnormality. The 1414G>A nucleotide variant was not previously described in MODY5 patients or in single nucleotide polymorphism databases (http://www.ncbi.nlm.nih.gov/SNP). A valine at position 472 in *HNF1β* is conserved in many species including mammalian, rodent and *Xenopus*. Furthermore, valine at position 472 is conserved in the HNF1α transactivation domain. However, in fish (salmon, zebrafish and tilapia) an isoleucine is found at an equivalent position and

Table 1. Clinical and pathological characteristics of the patients with chromophobe and clear cell renal carcinoma

Patient ID	Case number	Age/ gender	Diabetes mellitus	Familial history of renal cancer	Number of macroscopic nodules/ largest size (mm)	Histological subtype	Grade (Furhman)	Main associated diseases
Patient 1	1T	65/F	−	−	1/90	Chromophobe	II	
Patient 2	2T	37/F	−	−	5/27	Chromophobe	II–III	Renal cysts, pancreatic atrophy, renal abnormalities in children
	13T[a]				1/80	Chromophobe	II–III	
Patient 3	3T	56/M	−	−	1/50	Chromophobe	II–III	Hypercholesterolemia
Patient 4	4T	74/M	−	−	2/20	Chromophobe	III	Meningioma
Patient 5	5T	53/F	−	−	1/110	Chromophobe	II	Benign ovarian cysts
Patient 6	6T	76/F	−	−	1/45	Clear cell	III	—
	7T				1/40	Chromophobe	III	
Patient 8	8T	58/M	−	−	1/18	Chromophobe	I	—
Patient 9	9T	77/M	−	−	1/44	Chromophobe	II	Transitional cell carcinoma of the bladder
Patient 10	10T	67/F	−	−	1/40	Chromophobe	II	Thyroidectomy
Patient 11	11T	70/F	−	−	1/20	Chromophobe	II	Thyroid goiter
Patient 12	12T	54/F	+	−	1/35	Chromophobe	II	Bicornuate uterus, chronic renal failure, ovarian carcinoma
Patient 14	14T	79/M	NA	NA	1/50	Clear cell	III	NA
Patient 15	15T	73/M	−	−	1/43	Clear cell	III	
Patient 16	16T	78/M	+	−	1/90	Clear cell with TFE3 Hyperexpression	III	
Patient 17	17T	70/F	−	−	1/40	Clear cell	III	Asthma
Patient 18	18T	69/M	NA	NA	Multifocal/80	Clear cell	III	NA
Patient 19	19T	75/F	−	−	1/45	Clear cell	I–II	HCV-cirrhosis
Patient 20	20T	75/M	+	−	1/65	Clear cell	II–III	BMI = 33 kg/m^2
Patient 21	21T	56/M	−	−	1/42	Clear cell	I–II	—
Patient 22	22T	30/M	−	+ (VHL)	4/22 + 3 Cysts	Clear cell	I–II	VHL
Patient 23	23T	57/F	+	−	1/50	Clear cell	III–IV	SLE, Steroid-induced
Patient 24	24T	64/F	−	−	1/45	Clear cell[b]	III	—
Patient 25	25T	78/M	−	−	1/45	Clear cell[b]	III	Urolithiasis, hypertension

NA, non-available; VHL, von Hippel-Lindau disease; SLE, systemic lupus erythematosus.
[a]13T, relapse of patient 2.
[b]Initial diagnosis, chromophobe RCC.

a valine to isoleucine substitution is a conservative change that is not predicted to modify HNF1β function.

Using RT–PCR, we also searched for expression of abnormal mRNA species or aberrant splicing in 12 chromophobe renal cell samples. Both normal isoforms A and B of *HNF1β* were expressed in tumors with no aberrant mRNAs. Finally, no additional mutations or variants of sequence were identified either in the clear cell carcinomas and oncocytomas or in the 20 ovarian tumors.

RNA expression level of *HNF1β* and targeted genes

We quantified the expression level of *HNF1β* mRNA in 24 renal tumors and in matching non-tumor kidney samples of 10 of these cases (excluding mutated cases), using a test that detects all of the described *HNF1β* isoforms (Fig. 3A). *HNF1β* mRNA was significantly down-regulated in chromophobe cancer, oncocytoma and clear cell carcinoma (4-, 2- and 1.5-fold, respectively) when compared with normal renal tissues (Kruskal–Wallis test $P = 0.0001$, Fig. 3A). In each group of tumors, no individual sample showed a complete lack of *HNF1β* expression, indicating the absence of somatic alterations of *HNF1β* regulating sequences in these samples.

To explore the molecular consequences of *HNF1β* inactivation in renal tumors, we quantified the mRNA level of genes previously described to be regulated by HNF1β in mouse kidney (18,19). *PKHD1* transcript was down-regulated in chromophobe RCC, oncocytoma and clear cell renal carcinoma (30-, 40- and 4-fold, respectively) when compared with normal renal tissues (Kruskal–Wallis test $P < 0.0001$ in all cases, Fig. 3A). Linear regression analysis showed that the amount of *PKHD1* transcripts clearly correlated with the expression levels of *HNF1β* mRNA ($R^2 = 0.59$, $P < 10^{-6}$, Fig. 3B) in tumors without *HNF1β* mutation, whereas in tumors carrying a *HNF1β*-mutation, *PKHD1* mRNA expression was suppressed. *UMOD* was profoundly down-regulated in all tested tumors; 10^3- to 10^5-fold, when compared with normal renal tissues (Fig. 3A). A significant correlation between *HNF1β* and *UMOD* mRNA level of expression was also observed ($R^2 = 0.39$, $P = 0.0001$, Fig. 3C) in the non-tumor renal samples, whereas in *HNF1β*-mutated cases *UMOD* expression was also turned off (Fig. 3A). In contrast, *PKD2* was expressed in all tumor and non-tumor tissues at a

