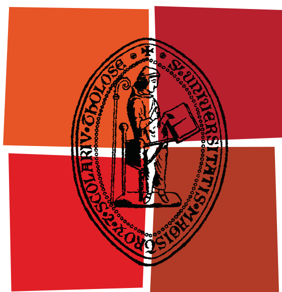

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-

ROLE D'HNF-1Beta DANS L'INSUFFISANCE RENALE AIGUE EXPERIMENTALE

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CARACTERISATION PHENOTYPIQUE ET GENOTYPIQUE DE LA NEPHROPATHIE LIEE AUX MUTATIONS DU FACTEUR DE TRANSCRIPTION HNF-1β

-

ROLE D'HNF-1β DANS L'INSUFFISANCE RENALE AIGUE EXPERIMENTALE

Thèse de Science

Stanislas Faguer

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La maladie liée aux mutations du gène *HNF1B* appartient au groupe des maladies du développement. Celles-ci sont intégrées aux malformations congénitales ou anomalies constitutionnelles, qui sont reconnues chez environ 3 p. cent des individus à la naissance, et dont les causes se répartissent en trois groupes de fréquence sensiblement identiques : aberrations chromosomiques, mutations géniques (dont la maladie liée à *HNF1B*) et embryopathies. La gravité des maladies héréditaires du développement varie amplement. Certaines formes majeures sont incompatibles avec la vie, ou associées à un handicap lourd immédiatement perceptible. Les malformations mineures peuvent se révéler sans conséquence sérieuse. Entre ces deux extrêmes, les manifestations révélatrices peuvent être décelées dans l'enfance, l'adolescence, ou plus tard au cours de la vie adulte. La maladie liée aux mutations de *HNF1B* est un paradigme de cette diversité phénotypique.

Les mutations du gène *HNF1B*, qui code pour le facteur de transcription éponyme HNF-1β, sont responsables d'une affection héréditaire de transmission autosomique dominante d'identification récente (1997). Le spectre clinique est extrêmement hétérogène et associe des anomalies morphologiques ou fonctionnelles des reins, du pancréas, du foie et du tractus génital.[1,2] La prévalence réelle de la maladie est inconnue, l'affection est reconnue avec une fréquence croissante, elle appartient au groupe des maladies rares, mais probablement pas des maladies ultra-rares, dont la fréquence est inférieure à 1/50 000.

A mon arrivée au laboratoire, aucun travail préalable n'avait été consacré localement à HNF-1β, et les efforts de recherche fondamentale menés ailleurs depuis une dizaine d'années avaient permis d'identifier quelques gènes cibles d'HNF-1β, grâce à des modèles d'invalidation de l'orthologue murin d'*HNF1B*[3-5] et des expériences de mutagenèse dirigée chez la souris, le poisson-zèbre ou le xénope[6]. Les données cliniques étaient également très peu abondantes, mais avec une implication forte des médecins néphrologues d'enfants ou d'adultes du CHU de Toulouse[1,2,7] : deux études étaient disponibles chez l'enfant et offraient un panorama descriptif essentiellement transversal[7,8]. Chez l'adulte, les données cliniques rassemblées restaient parcellaires et se résumaient à trois travaux dont les avancées principales concernaient : 1) la première description systématisée du spectre des conséquences tissulaires, morphologiques et fonctionnelles des mutations de *HNF1B*[1], 2) une épidémiologie balbutiante indiquant la fréquence et la variété des mutations d'*HNF1B* dans une population de patients sélectionnés sur l'existence d'une néphropathie malformative congénitale non étiquetée[9] et 3) la génétique de la maladie, puisqu'un tiers à la moitié des patients dont le gène *HNF1B* est muté ont en réalité une vaste délétion de la région 17q12 emportant *HNF1B*[10].

Mes travaux de thèse ont en conséquence été entrepris selon trois axes de recherche, clinique, génétique et physiopathologique, dont les objectifs assignés étaient les suivants : d'une part, compléter

la description de la néphropathie humaine, ses caractéristiques fonctionnelles et son histoire naturelle notamment à l'âge adulte ; d'autre part, développer les méthodes d'analyse génétique d'*HNF1B*, identifier d'éventuelles corrélations génotype-phénotype, améliorer les indications du dépistage génétique, et explorer la contribution potentielle d'autres gènes impliqués dans le réseau transcriptionnel dépendant d'HNF-1β–; et enfin de tester le rôle éventuel d'HNF-1β à l'âge adulte : à cette fin, les mécanismes de régulation d'HNF-1β et son rôle propre dans des modèles murins d'agression rénale aiguë ischémique ou septique ont été disséqués.

Sur le versant clinique, mes travaux de thèse ont débuté par la caractérisation génotypique et phénotypique précise de la néphropathie associée à *HNF1B* chez 27 adultes issus de 20 familles indépendantes. L'analyse moléculaire montre que l'anomalie génétique causale consiste en une délétion complète du gène *HNF1B* dans 11/20 familles, et une mutation ponctuelle dans les autres cas. La preuve d'une mutation *de novo* est apportée dans la moitié des familles testées. L'atteinte rénale est hétérogène, avec les caractéristiques d'une néphropathie tubulo-interstitielle chronique pauci-kystique ($\leq$ 5 kystes par rein chez 62 p. cent des patients), et une progression lente de l'insuffisance rénale sur plusieurs décennies. Nous avons montré qu'un abaissement des concentrations de potassium ou de magnésium est observé chez la moitié des patients, et résulte d'une fuite tubulaire rénale de ces ions. Deux patients ont un syndrome de Fanconi (tubulopathie proximale). Les troubles ioniques suggèrent que chez l'adulte le facteur de transcription HNF-1β contribue à l'homéostasie électrolytique.

Par ailleurs, nous avons établi chez deux jumelles monozygotes qu'un phénotype de type polykystose rénale autosomique dominante associé à une délétion d'*HNF1B* peut être exceptionnellement observé ; enfin j'ai contribué à caractériser les conséquences morphologiques et fonctionnelles rénales d'une cohorte de 75 patients, principalement des enfants, dont 56 propositus avec anomalies anténatales.

Ces données ont fourni la matière utile à deux travaux complémentaires de génétique. D'abord, l'élaboration d'un score prédictif de mutation d'*HNF1B*, visant à réduire le nombre de tests génétiques réalisés en optimisant la sélection phénotypique. Le score a été élaboré par attribution de points selon un barème prenant en considération la présence ou l'absence de symptôme rénal et extra-rénal propre à la maladie liée à *HNF1B*. Ce score, établi rétrospectivement, a été validé prospectivement à l'aide des prélèvements adressés dans le Service de Génétique du CHU de Toulouse, et ses conclusions sont diffusées aux prescripteurs dans l'Hexagone. Sa valeur prédictive négative est supérieure à 99 p. cent, et nous avons montré que son adoption par les cliniciens prescripteurs pourrait diminuer de 38 p. cent le nombre de tests effectués. Ce score est en attente d'une validation externe, qui est entreprise en collaboration avec d'autres laboratoires de Génétique dépistant les mutations d'*HNF1B*.

A l'initiation de cette thèse, les données concernant le rôle d'HNF-1β dans le rein mature restaient totalement méconnues. Ceci s'associait à une mauvaise corrélation entre le phénotype de la maladie liée à *Hnf1b* chez la souris obtenu par invalidation anténatale (polykystose rénale, insuffisance rénale rapidement progressive, transmission récessive) et celle observée chez l'homme en post-natale (néphropathie interstitielle chronique, atteinte paucikystique peu évolutive, transmission dominante). Nous avons en conséquence exploré le rôle d'HNF-1β et ses mécanismes de régulation dans le rein mature.

En premier lieu, nous avons entrepris d'analyser l'expression dans le rein humain des gènes cibles d'HNF-1β précédemment identifiés chez la souris. Classiquement, la biopsie rénale n'est pas requise dans l'exploration d'une néphropathie liée à HNF-1β. En l'absence de matériel exploitable, le recours à la quantification de l'expression de ces gènes dans le culot cellulaire urinaire (considéré comme succédané au tissu rénal) a permis de montrer que dans le rein mature de patients porteurs d'une mutation d'*HNF1B*, l'expression des cystogènes *PKHD1*, *PKD2*, *CDH16*, *UMOD* et COLLECTRIN n'était pas différente de celle d'individus témoins. L'expression du gène *ATP1A1*, codant pour la sous-unité alpha de la Na^{+}-K^{+}-ATPase et impliqué dans la régulation de la réabsorption tubulaire de magnésium , était en ravanche nettement augmentée.

Enfin, le recours à deux modèles d'agression rénale aiguë (ischémique par choc hémorragique contrôlé et septique par injection d'endotoxine), nous a permis de caractériser *in vitro* la réponse d'HNF-1β à des signaux extra/intra-cellulaires incluant l'hypoxie et l'activation de la voie NF-κB via la stimulation du TLR4 (lipopolysaccharide). *In vivo*, nous avons pu montrer qu'HNF-1β contribue à réguler la réponse cellulaire à une agression aiguë en modulant les voies de signalisation IL6/IL6-R, HGF-R, EGF-R au travers de la régulation de l'expression *Socs3*.

Le plan retenu pour la rédaction de cette thèse compte donc quatre parties:

1) La caractérisation du facteur HNF-1β (structure, rôle, réseau transcriptionnel)
2) La caractérisation phénotypique de la maladie liée à HNF-1β (modèles animaux et phénotype chez l'homme)
3) L'approche génétique de la maladie chez l'homme
4) La caractérisation des mécanismes de régulation d'HNF-1β et le rôle de celui-ci dans les situations d'agression puis de régénération épithéliale.

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Rôle d'Hnf-1β dans la formation du pro/mesonephros

Rôle d'Hnf-1β dans la formation du metanephros

- Induction du canal urétéral
- Formation du système collecteur : effet sur la voie HGF-Jak/Stat via l'inhibition de *Socs3*
- Différenciation néphronique et polarisation planaire : effets sur la voie Wnt et sur les cystogènes

Rôle d'Hnf-1β à l'âge adulte : axe *SNAIL1 – Hnf-1β – KSP-Cadherin* et rôle dans l'homéostasie du magnésium

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A) Rôle physiologique du facteur de transcription HNF-1β

1 - Le facteur de transcription HNF-1β (hepatocyte nuclear factor-1Beta) : structure, fonction

a. le gène *HNF1B*

Le gène *HNF1B*, anciennement appelé *TCF2* (*Transcription Factor 2*), *LFB3* ou *vHNF1* est porté par le chromosome 17 chez l'homme, en position 17q12 (position 17: 36,046,434-36,105,237 ; Ensembl).

HNF1B forme avec son homologue *HNF1A* (anciennement TCF1) une famille digénique propre aux vertébrés, vraisemblablement apparue par duplication d'un gène ancestral commun. Les premières publications concernant HNF-1β ne faisaient référence qu'à un « variant » de HNF-1α (donc nommé vHNF1) du fait de l'homologie des 315 premiers acides aminés avec ce dernier (Figure 1)[11,12]. HNF-1α a été initialement isolé dans le noyau d'hépatocytes où il se lie aux promoteurs de divers gènes, notamment ceux codant pour la chaîne β du fibrinogène et pour l'α_1-antitrypsine[13], ainsi qu'au promoteur du gène codant pour l'albumine[14] d'où son abondance dans l'hépatocyte. Secondairement, deux études ont permis d'identifier dans des hépatocytes en différenciation un second facteur de transcription reconnaissant les mêmes motifs qu'HNF-1α, nommé HNF-1β, et nécessaire à la différenciation de ce type cellulaire.[14,15]

Le clonage du gène a été réalisé en 1991[16] permettant de confirmer qu'HNF-1α et HNF-1β sont les produits de deux gènes distincts, *HNF1A* étant porté par le chromosome 12 et *HNF1B* par le chromosome 17. Au niveau moléculaire, *HNF1B* est composé de 9 exons. Le transcrit primaire est composé de 59 236 paires de bases. Les gènes contigus et ceux localisés à proximité d'*HNF1B* (*LHX1, AATF, ACACA, TADA2L, DUSP14, DDXS2 et AP1GBP1*) n'ont pas de rôle physiologique connu, à l'exception de *LHX1* (gène *Lim1* chez la souris) dont le rôle dans le développement rénal et cérébral a été récemment démontré[17,18].

b. Le facteur de transcription HNF-1β

HNF-1β est un facteur de transcription (FT) de 557 acides aminés (AA), avec un poids moléculaire de 61 324 Da, de localisation intra-nucléaire. Il fait partie de la famille des FT avec domaine de liaison à l'ADN de type homeobox (structure hélice-boucle-hélice). Il se fixe sous forme d'homo- ou d'hétérodimère (HNF-1β — HNF-1β ou HNF-1α— HNF-1β) au promoteur de ses gènes cibles.

Trois domaines peuvent être individualisés (Figure 1) :

- Un domaine de dimérisation à la partie N-terminale (AA 1 à 31).
- Un domaine de liaison à l'ADN, composé de deux sous domaines : POU-A (AA 88 à 178) et POU-B (homeobox, AA 231 à 311) et d'un segment de transition. Il reconnaît le motif palindromique **5'-GTTAATNATTAAC-3'**. La séquence des AA 229 à 235 (KKMRRNR) représente le signal de localisation nucléaire[19].

- Un domaine de transactivation à la partie C-terminale. Le potentiel de transactivation d'HNF-1β est dépendant d'une interaction physique de son domaine C-terminal avec différentes protéines co-régulatrices : l'histone acetyltransferase CREB-binding protein (CBP), la p300 CBP-associated factor (PCAF) et l'histone-dé-acétylase 1 (HDAC-1)[20]. L'association fonctionnelle d'HNF-1β avec deux autres protéines régulatrices, ZFP36L1 (prédominant dans le cytoplasme, activatrice) et E4F1 (prédominant dans le noyau, inhibitrice), a récemment été caractérisée[21].

HNF-1β compte deux résidus phosphosérine en positions 75 et 80 dont le rôle dans sa régulation n'est pas connu. Par analogie avec les mécanismes de régulation du facteur de transcription SALL1[22], la phosphorylation de ces résidus pourraient être un signal d'activation ou d'inhibition d'HNF-1β.

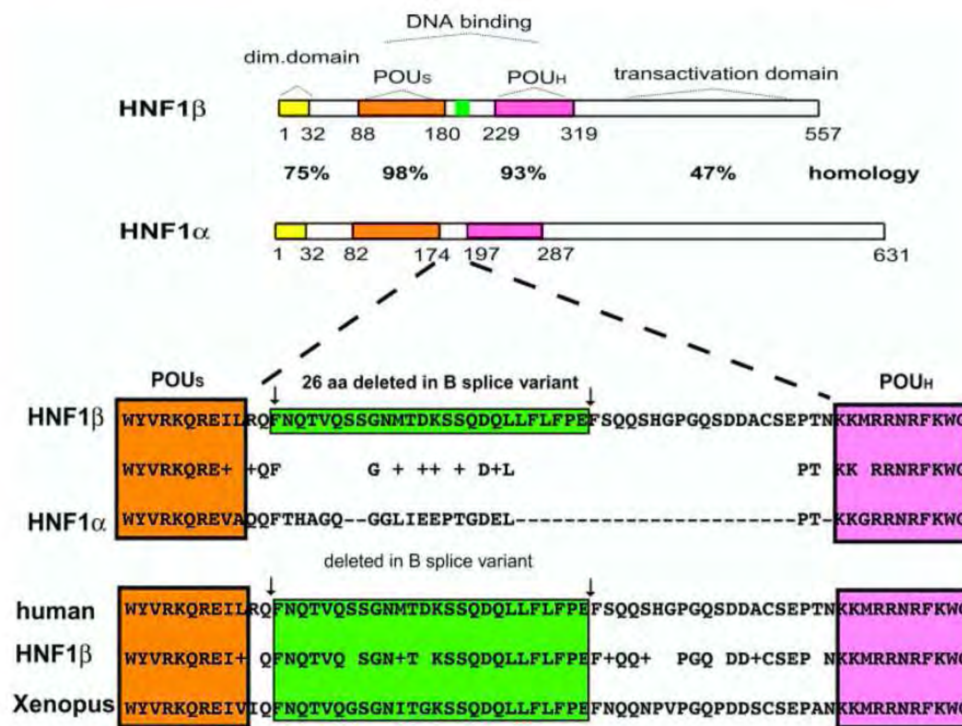

Figure 1 : Représentation du facteur de transcription HNF-1β, avec ses différents domaines (de dimérisation, de liaison à l'ADN et de transactivation) et analyse comparative d'*HNF1B* et *HNF1A* (homologie des deux premiers domaines de 75 à 98%).

Trois isoformes d'HNF-1β ont été identifiées dans les hépatocytes humains et dénommées isoformes A, B et C (Figure 2)[23]. L'isoforme B présente une délétion de 26 AA de la zone de transition (AA 183 à 208) secondaire à un épissage alternatif. L'isoforme C présente une extrémité C-terminale différente, secondaire à une délétion non en phase.

Ces isoformes présentent des caractéristiques fonctionnelles différentes : transactivation pour l'isoforme A versus transinhibition pour l'isoforme B[23]. Ceci a été confirmé par Wu et al[19] dans un

modèle de xénope. Le ratio isoforme A/isoforme B n'influence pas l'embryogenèse précoce (différenciation de l'endoderme viscéral à partir des cellules souches embryonnaires)[24]. Pour autant, la constatation que le ratio ne varie pas au cours du développement rénal suggère un rôle de la régulation de la synthèse de ces isoformes à un stade plus tardif[25]. Le rôle de l'isoforme C n'est pas connu.

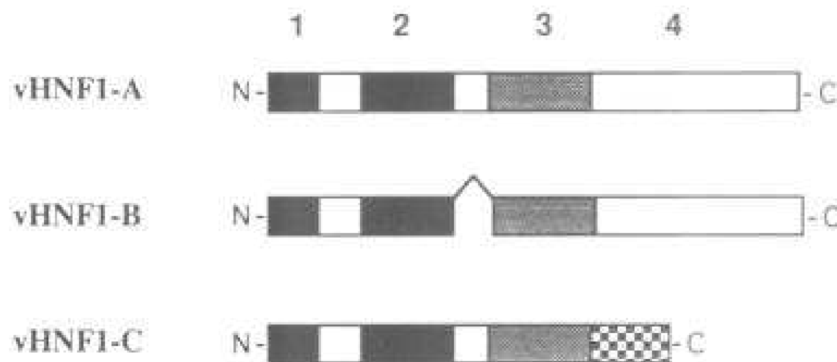

Figure 2: représentation des trois isoformes A, B et C de Hnf1b (souris) (d'après Bach et al[23])

2 - Rôle biologique du facteur de transcription HNF-1β

a. Profil d'expression d'*Hnf1b*

Embryogenèse précoce : effet sur *Ipf1*, *Shh* et *Ihh*

Après l'implantation dans le mur utérin, l'embryon subit de profonds changements dans sa structure qui aboutissent à un corps embryonnaire composé de 3 feuillets primitifs : l'ectoderme, le mésoderme et l'endoderme. De multiples interactions réciproques entre les feuillets primitifs induisent la spécification régionale.

Chez le poisson zèbre, l'expression d'*Hnf1b* débute précocement au cours de l'embryogenèse : entre le stade 256 et 1000 cellules[26]. A ce stade, HNF-1β régule l'expression de gènes nécessaires à la spécification régionale des tissus : endoderme viscéral et ses dérivés (pancréas, foie), pronéphros et partie postérieure de la jonction rhombomérique r4/r5 (système nerveux central).

Chez la souris, HNF-1β est requis pour la différenciation de l'endoderme viscéral[27,28]. Ainsi les souris avec une double délétion de *Hnf1b* meurent au 5ème jour de vie embryonnaire avec une absence complète de différenciation de ce feuillet. Après le stade de gastrulation, l'expression d'*Hnf1b* est limitée au tube neural en formation, à l'intestin primitif et secondairement au méso- et métanéphros en développement[27] (Figure 3).

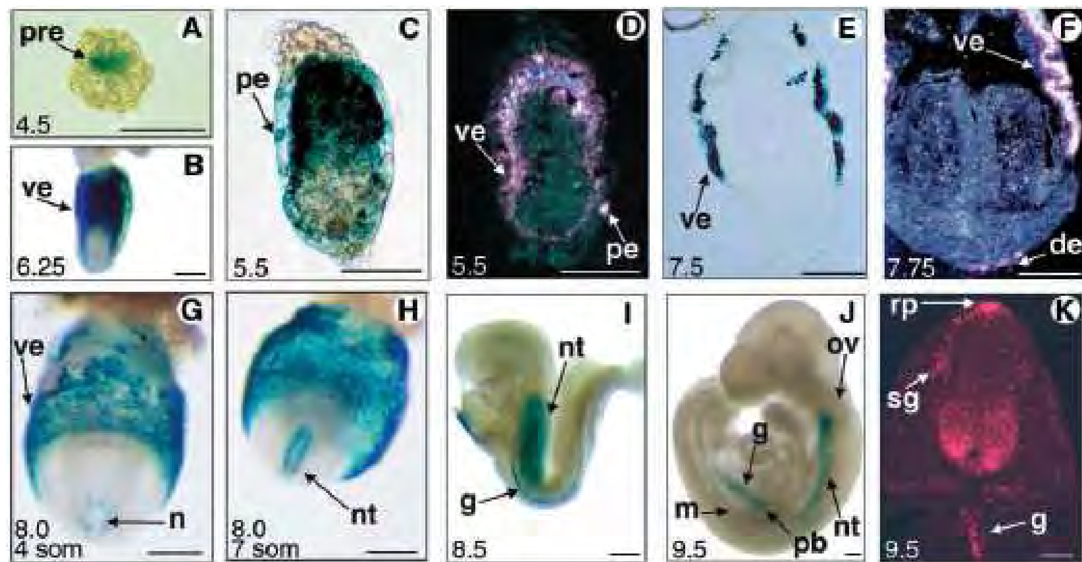

Figure 3 : Expression d'*Hnf1b* dans l'endoderme visceral (ve) et le mésoderme intermédiaire. *g* gut, *nt* notochorde, *m* mésonéphros (d'après Barbacci et al[27]). Le marquage bleu montre les zones où *Hnf1b* est exprimé.

- **Dans l'endoderme viscéral**, les gènes *Ipf1* et *Shh* (sonic hedgehog) se répriment mutuellement par l'intermédiaire d'une boucle régulatrice[29,30] permettant une expression spatiale restreinte de ces deux gènes favorisant le développement du pancréas[31]. HNF-1β régule *Ipf1*[32,33], *Shh*[26] et *Ihh* de manière directe ou indirecte, suggérant un rôle essentiel dans l'induction et le contrôle du développement du pancréas, et la régionalisation de l'intestin primitif (Figure 4)[5]. Plus récemment, les rôles respectifs d'HNF-1α et HNF-1β dans la différenciation terminale des entérocytes et des cellules sécrétrices intestinales, via la régulation fine de la voie Notch (régulation directe de l'expression des gènes *Jagged1* et *Atoh1*) ont été établis.[34]

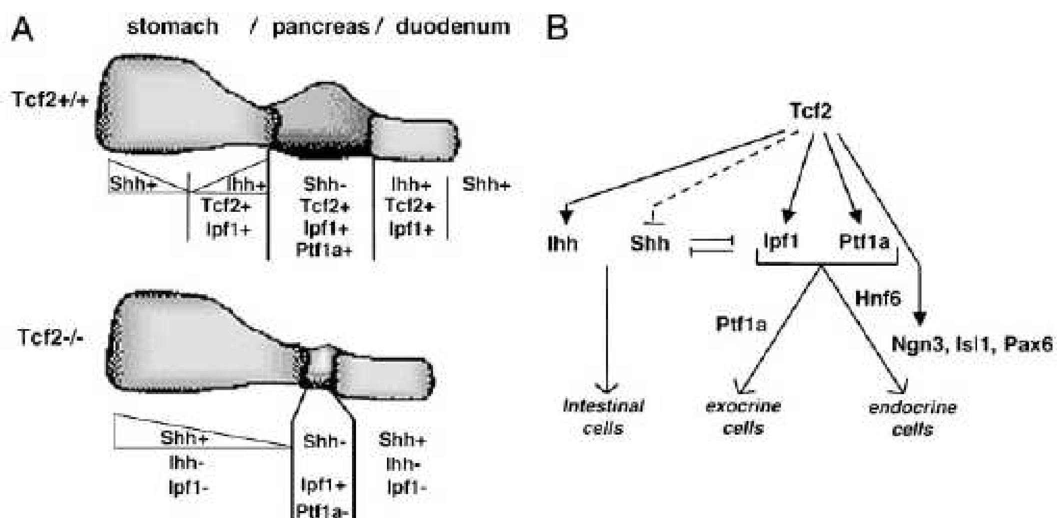

Figure 4: Rôle d'HNF-1β (ex-*Tcf2)* dans le développement du pancréas et de l'intestin chez la souris (d'après Haumaitre et coll[5])

- **Dans l'encéphale caudal**, HNF-1β semble être un acteur essentiel de la segmentation rhombomérique. Il est exprimé spécifiquement à la jonction des rhombomères 4 et 5, dans la partie postérieure. Sa présence est requise pour induire l'expression des facteurs de transcriptions *MafB* et *KROX20*[35,36]. De façon intéressante, l'expression d'*Hnf1b* dans cette région est sous la dépendance de l'acide rétinoïque[36,37]. Pouilhe et coll. ont montré que le complexe RAR-RXR induit par la liaison de l'acide rétinoique à son récepteur pouvait se fixer à un motif RARE (Retinoic Acid Responsive Element) situé dans une région régulatrice (en *cis*) dans le 4ème intron d'*Hnf1b*[38]. Secondairement, la boucle d'amplification *HNF-1β-MafB* permet de maintenir l'expression d'*Hnf1b* par l'intermédiaire d'une séquence T-MARE (MAF- Responsive Element) située dans la même région que la séquence RARE (4ème intron). Cette voie de signalisation acide rétinoïque–*Hnf1b* a également été mise en évidence au cours du développement du pancréas[39] (Figure 5) suggérant une voie commune et pléïotrope. Chez la souris avec délétion bi-allélique du gène codant pour le récepteur de l'acide rétinoïque, des malformations uro-néphrologiques et du tractus génital sont observées[40]. Enfin, chez la souris avec invalidation bi-allélique de *MafB* on observe une dysgénésie tubulaire rénale associée à une anomalie de la différenciation podocytaire[41]. Les données concernant l'expression d'*Hnf1b* dans ce modèle sont malheureusement manquantes. Nous avons récemment recherché des variations génétiques dans les séquences T-MARE et RARE de 51 individus présentant différentes anomalies rénales compatibles avec une mutation d'*HNF1B* mais sans mutation identifiée (Annexe, article 5). Aucune mutation dans ces motifs n'a pu être identifiée suggérant que cette voie d'activation est probablement minoritaire dans le rein humain.

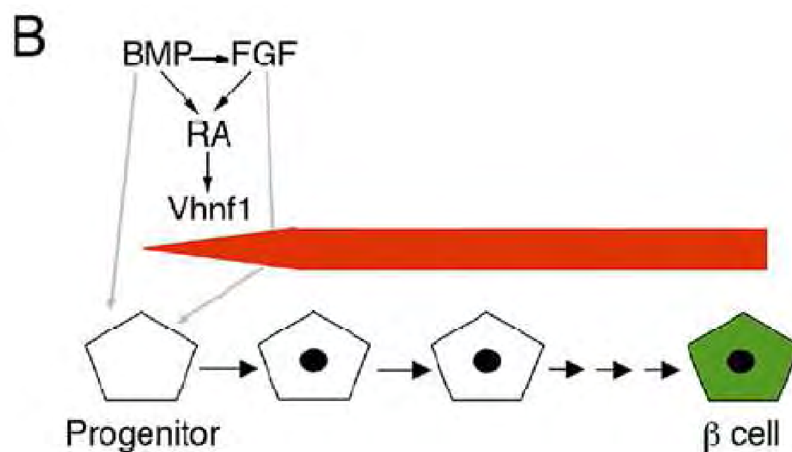

Figure 5 : Modèle d'intégration du signal de Bmp, Fgf, Acide rétinoïque (RA) et Hnf1b (Vhnf1) dans le développement des ilôts β pancréatique (d'après Song et coll[39]).

Rôle d'HNF-1β dans le développement du mésoderme intermédiaire

Chez les mammifères, le développement rénal se déroule en trois étapes. Le mésoderme intermédiaire donne naissance à deux structures transitoires, le pronéphros et le mésonéphros, et une définitive, le métanéphros. La transition d'un phénotype mésenchymateux vers un phénotype épithélial des cellules du mésoderme intermédiaire permet la formation du canal et des tubules mésonéphrotiques. Le canal de Wolff (ou canal mésonéphrotique) est une extension du canal pronéphrique, qui s'étend vers la région caudale au travers du mésenchyme mésonéphrique (mésoderme intermédiaire) et induit la formation du mésonéphros. Le canal de Wolff atteint finalement le cloaque et forme la partie postérieure de la vessie (Figure 6).