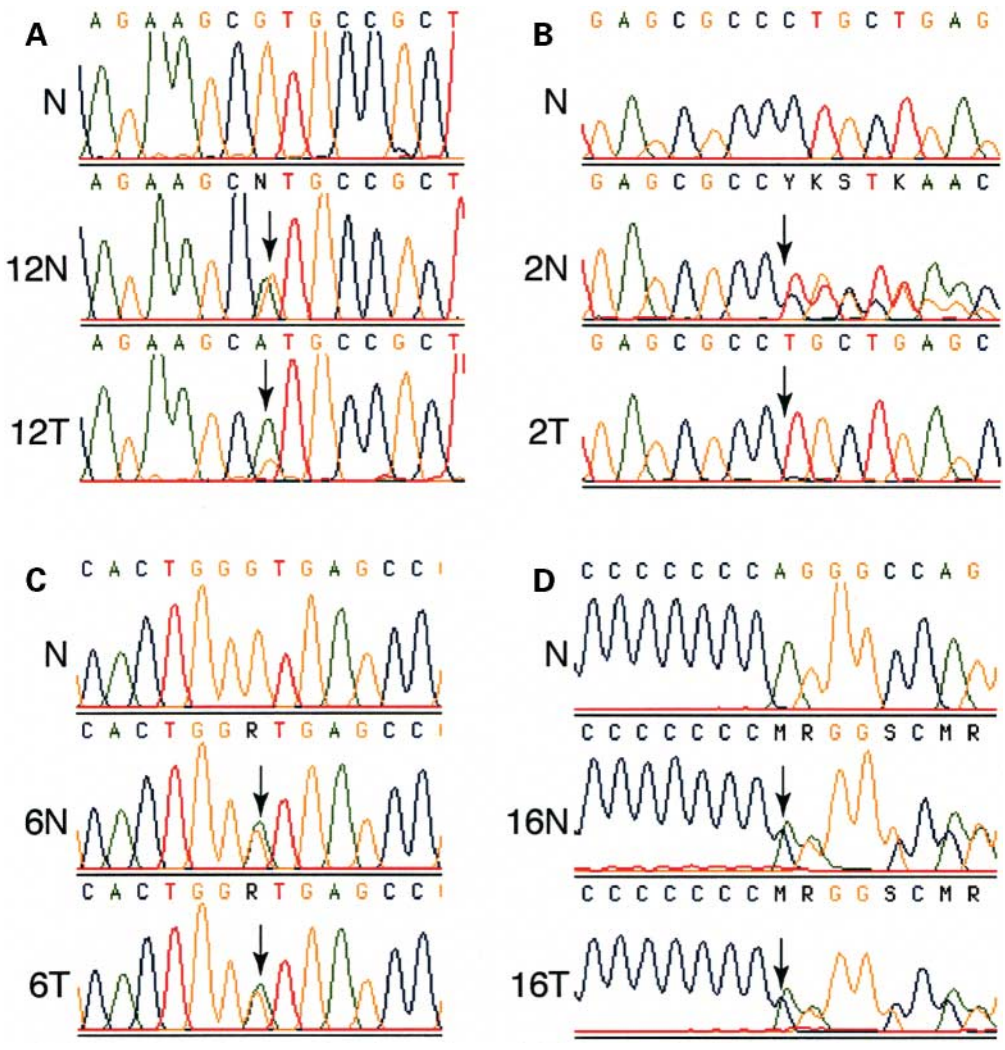

Figure 1. *HNF1β* and *HNF1α* mutations. In case 12 (**A**), *HNF1β* mutation leading to R165H amino acid substitution, in case 2 (**B**), *HNF1β* mutation leading to L16fsX17 frameshift. In tumor 12T and 2T, normal allele was lost. In case 6 (**C**), *HNF1α* mutation leading to G31D amino acid substitution, in case 16 (**D**), *HNF1α* mutation leading to P291fsX316 frameshift. In all panels, N is a normal reference sequence; 12N, 2N, 6N and 16N are sequences from non-tumor DNA; 12T, 2T, 6T and 16T were obtained from tumor samples.

similar level including the two *HNF1β*-mutated tumors (Fig. 3A). Finally, no significant relation was observed between *HNF1β* and *PKD2* mRNA level of expression (Fig. 3D). These results indicate that HNF1β may control the level of transcription of *PKHD1* and *UMOD* in human renal tissues, but not of *PKD2*.

Level of expression of *HNF1α*, *HNF4α* and targeted gene in renal tumors

To investigate the possible involvement of *HNF1α* and its network of transcription targets in renal tumors, screening for *HNF1α* mutation was performed in the 35 kidney tumors (Table 2). A monoallelic mutation, of germline origin, was found in the three tumors and in the non-tumor renal parenchyma originating from two patients. Patient 6 is a female with a 92G>A mutation leading to G31D amino acid substitution (Fig. 1C), a mutation previously described in MODY3 patients (20) and not found in more than 200 French controls screened in our laboratory. This patient was incidentally diagnosed as having two renal masses of the left kidney and a single renal cyst of the right kidney at age 75. One tumor was of clear cell subtype (Furhman grade 3, pT1), while the other met distinctive features of chromophobe renal carcinoma (Furhman grade 3, pT3a). By age 77, her fasting blood glucose was 4.5 mmol/l, and a follow up CT-scan failed to show evidence of relapse. None of her relatives had a history of diabetes or renal carcinoma. Analysis of both tumors failed to disclose a mutation or deletion of the second *HNF1α* allele. For patient 16, we identified a germline *HNF1α*-mutation consisting of a cytosine insertion leading to a frame shift at codon 291, P291fsX316 (Fig. 1D). This mutation is most frequently found in MODY3 patients, accounting for 15% of cases. This patient was diagnosed as having diabetes mellitus in his sixth decade by routine plasma glucose measurement. He was lean (BMI at diagnosis, 23 kg/m^2),

Table 2. *HNF1α* and *HNF1β* mutations in renal tumors

	Sample ID	Allele 1	Allele 2	Status
HNF1β mutations	12T	494G>A, R165H[a]	LOH	Biallelic mutation
	2T and 13T	46delC, L16fsX17[a]	LOH	Biallelic mutation
	6T, 7T and 28T	−67C>T	Non-mutated	Variant
	36T	1474G>A, V472I	Non-mutated	Variant
HNF1α mutations	6T and 7T	92G>A, G31D[a]	Non-mutated	Monoallelic mutation
	16T	872_873insC, P291fsX316[a]	Non-mutated	Monoallelic mutation

[a]Germline.

and metabolic control was easily attained by diet and oral hypoglycemic agents. At age 78, a 9 cm sized tumor was recognized in the left kidney. HbAIc was 6.2%. A diagnosis of clear cell renal carcinoma was confirmed following radical nephrectomy. None of his relatives had a diagnosis of renal carcinoma or diabetes. Screening for *HNF1α* mutation in the tumor and non-tumor tissue samples showed the monoallelic germline *HNF1α* alteration mentioned previously, without mutation or deletion of the second allele (Table 2).

The level of expression of *HNF1α* mRNA was quantified using quantitative RT–PCR. *HNF1α* was expressed in normal kidney at a level similar to that found in normal liver without important variation between assays (coefficient of variation = 38%, data not shown). As compared to normal renal tissues, *HNF1α* mRNA expression was similar in the clear cell renal carcinoma but 6-fold, down-regulated in chromophobe carcinoma and oncocytoma (Kruskal–Wallis $P = 0.0002$, Fig. 4A). In an attempt to demonstrate the functional consequences downstream of HNF1α, we analyzed expression databases (GeneNote at http://genecards.weizmann.ac.il) and selected two genes *UGT2B7* and *FABP1*, known to be regulated by HNF1α in liver and expressed in normal renal tissues. In normal renal samples, significant correlations were found between *HNF1α* and *FABP1* or *UGT2B7* expression levels, with R^2 for the regression equal to 0.41 ($P = 0.002$) and 0.56 ($P = 0.0002$), respectively, indicating that expression of *FABP1* and *UGT2B7* may be regulated by HNF1α in normal renal tissues (Fig. 4B and C). Expression of *UGT2B7* and *FABP1* in renal tumors was dramatically down-regulated as compared to normal renal tissues (Kruskal–Wallis $P = 0.0001$). However, no specific suppression of expression was observed in the two *HNF1α*-mutated cases (Fig. 4A).