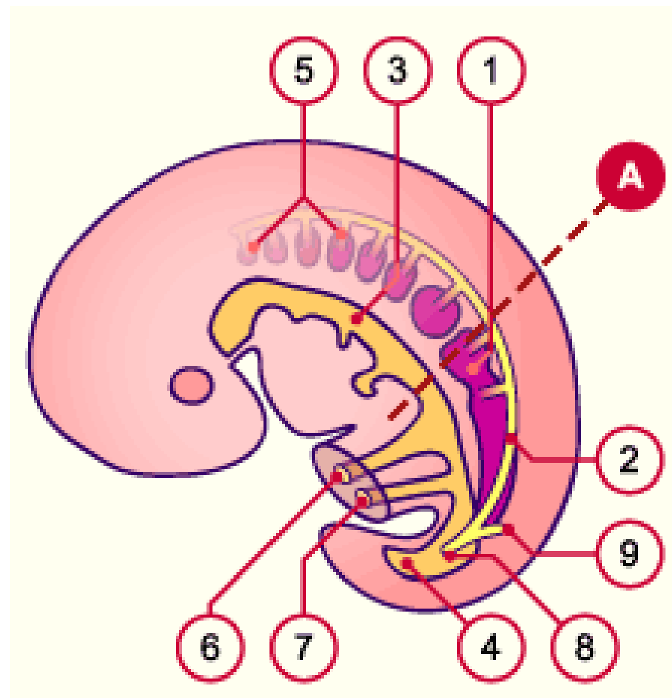

Figure 6 : Représentation schématique du mésonephros. 1 mésonephros, 2 Canal de Wolff (= canal mésonéphrotique), 3 intestin, 4 cloaque, 5 pronéphros en régression, 9 ébauche du canal urétéral (qui va envahir le blastème métanéphrogène). [adapté de www.embryology.ch]

A l'extrémité caudale de ce canal, des interactions moléculaires réciproques entre le mésenchyme métanéphrique et une excroissance du canal de Wolff, le canal urétéral, sont à l'origine de la formation du métanéphros. On observe initialement une croissance du canal urétéral sous l'influence de la voie GDNF-RET-GDNFRα[42] (Figure 7). Secondairement, le canal urétéral s'arborise (futur réseau collecteur) et on observe une condensation du mésenchyme puis une différenciation épithéliale du néphron sous l'influence des facteurs de transcription *PAX2*, *WT1* et *SALL1*[43,44].

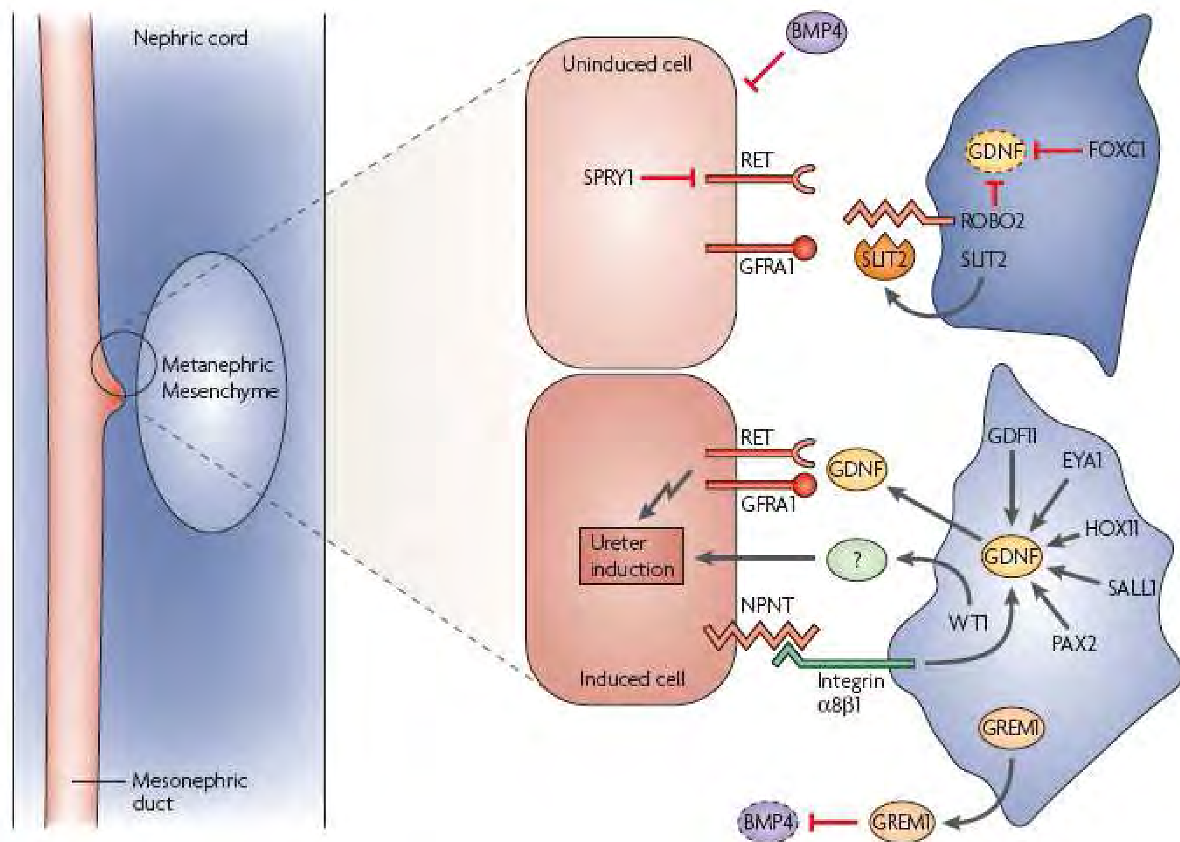

Figure 7: Voie de signalisation moléculaire contrôlant la tubulogenèse rénale (d'après Schedl et al.)[42]. On note l'induction réciproque entre le blastème métanéphrique (cellules mésenchymateuses sécrétant le GDNF) et le canal urétéral (cellules de type épithélial portant en surface le récepteur du GDNF (RET-GNDFR)).

Rôle d'HNF-1β dans la formation du pro/mésonéphros

Le rôle d'HNF-1β au cours de la formation du pronephros a été élucidé grâce à l'étude de modèles animaux (poissons-zèbres mutés pour *Hnf1b* et embryons de xénope). Chez les poissons-zèbres, obtenus par mutagenèse dirigée, les tubules pronéphriques sont interrompus, aboutissant secondairement à des formations kystiques dans la région du pronephros. Les domaines d'expression de *Pax2* et *Wt1* sont modifiés (abolis et étendus, respectivement)[26].

La mutation P328L329fsdelCCTCT (pathologique chez l'homme), qui confère un gain de fonction de transactivation à Hnf1b, induit une agénésie du pronephros quand elle est surexprimée dans des embryons de xénope. A l'inverse, la mutation R137-K161del, qui est douée d'un effet dominant-négatif, affecte de manière moins fréquente la formation du pronéphros. Ainsi, les gènes cibles sont extrêmement sensibles à la concentration d'HNF-1β efficace présente dans leur environnement.

Chez le poisson-zèbre mutant pour *Hnf1b*, on observe une disparition de l'expression de *Pax2*[26]. Chez la souris avec mutation bi-allélique de *Pax2* l'absence de différenciation épithéliale des

cellules du mésenchyme de la corde mésonéphrotique s'accompagne d'une absence de canal de Wolff et donc de métanephros[45]. Enfin, *Pax2* régule la prolifération cellulaire du canal urétéral en contrôlant la voie GDNF-RET-GDNFRα et l'apoptose épithéliale[46]. Pax2 se lie à un élément régulateur en amont du promoteur des gènes *Gdnf* et *Ret* et peut transactiver l'expression de ces gènes[46,47]. D'autre part, les cellules du canal urétéral des souris *Pax2*$^{+/-}$ présentent une apoptose excessive malgré la présence d'un signal de prolifération induit par RET[48]. Ainsi, *Pax2* serait un « décodeur » du signal prolifération–différenciation–survie et *Hnf1b* pourrait réguler l'expression de ce décodeur. Au total, chez le poisson-zèbre HNF-1β semble contrôler la transformation épithéliale des mésenchymes pronéphrique et mésonéphrique par l'intermédiaire de *Pax2*. Récemment, ces données ont été élégamment confirmées dans un modèle murin *Hnf1b*$^{-/-}$ obtenu à l'aide de chimère diploïde ou tétraploïde (voir ci-dessous).[49]

Rôle d'HNF-1β dans la formation du métanephros

- *Induction du canal urétéral*

En 2010, Lokmane et coll. ont pu montrer qu'HNF-1β contrôle directement la persistance et la croissance du bourgeon urétéral par la régulation directe de l'expression de trois facteurs de transcription majeurs de la néphrogenèse : PAX2, LIM1 (LHX1 chez l'homme) et WNT9b.[49] Il n'est pas nécessaire à l'induction du canal urétéral (dépendante du couple GDNF-RET) mais à sa croissance à partir du stade-T.

- *Formation du système collecteur : effet sur la voie HGF-Jak/Stat via l'inhibition de Socs3*

Dans le rein de souris, *Hnf1b* est exprimé dans le pronéphros, le mésonéphros puis dans le bourgeon urétéral (Figure 8). A l'âge adulte, il est exprimé essentiellement dans le cortex rénal (cellules épithéliales du tube proximal, de l'anse de Henlé, du tube distal et du canal collecteur) mais est totalement absent des vaisseaux et des glomérules[50].

La formation du canal urétéral implique des mécanismes de migration cellulaire, de prolifération cellulaire et de tubulogenèse. Ces événements, nécessaires à l'élaboration d'un système excréteur fonctionnel, sont dépendants de facteurs extra-cellulaire qui régulent la tubulogenèse, de manière positive pour la pleiotrophine, l'Hepatocyte Growth Factor (HGF), l'Epithelial Growth Factor (EGF) et les Fibroblast Growth Factors (FGF), ou négative pour les Bone Morphogenetic Proteins (BMP), les inhibiteurs de métalloproteases (TIMP2) et l'endostatine[51-58].

En particulier, HGF a le potentiel, *in vitro*, d'induire la tubulogenèse des cellules mésenchymateuses mésodermiques. Le signal induit par la liaison d'HGF à son récepteur, c-met[58,59] contrôle les réarrangements du cytosquelette dépendants de la phospho-inositide 3-kinase (PI 3K)[60] et les interactions cellule-matrice extra-cellulaire nécessaires à la migration cellulaire[61,62](Figure 9).

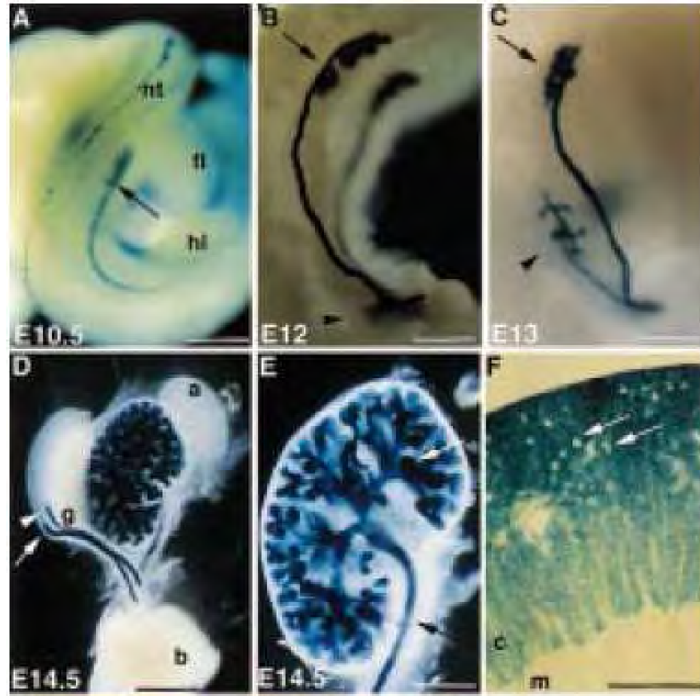

Figure 8 : Profil d'expression d'*Hnf1b* dans le mesonephros (A et B) puis le metanephros (C à F) (d'après Coffinier et al.[50]).

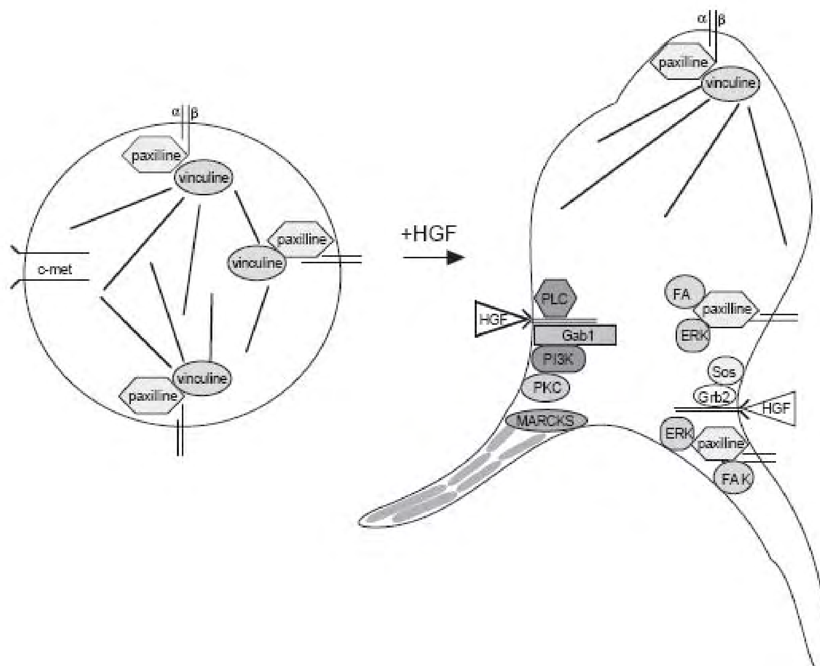

Figure 9 : Réarrangement du cytosquelette secondaire à la liaison d'HGF sur son récepteur c-met permettant de confier à la cellule indifférencié des capacités de tubulogenèse (d'après Cantley , Act Neph Necker 2003).

Récemment, Ma et al.[63] ont montré par une approche génomique combinée (analyse ChIP-chip, microarray RNA) que le promoteur du gène *Socs3* (pour suppressor of cytokine signaling-3) est

spécifiquement reconnu par HNF-1β avec une inhibition spécifique de *Socs3* parmi les gènes de la famille *Socs*[63]. L'augmentation de l'expression de *Socs3* dans les cellules *Hnf1b*$^{-/-}$ se traduit par une répression de la tubulogenèse induite par HGF secondaire à un défaut de phosphorylation d'ERK et de STAT3 (Figure 10).

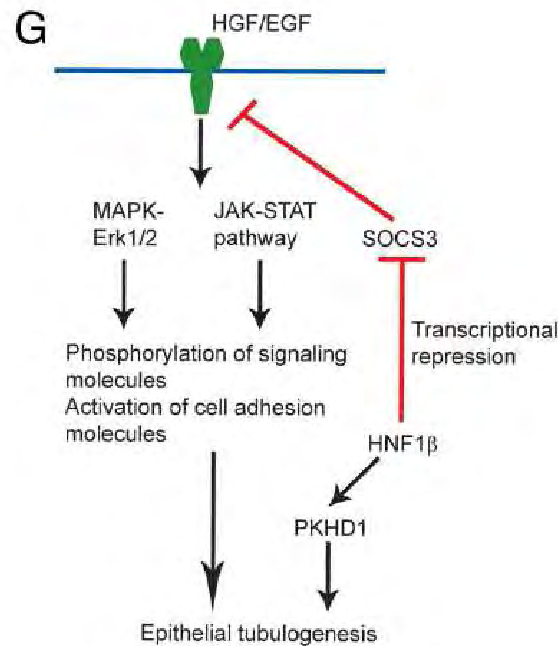

Figure 10: Régulation de *SOCS3* et des signaux de prolifération cellulaire dépendants d'HGF par HNF-1β (d'après Ma et al.[63]).

Par ailleurs, on peut ajouter que Socs3 inhibe d'autre signaux issus de facteurs de croissance ou de cytokines comme l'EGF, l'insuline-like growth factor et l'angiotensine II, dont plusieurs jouent un rôle majeur dans la morphogenèse tubulaire[53,64]. HNF-1β régule donc positivement, via *Socs3*, un ensemble de voies de signalisation nécessaires à la tubulogenèse rénale.

Enfin, il est établi que HGF et ses effecteurs, comme Erk, ont un rôle dans la polarisation cellulaire[65], celle-ci étant un des paramètres importants de la capacité de polarisation planaire des cellules épithéliales (orientation de la division cellulaire dans l'espace). Dans un modèle de souris avec invalidation conditionnelle rénale d'*Hnf1b*, une perte de la polarisation planaire des cellules épithéliales tubulaires a pu être démontrée[66]. Ce mécanisme pourrait participer à la genèse des kystes observés dans les reins de ces souris.

De même, il est bien établi qu'HNF-1β induit l'expression du gène codant pour la protéine cadherin16, également appelé KSP-cadherin (pour kidney specific protein-cadherin)[6]. La KSP-cadherin appartient à la famille des cadhérines, protéines composant les jonctions adhérentes inter-cellulaires, dont le rôle dans les mécanismes d'adhésion cellule-cellule, de différenciation cellulaire et de polarisation planaire et apicobasale est bien identifié[67].

Au total, HNF-1β joue un rôle essentiel dans la régulation de la formation du système collecteur en contrôlant les signaux issus des facteurs de croissance extra-cellulaire qui dirigent la tubulogenèse. A ce stade, on observe une arborescence du canal urétéral dans le mésenchyme métanéphrique (Figure 11).

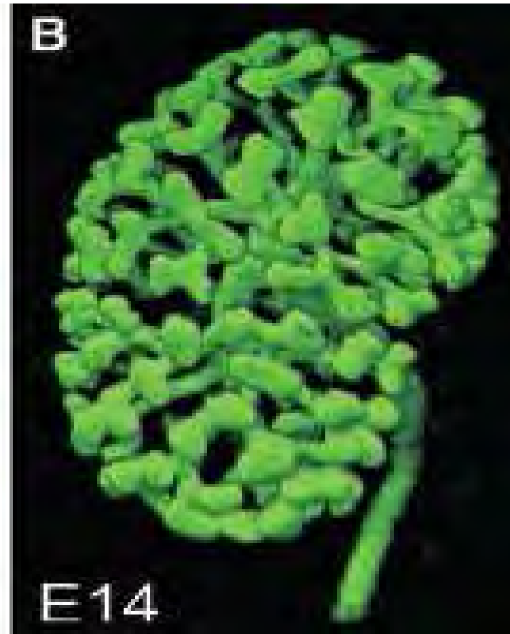

Figure 11: Arborescence induite par la prolifération et la division segmentaire du canal urétéral formant à terme les cavités excrétrices du rein.

- *Différenciation néphronique et polarisation planaire : effets d'HNF-1β sur la voie Wnt et sur les cystogènes*

- HNF-1β et voie Wnt

La croissance du canal urétéral et ses divisions successives suivent le gradient de GNDF sécrété par les cellules mésenchymateuses. De façon réciproque, le canal urétéral va induire la transformation épithéliale des cellules mésenchymateuses[42]. On observe successivement une condensation des cellules (vésicule rénale), un corps en virgule, un corps en s puis le néphron dans sa forme définitive (Figure 12)[42].

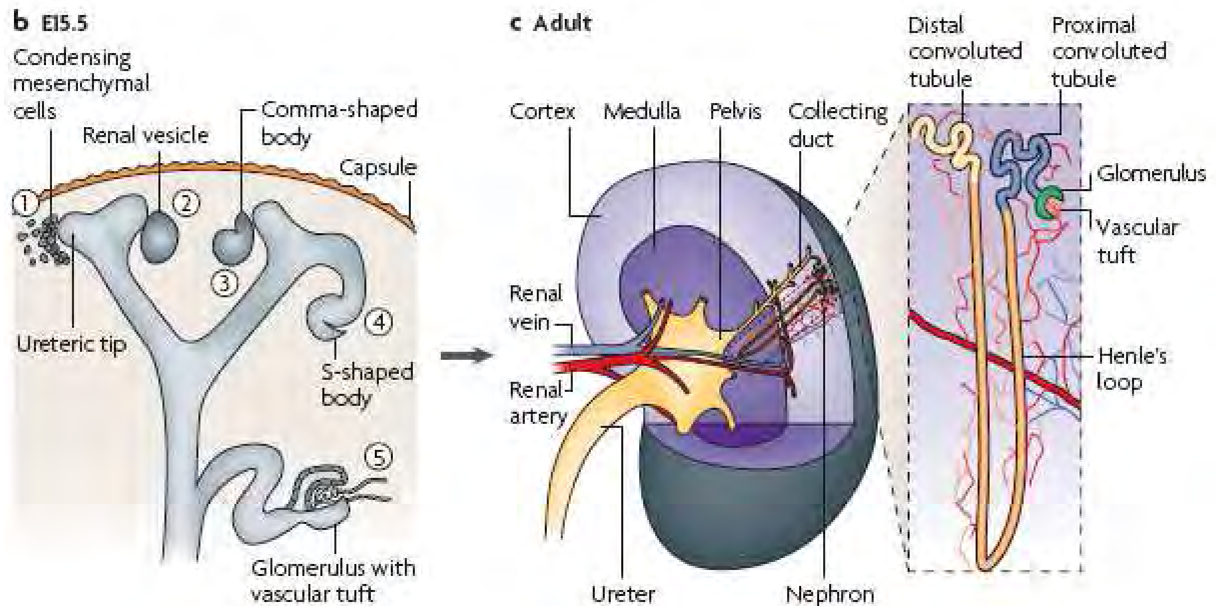

Figure 12: Développement du néphron chez l'homme (b embryon à 15 jours de vie embryonnaire avec les 3 stades de différenciation : vésicule rénale, corps en virgule puis corps en s ; c âge adulte) (d'après Schedl et al[42]).

La voie de signalisation WNT-β-caténine joue un rôle essentiel au cours de ce processus, comme le démontrent les modèles de souris invalidées pour *Wnt9b*[68]. Celles-ci n'expriment pas les marqueurs précoces de la formation néphronique *Lim1*, *Pax8*, *Fgf8* et *Wnt4*. De même, l'expression de *Wnt4* est suffisante pour diriger la transition mésenchyme-épithélium et induire la néphrogenèse. *Wnt4* est régulé par *Pax2*, *Wt1* et *Fgf8*[48,69,70]. Ainsi, HNF-1β pourrait coordonner la néphrogenèse par l'intermédiaire d'une cascade de régulation moléculaire comprenant *Pax2, Wt1* et la voie canonique *Wnt*. Cette hypothèse est désormais validée chez la souris.[49,71] Après la phase d'induction du bourgeon urétérale, HNF-1β stimule directement l'expression de *Wnt9b*, *Lim1* et *Pax2*. Les reins de souris chimériques *Hnf1b*$^{-/-}$ ont une expression réduite de ces 3 facteurs mais également de leurs gènes cibles respectifs ou des voies de signalisation qu'ils activent habituellement (*Wnt4*, *Fgf8* et *Pax8),* molécules indispensable à une tubulogenèse efficace.

Le facteur de transcription *Lim1* n'est quant lui pas requis pour l'induction de la différenciation du mésenchyme métanéphrique en vésicule rénale, mais il est nécessaire à la bonne segmentation ultérieure du tubule. HNF-1β régule donc la tubulogenèse et la segmentation tubulaire au cours de la néphrogenèse via la régulation directe de différents réseaux transcriptionnels.

- HNF-1β et cystogènes

Deux modèles animaux mutés pour *Hnf1b* ont été générés, l'un par construction d'un transgène exprimant une forme mutée d'*Hnf1b* murine équivalente à une mutation humaine[6], l'autre par construction d'un modèle d'invalidation rénale conditionnelle d'*Hnf1b* (système Cre-Lox sous la

dépendance du promoteur de la KSP-Cadherin)[4]. Ce dernier modèle a permis d'identifier certains gènes sous la dépendance d'HNF-1β. Parmi ceux-ci on note la présence de gènes activés (*Umod, Pkhd1, Pkd2, Polaris/Tg737, Nphp1)* et de gènes inactivés (*Aqp2, Avpr2*) (Figure 13)[4]. Les gènes activés font partie de la famille des cystogènes, impliqués dans des maladies kystiques murines. Par méthode d'immunoprécipitation de chromatine, il a été établi qu'HNF-1β se fixe directement au promoteur des gènes *Umod*, *Pkhd1* et *Pkd2*.

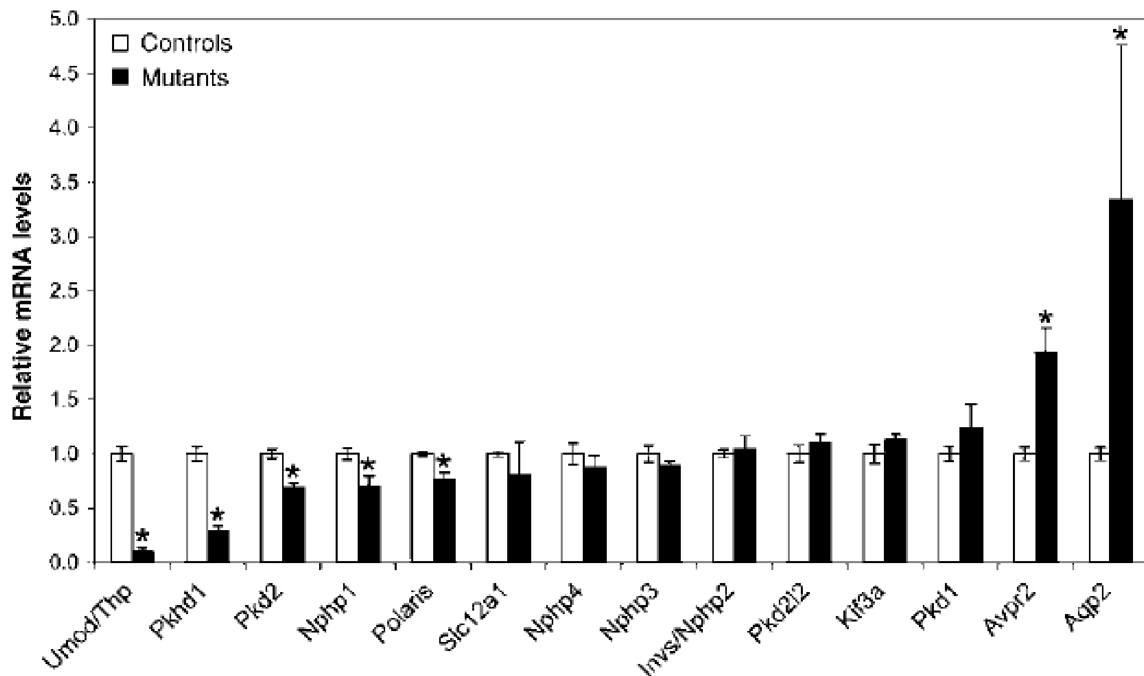

Figure 13 : expression rénale des gènes cibles potentiels d'HNF-1β chez les souris KspCre, Hnf1b$^{flox/+}$ (d'après Gresh et al.[4]) * : valeurs significatives.

Les mutations des gènes orthologues humains d'*Umod*, *Pkhd1* et *Pkd2* sont responsables de néphropathies kystiques rénales humaines : *UMOD* pour la néphropathie hyperuricémique juvénile familiale (ou medullary cystic kidney disease type 2), *PKHD1* pour la polykystose rénale autosomique récessive et *PKD2* pour la polykystose rénale autosomique dominante de type 2.

De nouveaux gènes cibles d'HNF-1β ont été mis en évidence dans les cellules épithéliales tubulaires rénales de souris. On peut citer les deux gènes suivants : *Collectrin* (un membre de la famille des gènes de l'enzyme de conversion de l'angiotensine)[72] et *Kif12* (un membre de la famille des kinésines).[73] Ces deux gènes codent également pour des protéines impliquées dans la formation et le maintien de la structure du cil primaire des cellules épithéliales tubulaires rénales. *Collectrin* est également co-exprimé avec *Hnf1b* dans les cellules β pancréatiques. La collectrine, produit du gène homonyme, est une protéine « chaperonne » encadrant l'adressage de protéines membranaires du réticulum endoplasmique vers la surface membranaire apicale permettant une polarisation cellulaire. De façon intéressante, la collectrine 1) colocalise avec l'α-tubuline acétylée qui est un constituant du cil primaire et 2) forme un complexe avec la polycystine-2 (codée par le gène *Pkd2*) et Polaris/Tg737

(Figure 14). De même, une inactivation de l'expression de *Collectrin* par RNA interférence induit une diminution de la polycystine-2 membranaire, un défaut de formation du cil primaire puis une augmentation de la prolifération cellulaire et de l'apoptose. Cette diminution semble secondaire à un défaut d'adressage de la polycystine-2 vers le cil primaire[72]. *Collectrin* est également un gène cible d'HNF-1α[74].