We also quantified the expression of HNF4α, as this gene is known to regulate HNF1α transcription in pancreas, liver and kidney. By analyzing results in all tumors and non-tumor specimen subgroups, we identified a close relation between mRNA expression of *HNF1α* and *HNF4α* ($R^2 = 0.66$, $P < 10^{-6}$, Fig. 4D). Finally, we analysed the mRNA quantification results obtained for all tested genes in a hierarchical clustering analysis (Fig. 5). Using this method, two related clusters of co-regulated genes were identified associating *HNF1β*, *PKHD1* and *UMOD* in the first group and *HNF1α*,

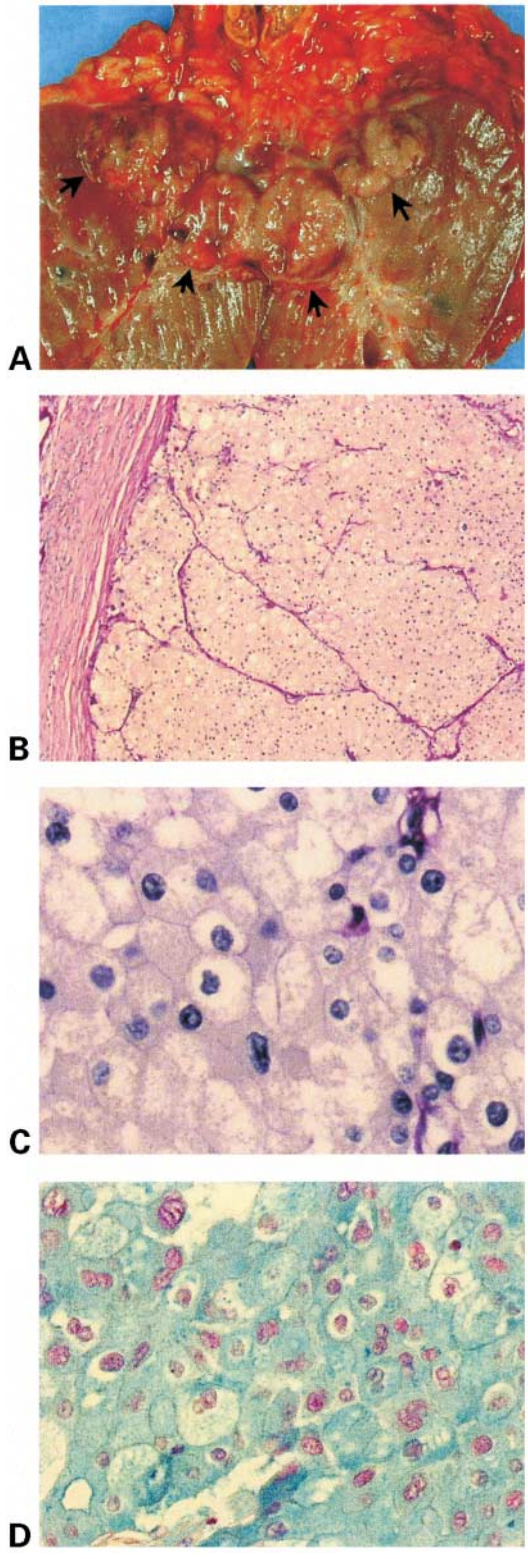

Figure 2. Chromophobe cell carcinoma mutated for *HNF1β* exhibited a typical aspect (patient 2). (**A**) Macroscopic examination showed two homogeneously tan colored intra-renal tumors of the upper pole of the kidney. (**B** and **C**) Chromophobe cells arranged along vascular channels showing binucleations, perinuclear halos, granular cytoplasm and prominent cell membranes (PAS magnitude 10× and 40×). (**D**) Hale's colloidal iron stain positivity in the cytoplasm.