Kif12 (Kinesin family member 12) est quant à lui un gène modificateur du phénotype des souris *cpk* caractérisée par une polykystose rénale. Kif12 contrôle l'orientation dans l'espace de la division des cellules épithéliales rénales et pourrait donc être le lien moléculaire entre HNF-1β et la fonction de polarisation planaire.[75]

Plus récemment, Verdeguer et coll. ont pu identifier trois nouveaux gènes cibles d'HNF-1β impliqués dans la polarisation cellulaire et/ou la kystogenèse : *Bicc1*, *Crb3*, *Tcfap2b*.

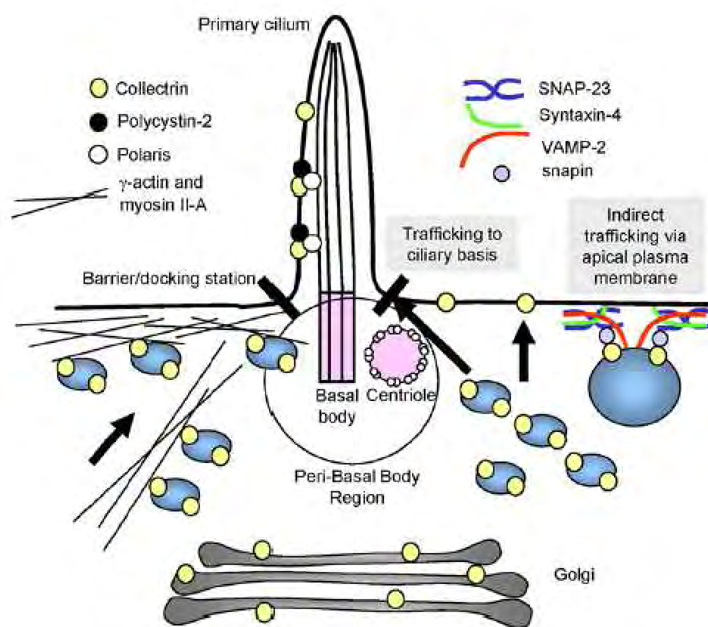

Figure 14 : Modèle de transport des protéines ciliaires intactes au niveau des cellules tubulaires rénales. Rôle de la collectrine (d'après Zhang et coll.[72]).

En résumé, chez la souris, HNF-1β semble contrôler l'élaboration et le maintien de la structure du cil primaire à deux niveaux :

1) <u>régulation transcriptionnelle</u> de certains gènes ciliaires (*Pkhd1, Pkd2*).

2) <u>régulation post-traductionnelle</u> : régulation de l'homéostasie de certaines protéines de la membrane du cil primaire, dont la polycystine-2, par le biais du contrôle de l'expression du gène *Collectrin*.

Cette polarisation induite par HNF-1β au niveau cellulaire présente un pendant architectural : la polarité planaire. Pontoglio et coll.[66] ont récemment montré, à l'aide d'une analyse architecturale en trois dimensions du rein de souris présentant une inactivation conditionnelle rénale d'*Hnf1b*, que l'orientation de la division cellulaire au cours de la mitose des cellules épithéliales tubulaires rénale est finement régulée et conditionne le maintien d'un diamètre tubulaire constant. Les souris n'exprimant pas *Hnf1b* dans le rein présentent des dilatations kystiques tubulaires et un épithélium pluri-stratifié secondaires à une prolifération cellulaire anarchique[4].

<u>Rôle d'*Hnf1b* à l'âge adulte</u>

Chez la souris, *Hnf1b* continue d'être exprimé après la période embryonnaire[50]. Boutet et coll. ont montré que le promoteur d'*Hnf1b* est un site de liaison au facteur de transcription *Snail1*[76]. Ce facteur est un puissant inducteur de la transition épithélio-mésenchymateuse. A l'état basal, chez la souris adulte, son expression est nulle. Lorsque les auteurs induisent son expression, ils observent en 48 heures une perte des caractéristiques épithéliales des cellules tubulaires (perte d'expression de la KSP-cadherin) et l'apparition de marqueurs mésenchymateux (expression de la vimentine) puis l'évolution vers la fibrose. La dédifférenciation cellulaire est concomitante de la répression par Snail1 d'*Hnf1b* et, de fait, de la perte d'expression de la KSP-cadherin (qui est sous la dépendance directe d'HNF-1β).

Plus récemment, l'implication d'HNF-1β dans la régulation de l'homéostasie du magnésium après la période anténatale a bien été identifiée par l'analyse du phénotype des patients porteurs d'une mutation d'*HNF1B* (cf. infra).[77] Par une approche d'immunoprécipitation de chromatine, deux équipes ont pu mettre en évidence un site de liaison d'Hnf1b dans le premier intron du gène *Fxyd2* qui code pour la sous unité gamma de la Na^+-K^+-ATPase.[77] Les mutations de *FXYD2* sont associées chez l'homme à un tableau d'hypomagnésémie (pertes rénales de magnésium) et d'hypocalciurie. Le rôle d'HNF-1β serait alors de réguler l'expression des différents isoformes de *FXYD2*, suggérant également qu'HNF-1β puisse participer à la segmentation du tubule rénal à l'âge adulte et à différentes fonctions d'homéostasie.

Enfin, le recours à un modèle murin d'invalidation conditionnelle d'*Hnf1b* (sous la dépendance d'un promoteur sensible à l'interferon) et limitée au rein a permis d'identifier une nouvelle caractéristique de ce facteur de transcription[78]. Celui-ci a la capacité de rester fixer à la chromatine au cours des mitoses permettant ainsi le maintien de l'expression de certains gènes cibles indispensables au maintien de la polarisation planaire des cellules épithéliales rénales. Ainsi, on distingue deux types de gènes cibles : ceux dont l'expression nécessite la présence d'HNF-1β quel que soit l'état d'activation de la cellule (par exemple *Umod*) et ceux pour lesquels l'expression normale d'HNF-1β n'est requise qu'au cours des états d'activation (prolifération cellulaire ; par exemple *Pkhd1*).

b. **Apports des lignées cellulaires dans l'identification d'autres gènes cibles d'*HNF-1ß***

Des données préliminaires obtenues par oligonucleotides microarrays sur des lignées de cellules rénales embryonnaires (HEK293) ont permis de caractériser 25 gènes régulés par HNF-1β et 9 gènes régulés par HNF-1α[79]. Certains de ces gènes ont un intérêt tout particulier en physiopathologie rénale :

1) *CD24* code pour une protéine de surface cellulaire récemment identifiée comme un marqueur des cellules progénitrices rénales dans le mésenchyme métanéphrique non induit[80].
2) *DPP4* code pour la DiPeptidyl-Peptididase IV, enzyme impliquée dans la régulation de l'activité biologique de différentes hormones comme le glucagon-like peptide 1 qui joue un rôle dans l'homéostasie du glucose. *DPP4* est fortement exprimé dans la bordure en brosse du tube proximal[81].
3) *ACE2* code pour l'enzyme de conversion de l'angiotensine I de type 2, qui est également exprimé dans les bordures en brosse du tube proximal[82]. Une altération de l'expression d'*ACE2* a été observée dans différentes maladies rénales chroniques mais son implication dans l'évolution de ces néphropathies n'est pas établie[83].
4) *Osteopontin* code pour une protéine sécrétée au niveau de la branche ascendante large de Henlé. Une immunisation vis-à-vis de l'ostéopontine prévient la tubulogenèse normale chez le rat. Cette protéine serait affiliée aux intégrines[83]. Ce gène est également régulé par *Hnf1a* dans les cellules HEK293.

En conclusion, HNF-1β est un facteur de transcription dont on mesure le rôle fondamental mais complexe au cours du développement murin embryonnaire précoce (spécification de l'endoderme viscéral et du mésoderme intermédiaire) ou tardif (spécialisation et croissance du rein définitif, du foie, du pancréas, du tube digestif et du tube neural), puis ultérieurement dans le maintien de l'architecture de différents épithéliums polarisés (reins, appareil urogénital, poumon, foie, pancréas). Il régule chez l'animal un grand nombre de gènes dont la liste n'est pas close (voir Tableau 1).

c. Expression d'*HNF1B* dans l'espèce humaine

Les données sont parcellaires et limitées. Au cours du développement rénal, une seule étude s'est attachée à analyser l'expression d'*HNF1B*.[84] Par RT-PCR et hybridation in situ de l'ARNm d'*HNF1B*, sur du tissu rénal de fœtus âgé de 53, 63, 84 et 91 jours de gestation, ces auteurs ont pu montrer que :

1) *HNF1B* est exprimé avec l'intensité la plus forte dans les cellules épithéliales des canaux collecteurs médullaires et corticaux (dérivés du canal urétéral). Un signal de faible intensité est détecté dans les glomérules immatures, les néphrons primitifs et le reste du mésenchyme

cortical (Figure 15a et b). L'expression glomérulaire décrite à l'aide de cette méthode reste d'interprétation délicate, aucune autre étude n'étant venue corroborer ces résultats.

2) *HNF1B* est également exprimé dans les cellules épithéliales polarisées de différents tissus (poumons, foie, pancréas et estomac).

Récemment, nous avons partiellement confirmé les données obtenues par l'équipe de Marco Pontoglio chez la souris invalidées tardivement pour *Hnf1b* en étudiant l'expression d'*HNF1B* et de certains de ces gènes cibles dans le sédiment urinaire de patients porteurs d'une mutation d'*HNF1B*[85] : l'expression relative de *PKHD1*, *COLLECTRINE*, *PKD2* n'est pas différente de celle observée chez les apparentés au premier degré indemne de mutation (Annexe, article 7).

Chez l'homme adulte, comme chez la souris, *HNF1B* est exprimé dans le rein normal[76,86]. En situation pathologique, on observe également qu'*HNF1B* est réprimé dans les zones de fibrose où *SNAIL1* s'exprime[76]. Ces différentes données suggèrent qu'*HNF1B* est également nécessaire au maintien de la structure épithéliale à l'âge adulte chez l'homme.

Organe	Gène	Référence
Rein	*Pkhd1*	Gresh et al EMBO 2004
	Pkd2	
	Polaris	
	Umod	
	Aqp2	
	Avpr2	
	Nphp1	
	Lim1	Lokman et al. Development 2010
	Pax2	
	Wnt9b	
	Collectrin	Zhang et al, PLoS 2007
	Osteopontin	Senkel et al, Bioch Bioph Acta 2005
	DPP4	
	CD24	
	ACE2	
	Urat1	Kikuchi et al, Mol Pharmacol 2007
	KSP-Cadherin	Bai et al, AJP_Renal Physiol 2002
	Socs3	Ma et al, PNAS 2007
	Bicc1	Verdeguer et al, Nature Med 2010
	Cbr3	
	Tcfap2b	
	Clcn5	Tanaka et al, AJP_Renal Physiol 2010
	Fxyd2	Adalat et al. JASN 2009
Foie	*Hnf1a*	Tanaka et al, Nucleic Acids Res 2004
	Hnf3g	
	Hnf6	
Tube digestif	*Claudin15*	Bagnat et al, Nat Cell Biol 2007
	Jagged1	D'Angelo et al. Development 2010
	Atoh1	
	Slc26a3	
Tube neural antérieur	*Mafb*	Pouilhe et al, Dev Biology 2007
Pancréas	*Sox9*	Lynn et al, PNAS 2007
	Hnf4a	Wang et al, Endocrinology 2004
	Hnf6	Poll et al, Diabetes 2006
	Ipf1	Gerrish et al, JBC 2001
	Shh	Sun et al, Genes Dev 2001
	Ihh	

Tableau 1: Gènes directement régulés par HNF-1β chez la souris et/ou dans des lignés cellulaires.

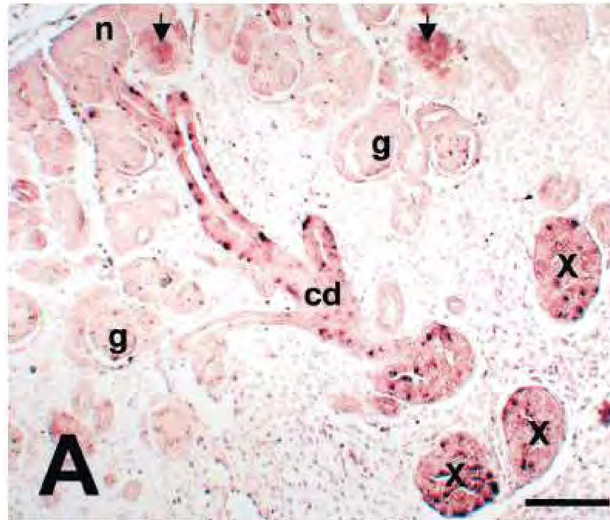

Figure 15a: expression d'*HNF1B* (en pourpre) au cours du développement rénal chez l'homme (d'après Kolatsi et al [84]). cd canal collecteur, g glomérule primitif, n néphron primitif (Hybridation in situ ARNm *HNF1B*).

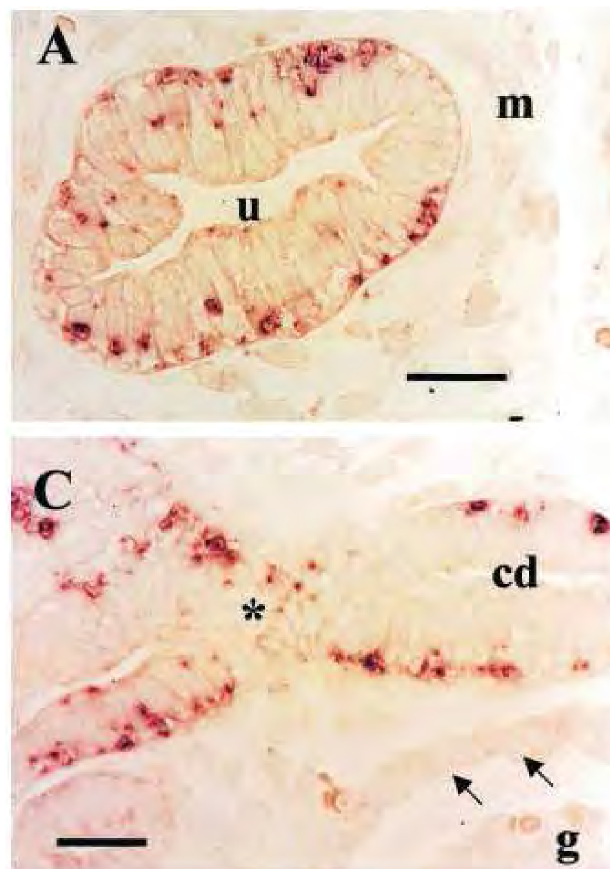

Figure 15b: Expression d'*HNF1B* dans les cellules épithéliales du canal urétéral et des canaux collecteurs (d'après Kolatsi et al.[84]). m mésenchyme ; u canal urétéral ; cd canal collecteur (Hybridation in situ ARNm *HNF1B*).

B) Phénotypes animaux et humains associés aux mutations des gènes *Hnf1b/HNF1B*

1 - Phénotype chez l'animal

Phénotype rénal

- *Souris avec inactivation rénale spécifique de Hnf1b*[4]

Il s'agit d'un modèle d'inactivation bi-allélique. La majorité des souris meurt entre le 10 et le 21ème jour de vie post-natale d'insuffisance rénale terminale. Sur le plan morphologique, les animaux présentent une dilatation urétérale et calicielle non obstructive. Dans le parenchyme rénal, on note une dilatation des tubules médullaires et la présence de kystes à prédominance médullaire (Figure 16).

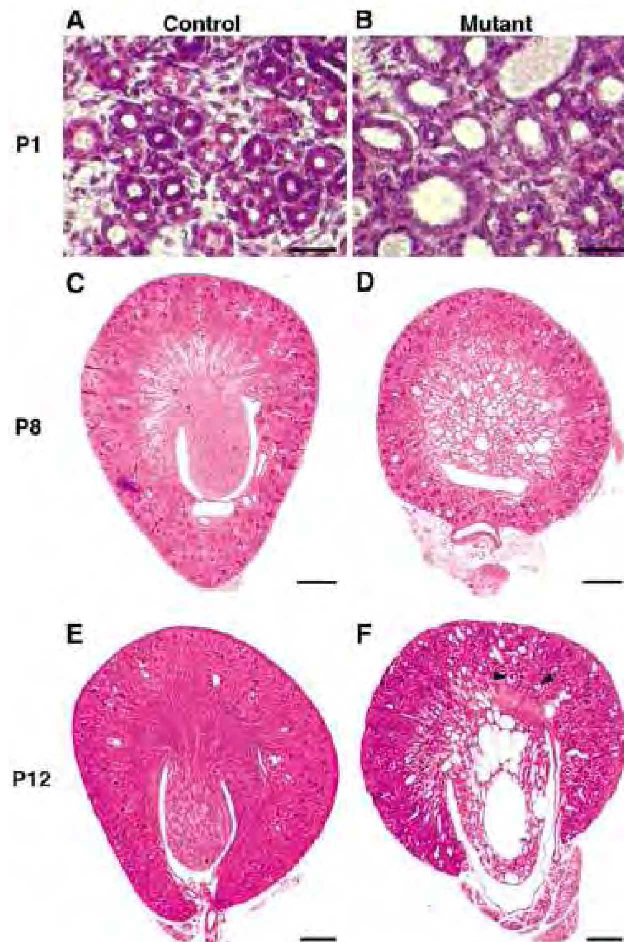

Figure 16 : Coupe histologique de reins de souris contrôle vs. souris avec inactivation spécifique rénale d'*Hnf1b* (d'après Gresh et coll.[4])

Tardivement, la chambre urinaire glomérulaire peut apparaître élargie donnant un aspect glomérulokystique. Enfin, il existe une augmentation de la prolifération cellulaire au niveau de l'épithélium tubulaire dont l'aspect est pluristratifié.

Des constatations histologiques similaires ont été colligées dans un modèle de souris transgénique présentant une délétion de la partie C-terminale d'HNF-1β[6].

- *Xénope*

La technique de microinjection d'ARNm d'*Hnf1b* muté dans des blastomères de xénope au stade 2-cellules a été utilisée par deux équipes différentes[19,87]. Bohn et coll. ont montré que les délétions emportant le site de liaison à l'ADN se compliquent dans ce modèle d'un élargissement du pronéphros. En revanche, si ce site est présent dans la protéine mutée on observe une réduction de la taille du pronéphros[87]. Wu et coll. ont précisé ces données en concluant que trois domaines doivent être présents dans la protéine mutée pour obtenir un phénotype de réduction néphronique : le domaine de dimérisation, l'homéodomaine et la séquence de 26 acides aminés spécifique de l'isoforme B d'HNF-1β[19]. Ils ont également montré qu'*Hnf1b* présente, notamment, une activité antagoniste de celle de *Pax8* et *Lim1*, deux gènes exprimés précocement dans le développement du pronéphros[19].

- *Poisson-zèbre*

Sun et coll. ont étudié le rôle d'HNF-1β dans le développement du zebrafish en étudiant des poissons modifiés par mutagenèse dirigée[26]. Des malformations du pronéphros sont mises en évidence dès les premiers stades de leur développement : absence de communication des tubules puis kystes d'origine tubulaire. Ces anomalies sont corrélées à une perte d'expression de *Pax2*.

Phénotype pancréatique

Chez la souris, l'invalidation pancréatique conditionnelle d'*Hnf1b* (stade précoce) aboutit à une agénésie pancréatique[5].

En utilisant un modèle murin d'invalidation pancréatique (îlots β-pancréatiques) conditionnelle d'*Hnf1b* (technique *Cre/Lox*), Wang et coll. ont pu étudier le rôle d'HNF-1β dans l'homéostasie du glucose[88] et ont montré les faits suivants :

1) il existe un abaissement de la sécrétion d'insuline provoquée par l'apport de glucose, responsable d'une intolérance au glucose ;
2) la sécrétion d'insuline induite par d'autre sécrétagogues comme l'arginine est maintenue.

Atteinte digestive

Récemment, le rôle complémentaire voire redondant des facteurs de transcription HNF-1α et HNF-1β a été mis en évidence dans le tube digestif murin en développement.[34] Seules les souris avec une double invalidation *Hnf1a*$^{-/-}$ *Hnf1b*$^{-/-}$ présentent des troubles létaux du développement digestif.. Ils régulent tout deux la voie de signalisation *Notch* ainsi que le canal chlore *Slc26a3*. HNF-1β régule donc la différenciation de l'épithélium digestif mais également certaines de ses fonctions à l'âge adulte.

Chez le poisson-zèbre présentant une mutation d'*Hnf1b*, on note également une diminution de l'expression du gène *claudin15* au niveau du tube digestif[89]. Claudin15 est une protéine mise en évidence dans le rein, le tube neural antérieur et l'intestin. Sa fonction reste imparfaitement connue.

Elle semble être un pore impliqué dans le transport paracellulaire d'ions ou de fluide. Les auteurs font l'hypothèse que l'accumulation de fluide induite par l'expression de ce pore permet de créer la force nécessaire à la coalescence des lumières tubulaires (Figure 17). Les poissons mutés sont dans l'incapacité de former un tube digestif uni-luminal. Les poissons invalidés pour *Claudin15* ne présentent pas d'anomalie du pronéphros ou du tube neural antérieur, suggérant l'existence d'une redondance moléculaire.

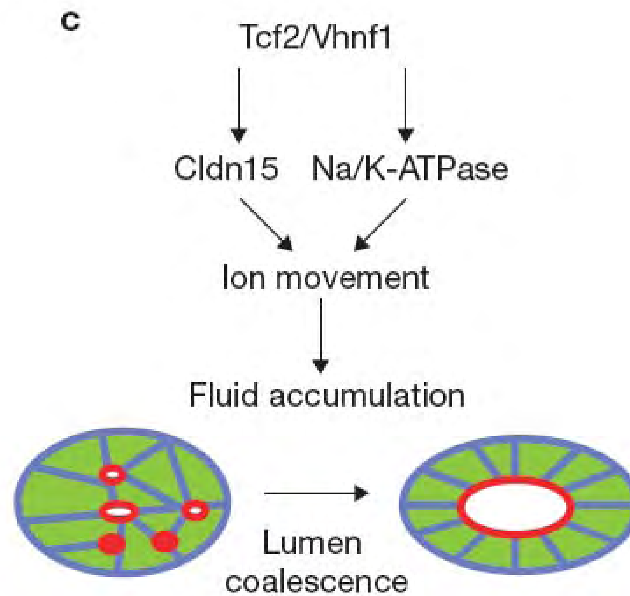

Figure 17 : Rôle d'Hnf-1β (Tcf2/Vhnf1) dans la formation du tube digestif (d'après Bagnat et al [89]). *Cldn15, claudin15.*

Atteinte hépatique

A l'aide d'expériences élégantes utilisant des embryons *Hnf1b*$^{-/-}$ tétraploïdes, Lokmane et coll. ont pu montrer le rôle prépondérant d'HNF-1β dans la différenciation précoce de l'endoderme viscéral permettant à la fois la formation du canal hépatique et la spécification intestinale.[90]

A nouveau, c'est le recours à une invalidation spécifique d'organe (hépatocytes et cellules épithéliales des voies biliaires) qui a permis de bien caractériser chez la souris le rôle d'HNF-1β dans le développement plus tardif du foie.[3] Ces souris développent un ictère en rapport avec une anomalie de la vésicule biliaire et une diminution du nombre des voies biliaires intra-hépatiques. De plus, ces souris sont caractérisées par un déficit en artères hépatiques inter-lobaires, confirmant le lien fonctionnel entre les cellules épithéliales et endothéliales au cours du développement hépatique.

2. Phénotypes liés aux mutations d'*HNF1B* chez l'homme

Les mutations d'*HNF1B* ont initialement été mises en évidence chez des patients japonais présentant un diabète précoce autosomique dominant de type MODY5 (Maturity Onset Diabetes of the Young type 5)[91]. Secondairement, il est apparu que les manifestations rénales sont prépondérantes et contribuent largement au pronostic de la maladie.[1,2,5,92] La majorité des organes où *HNF1B* est exprimé est l'objet d'anomalies morphologiques ou fonctionnelles.

a. Manifestations rénales

La première description du phénotype rénal a été faite par Loirat et coll. en 1982[93], le diagnostic moléculaire ayant été établi *a posteriori*. Les deux patientes (mère-fille) décrites présentaient des reins hypoplasiques et glomérulokystiques avec une absence de papilles rénales. Ces anomalies structurales étaient accompagnées d'une insuffisance rénale chronique précoce mais peu évolutive. Par la suite de nombreux cas isolés ont été rapportés permettant d'élargir le phénotype rénal des mutations d'*HNF1B* mais les données restent éparses.

Trois situations peuvent être individualisées : la période anténatale, l'âge pédiatrique et l'âge adulte.

- *Période anténatale*

Dans une cohorte de 62 fœtus présentant des reins hyperéchogènes[7], l'équipe de néphrologie pédiatrique de l'Hôpital des Enfants du CHU de Toulouse a rétrospectivement analysé le gène *HNF1B* : 18 patients (29%) présentaient une mutation. Deux phénotypes échographiques ont pu être mis en évidence : 1) deux reins hyperéchogènes de taille normale avec ou sans microkystes corticaux ou 2) un rein hyperéchogène de taille normale avec un rein controlatéral de taille augmentée avec des kystes volumineux. Aucun fœtus ne présentait d'oligohydramnios, de dédifférenciation corticomédullaire ou de néphromégalie supérieure à [+3 DS]. En période post-natale on note une atrophie rénale progressive chez 16 enfants et une augmentation progressive du nombre et de la taille des kystes.

Plus récemment, l'analyse rétrospective des données anténatales de 56 individus avec une mutation d'*HNF1B* a confirmé la forte prévalence des reins hyperéchogènes de taille normale ou légèrement augmentée (n=38) (Annexe, article 3). Les autres anomalies associaient une dysplasie multikystique uni ou bilatérale (n=8 et 2, respectivement), des macrokystes (n=3), une hypoplasie unilatérale (n=1) ou une dilatation des cavités pyélocalicielles (n=1).[94]

- *Age pédiatrique*

L'étude d'une cohorte de 80 enfants, âgés de 0 à 14 ans, présentant une anomalie rénale sur la première échographie post-natale (kystes rénaux, hypoplasie ou agénésie rénale ou dysplasie kystique) a permis d'estimer la prévalence des mutations ponctuelles d'*HNF1B* à 31% dans ces situations (25

enfants)[8]. La majorité des enfants mutés présentaient des kystes rénaux (21/25, 84%), avec une atteinte bilatérale chez 16 enfants. Les hypoplasies rénales bilatérales isolées sont rares : seuls 25% de ces patients ont une mutation d'*HNF1B*. Enfin aucun enfant muté ne présentait d'agénésie rénale unilatérale. La fonction rénale était indépendante de la présence d'une mutation d'*HNF1B* (81.7±6.97 vs. 80.7±5.69ml/min) avec un taux d'insuffisance rénale (définie par un DFG inférieur à 80ml/min/1,73m²) de 44 et 40%, respectivement (p non significatif). La dégradation de la fonction rénale semble davantage liée à l'intensité de la réduction néphronique initiale qu'à la sévérité de l'atteinte kystique.

Ces données ont été confirmé par les résultats de l'étude ESCAPE[95], dans laquelle 99 enfants présentant une hypo/dysplasie rénale ont été testés pour différents gènes du développement rénal : *HNF1B, PAX2* (syndrome Rein-Colobome), *SALL1* (syndrome de Townes-Brocks, OMIM 107480), *SIX1* et *EYA1* (syndrome branchio-oto-rénal, OMIM 113650). Une mutation d'*HNF1B* a été mise en évidence chez 8 enfants (8%) dont 6 étaient porteurs de kystes rénaux. L'atteinte était bilatérale chez les 8 patients. On notait chez ces enfants deux cas de reflux vésico-urétéral, un rein en fer à cheval et une agénésie rénale. Dans cette étude, les données concernant le débit de filtration glomérulaire ne sont pas disponibles.

Plus récemment, une hypomagnésémie a été identifiée chez 8/18 (48%) enfants britanniques avec une mutation d'*HNF1B* contre 1/48 (2%) chez les patients indemnes de mutation pour ce gène.[77] L'association fréquente à une hypocalciurie a permis de soulever l'hypothèse d'une régulation directe par HNF-1β du gène *FXYD2*, dont les mutations sont associées à un tableau d'hypomagnésémie-hypocalciurie.