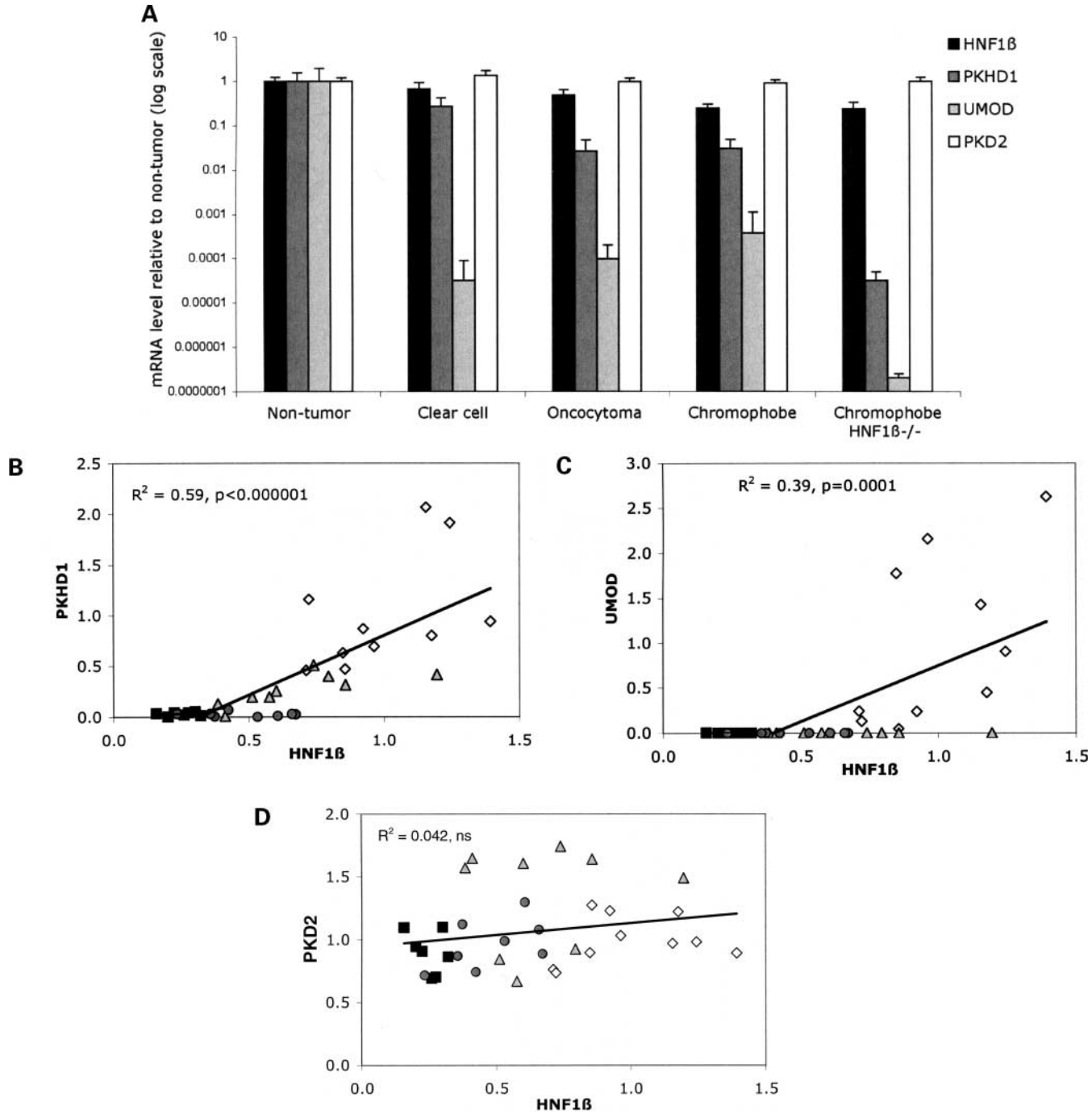

Figure 3. Graphical representation of relative mRNA expression of *HNF1β* and putative targeted genes. Relative mRNA expression is given normalized to R18S and relatively to non-tumor tissues tested as *n*-fold of the non-tumor values. (**A**) Tested tissues for *HNF1β, PKHD1, UMOD* and *PKD2* expression were non-tumor renal tissues ($n = 10$), clear cell carcinoma ($n = 9$), oncocytoma ($n = 8$), chromophobe carcinoma non-mutated for *HNF1β* ($n = 7$) and mutated for *HNF1β* ($n = 2$). The mean level of expression is indicated in each group of tumor together with standard deviation. (**B**–**D**) Linear regression between *HNF1β* level of expression and *PKHD1* (B), *UMOD* (C) and *PKD2* (D). Values corresponding to relative mRNA level of expression for each sample are represented in non-tumor (white rhombus), chromophobe carcinomas (black square), clear cell carcinomas (gray triangle) and oncocytoma (gray circle). For each comparison, R^2 and *P*-value calculated using linear regression test are indicated.

HNF4α, *FABP1* and *UGT2B7* in the second group, respectively. *PKD2* expression was independent of these groups. Moreover, tissue samples were also well classified according to their pathological characteristics (Fig. 5).

DISCUSSION

In this study, we found biallelic inactivation of *HNF1β* in two out of 12 patients with chromophobe RCC, i.e. 15% of the

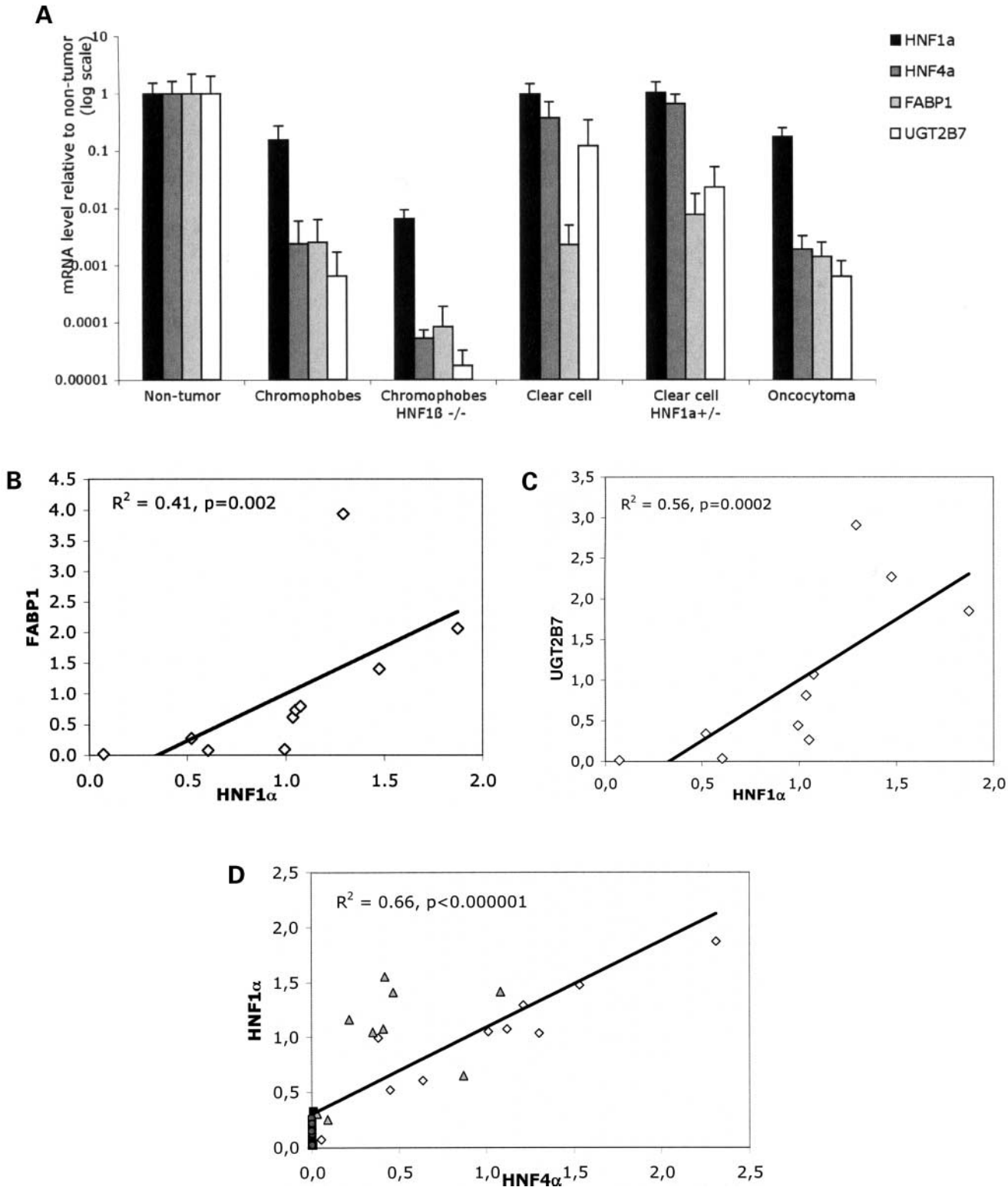

Figure 4. Graphical representation of relative mRNA level of expression of *HNF1α* and putative targeted genes. Relative mRNA level of expression are given normalized to R18S and relatively to non-tumor tissues tested as *n*-fold of the non-tumor values. (**A**) Tested tissues for *HNF1α, HNF4, FABP1* and *UGT2B7* expression were non-tumor renal tissues ($n = 10$), clear cell carcinoma non-mutated for HNF1α ($n = 7$) and mutated for *HNF1α* ($n = 2$), oncocytoma ($n = 8$) and chromophobe carcinoma non-mutated for *HNF1β* ($n = 7$) and mutated for *HNF1β* ($n = 2$). The mean level of expression is indicated in each group of tumor together with standard deviation. (**B**–**D**) Linear regression between *HNF1α* level of expression and *FABP1* (B), *UGT2B7* (C) and *HNF4* (D). Values corresponding to relative mRNA level of expression for each sample are represented in non-tumor (B and C) or in all samples (D), i.e. non-tumor (white rhombus), chromophobe carcinomas (black square), clear cell carcinomas (gray triangle) and oncocytoma (gray circle). For each comparison, coefficient of regression, R^2 and *P*-value calculated using linear regression test are indicated.