Dans un nombre apparemment très restreint d'observations, le phénotype rénal peut être extrêmement sévère : dans la littérature on note deux observations d'insuffisance rénale néonatale évoluant précocement vers l'insuffisance rénale terminale (4 mois et 3 ans) et nécessitant la réalisation d'une transplantation rénale[96,97].

Les données prospectives concernant l'évolution de la fonction rénale chez les enfants mutés pour *HNF1B* sont rares et ne permettent pas de définir la pente moyenne de décroissance annuelle du débit de filtration glomérulaire.

- *Anatomopathologie*

Chez un enfant porteur d'une mutation d'*HNF1B* avec reins hyperéchogènes en période anténatale persistant en post-natal et avec hypoplasie rénale bilatérale, une histologie rénale est disponible : on note un aspect glomérulokystique (Figure 18)[98].

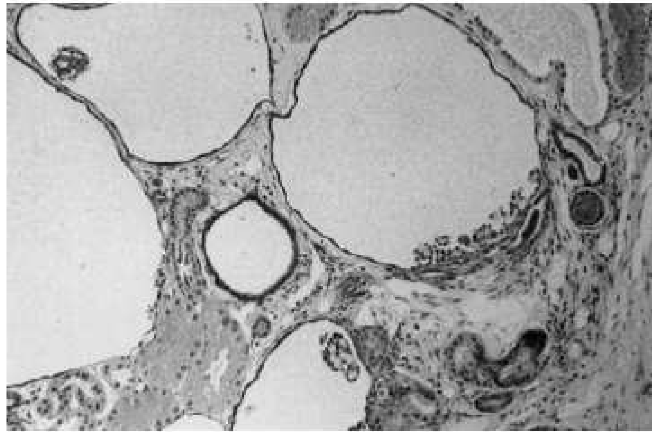

Figure 18 : Biopsie rénale d'un enfant muté pour *HNF1B* avec aspect de kyste glomérulaire cortical (d'après Mache et coll.[98])

Par ailleurs, en 1999, Lindner et coll ont décrit une famille présentant un MODY à transmission autosomique dominante en rapport avec une délétion de 75 paires de base dans l'exon 2 d'*HNF1B* (R137-K161del)[99]. Dans cette famille, une description histologique est disponible chez un adolescent de 14 ans dont les principales caractéristiques sont les suivantes : dilatation tubulaire proximale, glomérulomégalie, effacement segmentaire des pieds des podocytes et amincissement modéré des membranes basales glomérulaires (Figure 19).

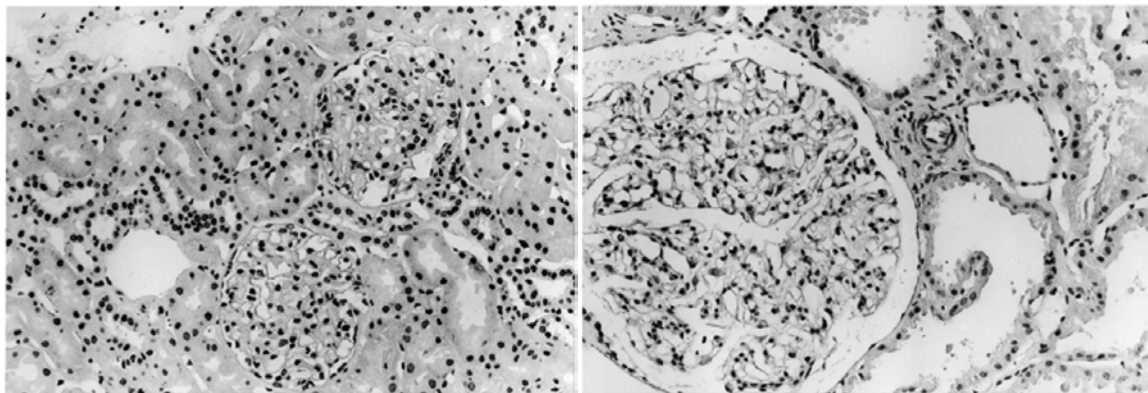

Figure 19 : Histologie rénale d'un patient présentant une mutation d'*HNF1B* (R137-K161del), d'après Lindner et coll[99].

- *Age adulte*

Comme nous l'avons dit précédemment, le premier adulte rapporté avec mutation d'*HNF1B* présentait une insuffisance rénale chronique compliquant une maladie glomérulokystique[93].

En 2004, l'analyse de 8 patients, âgés de 13 à 38 ans, par Bellanné et coll. en 2004 a permis de préciser une part du phénotype rénal à l'âge adulte[1]. L'âge de découverte des anomalies rénales est de 18 à 41 ans. Tous les patients présentaient une hypoplasie rénale (taille 85–105mm) accompagnée de

kystes cortico-médullaires (atteinte pauci-kystique) et d'anomalies pyélo-calicielles de type fond de calice aplati ou dilatation pyélique sans cause d'obstruction (Figures 20 et 22). La fonction rénale est altérée (DFG 29–77ml/min) avec une pente de décroissance du DFG de 0 à 2 ml/min/an. Un examen histologique a été effectué chez 6 patients : on note une fibrose interstitielle chez la totalité des patients, des glomérules de grande taille chez quatre d'entre eux avec un aspect glomérulokystiques chez deux et un aspect d'oligoméganéphronie chez un autre. Une atteinte rénale a été mise en évidence chez un apparenté à l'âge de 4 ans. Elle associait une agénésie rénale droite avec une dilatation modérée pyélique gauche.

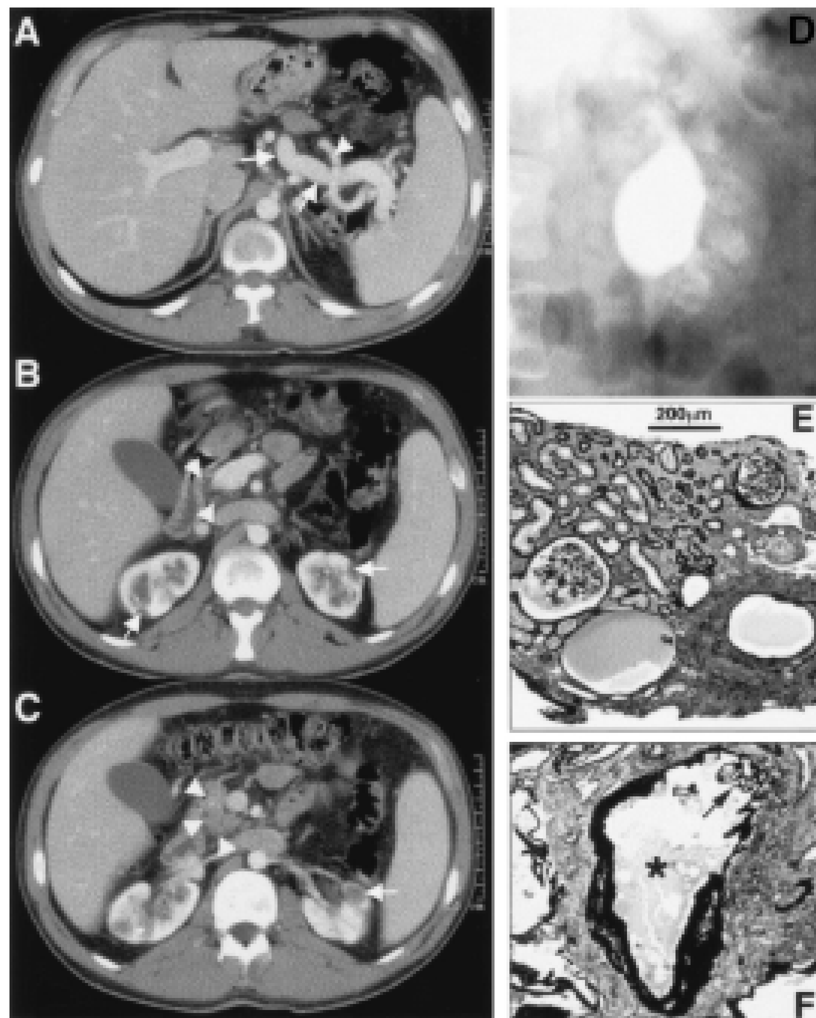

Figure 20 : Patient adulte avec une mutation d'*HNF1B.* A, B, C scanner abdominal montrant une atteinte rénale paucikystique. D UIV chez ce même patient avec aspect de papille en matraque et dilatation modérée du pyélon. E, F histologie rénale montrant l'aspect glomérulokystique (étoile) associé à une dilatation des canaux collecteurs (d'après Bellanné et al[1]).

En parallèle, Bingham et coll. ont confirmé le rôle d'*HNF1B* dans certaines maladies glomérulokystiques non syndromiques[100]. Les anomalies pyélo-calicielles sont similaires. Cette même équipe a étudié la prévalence des mutations d'*HNF1B* dans une population de 160 patients avec néphropathie indéterminée[9,101]. Les patients étaient classés en cinq catégories : 1) maladie rénale glomérulokystique, 2) néphropathie hyperuricémique juvénile familiale atypique (FHJN), 3) syndrome diabète et kystes rénaux (RCAD syndrome), 4) dysplasie rénale et 5) malformations rénales diverses. Une mutation d'*HNF1B* a été mise en évidence chez 38 patients (24%), dont 23 présentant une dysplasie kystique ou des kystes rénaux (61%), huit patients avec une maladie glomérulokystique (21%), trois avec une agénésie rénale (8%), deux avec FHJN atypique (5%) et un avec dysplasie rénale (2%). Malheureusement, la description phénotypique clinique, radiologique et biologique est très pauvre dans ce travail et ne donne pas une perspective synthétique pratique.

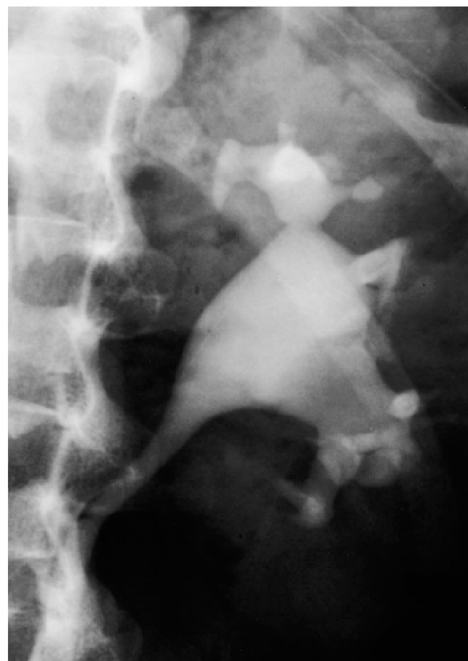

Figure 21 : UIV chez un patient de 30 ans avec un rein unique gauche présentant une dilatation modérée du pyélon et un aspect en boule des calices (d'après Bingham et al[2]).

Le spectre de la maladie rénale liée à *HNF1B* est déroutant. Chez deux sœurs jumelles, nous avons décrit pour la première fois une atteinte rénale atypique ressemblant à une polykystose rénale autosomique dominante évoluant vers l'insuffisance rénale terminale dans la 3ème décennie (Annexe, article 2).[102] Chez ces deux jeunes patientes, c'est l'absence d'histoire familiale de PKRAD, l'apparition d'un diabète et la détection d'une hypomagnésémie qui ont orienté le diagnostic vers une mutation d'*HNF1B*.

Il n'est pas aisé de comprendre l'impact d'HNF-1β sur le métabolisme de l'acide urique. Chez la souris, HNF-1β contrôle l'expression d'*Umod* (cf supra), orthologue murin du gène muté dans une variété de néphropathie familiale hyperuricémique juvénile (FHJN) qui est caractérisée par la présence de kystes de la médullaire rénale (MCKD)[103]. Chez l'homme, nous ignorons si HNF-1β contrôle également *UMOD*. Cette possibilité est suggérée par la constatation d'une hyperuricémie avec diminution de la fraction excrétée de l'acide urique chez environ 20% des patients britanniques mutés pour *HNF1B*[103]. A l'extrême, un phénotype rénal simulant une mutation d'*UMOD* peut être observé. Pour autant, la souris invalidée pour *Umod* ne présente pas de tableau de néphropathie kystique mais un trouble de concentration des urines et une propension à développer une infection urinaire à E.Coli.[104] D'autre part, HNF-1β régule positivement l'expression du transporteur d'acide urique *Urat1*.[105] On s'attendrait donc dans cette dernière hypothèse à une fuite rénale d'acide urique. Finalement, le mécanisme moléculaire précis à l'origine de l'hyperuricémie et des crises de goutte précoce chez les patients avec mutation d'*HNF1B* reste inconnus.

A l'âge adulte, l'évolution vers l'insuffisance rénale terminale est loin d'être constante. Dans une revue parue en 2004, Bingham et coll. rapportent une incidence de 15% d'insuffisance rénale chronique terminale (IRCT) traitée par épuration extra-rénale ou transplantation rénale dans une cohorte de 22 patients mutés pour *HNF1B*[2]. Dans une cohorte parisienne, la doyenne des patients a une clairance de la créatininémie à 66 ml/min à l'âge de 77 ans[1].

Deux remarques supplémentaires sur les conséquences rénales de la maladie liée à HNF-1β : 1) de façon intrigante, malgré l'existence d'un diabète de type MODY, l'histologie rénale ne met jamais en évidence de glomérulopathie diabétique et 2) la survenue d'un cancer chromophobe du rein a été documentée chez deux patientes mutées pour *HNF1B*[86]. Le risque oncogénique des patients *HNF1B* est discuté plus bas.

Nous avons personnellement précisé le phénotype de 27 patients porteurs d'une mutation d'*HNF1B* et suivi en Néphrologie adulte (Annexe, article 1). Ceux-ci étaient suivis dans les CHU de Toulouse, de Clermont-Ferrand, à l'hôpital Tenon (AP-HP, Paris) et à la clinique universitaire Saint Luc (Bruxelles, Belgique), et ont été caractérisés sur le plan génotypique et phénotypique.[92] Chez 14 individus, la première anomalie identifiée était rénale (61 %) et ceci en période anténatale (n=6), dans l'enfance (n=4) ou à l'âge adulte (n=4). Les anomalies rénales à l'âge adulte se répartissaient grossièrement en quatre catégories (1) reins normaux (pas de kystes et taille normale) (n=3, 12%), (2) rein unique (n=5, 25%), (3) kystes rénaux (n=15/24, 62%) le plus souvent en faible nombre et (4) anomalies urologiques : néphrocalcinose, lithiase rénale, reflux vésico-urétéral, hydronéphrose.

Dans cette cohorte, le sédiment urinaire était normal. La protéinurie était nulle (n=20) ou inférieure au gramme par litre (n=7). Une hypomagnésémie (Mg^{2+} < 0.75 mmol/l) et une hypokaliémie (K^{+} < 3.5 mmol/l) étaient identifiées chez 62 et 46% des individus testés. Dans ces deux situations, la perte urinaire de magnésium ou de potassium était inadaptée indiquant une fuite tubulaire, et l'apport

oral en potassium et en magnésium ne permettait pas de normaliser les taux sanguins. Le mécanisme de l'hypokaliémie est incertain. L'anomalie biologique n'ayant pas été repérée chez les enfants, la contribution de l'hypomagnésémie chronique ne peut être écartée, mais certains patients ont une hypokaliémie sans hypomagnésémie de sorte qu'une explication moléculaire plus subtile n'est pas écartée. La fréquence de ces anomalies ioniques était similaires quelque soit le statut génotypique (délétion ou mutation ponctuelle). Curieusement, deux patients présentaient un tableau de tubulopathie proximale (protéinurie de bas poids moléculaire, aminoacidurie, glycosurie, phosphaturie, hypokaliémie et acidose tubulaire incomplète). Ces deux patients étaient indemnes d'une mutation concomitante d'*HNF1A* que l'on sait être responsable chez la souris d'une dysfonction tubulaire proximale[106], suggérant que les dimères HNF-1α-HNF-1β sont fonctionnellement défaillants.

Les différentes anomalies rénales (morphologiques, fonctionnelles et histologiques) liées aux mutations d'*HNF1B*, extraordinairement hétérogènes, sont résumées dans le tableau 2. Les fréquences sont indicatives et issues des données de séries incluant au moins cinq patients.

Examen	Anomalies	Fréquence
Echographie / TDM	Hyperéchogénicité rénale anténatale	Très Fréquent
	Kystes corticomédullaires	
	Enfants (kystes nombreux)	75-84 %
	Adultes (kystes peu nombreux)	62-100 %
	Hypoplasie rénale uni ou bilatérale (enfance)	50-70%
	Rein en fer à cheval	<5%
	Rein en position pelvienne	<5%
	Agénésie rénale	0-20%
	Gros reins polykystiques évoquant une polykystose dominante	<5%
	Tumeur solide	Rare
Urographie intra-veineuse	Dilatation pyélique	Indéterminée
	Papille "en matraque"	Indéterminée
	Reflux vesico-urétéral	Indéterminée
Test sanguins / urinaires	Hypomagnésémie (enfants et adultes)	48-62 %
	Hypokaliémie (adultes)	46 %
	Hypocalciurie	Indéterminée
	Tubulopathie proximale type Fanconi	8 %
Histologie	Maladie glomérulo-kystique	Indéterminée
	Kystes d'origine tubulaire	Indéterminée
	Fibrose interstitielle	Indéterminée
	Oligoméganéphronie	Indéterminée

Tableau 2 : Résumé des modifications rénales liées aux mutations d'*HNF1B*.

Il faut souligner ici les analogies mais surtout les différences importantes du phénotype rénal observées entre souris avec invalidation rénale conditionnelle $Hnf1b^{-/-}$ et la maladie humaine à transmission dominante

b. Manifestations pancréatiques

Elles peuvent concerner le pancréas endocrine (diabète) et le pancréas exocrine.

En ce qui concerne la fonction endocrine, la première mutation d'*HNF1B* a été décrite dans une famille dont plusieurs membres présentaient un diabète de type MODY avec une transmission « verticale » c'est-à-dire autosomique dominante[91]. Les diabètes de type MODY (Maturity Onset Diabetes of the Young) présentent un phénotype combinant classiquement [107]:

1) un diabète familial à transmission autosomique dominante ;
2) une hyperglycémie de survenue précoce (classiquement avant 25 ans, mais souvent dès l'enfance ou à l'adolescence) sans insulinodépendance ;
3) enfin, une anomalie primaire de l'insulinosécrétion.

A l'heure actuelle, des mutations à l'origine d'un diabète de type MODY ont été identifiées dans six gènes : *HNF-4α*/MODY1[108], *Glucokinase*/MODY2[109,110], *HNF-1α*/MODY3[111], *PDX1*/MODY4[112], *HNF-1β*/MODY5 et *β2/NeuroD1*/MODY6[113]. Par ailleurs, entre 20 et 45% des MODYs n'ont pas d'explication moléculaire[114].

Des différences significatives du phénotype pancréatique existent entre les modèles murins avec mutation d'*Hnf1b* et la maladie humaine liée à *HNF1B*. Chez l'homme, il existe une variabilité importante de l'âge de début des symptômes, les anomalies morphologiques du pancréas sont fréquentes et l'hyperglycémie est précoce. Ces différences inter-espèces peuvent s'expliquer d'au moins trois façons : une expression faible et retardée du promoteur de la Cre-recombinase dans le modèle Cre/Lox, des fonctions différentes d'HNF-1β entre les deux espèces, ou encore une perturbation fonctionnelle entre cellules β du pancréas et cellules exocrines

.

L'analyse phénotypique chez l'homme est donc cruciale. Une analyse comparative des caractéristiques endocrinologiques des MODY3 (mutations d'*HNF1A*) et 5 est maintenant disponible[115,116]. Les patients avec un MODY5 présentent une réponse au tolbutamide similaire aux patients diabétiques de type 2, le peptide C est abaissé mais reste dosable. La fonction des cellules β des îlots de Langerhans, mesurée par technique HOMA (homeostasis method assessment) reste normale dans cette étude, ce qui tranche avec les études antérieures[116] où elle apparaissait abaissée, confirmant la variabilité inter-individuelle des MODY5. L'hypothèse d'une insulinorésistance est également soulevée du fait de l'association d'une dyslipidémie fréquente (hypertriglycéridémie, HDL-cholestérol bas), d'une cytolyse hépatique et d'une hyperuricémie, triade classique des états d'insulinorésistance.

Les caractéristiques évolutives du diabète MODY5 sont les suivantes :

1) progression lente vers l'insulinorequérance mais doses d'insuline requises faibles (0,26 à 0,45U/kg/j)[99,116] ;
2) sécrétion résiduelle de peptide C ;
3) index de masse corporelle normal ;
4) rareté de la rétinopathie et de la glomérulosclérose diabétique.

La pénétrance du diabète est progressive chez les patients avec mutation d'*HNF1B*. Une intolérance aux hydrates de carbone est présente chez 4% des patients. L'âge de début du diabète est variable : de la période néonatale [97] à l'âge de 61 ans[1], avec un âge moyen de 26 ans[116]. Le diagnostic est le plus souvent fortuit mais une acido-cétose inaugurale n'est pas impossible, éventuellement dès l'enfance.[92,97] Un diabète (sévère) peut apparaître après transplantation rénale.[117] Enfin, la coexistence d'un diabète et d'une insuffisance rénale terminale chez un patient muté pour *HNF1B* est une indication logique de greffe rein-pancréas. Le bien fondé de cette approche a été illustré récemment dans une courte série de huit patients [118]. A Toulouse, trois patients adultes ont bénéficié d'une transplantation rein-pancréas. Deux d'entre eux ont développé une cholestase sévère inexpliquée (Esposito et coll. Abstract ATC 2012). Une telle cholestase post-greffe a déjà été après transplantation rénale isolée [117], suggérant un impact du traitement immunosuppresseur sur les hépatocytes ou les cholangiocytes.

La particularité des atteintes pancréatiques liées à *HNF1B* tient à l'atteinte conjointe du pancréas exocrine : une atrophie diffuse ou partielle du pancréas est constatée chez la majorité des patients adultes. Des calcifications de la tête pancréatique ont également été observées.[92,119] Les conséquences fonctionnelles de cette atrophie sont notées chez 80 à 90% des patients adultes testés qui ont une: diminution de l'élastase fécale. Cette anomalie demeure le plus souvent asymptomatique, mais peut justifier une supplémentation enzymatique.

c. Manifestations génitales

Leur fréquence est incertaine : une malformation génitale est mise en évidence chez 11% (6/55) des patients britanniques[2] mais 65 % d'une courte série de propositus français (5/8)[1]. Chez l'homme, une infertilité par atteinte des résidus du canal de Wolff est observée : agénésie des canaux déférents dans deux observations, kystes bilatéraux de l'épididyme[1], asthénospermie ou hypospadias.[92] La fréquence des anomalies d*HNF1B* dans les infertilités masculines n'est pas connue. Chez la femme, les résidus du canal de Müller sont affectés : utérus bicorne ou didelphe, utérus rudimentaire, hémiutérus avec ovaire unique et agénésie vaginale ou duplicité vaginale[2]. Des mutations d'*HNF1B* ne sont identifiées que chez les patientes avec une atteinte des deux organes (utérus et reins) et pas chez

les patientes avec atteinte génitale isolée.[120] Ces atteintes génitales sont probablement sous-estimées, faute d'évaluation systématique.

d. Manifestations hépatiques

Chez l'adulte, une atteinte hépatique infra-clinique, caractérisée par l'élévation intermittente des enzymes (ALAT et γGT), sans ictère ni insuffisance hépato-cellulaire est décelée chez deux tiers des patients environ. Le parenchyme hépatique est morphologiquement indemne de kystes ou d'anomalie des voies biliaires[1]. Une biopsie du foie a été réalisée chez quatre patients : aucune anomalie histologique hépatique n'était visible sur le parenchyme prélevé[1]. Chez l'enfant, un cas d'ictère néonatal a été rapporté. Le diagnostic initial de syndrome d'Alagille a secondairement été corrigé devant l'apparition d'un diabète de type MODY5[121]. Les anomalies hépatiques après greffe ont été décrites ci-dessus.

e. Risque néoplasique : *HNF1B*, un anti-oncogène ?

L'observation initiale d'un cancer ovarien chez une femme présentant un MODY5[1], ayant secondairement développé un cancer rénal chromophobe a incité a analysé *HNF1A* et *HNF1B* dans 20 cancers ovariens et 35 cancers rénaux (12 cancers chromophobes, 13 cancers à cellules claires et 10 oncocytomes)[119]. Deux patients avec un cancer chromophobe présentaient une mutation bi-allélique d'*HNF1B* dans le tissu tumoral : association d'une mutation germinale (R165H et L16fsX17) et d'une délétion complète du deuxième allèle. Chez une patiente, une récidive est apparue 5 ans après : les anomalies moléculaires étaient identiques. Au final, 15% des patients avec cancer chromophobe (2/12) présentaient dans cette étude un MODY5.

L'association d'une mutation germinale d'*HNF1B* et d'une délétion somatique du deuxième allèle rappelle ce qui est observé dans les adénomes hépatiques et les carcinomes hépatocellulaires en l'absence de cirrhose, dans lesquels HNF1A est inactivé par une séquence en deux temps[122,123]. La question de considérer *HNF1B* comme un gène suppresseur de tumeur est débattue.

Le cancer chromophobe, est une forme rare (10 %) de cancer rénal. L'étude de la prévalence des mutations de l'anti-oncogène *p53*, de *BHD* et d'*HNF1B* dans une série de 92 cancers du rein sporadiques (dont 46 cancers chromophobes) doit faire pondérer le potentiel anti-oncogène de ce gène[124]. En effet, dans cette série, ces trois gènes étaient mutés chez 6, 13 et 0 patients, respectivement (le cancer associé à ces mutations était un cancer chromophobe dans 84% des cas).

Dans le domaine des cancers ovariens, Terasawa et coll. ont montré récemment qu'une inactivation épigénétique d'*HNF1B,* par le biais d'une méthylation d'un domaine riche en CpG dans la région 5', était présente dans plus de 50% des lignées cellulaires issues de cancers ovariens et dans 26% des cancers ovariens eux-même (analyse tissulaire)[125]. Cette méthylation aberrante n'était retrouvée dans aucun tissu ovarien sain. Par RT-PCR quantitative, les auteurs confirmaient la répression de l'expression d'*HNF1B* par la méthylation de sa région 5'. Celle-ci est également

associée à une acétylation faible des histones H3. De façon intéressante, la méthylation d'*HNF1B* était corrélée au type histologique : elle est présente dans 41% des cas de cancers séreux, 25% de cancers mucineux, 25% de cancers indifférenciés mais jamais dans les cancers à cellules claires (p=0.005). Enfin, des données similaires sont observées dans des lignées cellulaires de tumeurs colorectales, gastriques et pancréatiques, suggérant un rôle général de l'inactivation épigénétique d'*HNF1B* dans les mécanismes de tumorigenèse[125].

D'un point de vue moléculaire, deux situations peuvent donc amener à une inhibition complète de *HNF1B* et au développement d'un cancer :

1) inactivation épigénétique par méthylation de l'ADN et dé-acétylation des histones H3 de la région 5' ;
2) mutation germinale puis deuxième mutation somatique.

Inversement, Tsuchiya et coll. ont montré à l'aide d'une technique de microarrays d'oligonucléotides que les carcinomes ovariens à cellules claires présentaient une surexpression d'*HNF1B*[126]. La normalisation de l'expression d'*HNF1B* par méthode de RNA interférence induisait une apoptose des cellules cancéreuses. Le caractère hypométhylé des ilots CpG du gène *HNF1B* dans les cellules de cancers ovariens à cellules claires a été également observé par Kato et coll.[127] confirmant la surexpression d'HNF-1β dans ces cellules.

Enfin, Gudmunsson et coll ont pu montrer en réalisant une étude de l'ensemble des polymorphismes portant sur un nucléotide (genome-wide SNP (single nucleotide polymorphism) association study) chez 1501 patients masculins souffrant d'un cancer de la prostate, qu'un variant d'*HNF1B* (polymorphisme dans l'intron 1 : rs4430796A) est associé de manière significative avec le risque de développer ce type de cancer[128]. Ce constat a secondairement été confirmé par plusieurs études.

AInsi, une dérégulation d'*HNF1B* est possiblement associé à un risque néoplasique. Dans la maladie liée à HNF-1β, à l'opposé de maladies autosomiques dominantes impliquant authentiquement un gène suppresseur de tumeur (maladie de Von Hippel-Lindau par exemple), le petit nombre de patients mutés développant une tumeur par délétion bi-allélique d'*HNF1B* et le caractère non-multiple de ces tumeurs ne permet pas de recommandation ferme de dépistage tumoral (rein ou appareil génital). Il reste à définir si une anomalie d'*HNF1B* est suffisante pour initier un processus de tumorigenèse.

En conclusion, la maladie liée à *HNF1B* dans sa forme complète associe une atteinte rénale, pancréatique, génitale et hépatique. Le pédiatre doit l'évoquer 1) en période anténatal devant des reins hyperéchogènes (souvent de grande taille et avec des kystes corticaux) et 2) après la naissance devant des reins de petite taille avec quelques kystes ou un rein multikystique dysplasique en involution avec rein controlatéral hyperéchogène et non hypertrophié. L'atteinte rénale est souvent isolée dans cette tranche de vie. Pour le néphrologue adulte, la constatation d'une insuffisance rénale chronique lentement progressive avec reins hypoplasiques et kystes corticaux, ou hypokaliémie/hypomagnésémie doit faire rechercher un diabète et une hypoplasie pancréatique, évocateurs d'une mutation d'*HNF1B*. La découverte d'une infertilité ou d'une anomalie morphologique du tractus génital facilite le diagnostic. Les antécédents familiaux peuvent grandement faciliter le diagnostic. La gravité de la maladie varie d'un individu à l'autre, la forme la plus sévère étant une dysplasie multikystique anténatale avec oligohydramnios et hypoplasie pancréatique. La forme la plus bénigne se résumant à une insuffisance rénale modérée dans la 8ème décennie.

C) Mutations du gène *HNF1B* : méthodes d'analyse, caractéristiques moléculaire et corrélation phénotype-génotype

1 – Méthodes d'analyses

Trois méthodes d'analyse ont été développées pour analyser le gène *HNF1B* : 1) le séquençage direct, 2) la QMPSF (Quantitative Multiplex PCR of Short Fragments, Figure I.1), 3) la MLPA (Multiplex Ligation-dependant Probe Amplification). La première permet de détecter les mutations ponctuelles, les deux suivantes les délétions ou duplications exoniques.

Tous les types de mutations ont été rapportés :

- mutation ponctuelle : insertion, délétion ou substitution, anomalie d'épissage[9]
- délétion ou duplication d'un exon (exon 4 ou exon 5)[10,129]
- délétion complète du gène étendue sur 1,2 à 3,4 Mb et emportant plusieurs gènes contigus, dont les fonctions sont inconnues.[10,101]

L'étude de trois cohortes a permis de mettre en évidence une forte contribution des délétions complète d'*HNF1B*, avec une prévalence de 33% chez l'adulte[10] et 64% chez l'enfant[7,8]. En conséquence, la recherche d'une délétion complète par QMPSF est désormais la première étape de l'analyse moléculaire (Figure 22), le séquençage direct étant réalisé par la suite en l'absence de délétion.

Les mutations ponctuelles sont quant à elles dispersées sur l'ensemble des exons, avec une prédominance dans le domaine de liaison à l'ADN.[9,94]

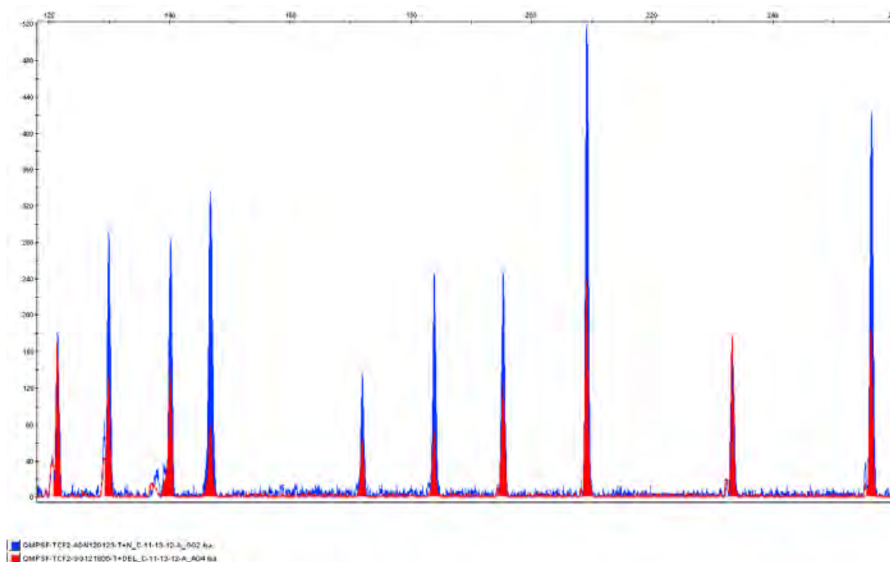

Figure 22: Exemple de résultats obtenus par QMPSF au laboratoire chez un patient avec une délétion complète d'*HNF1B* (panel rouge) ou chez un témoin (panel bleu)

2 – Corrélation génotype - phénotype

Le MODY5 est une maladie à transmission autosomique dominante. La maladie est également caractérisée par une vaste hétérogénéité intra-familiale. La caractérisation des antécédents familiaux (néphrologiques, diabétiques…) revêt donc une importance capitale pour l'identification diagnostique. Cependant l'étude des cohortes pédiatriques a montré une fréquence élevée et concordantes de mutations *de novo* : 9/12 soit 60 % (dont 7 délétions complètes) dans une cohorte pédiatrique française[8] et 7/12 soit 60 % (dont 6 délétions complètes) dans la cohorte anténatale toulousaine[7]. Dans une large cohorte de 377 individus (d'âge plutôt pédiatrique) avec anomalies rénales variées, une mutation d'*HNF1B* a été identifiée chez 75 d'entre eux. Parmi les 39 individus dont l'analyse parentale est disponible, une mutation *de novo* est identifiée chez 20 soit 51 %.[94]

Aucune corrélation phénotype–génotype évidente n'est observée. Les délétions complètes de *HNF1B* pourraient être responsables de formes plus sévères et plus précoce comme le suggère la grande fréquence de cette anomalie chez les patients identifiés en période anténatale[2] mais les données épidémiologiques sont entachées de biais, et l'absence de suivi longitudinal de ces jeunes patients interdit une appréciation correcte.

Récemment, Mefford et coll. ont décrit un microréarrangement chromosomique (microdélétion ou microduplication) récurrent en 17q12, mis en évidence par CGH-array (hybridation comparative génomique par microarrays), emportant le gène *HNF1B*, chez des fœtus présentant une dysplasie rénale multikystique [130]. La microduplication est observée aussi bien chez des patients atteints d'épilepsie et/ou de retard mental, ou indemnes de ces anomalies. La microdélétion en 17q12 est médiée par la duplication des segments flanquants obéissant à un mécanisme de recombinaison homologue non-allélique. Ce mécanisme de réarrangement chromosomique est fréquent, de l'ordre d'une naissance pour mille[130] et permet d'expliquer la fréquence élevée des délétions *de novo* d'*HNF1B* dans les cohortes pédiatriques de maladies rénales et de MODY5. De même, le début précoce de la maladie rénale chez les patients présentant une délétion complète d'*HNF1B* pourrait être expliquée par l'haploinsuffisance combinée d'*HNF1B* et de *LHX1* secondaire à la microdélétion (syndrome de gènes contigus). En effet, *LHX1* est un gène à homéodomaine important dans le développement rénal chez la souris[18]. Dans une famille rouennaise que nous avons récemment étudiée sur le plan moléculaire, les individus vivant avec cette microdélétion 17q12 présentent une hétérogénéité phénotypique : l'analyse par CGH-array a été effectuée chez un enfant atteint d'un retard psycho-moteur sévère, d'une épilepsie et d'une maladie kystique rénale, la délétion a été confirmée dans notre laboratoire par QMPSF chez l'enfant et chez sa mère. Cette dernière ne présente qu'une atteinte rénale paucikystique et un retard mental modéré. Une microdélétion en mosaïque n'est pas exclue dans cette famille.

Enfin, il est probable que des remaniements chromosomiques plus vastes que la simple microdélétion/duplication de la région 17q12 précédemment décrite, emportant *HNF1B*, puissent rendre compte de phénotypes polymalformatifs plus complexes que ceux décrits avec les mutations isolées de ce gène, associant des traits cliniques propres aux mutations d'*HNF1B* et des manifestations qui n'appartiennent pas au spectre habituel (dysmorphie, troubles neurologiques…). Une délétion de 3.4Mb emportant *HNF1B* a par exemple été rapportée[131]. Le phénotype associait un diabète de type MODY, une goutte précoce, une insuffisance rénale progressive terminale à l'âge de 46 ans avec hypoplasie rénale et urétérohydronéphrose bilatérale, une mégavessie, une cryptorchidie bilatérale et une absence complète des muscles de la paroi abdominale imitant un syndrome de Prune-Belly. Enfin, on peut souligner trois cas rapportés de délétion d'*HNF1B* associées à des troubles neurologiques (retard mental ou trait autistique) qui s'accordent bien avec les conséquences de la microdélétion fréquente en 17q12.[132]

3 – Score HNF1B : Qui (ne pas) tester ?

Comme préciser précédemment, l'hétérogénéité phénotypique importante, des anomalies parfois asymptomatiques ou d'apparition tardive, et la fréquence des néo-mutations rendent particulièrement difficile l'identification des patients à haut risque de porter une mutation d'*HNF1B*. Ainsi, le taux de détection des mutations d'*HNF1B* est faible et varie selon les critères d'inclusion et l'âge des individus testés entre 0.6 et 29%.[1,94,133] Outre l'implication économique indéniable, l'évocation d'un risque potentiel de diabète n'est pas sans conséquence. Nous avons donc développé et testé un score composite clinique, biologique et radiologique permettant de préciser la population à tester (manuscrit en préparation ; Annexe, article 4). Ainsi, l'application de ce score à l'ensemble des tests d'*HNF1B* réalisés dans le laboratoire de Génétique médicale du CHU de Toulouse entre le 1 janvier 2007 et le 1 juillet 2012, aurait permis de réduire le nombre de tests de 433 à 277 (soit une économie d'environ 127.000 euros).

4 – Place du conseil génétique

Le phénotype lié aux mutations d'*HNF1B* ségrége sur un mode autosomique dominant : la transmission est dite « verticale », chaque génération est touchée, une transmission père-fils est observable et le risque de récurrence chez les enfants d'un patient porteur de la mutation est de 50%. Lorsqu'une néo-mutation est identifiée chez un enfant, quel est le risque de récurrence dans sa fratrie ? Celui-ci est proche de zéro si les deux parents sont indemnes de mutations dans les lymphocytes périphériques. Néanmoins, une mosaïque germinale a pu être documentée [97] et ne peut pas être formellement écartée : cette possibilité nécessite d'informer les parents du risque non nul de récurrence. La fréquence de ces mosaïques n'est pas connue. Les grossesses suivantes doivent donc

être surveillées de manière rapprochée, en ajoutant éventuellement une échographie supplémentaire entre la 24ème et la 34ème semaine.

La variabilité phénotypique majeure inter et intra-familiale et l'absence de corrélation génotype-phénotype empêchent une prédiction précise de l'évolution de la fonction rénale ou de l'émergence et de la sévérité d'un diabète. La majorité des fœtus présentent un liquide amniotique de volume normal[7]. Des tentatives préliminaires d'élaboration de critères prédictifs, utilisant le dosage de la β2-microglobuline dans le liquide amniotique ou la mesure du volume de liquide amniotique ont échoué[7]. Au regard de la législation française, l'impact usuel de la maladie liée aux mutations d'*HNF1B* ne classe pas cette maladie rénale rare dans le champ des indications d'interruption médicale de grossesse (IMG) pour risque fœtal grave ou anomalie d'organe à risque de déficit neurologique ou rénal létal à brève échéance. A titre exceptionnel, une IMG a été réalisée en Grande-Bretagne en raison d'une présentation rénale anténatale particulièrement péjorative. En France, l'équipe de néphrologie pédiatrique de l'Hôpital des Enfants au CHU Toulouse a également recommandé une IMG chez une jeune femme ayant une mutation d'*HNF1B* dont le fœtus présentait une dysplasie kystique sévère compliquée d'anamnios (données personnelles). Cette recommandation, conforme au souhait des parents a été suivie par le centre de diagnostic anténatal. La problématique est particulièrement délicate et mal résolue dans les familles présentant une microdélétion chromosomique de grande taille de la région 17q12 où l'on connaît le risque d'atteinte malformative et/ou de troubles neurologiques associées .[7,132] Dans ces situations l'impact familial antérieur est pris en considération et l'avis des parents est prépondérant.

Enfin, le risque néoplasique, s'il est confirmé, impose le dépistage des apparentés afin de proposer le dépistage prospectif d'un cancer rénal.

En résumé, la variabilité phénotypique inter et intra-familiale est une composante importante de la maladie liée à *HNF1B,* comme pour de nombreuses maladies à transmission autosomique dominante. L'une des singularité génétique de l'affection concerne la fréquence élevée des néo-mutations (en particulier des délétions qui concernent peut être 50% des patients). En conséquence, l'absence d'histoire familiale ne doit pas faire récuser le diagnostic. En routine, l'analyse moléculaire d'*HNF1B* s'attache en premier lieu à détecter une délétion complète du gène (QMPSF) et si cette étape est négative, à identifier une mutation ponctuelle (séquençage direct). Actuellement, aucune corrélation génotype–phénotype n'a pu être caractérisée et, pour les grossesses à risque, c'est l'échographie anténatale, réalisée dans un centre de niveau 3, qui permet d'identifier les rares situations où une interruption de la grossesse est susceptible d'être évoquée entre les parents et une équipe médicale pluridisciplinaire, en accord avec la législation.

D) Rôle d'HNF-1β en situation pathologique : agression / réparation épithéliale

Le rôle d'HNF-1β au cours des différentes étapes du développement rénal et dans le maintien de la différenciation tubulaire à l'âge adulte nous a incité à explorer le rôle de ce facteur au cours des étapes de réparation de l'épithélium tubulaire suivant une agression aiguë, qu'elle soit ischémique ou septique. En effet, la régénération épithéliale nécessite la ré-expression transitoire de gènes du développement rénal dont certains sont des cibles d'HNF-1β (par exemple *Pax2*, *Wnt4*), la mise en œuvre d'un cycle de dédifférentiation-différentiation (épithélium → mésenchyme → épithélium) avec prolifération des cellules tubulaires viables et la ré-épithélialisation de la membrane basale tubulaire mise à nu selon un axe parallèle au tubule (qui requiert le maintien de la polarisation planaire cellulaire (division mitotique orientée). Au cours du développement rénal normal, HNF-1β régule ces différents évènements (cf. supra) et pourraient donc être également un intervenant majeur de la réparation épithéliale rénale post agression.

Plusieurs données de la littérature corroborent cette hypothèse :

- Les souris dont l'invalidation d'*Hnf1b* spécifique du rein est provoquée à l'âge adulte ne présente pas de symptomatologie rénale évidente sauf si elles sont soumises à une agression rénale aiguë ischémique (clampage temporaire d'une artère rénale).[78] Dans cette situation, le rein lésé présente une évolution kystique sévère dans les 3 semaines suivant le clampage, en rapport avec un trouble de l'orientation de la division mitotique des cellules épithéliales régénérant après l'agression initiale. L'absence d'HNF-1β au cours de la vague de prolifération cellulaire suivant l'ischémie rénale ne permet pas la réexpression immédiate des cystogènes *Pkhd1*, *Pkd2*. Dans cette étude, l'expression et le rôle d'HNF-1β dans les phases précoces de la régénération n'ont pas été étudiés.
- Certains gènes cibles d'HNF-1β (*Socs3*, *Pax2*, *Wnt4*…) sont des acteurs clés de la réparation de l'épithélium rénal après agression.
- Le rôle crucial du facteur de transcription HNF-1α, homologue d'HNF-1β et qui agit en homo ou hétérodimère avec HNF-1β, est bien documenté au cours de la réparation des hépatocytes après agression aiguë.[134]
- La phase de réparation suivant une agression hépatique chimique par tétrachloride CCl_4^- s'accompagne d'une surexpression d'HNF-1β responsable d'une augmentation de la prolifération cellulaire mais également d'une instabilité génomique.[135]

Pour étudier le rôle d'HNF-1β dans les phases précoces de l'agression et de la réparation de l'épithélium rénal, nous avons donc utilisé deux modèles murins : un modèle d'agression rénale aiguë ischémique induite par un choc hémorragique contrôlé (Annexe, article 6) et un modèle d'agression rénale aiguë septique (injection d'endotoxine LPS).

1- Agression rénale aiguë ischémique :

L'agression rénale ischémique a été étudiée à l'aide d'un modèle murin de choc hémorragique contrôlé récemment développé par Nicolas Mayeur dans l'unité INSERM U1048 à Toulouse.[136] Il consiste en une déplétion sanguine transitoire (2 heures) avec un objectif prédéfini de pression artérielle sanglante (cible à 30 mmHg). A l'issue des 2 heures de déplétion, le sang est reperfusé et combiné à une expansion volémique supplémentaire par du soluté salé à 0.9%. Dans ce modèle, l'insuffisance rénale aiguë organique est démontrée par la mesure de la clairance de l'inuline, la mesure de l'expression de la molécule KIM-1 et par l'analyse histologique à J2, J6 et J21 (Figure 23). A J21, la récupération fonctionnelle est intégrale mais on note la présence de quelques coulées fibreuses attestant de la présence de séquelles à distance de l'agression aiguë.

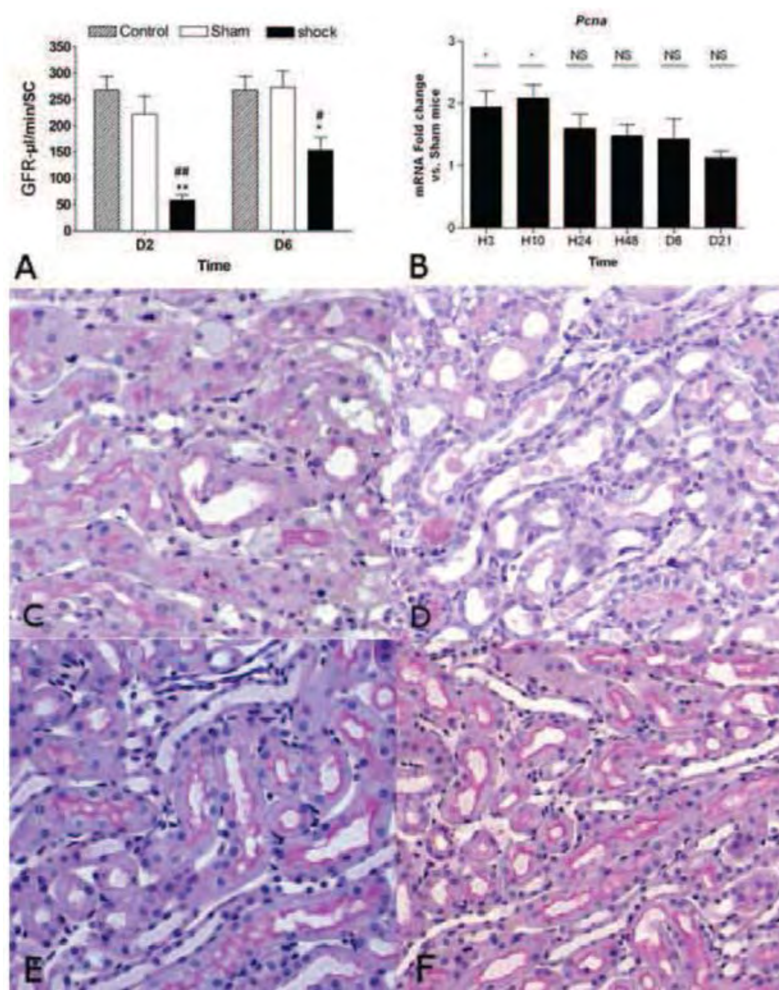

Figure 23 : Variation du débit de filtration glomérulaire (clairance de l'inuline à J2, J6, J21), de l'expression génique de *Pcna* (Proliferating cells nuclear antigen) et modifications histologiques (coloration à l'acide périodique de Schiff à J2 et J6) induites par 2 heures de choc hémorragique contrôlé.

Dans ce modèle, l'expression d'HNF-1β (ARN et protéines) a été étudiée à différents temps suivant l'agression (H3, H10, H24, J2, J6 et J21), c'est-à-dire au cours des différentes étapes de réparation de l'épithélium tubulaire. En ARN, nous montrons que l'expression d'HNF-1β observe une cinétique biphasique : diminution de l'expression de H3 à H10, surexpression à H24 puis normalisation à partir de J2. Ces données sont confirmées par la quantification du facteur de transcription par western blot (Figure 24).

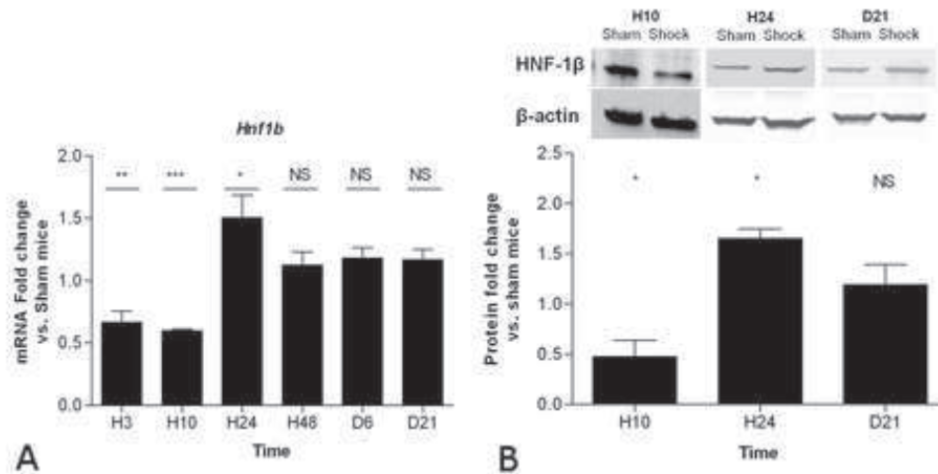

Figure 24 : Expression rénale (ARNm (A) et protéine (B)) d'HNF-1β après deux heurs de choc hémorragique contrôlé.

En parallèle, nous avons observé que l'expression de *Socs3*, normalement inhibée par HNF-1β, est fortement augmentée à H3 et H12 puis se normalise. L'expression de *Pkhd1* et *Cdh16*, cibles induites par HNF-1β, est diminuée dans les premiers temps de la réparation (H3-H12) puis se normalise progressivement (Figure 25).

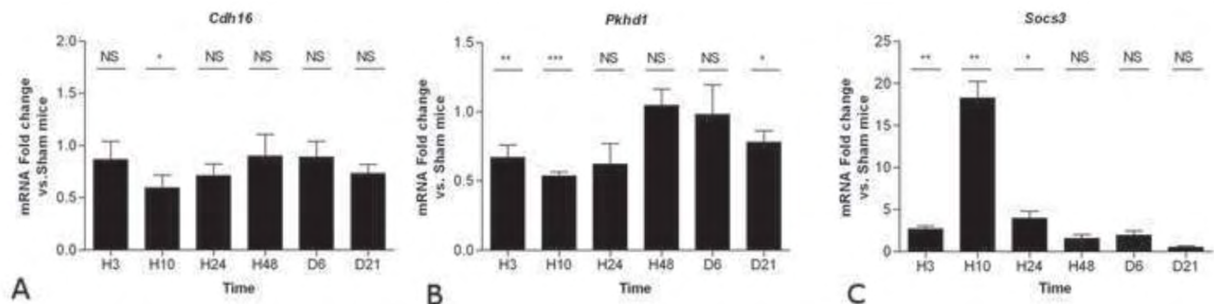

Figure 25 : Expression rénale de *Cdh16*, *Pkhd1* et *Socs3* après deux heures de choc hémorragique contrôlé.

On peut également noter que la cinétique d'*Hnf1a* est parallèle aux phases de prolifération et de dédifférenciation-différenciation épithéliales, jaugées sur l'expression de *Pcna* (proliferating cells nuclear antigen) et *Lrp2* / *Cubln* (complexe mégaline – cubiline), ces derniers servant de marqueurs succédanés de la différenciation tubulaire proximale (Figure 26).

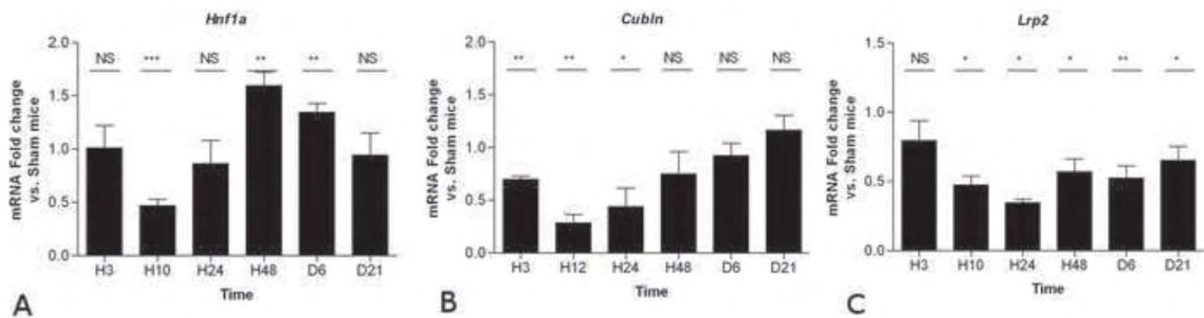

Figure 26 : Expression génique d'*Hnf1a*, *Cubln* et *Lrp2* après deux heures de choc hémorragique contrôlé.

Ces données, suggérant qu'HNF-1β pourrait réguler finement l'expression de *Socs3* au cours de la réparation de l'épithélium tubulaire rénal, comme relais moléculaire intégrant les signaux extra-cellulaires de danger (IL-6) ou de réparation épithéliale (IL-6, HGF/EGF-R), nous avons entrepris de disséquer les mécanismes régulant l'expression d'HNF-1β, en particulier le rôle de l'hypoxie.

Nous avons entrepris des études d'hypoxie cellulaire *in vitro* dans une lignée cellulaire de tube proximal, HK-2) dont les résultats sont les suivants (Figure 27 et 28). L'expression protéique d'HNF-1β augmente significativement dès 30 minutes d'hypoxie, reste élevée jusqu'à 24 heures d'hypoxie, se normalise à H48 puis diminue si l'hypoxie persiste 72 heures. En revanche, L'expression génique d'*HNF1B* évaluée par la quantification de son ARNm montre une diminution précoce de la transcription d'*HNF1B*, dès H3, se prolongeant jusqu'à H24. Nous avons donc identifié une régulation différentielle d'HNF-1β par l'hypoxie : régulation post-traductionnelle à la phase aiguë de l'hypoxie et transcriptionnelle en phase chronique. Après 2 heures d'hypoxie, le retour en normoxie permet en revanche la normalisation de l'expression d'HNF-1β à la 24ème heure. Ces données suggèrent que la surexpression d'HNF-1β pourrait représenter une réponse adaptative à l'hypoxie à la phase aiguë tandis qu'une hypoxie persistante pourrait s'associer à une dysfonction épithéliale caractérisée par une perte d'expression d'HNF-1β.

Afin de mieux caractériser les mécanismes moléculaires aboutissant à la surexpression précoce (dès 30 minutes) d'HNF-1β en situation d'hypoxie, nous avons entrepris d'analyser l'impact du facteur de transcription sensible à l'hypoxie (HIF-1α) sur HNF-1β. sur La surexpression rapide d'HNF-1β suit la cinétique mais l'induction d'HIF-1α indépendamment d'une hypoxie cellulaire (par le biais d'une inhibition des prolylhydroxylases spécifiques d'HIF-1α) ne s'accompagne pas de

surexpression d'HNF-1β. Celle-ci est donc induite par l'hypoxie mais est indépendante d'HIF-1α. Le mécanisme exact aboutissant à la surexpression transitoire d'HNF-1β reste à définir.