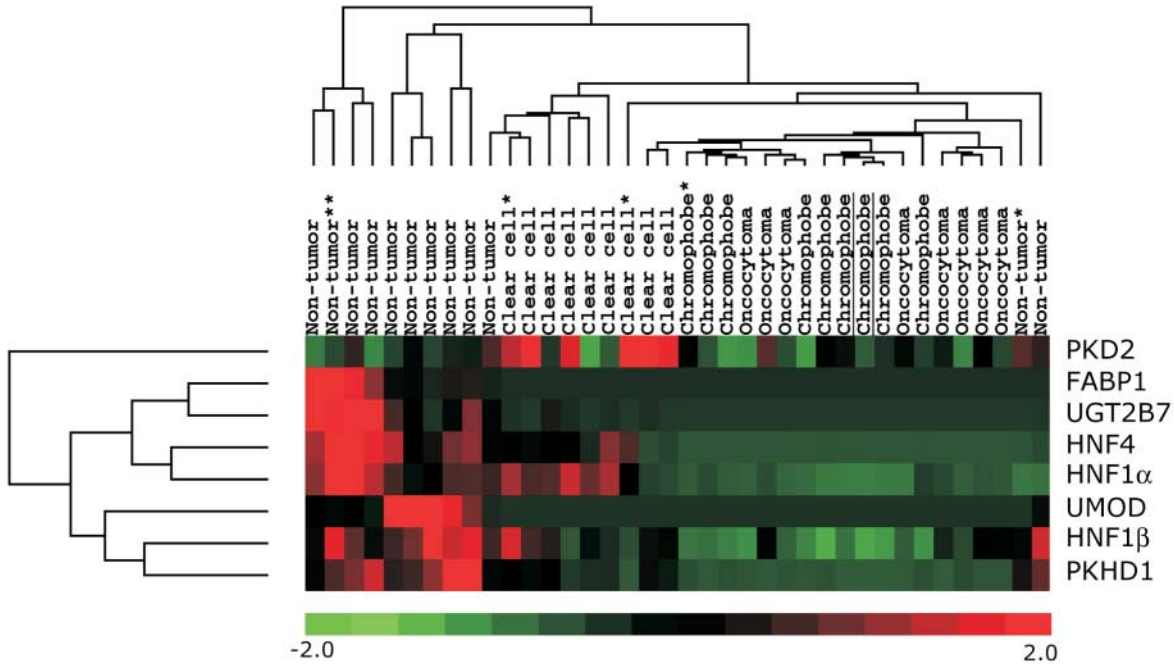

Figure 5. Quantitative RT–PCR results analyzed using hierarchical clustering. Tissues subtype of all tested samples are indicated, *heterozygously mutated for *HNF1α*, **heterozygously mutated for *HNF1β*, underlined: biallelic *HNF1β* mutation. Genes down-regulated and up-regulated are in green and red, respectively.

tested individuals. Both patients harbored a germline *HNF1β* mutation. One patient (patient 12) was known to be affected by MODY5 when her renal cancer was recognized at age 54. In contrast, in the other case (patient 2), recognition of the germline *HNF1β* mutation followed tumor analysis, with additional pathology findings limited to a few renal cysts and pancreatic atrophy; MODY and liver or genital abnormalities were lacking at age 37. Although genetic testing could not be carried out in the relatives in both cases, the lack of diabetes and/or renal failure in the parents is suggestive of a *de novo* mutation, a finding similar to two out of eight probands in our early experience (13). Both patients also had somatic deletion of the wild-type *HNF1β* allele. The association of germline and somatic inactivation is closely reminiscent to that observed in liver adenomatosis and liver carcinoma in the absence of liver cirrhosis in which *HNF1α* is inactivated through a two-hit sequence (14–16).

The finding of a biallelic *HNF1β* mutation in chromophobe renal carcinoma suggests that *HNF1β* plays a role of tumor suppressor gene in collecting tube cells in humans. This finding is in sharp contrast with rodent models, as mice with renal-specific inactivation of *HNF1β* and transgenic mice expressing a dominant-negative mutation develop renal cysts (18,19). Taken together, the data in humans and rodents argue that complete inactivation of *HNF1β* leads to an increased rate of cellular proliferation in renal epithelial cells, with different phenotype outcomes in the two species.

Chromophobe RCC accounts for 4–5% of all kidney cancer (21,22). The major histologic characteristic of this carcinoma is the voluminous cell cytoplasm, which has a pale, finely reticular quality contrasting with well-defined cell-borders. Ultrastructurally, it is characterized by numerous cytoplasmic vesicles that resemble those observed in normal intercalated B cells of the collecting ducts, so it has been suggested that chromophobe RCC originated from the latter (23–25). Chromosomal and genomic DNA analyses repeatedly demonstrated frequent losses of many entire chromosomes such as 1, 2, 6, 10, 13, 17 and 21, leading to hypodiploidy (26). Two regions of chromosome 17 have already been implicated in chromophobe cell cancer tumorigenesis: first, the *p53* tumor suppressor gene has been found to be mutated in 30% of the sporadic forms of the neoplasm (27). Second, in an autosomal dominant form of genodermatosis characterized by benign tumors of hair follicles named Birt–Hogg–Dubé syndrome, 15–30% of affected individuals develop kidney cancers of strikingly different subtypes, including chromophobe RCC in one-third of cases (28). The *BHD* gene is localized on 17p11.2. Our data indicate that alterations in another region of chromosome 17 may account for tumorigenesis in some chromophobe RCC, as the *HNF1β* gene is localized on 17q12.

In *HNF1β*-mutated tumors, we showed suppression of *PKHD1* expression. Analysis of mRNA in tumoral and non-tumoral renal tissues demonstrated that *PKHD1* expression was closely correlated with *HNF1β* expression. Altogether, these results suggest that HNF1β may regulate the activity of the *PKHD1* promoter. It is consistent with the recent findings obtained in mouse models in which *Pkhd1* gene has been identified as specifically regulated by HNF1β and HNF1α in the kidney (18,19). In humans, mutations in *PKHD1* cause autosomal recessive polycystic kidney disease, a disorder characterized by the association of cysts in the renal collecting ducts and biliary dysgenesis, a ductal plate malformation that produces aberrant intrahepatic bile ducts and portal fibrosis (29). The gene encodes for

polyductin, a protein predicted to be an integral membrane protein, predominantly expressed in the renal collecting ducts with lower expression in proximal and distal tubules (29–31). Abundant polyductin expression is observed in the primary cilium, an organelle that controls the proliferation of tubular cells. In mice with renal-specific inactivation of *hnf1β*, two additional genes were identified as being down-regulated by *hnf1β*: *Umod* and *Pkd2* which are the mouse orthologs of the human genes mutated in medullary cystic kidney disease type 2 and in autosomal dominant polycystic kidney disease, respectively (18). In human kidney, we also observed a correlation between *HNF1β* and *UMOD* expression in normal kidney, consistent with a regulation of UMOD by HNF1β. *Umod*, was found to be regulated by hnf1β in mice (18), it is expressed at a low level in most renal tumors and completely suppressed in *HNF1β*-mutated samples. However, because UMOD expression is physiologically restricted to the thick ascending limb of the loop of Henle, the low level of expression in tumors may be because of their different tubular origin. Thus we cannot affirm that UMOD participates in the carcinogenetic process. Finally, *Pkd2* was shown to be directly controlled by hnf1β in mice (18), whereas the human *PKD2* gene did not appear to depend on HNF1β.