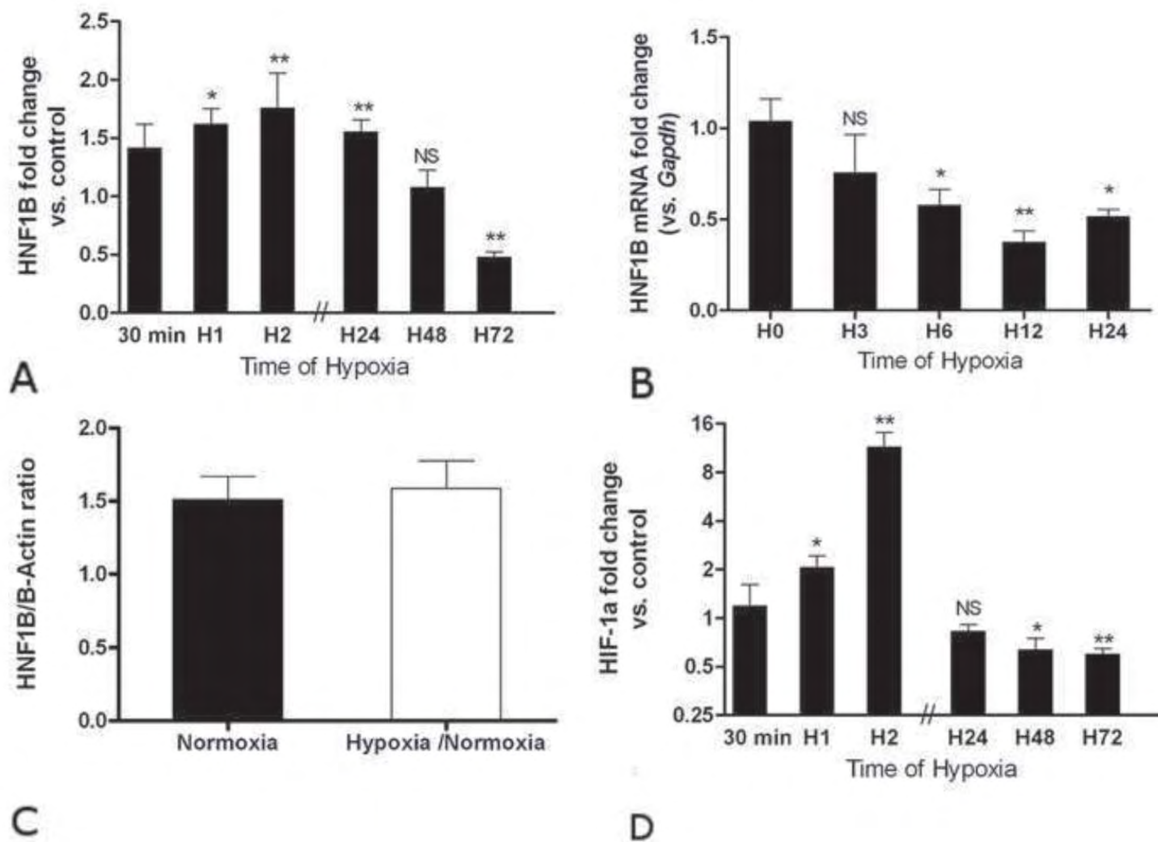

Figure 27 : Conséquences de l'hypoxie sur l'expression d'HNF-1β (protéine [A], ARNm [B]) et d'HIF-1α (protéine [D]) dans des cellules HK-2, et d'une séquence hypoxie (2 heures) – réoxygénation (24 heures) sur l'expression d'HNF-1β (protéine [C]).

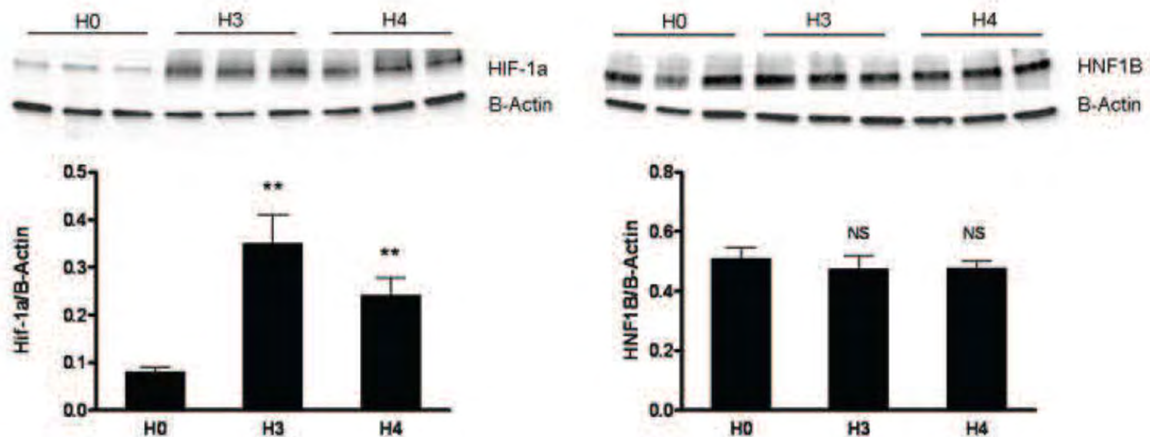

Figure 28 : Expression protéique d'HIF-1α et d'HNF-1β (protéine [A], ARNm [B]) des cellules HK-2 cultivées en présence d'un inhibiteur des prolyl-hydroxylases spécifiques d'HIF-1α.

En résumé, on peut souligner que l'hypoxie induit *in vitro* une surexpression transitoire d'HNF-1β et qu'*in vivo* HNF-1β semble orchestrer la régénération de l'épithélium tubulaire rénal succédant à une agression aiguë ischémique. Le rôle d'HNF-1β dans la réparation des autres épithéliums où il est exprimé (foie, tube digestif, pancréas…) n'est pas connu.

2- *Agression rénale endotoxinique*

L'injection d'entotoxines (lipopolysaccharide [LPS]) à des souris reproduit en partie le tableau de choc septique et de défaillance multi-viscérale observé au cours des infections à bacilles Gram-négatif chez l'homme. La défaillance multi-viscérale associe une insuffisance rénale aiguë par nécrose tubulaire, une hépatite cytolytique et cholestatique, une ischémie mésentérique et une pancréatite aiguë. Indépendamment du traitement de la cause de cette défaillance, celle-ci peut continuer à évoluer aboutissant à une insuffisance d'organe irréversible. Les mécanismes moléculaires sous-jacents sont multiples et encore mal compris : bas débit sanguin, hypoxie, toxicité directe des endotoxines, inflammation intra-tissulaire.

Afin de mieux appréhender l'impact d'un choc endotoxinique sur l'expression d'HNF-1β dans les différents organes où il est exprimé et son implication potentielle dans la défaillance multiviscérale, des souris C57/Bl6 ont été soumises à une injection intra-péritonéale de LPS (10 mg/kg) et caractérisées sur le plan histologique, fonctionnel et moléculaire à H6 et H24.

Brièvement, on observe une insuffisance rénale aiguë dès H6 (oligo-anurie et élévation de l'urée), puis une élévation des enzymes hépatiques (ASAT), de l'amylase et des LDH dès H24. Les souris sont prostrées. Les reins sont le siège d'une surexpression des gènes *Kim1* et *Il18*, marqueurs de lésions tubulaires aiguës organiques. On observe une dysfonction tubulaire proximale marquée par la perte de l'expression du complexe mégaline-cubiline (*Lrp2*/*Cubln*).

Dans le rein, l'expression d'HNF-1β est diminuée dès H6 (50%), diminution persistante à H24 (40%). L'expression de *Socs3* est significativement augmentée à ces temps.

Pour identifier le mécanisme moléculaire à l'origine de cette perte d'expression (action directe de l'endotoxine, dédifférenciation cellulaire, autre), des études *in vitro* ont été menées sur des cellules HK-2, un modèle de cellules tubulaires proximales. L'incubation de ces cellules avec du LPS (10 μg/mL) aboutit à une diminution d'expression précoce d'HNF-1β (H6-H24) attestant de la toxicité directe de l'endotoxine sur la cellule épithéliale.

Le LPS, en se liant à son récepteur spécifique Toll-like receptor-4 (TLR4) à la surface cellulaire, active plusieurs cascades de signalisation aboutissant à l'activation et à la translocation dans le noyau du facteur de transcription NF-κB. Pour disséquer le lien moléculaire entre NF-κB et HNF-1β, des inhibiteurs sélectifs des différentes voies de signalisation ont été utilisés. L'inhibition du facteur NEMO (activateur de NF-κB) ne modifie pas l'effet du LPS sur l'expression d'HNF-1β tandis

que l'inhibtion de Myd88, une molécule adaptatrice faisant le lien entre le TLR4 et la voie classique d'activation de NF-κB, diminue modérément mais de manière significative l'impact du LPS sur la baisse d'expression d'HNF-1β. Enfin, l'étude *in silico* (site Consite°) des facteurs de transcription se liant potentiellement sur ou à proximité du promoteur d'HNF-1β a permis d'identifier deux séquences cibles de NF-κB (en position -2110 et -100 en amont du site d'initiation de la transcription d'HNF-1β).

HNF-1β semble donc être un facteur de transcription régulé par la voie TLR4/Myd88/NF-κB. Afin de mieux caractériser le lien moléculaire entre l'activation de NF-κB et l'expression d'HNF-1β, des expérimentations d'immunoprécipitation de chromatine (ChIP) avec un anticorps anti-NF-κB sont actuellement menées au laboratoire.

Nous faisons l'hypothèse que la diminution de l'expression d'HNF-1β, au-delà de l'induction d'une potentielle dysfonction transitoire délétère des cellules tubulaires, pourrait être *in fine* un mécanisme de protection de la cellule épithéliale permettant de moduler les signaux extra-cellulaires induit par différentes cytokines (dont l'IL-6) et des facteurs de croissances (dont l'EGF et l'HGF), ou d'obtenir un gain en terme de protection contre la mort cellulaire. Les expérimentations permettant de répondre à cette question sont actuellement en cours au laboratoire.

E) CONCLUSION

La maladie liée à *HNF1B* est une maladie rénale monogénique au spectre déroutant. La mise à disposition en routine du diagnostic génétique de cette maladie a permis de nombreuses avancées cliniques: en médecine fœtale rénale, environ 30% des maladies kystiques des reins (ou des gros reins hyperéchogènes) ont une cause clairement identifiée ; simultanément, en néphrologie de l'enfant et de l'adulte, le champ des néphropathies tubulo-interstitielles chroniques héréditaires s'est enrichi d'un nouveau contributeur majeur, aux côtés de la maladie liée aux mutations d'*UMOD*, le gène codant pour l'uromoduline (ou protéine de Tamm-Horsfall).

Le travail clinique réalisé et présenté ici a contribué à une caractérisation plus fine du phénotype rénal liée aux mutations d'*HNF1B* à l'âge adulte : l'hétérogénéité phénotypique extrême suggère une remarquable précision dans le réglage des voies de signalisation dépendantes d'HNF-1β, et la possibilité de redondances de ces voies. L'algorithme décisionnel proposé pour la prescription du test génétique spécifique d'*HNF1B* rétrécit le champ des prescriptions inappropriées.

Cet effort n'est pas inutile, puisque les données colligées tout récemment dans le Centre de Référence Maladies Rénales Rares du Sud-Ouest (SORARE) indiquent que la prévalence de la maladie liée à *HNF1B* en Midi-Pyrénées et Limousin est étroitement convergente, respectivement à 1/62 000 et 1/67 000. L'affection est certainement plus fréquente que le syndrome d'Alport, et se hisse donc au deuxième rang des néphropathies héréditaires en fréquence.

Désigner un gène coupable, et tenter de comprendre la physiopathologie de la maladie en aval ne s'entend que par l'étude de son produit principal, le facteur de transcription HNF-1β. L'approche s'appuyant sur les modèles animaux, largement exploitée dans la littérature, a permis des avancées notables pour établir la grande variété de ses rôles physiologiques dans l'organogenèse épithéliale, la polarisation cellulaire planaire, le maintien de l'état de différenciation, et la réparation épithéliale… selon le type cellulaire et le stade de développement étudiés. Mais il faut concéder les limites des modèles : ceux-ci aboutissent notamment à une néphropathie assez éloignée de la maladie humaine.

Le travail fondamental réalisé personnellement et présenté ici explorait le rôle potentiel d'HNF-1β à l'occasion des épisodes d'agression et régénération épithéliales, une piste relativement négligée jusqu'ici. Nous avons montré que l'expression d'HNF-1β est finement régulée (tant au niveau transcriptionnel que post-traductionnel) après agression rénale aiguë, hypoxique ou septique. Ces modifications d'expression semblent participer à la dysfonction épithéliale transitoire succédant à l'agression et contrôler l'efficacité de la réparation d'un épithélium tubulaire. Des efforts supplémentaires sont nécessaires pour disséquer les voies de signalisation aboutissant à la régulation d'HNF-1β. Ce travail se poursuit dans le laboratoire et va s'étendre à la caractérisation du rôle d'HNF-1β à l'échelon tissulaire au cours des phases de réparation.

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G) ANNEXE

Ce chapitre recense l'ensemble des données nouvelles obtenues lors de ce travail de Thèse et exposées précédemment. Afin de faciliter la compréhension du lecteur, les articles scientifiques sont fournis ici selon un ordre thématique reprenant le plan de cette thèse :

1) Approche clinique de la maladie liée à *HNF1B* chez l'homme

Diagnosis, management, and prognosis of *HNF1B* nephropathy in adulthood. Faguer S, Decramer S, Chassaing N, Bellanné-Chantelot C, Calvas P, Beaufils S, Bessenay L, Lengelé JP, Dahan K, Ronco P, Devuyst O, Chauveau D. *Kidney International 2011* (Article 1)

Massively enlarged polycystic kidneys in monozygotic twin with hepatocyte nuclear factor-1beta (TCF2) whole-gene deletion Faguer S, Bouissou F, Dumazer P, Guitard J, Bellanné-Chantelot C, Chauveau D. *American journal of kidney disease 2007* (Article 2).

2) Approche génétique de la maladie liée à HNF1B chez l'homme

Spectrum of *HNF1B* mutations in a large cohort of patients harboring renal diseases Heidet L, Decramer S, Pawtowski S, Morinière V, Bandin F, Knebelmann B, Lebre AS, Faguer S, Guigonis V, Antignac C, Salomon R. *Clinical J Am Soc Nephrol 2010* (Article 3)

HNF1B score : a simple tool for both clinicians and geneticists Faguer S, Chassaing N, Bandin F, Prouheze C, Schanstra Joost-Peter, Calvas P, Decramer S and Chauveau D. *Manuscrit en cours de soumission* (Article 4).

Mutations in the RARE and MARE regulatory sequences of *HNF1B* are not a frequent cause of kidney/urinary tract malformation. Faguer S, Chauveau D, Decramer S, Chassaing N. *Clinical Kidney Journal (ex-NDT plus) 2009*. (Article 5)

3) Approche fondamentale du rôle d'HNF-1β dans le rein humain et murin

HNF-1β transcription factor is an early HIF-1α-independent marker of epithelial hypoxia and controls renal repair. Faguer S, Mayeur N, Pageaud AL, Courtellemont C, Cartery C, Casemayou A, Fournié GJ, Schanstra JP, Tack I, Bascands JL, Chauveau D. *PLoS One, Manuscript en revision favorable (*Article 6)

Expression of renal cystic genes in patients with *HNF1B* mutations. Faguer S, Decramer S, Devuyst O, Lengelé JP, Fournié GJ, Chauveau D. *Nephron Clinical Practice 2012* (Article 7).

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Diagnosis, management, and prognosis of *HNF1B* nephropathy in adulthood

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Mutations in *HNF1B* are responsible for a dominantly inherited disease with renal and nonrenal consequences, including maturity-onset diabetes of the young (MODY) type 5. While *HNF1B* nephropathy is typically responsible for bilateral renal cystic hypodysplasia in childhood, the adult phenotype is poorly described. To help define this we evaluated the clinical presentation, imaging findings, genetic changes, and disease progression in 27 adults from 20 families with *HNF1B* nephropathy. Whole-gene deletion was found in 11 families, point mutations in 9, and *de novo* mutations in half of the kindred tested. Renal involvement was extremely heterogeneous, with a tubulointerstitial profile at presentation and slowly progressive renal decline throughout adulthood as hallmarks of the disease. In 24 patients tested, there were cysts ($\leqslant$5 per kidney) in 15, a solitary kidney in 5, hypokalemia in 11, and hypomagnesemia in 10 of 16 tested, all as characteristics pointing to *HNF1B* disease. Two patients presented with renal Fanconi syndrome and, overall, 4 progressed to end-stage renal failure. Extrarenal phenotypes consisted of diabetes mellitus in 13 of the 27 patients, including 11 with MODY, abnormal liver tests in 8 of 21, diverse genital tract abnormalities in 5 of 13 females, and infertility in 2 of 14 males. Thus, our findings provide data that are useful for recognition and diagnosis of *HNF1B* disease in adulthood and might help in renal management and genetic counseling.

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KEYWORDS: adult patients; *HNF1*Beta; hypomagnesemia; MODY5; tubulointerstitial nephropathy

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HNF1B is a developmental gene coding for hepatocyte nuclear factor 1 homeobox B, a transcription factor first isolated in human hepatocytes.[1] During murine development and in adult mice, *Hnf1b* has tissue-specific expression in polarized epithelia of the pancreas, liver, renal, and genital tracts.[2–4] A direct hierarchy of *Hnf1b* upon the expression of renal cystic (*Pkhd1*, *Pkd2*, and *Umod*) and noncystic (*Fxyd2*) genes is established in mouse. In humans, heterozygous mutations of *HNF1B* are responsible for a dominantly inherited disease with both renal and extrarenal phenotypes.[5] Genetic changes consist of whole-gene deletion in $\sim$50% of the patients, whereas point mutations are detected in the majority of the remaining cases.[6–8] Extrarenal manifestations include maturity-onset diabetes of the young type 5 (MODY5);[9] exocrine pancreatic failure; fluctuating liver tests abnormalities; and genital tract abnormalities.[5,10]

Renal involvement has emerged as the earliest and most prevalent finding in *HNF1B* disease,[6] with phenotypic heterogeneity including renal malformations and tubular transport abnormalities. Prenatally, the most frequent presentation consists of bilateral hyperechogenic kidneys with or without cortical cysts,[11] whereas bilateral renal hypodysplasia with few or multiple cysts is more prevalent in early childhood.[12,13] Renal magnesium wasting affects up to 50% of children.[12]

HNF1B nephropathy may present only in adulthood. Diagnosis remains extremely challenging given that (1) *de novo* mutations are encountered in up to 30–50% of new cases;[5,7,11] (2) in kindred with evidence for autosomal-dominant inheritance, large intrafamilial variability of the renal phenotype is striking;[7,8] and (3) demonstration of phenocopies can be seen in some families.[5] In addition, all previous reports share serious limitations, being mostly devoted to syndromic presentations in isolated individuals or kindred,[3,12,14–21] and the description of novel mutations,[3,12,14–21] or biased by prespecifying MODY5 phenotype

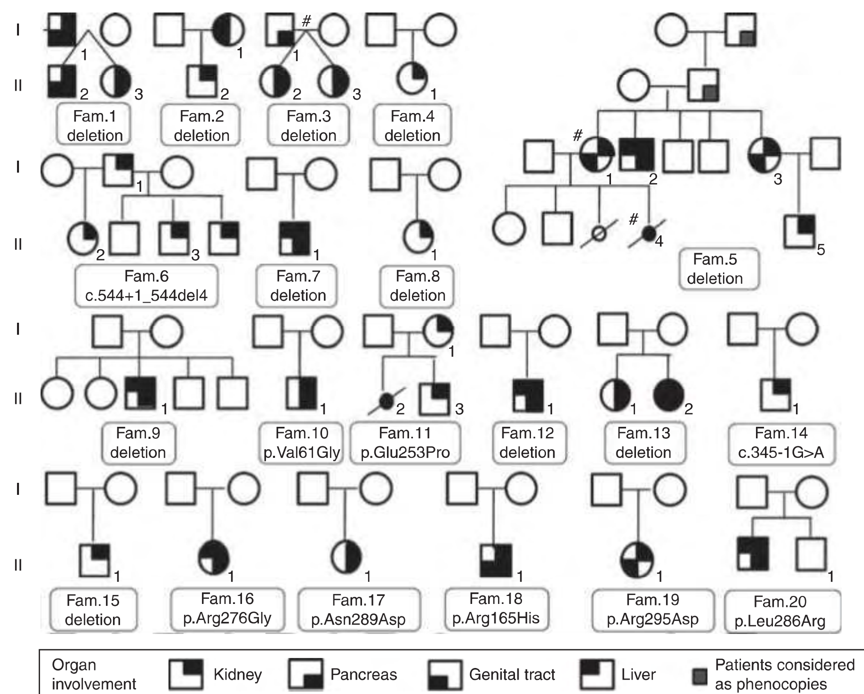

Figure 1 | Pedigrees of 20 families with *HNF1B* mutation. Filled quarters denote organ involvement. #These individuals had no molecular analysis, but their genotype was deduced from that of affected siblings (see text for details).

as one criterion for inclusion.[5,6] Therefore, a comprehensive assessment of phenotype and long-term outcomes of *HNF1B* nephropathy in adulthood is still lacking.

The current study was profiled to delineate the clinical presentation, molecular basis, and outcome in 27 adult patients with *HNF1B* nephropathy. Particular emphasis was placed upon detecting differences in the presentation and outcome in families with point mutation vs whole-gene deletion.

RESULTS

Within 20 families, we identified 35 individuals with *HNF1B* disease: 27 adults (14 males and 13 females) and 8 additional offspring lesser than 16 years of age (2 fetuses and 6 children; see Figure 1). In the adult group, genetic testing was made at the time of family screening in 11 individuals, including 9 (33%) for whom the proband was an affected child or fetus. In the 16 adult probands, half the presenting manifestations occurred in childhood whereas half occurred in adult life.

Mutation analyses: *HNF1B* alterations

All 27 individuals harbored a heterozygous molecular alteration of *HNF1B* (see Table 1 and Supplementary Table S1 online and Figure 1). Quantitative multiplex PCR of short fragments detected whole-gene deletion in 11/20 families (55%; 16 individuals). Direct DNA sequencing revealed point mutations in 9/20 families (45%; 11 individuals), including 7 previously reported in unrelated families (c.345-1G>A, c.544+1_544+4del4, p.Val61Gly, p.Arg165His, p.Gln253Pro, p.Arg276Gly, and p.Arg295Cys),[6,7,11] and two novel missense mutations (p.Leu286Val and p.Asn289Asp). None of the novel nucleotide variants could be detected in 100 control individuals.

Genotyping of apparently unaffected parents of adult probands was performed in 14 of the 20 families. *De novo* mutation was found in 7/14 (50%), including three kindred with whole-gene deletion (43%) and four with a point mutation (57%). Among the latter, we could demonstrate one patient with novel missense mutation (family 17) and one patient with splice site mutation (family 14).

Clinical phenotype

Data were collected at a median age of 35 years (16–74). The male-to-female ratio was 1.1:1. Four patients were symptom free when screening was performed between 21 and 46 years of age. Among the remaining 23 patients, the first manifestation of *HNF1B* disease was kidney involvement in 14 (61%), diabetes mellitus in 2 (9%), genital tract malformations in 4 (18%), and liver tests abnormalities in 3 (12%; Tables 1 and 2, and Figure 1).

Kidney involvement

Among the 14 patients who presented with renal disease, the first manifestation was noticed prenatally in 6, during childhood in 4, and during adulthood in 4 (aged 29–63 years; see Table 3 for details regarding antenatal and childhood presentation). In the latter, renal presentation consisted of chronic renal failure with small kidneys and few ($n=2$), multiple ($n=1$), or no cysts ($n=1$).

The 27 patients enrolled in the cohort were followed for a median of 5.5 years (1–29) and had a median age of 35 years (16–74) at last follow-up. Urinalysis was usually bland: none

Table 1 | Renal phenotype at last follow-up in 27 adult patients with *HNF1B* mutation

Family. patient	Gender	Age	eGFR	K^+	Mg^{++}	MgUE	Ca^{++}	CaUE	Renal imaging	Mutation
F1.1	M	40	52	3.7	0.6	7%	2.41	0.3%	Solitary RK, few cysts and pyelic dilatation	Deletion
F2.1	F	38	100	4	—	—	—	—	Normal	Deletion
F3.1	M	19	—	—	—	—	—	—	—	Deletion
F3.2	F	24	15	3.2	0.66		2.4		Mimicking ADPKD	Deletion
F3.3	F	23	28	3.3	0.67		2.35		Mimicking ADPKD	Deletion
F4.1	F	21	23	4	—	—	2.52		Solitary LK, few cysts (R-MCDK involution)	Deletion
F5.1	F	—	—	—	—	—	—	—	—	Deletion
F5.2	M	48	45	3.8	—	—	2.4		Hypoplastic RK	Deletion
F5.3	F	40	52	3	—	—	2.33	—	Normal	Deletion
F6.1	M	55	64	3.1	0.52	14%	2.23	1.8%	Few cysts	c.544+1_544+4del4
F6.3	M	31	ESRD	2.6	—	—	2.36	—	Bilateral hypoplasia and VUR	c.544+1_544+4del4
F6.4	M	29	65	—	—	—	—	—	Solitary LK, few cysts	c.544+1_544+4del4
F7.1	M	53	26	3.9	0.66	—	2.48	4%	Few cysts	Deletion
F8.1	F	57	45	3.4	—	—	2.49		Bilateral hypoplasia, few cysts	Deletion
F9.1	M	63	29	4	1	—	—		Many bilateral cysts	Deletion
F10.1	M	74	47	4.2	0.72*	8%	2.86[a]	1%	Few cysts, nephrocalcinosis	p.Val61Gly
F11.1	F	22	55	4	0.48	7%	2.4	0.4%	Few cysts	p.Gln253Pro
F12.1	M	27	33	3.8	—	—	2.4		Bilateral VUR and pyelic dilatation	Deletion
F13.1	F	46	94	3.9	1	—	2.2		—	Deletion
F13.2	F	48	ESRD	4	1	—	2.4		Hypoplastic RK with few cysts	Deletion
F14.1	M	16	23	3.4	0.7	22%	2.43	2.3%	Solitary RK (L-MCDK involution)	c.345-1G>A
F15.1	M	17	45	3.4	0.78	5%	2,46	0.2%	Bilateral hypoplasia, few cysts	Deletion
F16.1	F	38	ESRD	3.4	0.65	—	2.4	—	Bilateral nephrolithiasis and pyelic dilatation	p.Arg276Gly
F17.1	F	21	38	3.4	0.9	4%	2.25		Solitary LK (R-MCDK involution)	p.Asn289Asp
F18.1	M	18	ESRD	4	1	—	2.3	—	Bilateral Nx (age 7, bilateral MCDK)	p.Arg165His
F19.1	F	38	68	3.9	—	—	2.13	0.2%	Bilateral hypoplasia, few cysts, lithiasis	p.Arg295Cys
F20.1	M	32	23	3	—	—	1.9	—	Nephrocalcinosis, nephrolithiasis	p.Leu286Val

Abbreviations: ADPKD, autosomal-dominant polycystic kidney disease; Ca^{2+}, serum calcium level (mmol/l); CaUE, calcium urinary excretion (%; (U/P)[Ca]/(U/P)[creatinine]; $N > 1\%$); eGFR, estimated glomerular filtration rate (ml/min per 1.73 m^2; simplified Modification of Diet in Renal Disease (MDRD) formula); ESRD, end-stage renal disease; F, female; K^+, serum potassium level (mmol/l); L, left; LK, left kidney; M, male; MCDK, multicystic and dysplastic kidney; Mg^{2+}, serum magnesium level (mmol/l); MgUE, magnesium urinary excretion (%; (U/P)[Mg]/(U/P)[creatinine]; $N < 2\%$); Nx, nephrectomy; R, right; RK, right kidney; VUR, vesicoureteral reflux.

*Mg^{2+} oral supplementation.

[a]Primary hyperparathyroidism was evidenced in patient F10.1.

of the patients had microhematuria, 20 had no proteinuria, and 7 had a low-grade proteinuria (<1 g/l). Polyuria was not clinically evident. Only two individuals (7%) were hypertensive, including one (F13.1) with reflux nephropathy. On follow-up, hypokalemia or hypomagnesemia was detected in 15 patients (62%). Serum potassium levels were spontaneously <3.5 mmol/l in 11 of 24 tested individuals (46%). Hypokalemia resulted from renal loss (urinary K^+ concentration >30 mmol/l) and persisted despite worsening renal decline. Ten individuals with chronic kidney disease stage 3–5 had serum potassium levels <3.5 mmol/l, including one patient (F6.3) with a serum potassium concentration of 2.6 mmol/l at the start of dialysis. Serum magnesium levels were <0.75 mmol/l in 10 out of the 16 individuals tested (62%). Magnesium depletion also resulted from renal wasting (fractional urinary magnesium excretion (FE_{Mg}: 7–22%, $N<0.5\%$)). Oral supplementation with Mg^{2+} or K^+ did not restore either serum magnesium or potassium levels. The prevalence of hypokalemia and hypomagnesemia was roughly similar in patients with gene deletion and point mutation (Table 4). All but two patients with hypokalemia also had concurrent hypomagnesemia. In contrast, only five out of nine patients with hypomagnesemia had hypokalemia. Last, five out of eight individuals had low urinary calcium excretion.

Two unusual patients (F16.1 and F20.1) presented with generalized defects of proximal and distal tubular function, including tubular proteinuria, generalized aminoaciduria, renal glucosuria, phosphaturia, hypomagnesemia, hypokalemia, and incomplete renal tubular acidosis associated with hypercalciuria and recurrent nephrolithiasis. In both, genetic testing ruled out a concomitant mutation or deletion in *HNF1A*, the gene responsible for MODY3.

Renal imaging was available in 24 of the 27 patients (89%). At last follow-up, extreme heterogeneity was found, falling grossly into four categories with possible overlap: (1) normal kidneys, that is, two normally sized kidneys without detectable cysts (3/24, (12%)); (2) a solitary kidney seen in 5/24 individuals (21%); (3) renal cysts in 15/24 individuals (62%), including 11 with few (that is, $\leqslant 5$) cortical or medullary cysts per kidney (In most cases, cysts developed within the cortex, sparing the kidney outline. There was no correlation seen between the degree of renal cystic involvement and the severity of renal failure (data not shown). Of note, 9 patients (38%) had no renal cysts between 16 and 48 years of age.); and (4) a wide range of kidney and urinary tract abnormalities, including nephrocalcinosis, kidney stones, hydronephrosis or hydroureter, and vesicoureteric reflux (16%). One patient (F11.1) developed a renal tumor at age 20 years. She declined surgical treatment.

Table 2 | Individual extrarenal phenotype in 27 adult patients with *HNF1B* mutation

Family. patient	DM (age at diagnosis)	Initial presentation	Insulin therapy at last FU	Pancreas imaging	Liver test abnormalities	Genital tract abnormalities	Additional findings
F1.1	No	—	—	Tail hypoplasia	No	CBAVD, cryptorchidy	—
F2.1	No	—	—	Normal	Yes	Bicornual uterus	—
F3.1	MODY (19)	—	—	—	—	—	—
F3.2	MODY (21)	Slow	Yes	Normal	No	No	—
F3.3	MODY (21)	Slow	No	Normal	No	No	—
F4.1	No	—	—	Normal	No	No	—
F5.1	No	—	—	—	No	Bicornual uterus	—
F5.2	MODY (31)	Slow	Yes	—	Yes	—	Inguinal hernia
F5.3	Gestational	—	—	—	—	Bicornual uterus	—
F6.1	No	—	—	—	—	No	—
F6.3	No	—	—	—	—	—	Mental retardation, growth delay
F6.4	No	—	—	—	—	—	—
F7.1	MODY (41)	Slow	Yes	—	Yes	—	—
F8.1	No	—	—	—	—	—	—
F9.1	MODY (—)	Slow	No	—	Yes	—	—
F10.1	MODY (41)	Slow	Yes	—	No	—	—
F11.1	No	—	—	Normal	No	No, pre-eclampsia	Renal tumor (age 20)
F12.1	MODY (23)	Ketoacidosis	Yes	Normal	Yes	—	—
F13.1	MODY (43)	Slow	No	—	No	No, pre-eclampsia	—
F13.2	MODY (33)	Slow	Yes	Normal	Yes	Uterus absence, vaginal hypoplasia	Mental retardation, cervical cancer (age 19)
F14.1	No	—	—	—	No	—	—
F15.1	No	—	—	Normal	No	—	—
F16.1	Post-RT (37)	Early	Yes	Normal	Yes	—	—
F17.1	No	—	—	Tail hypoplasia	No	No	—
F18.1	Post-RT (8)	Early	Yes	Normal	No	Bilateral epidydimes and vas deferens cysts	Mental retardation, cortical and cerebellum hypoplasia, epilepsy
F19.1	No	—	—	Tail hypoplasia	No	Septate uterus, ovarian cyst	—
F20.1	MODY (23)	Ketoacidosis	Yes	Calcifications and tail hypoplasia	Yes	—	Epilepsy

Abbreviations: CBAVD, congenital bilateral absence of vas deferens; DM, diabetes mellitus; FU, follow-up; MODY, Maturity-Onset Diabetes of the Young; RT, renal transplantation.

Table 3 | Renal phenotype at presentation and at last follow-up in 10 adult patients with first renal manifestation during antenatal period (*n*=6) or in childhood (*n*=4)

Presentation	Last follow-up (adulthood)
Antenatal	
Bilateral cystic kidneys (*n*=2)	Massively enlarged cystic kidneys (*n*=2)
Unilateral MCDK (*n*=3)	MCDK involution (*n*=3) and few cysts (*n*=1)
Bilateral hypoplastic and microcystic kidneys (*n*=1)	Bilateral nephrectomy at age 6 (*n*=1)
Childhood	
Bilateral pyeloureteric junction syndrome (*n*=1)	Surgery at age 1, bilateral ureteric and caliceal dilatation at last follow-up (*n*=1)
Bilateral hypoplastic kidneys (*n*=1)	Bilateral hypoplastic kidneys with few cysts (*n*=1)
Cystic kidneys (*n*=1)	Cystic kidneys (*n*=1)
Right nephrectomy of unknown cause (*n*=1)	Few cysts (*n*=1)

Abbreviation: MCDK, multicystic and dysplastic kidney.

Table 4 | eGFR assessment and frequency of hypomagnesemia and hypokalemia in *HNF1B* patients according to genetic change

	Deletion	Point mutation	*P*-value
N	16	11	
Age at latest follow-up (years)	38.9 ± 14.6 (17–63)	36.9 ± 18.6 (16–74)	NS
eGFR (sMDRD, ml/min per 1.73 m^2)	36.6 ± 22.4 (0–100)	41.1 ± 29.6 (0–88)	NS
Low serum magnesium (*n*/*n* examined)	4/8 (50%)	6/8 (75%)	NS
Low serum potassium (*n*/*n* examined)	5/14 (36%)	5/10 (50%)	NS

Abbreviations: eGFR, estimated glomerular filtration rate; NS, not significant; sMDRD, simplified Modification of Diet in Renal Disease.
$P<0.05$ was considered as significant (Mann–Whitney test).

Kidney size was not available in two individuals, was normal in 13 (61%), decreased in 7 (30%; unilaterally in 2 and bilaterally in 5), and was massively enlarged in two homozygotic twins (9%) who harbored a renal phenotype mimicking autosomal-dominant polycystic kidney disease

(patients F3.2 and F3.3; for details, see Faguer *et al.*[17]). Figure 2 shows the kidney length at last follow-up according to age. In the six cystic individuals who underwent sequential imaging, none showed a progressive increase in the number of cysts over time.

At last evaluation, estimated glomerular filtration rate (eGFR) was available in 25 of the 27 individuals and ranged from 0 to 100 ml/min per 1.73 m^2 (median 38). According to chronic kidney disease classification, two patients were ranked stage 1, four stage 2, nine stage 3 (including one in her eighth decade), and six stage 4. Four patients (15%) progressed to end-stage renal failure at 7, 22, 29, and 31 years of age, respectively (Figure 3). In the 17 individuals not in end-stage renal failure for whom sequential assessment of eGFR was available over a median follow-up period of 5.5 years (1–16), the median yearly decline of eGFR was slow (−2.45 ml/min/year), with individual changes ranging between 0 and 19 ml/min/year. Three individuals had unexplained acute renal deterioration. No renal biopsy was performed in this series. Renal prognosis was not predicted by genetic changes (large deletion vs point mutation; see Figure 3 and Table 3). Renal transplantation was performed in three patients. By the end of the study, two grafts were still functional.

Gross kidney abnormalities and the course of renal disease within families were either homogeneous (family F3), or moderately (family f5) and even extremely heterogeneous (families F6 and F13).

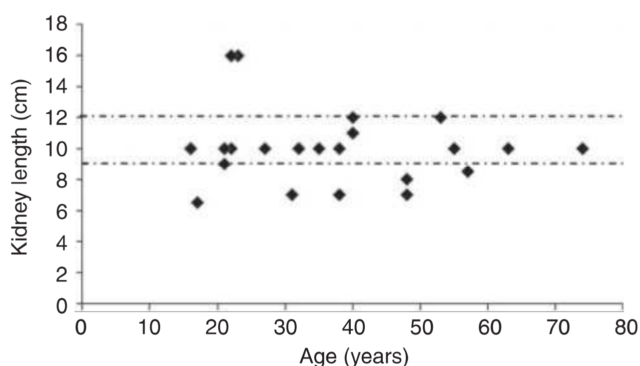

Figure 2 | Kidney length at last follow-up in 24 adult patients with *HNF1B* mutation.

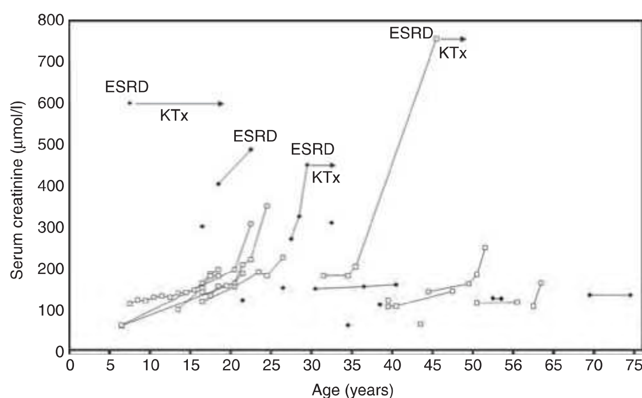

Figure 3 | Serial determination of serum creatinine in 27 adult patients with *HNF1B* mutation. Dark and white squares represent patients with point mutation and whole-gene deletion, respectively. ESRD, end-stage renal disease; KTx, kidney transplantation.

Extrarenal phenotype

With the exception of diabetes mellitus, extrarenal involvement related to *HNF1B* disease was not systematically searched for (Table 2 and Supplementary Table S2 online). Diabetes mellitus was diagnosed in 13/27 patients (48%): 11 presented with MODY and 2 with ketoacidosis. On follow-up, 9 patients required insulin therapy. None developed diabetic retinopathy or neuropathy. At last follow-up, the median age of MODY5 and nondiabetic individuals was similar (37 (18–74) vs 31 (16–57) years, $P = 0.31$).

Additional extrarenal findings consisted of pancreas hypoplasia (33%); liver test abnormalities (40%): serum titers of transaminases, alkaline phosphatase, and γ-glutamyl transferase fluctuated up to 10 times the upper limits of normal; genital tract malformation (5 of 12 (45%) tested females and 2 males); and mild-to-moderate mental retardation without concomitant hypomagnesemia (11%; see Table 2 and Supplementary Table S2 online, and Figure 4 for details).

DISCUSSION

Genetics

This study demonstrates the wide heterogeneity of both phenotype and genotype among adult patients with kidney involvement related to molecular alteration in *HNF1B*. The study was conducted in 27 middle-aged adults from 20 unrelated families. Large genomic rearrangement proved to be the most frequent genetic alteration (seen in 55% of all families). In *HNF1B* patients, whole-gene deletion encompasses a genomic region of at least 1.2 megabases,[6] and accounts for 36% of the genetic mutations in MODY5 adults,[6] and up to 83% in fetuses who present with renal abnormalities.[11] In the 11 families with whole-gene deletion, assignment of pathogenesis was straightforward, as well as in the four additional families with point mutations previously reported. In contrast, determining pathogenicity remains challenging in the absence of a functional assay for the novel point mutations identified in two families. We considered these variants as likely pathogenic given that (1) all mutations were localized in the DNA-binding domain where a known hot spot has been demonstrated;[7] (2) they affect highly conserved amino-acid residues between distant species and bioinformatics tools (PolyPhen: http://genetics.bwh.harvard.edu/pph/) predicted pathology; (3) they occurred *de novo* (family 17); and (4) they were not present in 200 chromosomes from white controls.

De novo mutation was a frequent finding, reaching 50% among the 14 families available for testing. This figure is close to the 53–58% rates found in two pediatric cohorts with renal abnormalities.[11,22] This finding has one ominous

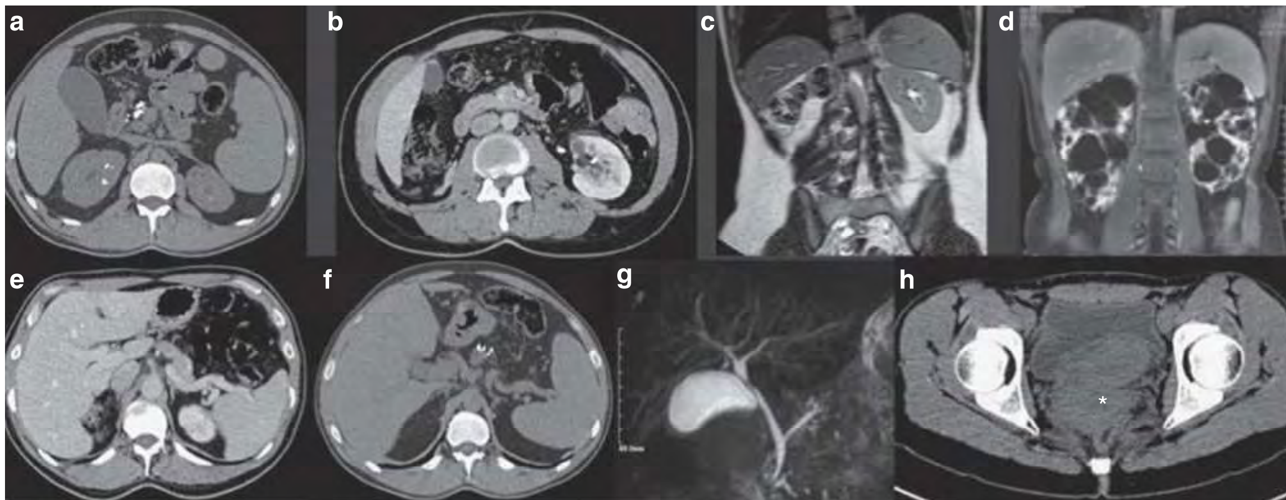

Figure 4 | The spectrum of abdominal imaging findings in *HNF1B* patients. (**a**, **b**) Abdominal computed tomography (CT) scan: bilateral small kidneys with nephrolithiasis (**a**, patient F19.1); solitary left kidney with few cortical cysts (**b**, patient F4.1). (**c**, **d**) Abdominal magnetic resonance imaging (MRI): left solitary kidney without cyst (**c**, patient F17.1); multicystic kidneys mimicking autosomal-dominant polycystic kidney disease (**d**, patient F3.2). (**e**, **f**) Abdominal CT scan: hypoplasia of pancreas tail (**e**, patient F1.1); calcification of pancreas head (**f**, patient F20.1). (**g**) Abdominal MRI (patient F20.1, chronic cholestasis): normal biliary tree. (**h**) Pelvic CT scan: septate uterus (star, patient F19.1).

consequence: in patients with a suggestive phenotype, the lack of family history should not prevent consideration of *HNF1B* disease as a possibility.

Renal phenotype

Previously, the description of *HNF1B* nephropathy was confined to sporadic cases,[3,20,21] cross-sectional studies in children,[3,7,9,13,20–22] or cohorts of MODY5 adult patients with limited follow-up.[5,6] This study, which represents the largest series of adults with *HNF1B* disease, highlights the heterogeneity of the renal involvement and provides unique landmarks to prompt early recognition. For the first time, we establish that the renal phenotype in adults with *HNF1B* disease is clearly one of chronic tubulointerstitial nephritis. Characteristic findings of bland urinalysis in 74% of patients, the universal absence of hematuria and urinary protein excretion <1 g/l in all tested patients, the low prevalence of hypertension (7%), and slowly progressive kidney failure (with a median yearly eGFR decline of -2.45 ml/min per $1.73\,m^2$) are the hallmarks of this disease. In adult patients with *HNF1B* mutation, renal biopsy may show nonspecific interstitial fibrosis and, to a lesser extent, enlarged glomeruli or oligomeganephronia.[5] Polyuria, characteristic of nephronophthisis,[23] was not observed in our adult patients with *HNF1B* mutation. Furthermore, in contrast with previous findings,[13,24] gouty attack was not a presenting manifestation in this cohort.

One of the strengths of this study is the systematic description of kidney imaging in 24 adult *HNF1B* mutation carriers. We observed a puzzling heterogeneity of gross kidney structural anomalies, evading easy classification. The most suggestive finding was a cystic phenotype, observed in 62% of this adult population. This figure is in keeping with previous studies in adults with MODY5,[5,6] where macroscopic cysts were a frequent (29/36, or 81%) but not a universal finding. Of note, most cystic patients harbored $\leqslant 5$ cortical cysts in each kidney, and with the exception of homozygotic twins,[17] the course of renal pathology was not characterized by a progressive increase in the number of cysts or in kidney size, a distinctive finding as compared with polycystic kidney disease, with either recessive or dominant inheritance. Another intriguing finding is the asymmetry of urinary tract involvement, with 21% of the patients presenting with a solitary kidney in adulthood. A single kidney in *HNF1B* patients may result from a progressive atrophy of a multicystic dysplastic or hypoplastic kidney.[11] However, a previous study found that *HNF1B* mutation accounts for only 8% of unilateral solitary kidney.[22] Lastly, subtle changes of the urinary tract consisting of clubbing or tiny diverticulae of calices and ureteric strictures were not found in this study, in contrast with our earlier experience with MODY5 patients.[5,12] These changes are best detected by intravenous pyelography or magnetic resonance urography and were possibly overlooked.

In this adult *HNF1B* cohort, we identified hypomagnesemia in 62% and hypokalemia in 46% of the tested patients. Both were related to inappropriate renal loss. The prevalence of hypomagnesemia is in line with the 44% rate recently reported in *HNF1B* children.[12] The kidney is involved in the fine-tuning of magnesium homeostasis; thus, *HNF1B* nephropathy should now be considered among the eight monogenic forms of renal hypomagnesemia identified at the molecular level.[25] The mechanism leading to hypomagnesemia is ascribed to direct control of *FXYD2* gene expression by *HNF1B*.[12] Mutations of *FXYD2* are also responsible for autosomal-dominant renal hypomagnesemia with hypocalciuria.[25] However, four patients in this study had hypomagnesemia without concomitant hypocalciuria.

A key finding in this report is that nearly half of our patients presented with hypokalemia (<3.5 mmol/l), despite the fact that the majority of cases were chronic kidney disease stage 3–5. The pathophysiology of hypokalemia in *HNF1B* patients is unknown. Hypokalemia was not previously mentioned in *HNF1B* children with hypomagnesemia, nor in previous pediatric series.[11,22] Whether renal potassium wasting in *HNF1B* adult patients results from a specific transcriptional *HNF1B* effect or from long-standing hypomagnesemia has not yet been established. However, refractory hypokalemia and potassium depletion are frequently associated with magnesium deficiency regardless of its mechanism, and may persist until the magnesium deficiency is restored. Recently, Yang *et al.*[26]pointed out that magnesium depletion modulates renal outer medullary potassium channel-mediated potassium secretion. Low serum magnesium and potassium may also be encountered in patients with Gitelman syndrome. In the latter, metabolic alkalosis and salt-losing tubulopathy are part of the metabolic changes, but were not observed in *HNF1B* patients. Furthermore, the genes responsible for Gitelman syndrome (that is, *SLC12A3* and *CLCNKB*) are not predicted to be target gene of *HNF1B*.[12] Future studies using a large-scale approach (that is, chromatin immunoprecipitation followed by DNA microarray or high-throughput sequencing) should test whether genes involved in the molecular machinery of renal potassium handling are involved in the *HNF1B* transcriptional network.

Surprisingly, two patients exhibited both distal tubular defects and a generalized dysfunction of proximal tubular function resulting in renal Fanconi syndrome. Renal Fanconi syndrome is a well-established complication of *HNF1A* mutations in both mice and humans.[27,28] In the latter, pancreatic involvement is responsible for MODY3. In our patients, digenism involving both *HNF1A* and *HNF1B* was ruled out. Whether *HNF1B* plays a direct role in the adult human proximal tubule is currently unknown. In rodents, *Hnf1b* binds DNA as a homodimer or heterodimer with the related factor *Hnf1a*. Activity of either partner may differentially affect the target genes.[29] Should this occur in humans, and if both patients carried a point mutation, a dominant-negative effect of *HNF1A/HNF1B* heterodimers could account for the phenotype. Alternatively, interacting genes may participate in phenotypic heterogeneity.

Chronic renal failure was found in 92% of the patients at a median age of 35 years. The severity of kidney failure was related neither to genetic changes (deletion vs point mutation) nor to MODY phenotype. A larger cohort should be assessed to confirm these findings. In this cohort, no renal biopsy was performed. Suggestive findings in *HNF1B* adults with MODY5 may include oligomeganephronia, glomerular cysts, or tubular dilatation.[5] Although the age at end-stage renal disease remains largely unpredictable, the slope of renal decline exhibited two main characteristics. First, the median decline was slow (−2.45 ml/min/year). This figure is nearly identical to our previous experience in 8 patients with MODY5 (−1.1 ml/min).[5] Second, *HNF1B* patients may experience acute unexplained worsening of renal failure. Whether a defective renal tubular repair following acute kidney injury is involved in human *HNF1B*-mutated kidney cells should be assessed. Indeed, mice with renal-specific *HNF1B* inactivation develop tubular cysts and renal failure within 3 weeks following acute kidney injury.[30] Altogether, the prevalence rate of end-stage renal disease in *HNF1B* adult patients was 3% among 28 MODY5 adults,[6] and 15% in this series.

HNF1B patients are good candidates for kidney transplantation. However, they are at risk of developing early new-onset diabetes post transplantation, as exemplified by two cases in this series. In nondiabetic individuals, a tailored immunosuppressive regimen should optimally avoid tacrolimus and reduce corticosteroid dosage to minimize the risk of developing diabetes mellitus.[31] In diabetic *HNF1B* patients with end-stage renal failure, simultaneous pancreatic and kidney transplantation should be considered.

Last, one female patient developed a renal solid tumor at age 20 years. The patient has so far declined surgery. Intriguingly, the occurrence of a chromophobe renal cancer has been previously described in two *HNF1B* patients at 34 and 54 years of age and regular screening was advocated.[32] In this cohort, no renal cancer was detected, even in renal graft recipients.

Extrarenal phenotype

Extrarenal manifestations are efficient tools for diagnosing many renal disorders, including inherited conditions. Given the wide range of renal phenotypes in *HNF1B* disease, recognition of extrarenal signs is critical. Although no systematic assessment was performed in this retrospective study, extrarenal involvement deserves four comments. First, liver test abnormalities were identified in 40% of cases as compared with 85% in adult patients with the MODY5 phenotype.[5,6,10] Whether the hepatic course will remain indolent in the long term is unknown. Indeed, liver-specific *Hnf1b* deletion in mice results in severe jaundice, paucity of intrahepatic bile ducts, and lack of interlobular arteries.[33]

Second, both exocrine and endocrine pancreatic function can be affected. The rate of pancreatic atrophy in a previous study of adults with MODY5 was 83%.[5] In this series, 30% were found to have pancreatic atrophy, while diabetes mellitus was found in only 48% of the patients. Thus, diabetes mellitus should no longer be regarded as a prerequisite to diagnose *HNF1B* disease, even in adulthood. The presentation of diabetes was not uniform. Two patients came to attention because of diabetic ketoacidosis, while 11 presented with typical MODY. On follow-up, diabetes mellitus had a slowly progressive course. By the last visit, 69% of diabetic individuals required insulin but none developed diabetic retinopathy or neuropathy, suggesting that *HNF1B* patients may be protected against microvascular disease as previously noted in MODY5 individuals.[5,10] In keeping with this hypothesis, diabetic glomerulopathy has not been

observed in kidney specimens from MODY5 patients.[5,10] Whether all *HNF1B* individuals will ultimately develop diabetes mellitus is not yet known.

Third, we found a 42% rate of genital tract abnormalities in *HNF1B* females, with bicornuate uterus emerging as the most frequent manifestation. Bicornuate, rudimentary uterus, single or absent ovaries, vaginal atrophy, and absent genitourinary tracts have all been previously reported.[5,10,21,34] Recently, Oram *et al.*[34] found an 18% rate of *HNF1B* mutations in patients with both renal and uterine malformations.

Finally, mild but obvious mental retardation was recognized in three patients, including two with missense mutations. Whole-gene *HNF1B* deletion or duplication may be associated with autistic features[35,36] or mental retardation with epilepsy and focal cortical dysplasia.[37,38] Whether neurological involvement is part of the spectrum of *HNF1B* disease is not yet elucidated, as other causes of cognitive impairment were not ruled out.

In summary, this unique series unveils a previously undescribed aspect of renal involvement in adult patients with *HNF1B* disease. Despite its shortcomings, data from this study should improve prompt recognition of *HNF1B* disease in adulthood and prove useful for renal management and genetic counseling.

MATERIALS AND METHODS

Recruitment

Participants were recruited from four adult renal units in France and Belgium (university hospitals in Toulouse, Clermont-Ferrand, and Paris (Tenon Hospital) and Cliniques Universitaires Saint-Luc in Brussels). Inclusion criteria included positive genetic testing for *HNF1B* and age $\geqslant$16 years. As no consensus has yet been reached on when to test for *HNF1B* mutation, indications for testing during the enrollment period included the presence of at least one of the following four conditions: (1) renal disease with multisystem involvement (diabetes with a MODY phenotype, pancreatic hypoplasia, unexplained elevated liver enzymes, or genital tract abnormalities); (2) renal disease of unknown origin but concomitant findings suggestive of autosomal-dominant inheritance ($\geqslant$1 first-degree relative with unexplained renal disease); (3) unexplained renal disease presenting with hypodysplasia and renal cysts; or (4) family screening in first-degree relatives of *HNF1B* mutation carriers.

Informed written consent was obtained from all subjects and tested relatives, and the study was conducted in agreement with the Declaration of Helsinki Principles. A total of 27 patients from 20 unrelated families with *HNF1B* mutations were identified. With the exception of two individuals from family 3 who exhibited an uncommon renal phenotype,[17] none of the 27 adult patients recruited for this study have been reported so far.

HNF1B genetic analysis

Genomic DNA was extracted from peripheral lymphocytes using standard procedures. Genetic analysis first assessed *HNF1B* (reference sequence NM_00458.2) rearrangement by quantitative multiplex PCR of short fragments, followed by direct sequencing of the 9 exons and exon–intron boundaries of the *HNF1B* gene, including individuals without quantitative multiplex PCR of short fragment abnormalities as recommended.[6] Genetic testing was conducted by three genetic laboratories in Paris, Brussels, and Toulouse. Family relationships were not tested in this study.

For this report, we excluded three families who presented with an *HNF1B*-like phenotype but had missense nucleotide variants in the *HNF1B* gene (p.Gly76Cys, $n=2$; p.Val25Leu, $n=1$) currently considered to be polymorphisms unlikely to cause *HNF1B* disease[12] (C Bellanné-Chantelot, personal communication).

Clinical evaluation

Renal and extrarenal involvement in the probands was recorded using a standardized assessment of hospital records. Hypertension was defined by blood pressure $>$140/90 mm Hg or the use of antihypertensive medication. Hematuria was assessed by urinary dipstick analysis. eGFR was estimated using the simplified Modification of Diet in Renal Disease (MDRD) formula.[39] Renal failure was defined by eGFR $<$60 ml/min per 1.73 m^2. In patients on renal replacement therapy, eGFR was arbitrarily set at 0. The rate of decline of renal function was analyzed in a subgroup of patients who had $\geqslant$2 eGFR measurements available more than 1 year apart, expressed as ml/min per 1.73 m^2 per year. Hypokalemia and hypomagnesemia were defined by serum potassium levels $<$3.5 mmol/l and serum magnesium levels $<$0.75 mmol/l, respectively. No proband was treated with any medication that could affect serum levels of potassium or magnesium. With the exception of presenting manifestation, gouty attack and uric acid values were not taken into consideration. Imaging studies of the kidney consisting of ultrasonography, computed tomography, or magnetic resonance imaging were reviewed. For the sake of clarity, the latest imaging test was considered in this report. However, consecutive imaging assessments provided sequential data on kidney morphology in six individuals. Renal imaging was categorized according to cystic involvement (0, $<$5, or $\geqslant$5 cysts/kidney) and renal length (increased ($>$2 s.d. for age), normal, or small ($<$2 s.d. for age)). Diabetes was diagnosed on the basis of documented treatment with insulin and/or oral hypoglycemic agents, or biochemical evidence of diabetes in accordance with the World Health Organization guidelines. To avoid misclassification related to phenocopies in relatives who were not available for genetic testing, two at-risk relatives presenting with diabetes above 40 years of age were considered noncarrier individuals (for details, see Figure 1, families F5, individuals specified with gray square). Genital tract abnormalities were assessed using magnetic resonance imaging or ultrasonography.

Family assessment

All probands were interviewed regarding the presence of renal disease or diabetes in at-risk relatives by the referring clinician. Genetic screening and minimal clinical testing (fasting plasma glucose and serum creatinine, and kidney ultrasonography) were offered to all first-degree relatives regardless of their clinical status.

Statistical analysis

Characteristics of the study cohort are expressed using descriptive statistics as median; minimum and maximum; and frequencies (%) for discrete measures. Continuous variables were compared using the Mann–Whitney test. Statistical significance was accepted as $P<0.05$.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Table S1. *HNF1B* missense mutations identified in this cohort.