Germline *HNF1α*-mutations were found in two patients with clear cell carcinoma among 13 screened cases. In these cases, the patients were 76 and 78 years old and the mutations were monoallelic not only in tumors but also in non-tumor renal parenchyma. This result contrasts with a previous published study in which no *HNF1α* mutation was found among 32 screened cases of clear cell carcinoma (32). However, both cases with *HNF1α*-mutations in our study were unusual: patient 6 presented two renal tumors, a clear cell carcinoma and a chromophobe tumor and patient 16 presented a particular clear cell carcinoma linked to Xp11.2 translocation with TFE3 over-expression, more commonly found in children and young adults (33). HNF1α and HNF4α loss of function at the protein level has previously been found in clear cell renal carcinoma (34,35). Here we show that level of mRNA expression of *HNF1α* and *HNF4α* is closely related, suggesting that transcription of the two genes is co-regulated in tumor and non-tumor renal tissues. However, the absence of clear-cut down-regulation of downstream targeted genes in tumors with *HNF1α* mutations, together with the monoallelic nature of the *HNF1α* mutation in these tumors, raised the question of the mechanism by which HNF1α alteration participates in the carcinogenetic pathway. This pattern is different from that observed in liver adenomas where mutation of *HNF1α* is biallelic (14). However, it resembles that observed in colon cancer (17) and endometrial carcinoma (36) in which almost all *HNF1α* mutations are monoallelic. These results suggest that HNF1α inactivation in renal, colon and endometrial tumors could contribute to carcinogenetic pathways through a haploinsufficiency mechanism. Heterozygous germline *HNF1α* mutation could also slightly predispose to renal tumor development through complex peripheral effect of the MODY3 disease as the two *HNF1α*-mutated clear cell carcinomas were observed in old MODY3 patients with or without clinical diabetes. In the absence of clear alteration of targeted gene expression in these tumors, the contribution of a monoallelic inactivation of *HNF1α* in tumors remains to be elucidated.

HNF1β is expressed along the length of the nephron, whereas HNF1α expression is restricted to proximal tubules. In mouse, PKHD1 is highly expressed in renal collecting ducts with lower level of expression in proximal and distal tubules. Moreover, Hiesberger *et al.* (19) showed that *pkhd1* mRNA expression was controlled by both hnf1α and 1β in mouse. In case of chromophobe carcinoma originating from intercalated cells of the collecting ducts, biallelic *HNF1β* mutation will lead to a complete extinction of the HNF1 pathway, as HNF1α is not expressed in collecting cells, as observed in our study. In fact, in these mutated tumors, *PKHD1* mRNA expression is suppressed consistent with the absence of HNF1 dependent expression. In contrast, in clear cell carcinoma originating in the proximal tube, *HNF1α* mutations are not sufficient to drastically down-regulate targeted genes, probably because of the monoallelic nature of *HNF1α* mutations and a continued expression of HNF1β. Fibrocystin/polyductin, the protein encoded by *PKHD1*, is predicted to be an integral membrane protein associated with primary cilia in renal epithelia (29,37). One prediction of our findings is that PKHD1 may participate in a tumor suppressor function in intercalated cells of the collecting tubes. However, further study will be required to determine mechanisms by which this protein controls cell proliferation in kidney and to evaluate its role in liver tumorigenesis.

In summary, the identification of two unrelated individuals with biallelic inactivation of *HNF1β* in chromophobe renal cell cancers enabled us to document *HNF1β* as a tumor suppressor gene. Our findings enlarge the spectrum of *HNF1β*-related nephropathy, with the potential for a hereditary form of renal carcinoma. The role of *HNF1α* alterations in renal carcinogenesis is less clear and future prospective studies should clarify the risk of renal carcinoma in MODY3 and MODY5 patients.

MATERIAL AND METHODS

Patients and tissue samples

The index case (patient 12) was previously described in Bellanne-Chantelot *et al.* (13). She was a female who presented with bicornuate uterus at age 20. Between age 40 and 54, her creatinine clearance declined from 29 to 20 ml/min. Kidney biopsy showed mild renal fibrosis. At age 45, a three decades history of mild, uncomplicated diabetes led to recognition of MODY. Fluctuating titers of liver enzymes were observed. MODY5 was ascertained by identification of a heterozygous germline *HNF1β* mutation at codon 165, leading to an amino acid substitution (R165H). At 48 years of age, she developed a left ovarian serous papillary carcinoma that was treated by surgery. While in remission, an incidental solid kidney mass was found at 54 years of age, with a pathological diagnosis of chromophobe RCC. For this patient, peripheral lymphocytes and chromophobe tumor tissue were available. The patient had no siblings, or children, and her parents could not be examined.

Thirty-four renal tumors were randomly selected from nephrectomy specimens, for which frozen tissue was

available, collected from 32 patients at the Necker hospital between January 2000 and 2003 (Table 1). Histological type was chromophobe cell carcinoma (11 cases), clear cell carcinomas (13 cases) and oncocytomas (10 cases). For each specimen, tumor tissue and surrounding non-tumoral tissue were cut into thin slices and immediately frozen in liquid nitrogen and stored at −80°C until used for molecular studies. Paraffin tissue sections were stained with HES (hematoxylin and eosin Safran). In difficult cases, a Hale's iron and immunohistochemical stains for pan-cytokeratin, CD10, CK7, EMA and vimentin were performed (Fig. 2). The pathological diagnosis of renal cell tumors was made on classical criteria (38).

Similarly, 20 ovarian tumors were collected during the same period from two referral centers from Paris (Hôpital Européen Georges Pompidou and Institut Curie). Among these tumors, two were benign (serous and mucinous cystadenomas), whereas the remaining 18 cases were ovarian carcinomas. The mean age of patients presenting ovarian cancer was 62 years (range 38–92). Tumors were well, moderately and poorly differentiated in nine, seven and two cases, respectively. The study was approved by the local Ethics Committee and informed consent was obtained in accordance with French legislation.

Mutation screening

We extracted DNA using a salt precipitation procedure (39). We amplified the nine *HNF1β* exons from tumor tissue by PCR using Qiagen HotStart DNA polymerase and purified them with a Millipore PCR purification kit as previously described in Bluteau *et al.* (14). The purified PCR products were directly sequenced using Big Dye Terminator Chemistry (Applied Biosystems) on an Applied Biosystems 3100 sequencer. Detailed PCR protocols and sequence of the primers are available upon request and at www.cephb.fr/tcf2. We analyzed sequences using Factura and Autoassembler software (Applied Biosystems). Germline origins of mutations were determined by screening DNA extracted from peripheral lymphocytes.

RT–PCR procedure

We extracted tumor RNA using the RNeasy kit (Qiagen). RT–PCR was performed using the omniscript kit (Qiagen) with random hexamer primers and Qiagen HotStart polymerase. In 12 cases of chromophobe carcinoma for which RNA were available, *HNF1β* transcripts corresponding to isoform A and B, were amplified using specific primers (see www.cephb.fr/tcf2 for detailed sequences and protocols). Amplified cDNA were analyzed by agarose gel electrophoresis followed by staining with ethidium bromide.

Reverse transcription quantitative PCR

RNAs were quantified by measuring UV absorbance at 260 nm. The quality of DNA and RNA was controlled by gel electrophoresis followed by staining with ethidium bromide and degraded samples were excluded. RNAs were accepted if the 28S/18S ratio was more than 1, which was the case for 9/12 chromophobe tumors, 10/13 clear cell carcinomas, 8/10 oncocytomas and 14/35 corresponding non-tumor renal tissues. One microgram of total RNA was reverse transcribed in a final volume of 100 µl using the High capacity Archive kit and random hexamers (Applied Biosystems). Reverse transcribed samples were diluted 5-fold in water and stored at −80°C. For each sample, 5 µl of cDNA, corresponding to ∼10 ng of reverse transcribed RNA, was analyzed by TaqMan PCR analysis, in duplicate, using the ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems). Previously developed sequence detection reagents specific for human *HNF1β, PKHD1, UMOD, PKD2, HNF4α, HNF1α, FABP1, UGT2B7* and *R18S* (Applied Biosystems), including forward and reverse primers as well as a fluorogenic TaqMan FAM-labeled NFQ MGB hybridization probes, were used in a 20 µl TaqMan universal PCR 1× master mix. Levels of RNA expression were determined using the SDS software version 2.1 (Applied Biosystems). The quality of cDNAs was assessed using a ribosomal *R18S* quantification by real time PCR. The relative amount of measured mRNA in samples was determined using the $2^{-\Delta\Delta CT}$ method (40), where $\Delta\Delta CT = (CT_{target} - CT_{R18s})_{sample} - (CT_{target} - CT_{R18s})_{calibrator}$. Briefly, expression results of a gene were normalized to internal control ribosomal 18S and relatively to a calibrator. The calibrator consisted of the mean expression level of the corresponding gene in non-tumor samples normalized to internal control ribosomal 18S. The values given in graphs express the *n*-fold ratio of the gene expression in a tested sample compared with the mean of non-tumor tissues. PCR efficiency was measured using LinRegPCR software (41). All gene assays demonstrated a PCR efficiency superior to 90%.

Statistical analysis

Statistical analysis was carried out using Stata 7.0 software (Stata Corp., College Station, TX, USA). Quantitative variables were compared using a linear regression test. An ANOVA analysis was used to compare mean value between different sample groups and a Bonferroni correction was applied for multiple-comparison tests. Qualitative and categorized quantitative variables were compared with each other using χ^2 test with Yates' correction when necessary. A Kruskal–Wallis non-parametric test was used to analyze quantitative RT–PCR results when variance was not comparable between groups. For all analyses, a threshold of 95% significance was retained. For hierarchical clustering analysis, dCHIP software was used (www.dchip.org).

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REFERENCES

1. Cereghini, S. (1996) Liver-enriched transcription factors and hepatocyte differentiation. *FASEB J.*, **10**, 267–282.
2. Courtois, G., Morgan, J.G., Campbell, L.A., Fourel, G. and Crabtree, G.R. (1987) Interaction of a liver-specific nuclear factor with the fibrinogen and alpha 1-antitrypsin promoters. *Science*, **238**, 688–692.
3. Frain, M., Swart, G., Monaci, P., Nicosia, A., Stampfli, S., Frank, R. and Cortese, R. (1989) The liver-specific transcription factor LF-B1 contains a highly diverged homeobox DNA binding domain. *Cell*, **59**, 145–157.
4. Cereghini, S., Raymondjean, M., Carranca, A.G., Herbomel, P. and Yaniv, M. (1987) Factors involved in control of tissue-specific expression of albumin gene. *Cell*, **50**, 627–638.
5. Rey-Campos, J., Chouard, T., Yaniv, M. and Cereghini, S. (1991) vHNF1 is a homeoprotein that activates transcription and forms heterodimers with HNF1. *EMBO J.*, **10**, 1445–1457.
6. Sladek, F.M., Zhong, W.M., Lai, E. and Darnell, J.E., Jr. (1990) Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Genes Dev.*, **4**, 2353–2365.
7. Li, J., Ning, G. and Duncan, S.A. (2000) Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. *Genes Dev.*, **14**, 464–474.
8. Fajans, S.S., Bell, G.I. and Polonsky, K.S. (2001) Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N. Engl. J. Med.*, **345**, 971–980.
9. Yamagata, K., Oda, N., Kaisaki, P.J., Menzel, S., Furuta, H., Vaxillaire, M., Southam, L., Cox, R.D., Lathrop, G.M., Boriraj, V.V. *et al.* (1996) Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). *Nature*, **384**, 455–458.
10. Horikawa, Y., Iwasaki, N., Hara, M., Furuta, H., Hinokio, Y., Cockburn, B.N., Lindner, T., Yamagata, K., Ogata, M., Tomonaga, O. *et al.* (1997) Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. *Nat. Genet.*, **17**, 384–385.
11. Yamagata, K., Furuta, H., Oda, N., Kaisaki, P.J., Menzel, S., Cox, N.J., Fajans, S.S., Signorini, S., Stoffel, M. and Bell, G.I. (1996) Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). *Nature*, **384**, 458–460.
12. Woolf, A.S. (2000) Diabetes, genes, and kidney development. *Kidney Int.*, **57**, 1202–1203.
13. Bellanne-Chantelot, C., Chauveau, D., Gautier, J.F., Dubois-Laforgue, D., Clauin, S., Beaufils, S., Wilhelm, J.M., Boitard, C., Noel, L.H., Velho, G. *et al.* (2004) Clinical spectrum associated with hepatocyte nuclear factor-1beta mutations. *Ann. Intern. Med.*, **140**, 510–517.
14. Bluteau, O., Jeannot, E., Bioulac-Sage, P., Marques, J.M., Blanc, J.F., Bui, H., Beaudoin, J.C., Franco, D., Balabaud, C., Laurent-Puig, P. *et al.* (2002) Bi-allelic inactivation of TCF1 in hepatic adenomas. *Nat. Genet.*, **32**, 312–315.
15. Bacq, Y., Jacquemin, E., Balabaud, C., Jeannot, E., Scotto, B., Branchereau, S., Laurent, C., Bourlier, P., Pariente, D., de Muret, A. *et al.* (2003) Familial liver adenomatosis associated with hepatocyte nuclear factor 1alpha inactivation. *Gastroenterology*, **125**, 1470–1475.
16. Reznik, Y., Dao, T., Coutant, R., Chiche, L., Jeannot, E., Clauin, S., Rousselot, P., Fabre, M., Oberti, F., Fatome, A. *et al.* (2004) Hepatocyte nuclear factor-1 alpha gene inactivation: cosegregation between liver adenomatosis and diabetes phenotypes in two maturity-onset diabetes of the young (MODY)3 families. *J. Clin. Endocrinol. Metab.*, **89**, 1476–1480.
17. Laurent-Puig, P., Plomteux, O., Bluteau, O., Zinzindohoue, F., Jeannot, E., Dahan, K., Kartheuser, A., Chapusot, C., Cugnenc, P.H. and Zucman-Rossi, J. (2003) Frequent mutations of hepatocyte nuclear factor 1 in colorectal cancer with microsatellite instability. *Gastroenterology*, **124**, 1311–1314.
18. Gresh, L., Fischer, E., Reimann, A., Tanguy, M., Garbay, S., Shao, X., Hiesberger, T., Fiette, L., Igarashi, P., Yaniv, M. *et al.* (2004) A transcriptional network in polycystic kidney disease. *EMBO J.*, **23**, 1657–1668.
19. Hiesberger, T., Bai, Y., Shao, X., McNally, B.T., Sinclair, A.M., Tian, X., Somlo, S. and Igarashi, P. (2004) Mutation of hepatocyte nuclear factor-1beta inhibits *Pkhd1* gene expression and produces renal cysts in mice. *J. Clin. Invest.*, **113**, 814–825.
20. Ellard, S. (2000) Hepatocyte nuclear factor 1 alpha (HNF-1 alpha) mutations in maturity-onset diabetes of the young. *Hum. Mutat.*, **16**, 377–385.
21. Kovacs, G., Akhtar, M., Beckwith, B.J., Bugert, P., Cooper, C.S., Delahunt, B., Eble, J.N., Fleming, S., Ljungberg, B., Medeiros, L.J. *et al.* (1997) The Heidelberg classification of renal cell tumours. *J. Pathol.*, **183**, 131–133.
22. Storkel, S., Eble, J.N., Adlakha, K., Amin, M., Blute, M.L., Bostwick, D.G., Darson, M., Delahunt, B. and Iczkowski, K. (1997) Classification of renal cell carcinoma: Workgroup No. 1. Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). *Cancer*, **80**, 987–989.
23. Thoenes, W., Storkel, S., Rumpelt, H.J., Moll, R., Baum, H.P. and Werner, S. (1988) Chromophobe cell renal carcinoma and its variants—a report on 32 cases. *J. Pathol.*, **155**, 277–287.
24. Crotty, T.B., Farrow, G.M. and Lieber, M.M. (1995) Chromophobe cell renal carcinoma: clinicopathological features of 50 cases. *J. Urol.*, **154**, 964–967.
25. Storkel, S., Steart, P.V., Drenckhahn, D. and Thoenes, W. (1989) The human chromophobe cell renal carcinoma: its probable relation to intercalated cells of the collecting duct. *Virchows Arch. B Cell. Pathol. Incl. Mol. Pathol.*, **56**, 237–245.
26. Bugert, P., Gaul, C., Weber, K., Herbers, J., Akhtar, M., Ljungberg, B. and Kovacs, G. (1997) Specific genetic changes of diagnostic importance in chromophobe renal cell carcinomas. *Lab. Invest.*, **76**, 203–208.
27. Contractor, H., Zariwala, M., Bugert, P., Zeisler, J. and Kovacs, G. (1997) Mutation of the p53 tumour suppressor gene occurs preferentially in the chromophobe type of renal cell tumour. *J. Pathol.*, **181**, 136–139.
28. Pavlovich, C.P., Walther, M.M., Eyler, R.A., Hewitt, S.M., Zbar, B., Linehan, W.M. and Merino, M.J. (2002) Renal tumors in the Birt–Hogg–Dube syndrome. *Am. J. Surg. Pathol.*, **26**, 1542–1552.
29. Ward, C.J., Hogan, M.C., Rossetti, S., Walker, D., Sneddon, T., Wang, X., Kubly, V., Cunningham, J.M., Bacallao, R., Ishibashi, M. *et al.* (2002) The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat. Genet.*, **30**, 259–269.
30. Xiong, H., Chen, Y., Yi, Y., Tsuchiya, K., Moeckel, G., Cheung, J., Liang, D., Tham, K., Xu, X., Chen, X.Z. *et al.* (2002) A novel gene encoding a TIG multiple domain protein is a positional candidate for autosomal recessive polycystic kidney disease. *Genomics*, **80**, 96–104.
31. Onuchic, L.F., Furu, L., Nagasawa, Y., Hou, X., Eggermann, T., Ren, Z., Bergmann, C., Senderek, J., Esquivel, E., Zeltner, R. *et al.* (2002) *PKHD1*, the polycystic kidney and hepatic disease 1 gene, encodes a novel large protein containing multiple immunoglobulin-like plexin-transcription-factor domains and parallel beta-helix 1 repeats. *Am. J. Hum. Genet.*, **70**, 1305–1317.
32. Lemm, I., Lingott, A., Pogge v Strandmann, E., Zoidl, C., Bulman, M.P., Hattersley, A.T., Schulz, W.A., Ebert, T. and Ryffel, G.U. (1999) Loss of HNF1alpha function in human renal cell carcinoma: frequent mutations in the *VHL* gene but not the *HNF1alpha* gene. *Mol. Carcinog.*, **24**, 305–314.
33. Argani, P., Lal, P., Hutchinson, B., Lui, M.Y., Reuter, V.E. and Ladanyi, M. (2003) Aberrant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions: a sensitive and specific immunohistochemical assay. *Am. J. Surg. Pathol.*, **27**, 750–761.
34. Clairmont, A., Ebert, T., Weber, H., Zoidl, C., Eickelmann, P., Schulz, W.A., Sies, H. and Ryffel, G.U. (1994) Lowered amounts of the tissue-specific transcription factor LFB1 (HNF1) correlate with decreased levels of glutathione S-transferase alpha messenger RNA in human renal cell carcinoma. *Cancer Res.*, **54**, 1319–1323.
35. Sel, S., Ebert, T., Ryffel, G.U. and Drewes, T. (1996) Human renal cell carcinogenesis is accompanied by a coordinate loss of the tissue specific transcription factors HNF4 alpha and HNF1 alpha. *Cancer Lett.*, **101**, 205–210.
36. Rebouissou, S., Rosty, C., Lecuru, F., Boisselier, S., Bui, H., le Frere-Belda, A., Sastre, X., Laurent-Puig, P. and Zucman-Rossi, J. (2004) Mutation of TCF1 encoding hepatocyte nuclear factor 1α in gynecological cancer. *Oncogene*, **23**, 7588–7592.

37. Zhang, M.Z., Mai, W., Li, C., Cho, S.Y., Hao, C., Moeckel, G., Zhao, R., Kim, I., Wang, J., Xiong, H. *et al.* (2004) PKHD1 protein encoded by the gene for autosomal recessive polycystic kidney disease associates with basal bodies and primary cilia in renal epithelial cells. *Proc. Natl Acad. Sci. USA*, **101**, 2311–2316.
38. Eble, J.N., Sauter, G., Epstein, J.I. and Sesterhenn, I.A. (2004) *Classification of Tumors. Pathology and Genetics of Tumors of the Urinary System and Male Genital Organs.* IARC Press, Lyon.
39. Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.*, **16**, 1215.
40. Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, **25**, 402–408.
41. Ramakers, C., Ruijter, J.M., Deprez, R.H. and Moorman, A.F. (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci. Lett.*, **339**, 62–66.