Table S2. Extrarenal involvement in 27 adult patients with *HNF1B* mutation.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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Massively Enlarged Polycystic Kidneys in Monozygotic Twins With *TCF2*/HNF-1β (Hepatocyte Nuclear Factor-1β) Heterozygous Whole-Gene Deletion

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TCF2, the gene encoding for hepatocyte nuclear factor 1β, is involved in early renal development. Mutations in *TCF2* lead to heterogeneous renal phenotypes. Antenatal ultrasonography may show unilateral/bilateral hyperechogenic or enlarged cystic kidneys. In children or adults, cystic renal hypoplasia/dysplasia is a common feature, occasionally associated with maturity-onset diabetes of the young type 5 and genital tract abnormalities. We report an unusual presentation characterized by massively enlarged polycystic kidneys mimicking autosomal dominant polycystic kidney disease in monozygotic twins. Bilateral enlarged cystic kidneys were discovered in week 13 of a gemellic pregnancy. Postnatally, kidney size increased in both children, reaching 16 cm at 20 years. Nephromegaly was associated with bilateral cysts and a slowly decreasing glomerular filtration rate (40 mL/min/1.73 m^2 at 20 years). There was neither pancreatic nor genital malformation. Non–type 1 diabetes mellitus was diagnosed incidentally in both twins at 20 years. Knowledge of early-onset diabetes (at age 19 years) in their father prompted us to search for the *TCF2* mutation. Genetic analysis showed complete *TCF2* heterozygous whole-gene deletion in both twins. Genetic testing could not be performed in the father. Bilateral massively enlarged polycystic kidneys mimicking autosomal dominant polycystic kidney disease in young adults may be related to *TCF2* mutation. Although uncommon, this new phenotype enlarges the clinical spectrum of kidney involvement associated with *TCF2* mutation. In this case, maturity-onset diabetes of the young–type diabetes paved the way to accurate diagnosis.
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INDEX WORDS: Hepatocyte nuclear factor-1β; HNF-1β; *TCF2*; polycystic kidney disease.

Hepatocyte nuclear factor-1β (variant hepatocyte nuclear factor 1) is a transcription factor that regulates tissue-specific gene expression in the kidneys, liver, pancreas, and genital tract.[1-3] Hepatocyte nuclear factor-1β is encoded by the *TCF2* gene. It is involved in early renal development. In humans, heterozygous mutations in *TCF2* produce a wide clinical spectrum encompassing maturity-onset diabetes of the young type 5 (MODY5), exocrine pancreatic failure and pancreatic atrophy, renal and genital malformations, and liver test result abnormalities.[4] MODY is a genetically heterogeneous form of monogenic diabetes mellitus characterized by: (1) early onset before age 25 years; (2) absence of features of type 1 diabetes mellitus, with C-peptide positivity; and (3) autosomal dominant inheritance across at least 3 generations. Currently, 7 genes have been implicated in MODY families. MODY5 is related to *TCF2* mutations. MODY5 may occur between the first and sixth decades, with progressive requirement for insulin therapy,[5] and de novo mutation is not uncommon.

Renal involvement related to *TCF2* mutation recently was established as the leading cause of bilateral renal hypoplasia/dysplasia in children.[6] However, clinical presentation is extremely heterogeneous and can be recognized in utero or in early childhood, presenting as unilateral or bilateral renal hypoplasia/dysplasia or unilateral or bilateral cystic (hyperechogenic) renal disease detected by means of fetal sonography.[7] In children with early presentation, progressive de-

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crease in renal size was documented in a historic cohort.[8] In adulthood, *TCF2*-related disease should be suspected when facing slowly progressive nondiabetic glomerulopathy with or without renal cysts and urinary tract malformation (unilateral kidney agenesis, pelviureteric junction obstruction, or ureteric stricture) in patients with MODY5[4] or poorly characterized dominantly inherited nephropathy, with or without early gout. Very rare patients with germinal mutation of *TCF2* may develop chromophobe renal cell carcinoma, and in 2 cases, biallelic inactivation of *TCF2* within the tumor was shown.[9]

TCF2 alterations responsible for human disease are heterogeneous. In a recent series of 28 families with MODY5 and positive test results, point mutation accounted for 18 of 28 cases (64%), whereas heterozygous whole-gene deletion was found in 9 of 28 patients (32%). In this series, gross rearrangements of *TCF2* were not associated with a specific phenotype.[10]

Here, we report monozygotic twins with a diagnosis of hyperechogenic kidneys in utero who presented at age 20 years with renal failure and massively enlarged cystic kidneys mimicking autosomal dominant polycystic kidney disease (ADPKD) caused by deletion of the *TCF2* gene.

CASE REPORT

A 22-year-old woman was referred at week 30 of her first pregnancy for evaluation of bilateral hyperechogenic kidneys in monochorionic (monozygotic) twins. Imaging study of both fetuses also showed increased kidney size (>2 SDs for age) and bilateral renal cysts within the cortex and medulla. Ultrasound study findings otherwise were normal (especially amniotic fluid). The parents were not consanguineous, and parental screening by means of ultrasonography failed to show renal cysts. The father had developed diabetes mellitus in his second decade, but later was lost to follow-up.

Postnatally, both twin sisters were hospitalized at 1 month of life for failure to thrive. Defective urinary concentration and moderate metabolic acidosis related to renal proximal tubular acidosis were found in both. Serum creatinine levels were 0.45 and 0.46 mg/dL (40 and 41 μmol/L), with glomerular filtration rate assessment of 63 and 72 mL/min/1.73 m^2 (1.05 and 1.20 mL/s/1.73 m^2) according to the Schwartz formula. Ultrasonography showed persistent increased-sized bilateral hyperechogenic polycystic kidneys. A diagnosis of autosomal recessive polycystic kidney disease was suspected. Liver test and liver sonography results in both children were normal. Treatment consisted of oral bicarbonate supplementation and water supply. At age 7 years, height development was normal, with stable renal function despite progressive increase in kidney size. Both sisters were then lost to follow-up.

Table 1. Biological Characteristics of Kidney, Liver, and Endocrine Pancreas in 20-Year-Old Monozygotic Twins With *TCF2* Heterozygous Whole-Gene Deletion

Biological Test Results (normal values)	Twin 1	Twin 2
Serum creatinine (65-120 μmol/L)	195	164
Uric acid (200-420 μmol/L)	440	457
Bicarbonate (21-30 mmol/L)	20	24
Proteinuria (dipstick)	0	0
Hematuria (dipstick)	0	0
Alanine aminotransferase (3-35 U/L)	19	15
Aspartate aminotransferase (5-45 U/L)	17	12
γ-Glutamyl transpeptidase (11-60 U/L)	17	15
Alkaline phosphatase (100-280 U/L)	157	139
Bilirubin (2-21 μmol/L)	10	11
Fasting glucose (4.1-5.1 mmol/L)	7	8.3
Hemoglobin A_{1c} (4%-6%)	5.7	6.3
Basal C-peptide (0-1 nmol/L)	1	1

Note: To convert serum creatinine in μmol/L to mg/dL, divide by 88.4; uric acid in μmol/L to mg/dL, divide by 59.48; glucose in mmol/L to mg/dL, divide by 0.05551; C-peptide in nmol/L to ng/mL, divide by 0.333; bicarbonate in mEq/L and mmol/L is equivalent.

At age 20 years, increased blood fasting glucose levels incidentally were found in each twin. Additional investigation prompted a diagnosis of diabetes mellitus in 1 twin and blood glucose intolerance in the other (Table 1), without diabetic retinopathy. Body mass index was not increased (24 kg/m^2), and immune markers for type 1 diabetes (anti-islet, antiglutamic acid decarboxylase, and anti-insulin antibodies) were negative. They were advised on diet and lifestyle modification.

Simultaneously, renal involvement was characterized by bilateral palpable cystic kidneys, normal blood pressure, and moderate renal failure without proteinuria or hematuria by means of dipstick testing (Table 1). Abdominal magnetic resonance imaging showed massively enlarged kidneys (maximal height in each twin, 145 and 160 mm) with multiple corticomedullary cysts modifying kidney contours and mimicking autosomal dominant polycystic kidneys (Fig 1, twin 1; imaging study was similar in her sister). Maximal diameter of the cysts reached 62 and 37 mm, respectively. One sister still showed distal renal tubular acidosis. Liver test results were normal in both twins, whereas magnetic resonance imaging failed to detect pancreas atrophy. Fecal elastase concentration was not assessed. No genital malformation was detected. On last follow-up, at age 21 years, serum creatinine levels reached 3.0 and 2.3 mg/dL (268 and 203 μmol/L; creatinine clearance according to the Cockcroft-Gault formula, 29 and 35 mL/min/1.73 m^2 (0.48 and 0.58 mL/s/1.73 m^2).

The association of early polycystic kidney disease and MODY phenotype prompted genetic testing of *TCF2*. After informed consent was received from the index cases and their mother, blood samples were tested first for the search of a large genomic rearrangement using the quantitative multi-

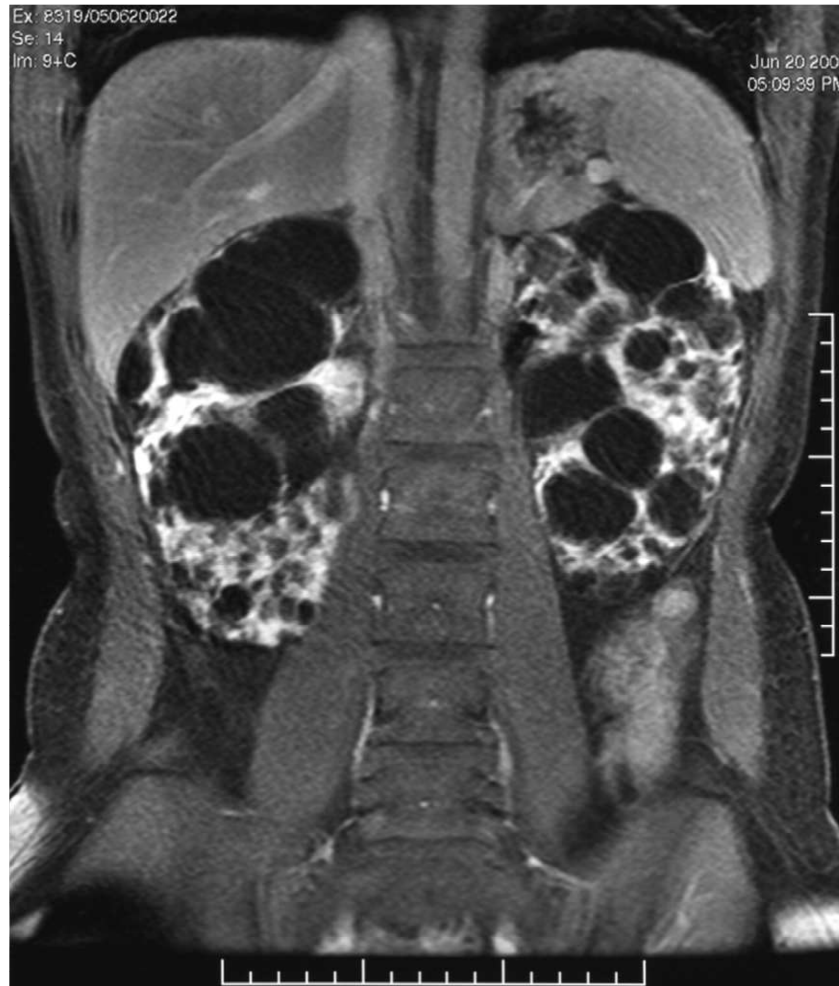

Figure 1. Abdominal MRI in a 20-year-old monozygotic twin with heterozygous *TCF2* whole-gene deletion showing massively enlarged polycystic kidneys. Note the absence of liver cyst.

plex polymerase chain reaction amplification of short fluorescent fragments technique described previously.[11] A complete deletion of the entire *TCF2* gene (p.Met1_Trp557del) was shown in the twins, but not their mother. Genetic testing could not be performed in the father. Neither *PKD1* nor *PKD2* was sequenced in this family.

DISCUSSION

Clinical presentation of renal involvement at age 20 years in these twins was highly suggestive of severe polycystic kidney disease. Given the increased size of both kidneys and bilateral cysts in the cortex and medulla, a diagnosis of ADPKD could be considered. However, such severe renal decline is very uncommon in this age range, in both *PKD1* and *PKD2* individuals. In addition, the lack of cystic kidney in the family was puzzling, although a 5% rate of de novo mutation is found in ADPKD families. Alternatively, increased kidney size, multiple cysts with significant renal failure, and the absence of family history suggested a diagnosis of autosomal recessive polycystic kidney disease.[12] In this setting, liver involvement on a pathological specimen is a constant finding, but some patients may lack specific symptoms and imaging abnormality results, and liver test results may be in the normal range.[13] In this family, the seminal finding of non–type 1 diabetes in the probands and their father prompted consideration of a diagnosis of MODY. Because MODY5 is the only monogenic diabetes with morphological kidney changes, we considered *TCF2* mutation as the more likely diagnosis in this family. Because *TCF2* heterozygous whole-gene deletion is responsible for 50% of mutations identified in patients with MODY5 and renal involvement,[10] we applied quantitative

Table 2. Differential Diagnosis of Large Cystic Kidneys in Infants and Children

Disease (inheritance)	Gene	Diabetes Mellitus
Polycystic kidneys without renal dysplasia		
ADPKD (AD)	*PKD1 (? PKD2)*	No
ARPKD (AR)	*PKHD1*	No
Tuberous sclerosis complex (AD)	*TSC2-PKD1* (contiguous gene syndrome)	No
TCF2/HNF1β-related nephropathy (AD)	*TCF2* (uncommon)	Yes (MODY5)
Cystic kidneys with multiple malformation syndrome		
Orofaciodigital syndrome, type 1 (X-linked)	*OFD1*	No
Bardet-Biedl syndrome (AR)	*BBS1-BBS8*	No
Meckel-Gruber syndrome (AR)	*MKS1* and *MKS3*	No
Zellweger syndrome (AR)	*PEX1*	No
Ivemark syndrome (AR)	Not identified	No
CDG syndrome (AR)	Multiple genes	No

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; CDG, congenital disorder of glycosylation syndrome; HNF1β, hepatocyte nuclear factor 1β; AD, autosomal dominant; AR, autosomal recessive.

multiplex polymerase chain reaction amplification of short fluorescent fragments as the first-line genetic test. A complete deletion of the entire *TCF2* gene (p.Met1_Trp557del) was shown in the twins. No further sequencing was applied to samples. Whether the deletion was a de novo mutation or inherited from the father could not be tested.

Interestingly, the phenotype of bilateral hyperechogenic kidneys incidentally found during pregnancy in the twins is now recognized as a common presentation in *TCF2*-mutated individuals (Table 2). For instance, in a French pediatric cohort including 25 patients with *TCF2* mutation–related multicystic kidneys on prenatal sonography, a *TCF2* heterozygous whole-gene deletion was found in 13 patients (52%), although the corresponding figure for ADPKD and autosomal recessive polycystic kidney disease was not provided.[7] Conversely, renal involvement leading to massively enlarged polycystic kidneys mimicking polycystic kidney disease was not yet reported in *TCF2*-mutated individuals and enlarges the clinical spectrum of renal involvement in adult patients. The latter includes unilateral kidney agenesis, a number of subtle changes of the urinary tract, glomerulocystic disease, and bilateral shrunken kidneys with or without cysts.

The finding of polycystic kidneys is not unexpected in patients with *TCF2* mutation. Gresh et al[14] showed that rodents with renal-specific biallelic inactivation of *Tcf2* developed cystic kidneys in vivo with downregulation of 2 cystic genes, *Pkd2* and *Pkhd1*, the latter being the ortholog of the gene involved in the autosomal recessive form of human polycystic kidney disease.[15] Whether such gene hierarchy also applies to humans with heterozygous autosomal dominant *TCF2* mutation requires further studies. However, it is interesting to emphasize that in $TCF2^{-/-}$ chromophobe cell carcinomas from patients carrying a germinal mutation, dramatic suppression of *PKHD1* and *UMOD* messenger RNA expression could be shown, whereas *PKD2* expression remained unaffected.[9]

In conclusion, our findings suggest that *TCF2* deletion may promote a huge cystic renal phenotype in teenagers and young adults, and this diagnosis may be considered in these patients.

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Spectrum of *HNF1B* Mutations in a Large Cohort of Patients Who Harbor Renal Diseases

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Background and objectives: Hepatocyte nuclear factor 1β (HNF1β) is a transcription factor that is critical for the development of kidney and pancreas. In humans, mutations in *HNF1B* lead to congenital anomalies of the kidney and urinary tract, pancreas atrophy, and maturity-onset diabetes of the young type 5 and genital malformations.

Design, setting, participants, & measurements: We report *HNF1B* screening in a cohort of 377 unrelated cases with various kidney phenotypes (hyperechogenic kidneys with size not more than +3 SD, multicystic kidney disease, renal agenesis, renal hypoplasia, cystic dysplasia, or hyperuricemic tubulointerstitial nephropathy not associated with *UMOD* mutation).

Results: We found a heterozygous mutation in 75 (19.9%) index cases, consisting of a deletion of the whole gene in 42, deletion of one exon in one, and small mutations in 32. Eighteen mutations were novel. *De novo* mutations accounted for 66% of deletions and 40% of small mutations. In patients who carried *HNF1B* mutation and for whom we were able to study prenatal ultrasonography (56 probands), isolated hyperechogenic kidneys with normal or slightly enhanced size were the more frequent (34 of 56) phenotype before birth. Various other prenatal renal phenotypes were associated with *HNF1B* mutations, at a lesser frequency. Diabetes developed in four probands. Hyperuricemia and hypomagnesemia, although not systematically investigated, were frequently associated.

Conclusions: This large series showed that the severity of the renal disease associated with *HNF1B* mutations was extremely variable (from prenatal renal failure to normal renal function in adulthood) and was not correlated with the genotype.

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Hepatocyte nuclear factor 1β gene (*HNF1B*) encodes a transcription factor that binds DNA as homodimer or as heterodimer with the related factor HNF1α. Heterozygous mutations of *HNF1B* were first described in maturity-onset diabetes of the young type 5 (1). Renal manifestations are frequently observed in patients with maturity-onset diabetes of the young type 5 and include a wide spectrum of phenotypes (2). More recently, *HNF1B* mutations were found to be associated with a subset of fetal bilateral hyperechogenic kidneys (3) and other kidney diseases diagnosed before birth (4). Besides diabetes, nonrenal anomalies involving Mullerian and Wolffian derivatives, liver and pancreas abnormalities, hyperuricemia with or without gout (5), and hypomagnesemia (6) have been reported.

HNF1B plays a crucial role in early development (7) and thereafter is involved in the organogenesis of several tissues, such as gut, pancreas, liver, lung, and kidney. The gene is also transiently expressed in the neural tube and in the epididymis, vas deferens, seminal vesicle, prostate, uterus, and oviduct (7,8). During kidney development, the gene is expressed in the ureteric bud, in the comma- and S-shaped bodies, and then in the proximal and distal tubules but not in the glomerulus (9). Kidney-specific inactivation of *Hnf1B* in the mouse leads to cystic disease, and HNF1β was shown to bind directly DNA elements that regulate the expression of genes whose mutations are responsible for cystic kidney diseases (*Nphp1*, *polaris, Umod*, *Pkhd1*, and *Pkd2*) (10) or of a gene identified as a candidate modifier in a mouse model of cystic kidney disease (*Kif12*) (11). Here we report on *HNF1B* mutation screening in a series of 377 unrelated patients who presented with various kidney phenotypes, giving special attention to the prenatal renal phenotypes.

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Materials and Methods

Patients

This is a retrospective study in which we included all cases that were not previously reported and were tested for *HNF1B* mutations in two reference centers for rare kidney diseases in France. Criteria for inclusion were hyperechogenic kidneys (but with size not more than +3 SD), uni- or bilateral multicystic kidney disease (MCD), renal agenesis, renal hypoplasia, cystic dysplasia, or hyperuricemic tubulointerstitial nephropathy not associated with *UMOD* mutation. Patients' samples, medical records, genealogy, and written informed consent from patient and/or parents were sent from Pediatric, Pediatric Nephrology, Nephrology, or Obstetric Departments. Genomic DNA was extracted from venous blood or tissues collected from 377 unrelated cases (271 children, 57 adults, and 49 fetuses), 221 male and 156 female.

Prenatal ultrasonographs were available for 245 probands (usually performed at 12, 22, and 32 weeks of amenorrhea) and had been considered as normal in only 11 cases. Renal phenotypes before birth were isolated hyperechogenic kidneys (not larger than +3 SD in size) in 55 cases, bilateral MCD (13 cases), unilateral MCD (74 cases), unilateral agenesis (34 cases), bilateral agenesis (13 cases), renal hypoplasia (25 cases), urinary tract dilation (11 cases), and cystic disease (nine cases). In 132 patients, either the result of the prenatal ultrasound was not known or ultrasound was not performed (patients born before 1980). Renal phenotypes after birth were hyperechogenic kidneys (23 cases), unilateral MCD (12 cases), unilateral agenesis (8 cases), renal hypoplasia (33 cases), urinary tract dilation (2 cases), hyperuricemic tubulointerstitial nephritis (18 cases), unclassified cystic disease (35 cases), and only extrarenal symptoms (diabetes and uterine abnormalities; one case).

Patients with renal cavity dilation and/or recurrent acute pyelonephritis had voiding cystourethrogram. GFR was estimated by the Modification of Diet in Renal Disease (MDRD) formula for adults and by the Schwartz formula for children who were younger than 16.

Molecular Analysis

Quantitative multiplex PCR amplification of short fluorescence fragments (12) was performed as described previously (13) for the search of deletion. When deletion was not found, the nine exons and the exon–intron boundaries of the gene were screened for mutations by direct sequencing as described previously (1).

Statistical Analysis

Testing for difference in proportions was performed using the χ^2. All tests were two sided. $P < 0.05$ was considered significant.

Results

Mutations

Heterozygous *HNF1B* alterations, which are thought to be pathogenic, were found in 75 probands (41 male and 34 female), leading to a mutation detection rate of 19.9% of tested index cases. They consisted of a heterozygous deletion of the entire gene in 42 cases (Table 1). Parent status was studied for 21 probands: deletions were *de novo* in 14 of 21 cases and inherited in seven of 21. Mutations that were not deletions of the entire gene are shown in Table 2. One patient was carrying a *de novo* heterozygous deletion of exon 4, which was previously reported (3,13). Twenty-four different heterozygous small mutations (11 missense, five nonsense, five frameshift, and three splice site mutations) were found in 32 probands. Parent status was studied for 20 of them. Mutation were shown to be *de novo* in eight of 20 cases and to be inherited in 12 of 20 cases; 18 were novel. Except for the mutation affecting the initiator codon, all missense mutations were localized in the DNA binding domain (Figure 1), were modifying a conserved amino acid, and were predicted to be probably damaging by the Polyphen program (14). In some families, there was a father-to-son transmission, in agreement with an autosomal dominant mode of inheritance (see proband 64 as an example).

Renal and Extrarenal Phenotype

Patients for Whom Prenatal Ultrasound Was Available

In 245 cases tested for *HNF1B* mutation, we were able to go back to the prenatal ultrasound. Mutations were identified in 56 of them.

Prenatal phenotype in patients with *HNF1B* mutation was isolated bilateral hyperechogenic kidneys with normal or moderately enlarged size in 34 cases, including one termination of pregnancy (TOP) because of an associated oligo-anamnios. Evaluation of these patients at last follow-up showed renal failure with GFR <80 ml/min per 1.73 m^2 (range 32 to 61 ml/min per 1.73 m^2) in eight patients (1 months to 14 years old), GFR >80 ml/min per 1.73 m^2 in 20 patients (1 to 17 years old), and unknown in five patients. Five patients experienced transitory renal failure at birth, and one developed diabetes at the age of 17.

Other prenatal phenotypes in patients with *HNF1B* mutation were bilateral MCD (leading to TOP) in two patients, unilateral MCD in eight patients, unilateral renal agenesis (with hypoplasia and/or cysts on the single kidney) in four patients, unilateral renal hypoplasia in one patient, renal macrocysts in three patients (with urinary tract dilation, pancreas hypoplasia, and TOP in one patient), and isolated upper urinary tract dilation in one patient (who developed small cortical cysts after birth). In three patients who presented with severe cystic dysplasia on early ultrasound, the prenatal ultrasounds were considered as normal. In all cases with unilateral MCD, patients developed postnatal anomalies on the contralateral kidney. In the case with unilateral hypoplasia, cysts developed on the hypoplastic kidney after birth

Patients for Whom Prenatal Ultrasound Was not Available

In 132 patients who were tested for *HNF1B* mutation, we were not able to go back to prenatal ultrasound (either the result of it was not known, or ultrasound was not performed). We found an *HNF1B* mutation in 19 of them, including 10 who were tested during adulthood, six of whom had a family history of renal diseases. Four adult probands had cystic renal hypoplasia (associated with hypomagnesemia, gout, and a diabetes that occurred at 42 years in one and with gestational diabetes in another). Two had hyperechogenic kidneys with microcysts. One had solitary kidney and early gout, and another one had hyperuricemic interstitial nephropathy. One female born from consanguineous parents developed unclassified renal cystic dysplasia with uterus agenesis, imperforated vagina, cleft palate, and mental retardation. One presented with diabetes at the age of 31 and bicornuate uterus. Four adults (aged 29 to 35 years) had normal renal function and five (aged 28 to 33 years) had reduced GFR (65 ml/min per 1.73 m^2

Table 1. Phenotypes in probands with complete *HNF1B* deletions

Probands	Prenatal Renal Phenotype	Postnatal Renal Phenotype	Deletion Inheritance
1	Normal ultrasound	Large kidneys with numerous bilateral cysts, preterminal renal failure at 8 months; father with renal hypoplasia, GFR unknown	ND (parents not tested)
2	Normal ultrasound	Hyperechogenic and cystic kidneys, normal GFR at 6 years	*De novo*
3	Bilateral hyperechogenic kidneys	Hyperechogenic, normal-sized kidneys, normal GFR at 10 months	ND (parents not tested)
4	Bilateral hyperechogenic kidneys	Hyperechogenic kidneys, multiple microcysts, CRF (GFR 29 at 6 years); mother with renal cysts.	Deletion in the mother
5	Bilateral hyperechogenic kidneys	Hyperechogenic, normal-sized kidneys, microcysts, normal GFR at 5 years	ND (parents not tested)
6	Unilateral hypoplasia	Unilateral hypoplasia with cysts, normal GFR at 2 years Father and paternal grandmother with renal cysts	*De novo*
7	Bilateral hyperechogenic kidneys	Small cystic kidneys, normal GFR at 3 years	ND (parents not tested)
8	Bilateral hyperechogenic kidneys	Normal-sized kidney with cortical cysts + left PUJO hyperuricemia, normal GFR at 5 years	ND (parents not tested)
9	Unilateral MCD, contralateral cysts	Unilateral MCD, cortical cysts on contralateral kidney, hyperuricemia, elevated liver enzymes, normal GFR at 10 years	ND (parents not tested)
10	Bilateral hyperechogenic kidneys	Bilateral cortical cysts, normal-sized kidney, normal GFR at 17 months; father with renal hypodysplasia, GFR unknown; paternal grandfather has CRF; previous TOP for MCD and anamnios in the mother	ND (parents not tested)
11	ND	Diabetes, bicornuate uterus TOP in the past because of anamnios, normal GFR at adult age; diabetes in sisters and father	Deletion in the father
12	Bilateral hyperechogenic kidneys	Few cysts, unknown GFR	*De novo*
13	Unilateral MCD, contralateral hyperechogenic kidney	Cysts in the single kidney, normal GFR at 9 years	ND (parents not tested)
14	Bilateral hyperechogenic kidneys, one cortical cyst	Hyperechogenic large (+2 SD) kidneys, CRF (unknown GFR) at 1 month	*De novo*
15	ND	Cystic kidney disease, uterine agenesis, imperforated vagina, mental retardation, normal GFR at 29 years	ND (parents not tested)
16	Bilateral pelvic dilation	Bilateral PUJO, unilateral small cortical cysts, normal GFR at 14 months	*De novo*
17	Bilateral hyperechogenic kidneys	Bilateral cortical cysts, normal GFR at 3 years; brother with pelvic kidney and PUJO; mother with normal kidneys and normal GFR, left hepatic agenesis, pancreas head hypoplasia, bicornuate uterus	Deletion in the mother

Table 1. continued

Probands	Prenatal Renal Phenotype	Postnatal Renal Phenotype	Deletion Inheritance
18	Bilateral hyperechogenic kidneys	Few cysts, normal-sized hyperechogenic kidneys, neonatal renal failure, normal GFR at 20 months; mother with cysts and gestational diabetes	Deletion in the mother
19	Bilateral hyperechogenic kidneys	Bilateral cortical cysts, normal GFR at 5 years; mother with renal cysts and severe cholestasis	Deletion in the mother
20	ND	Bilateral cysts, normal GFR at 11 years	ND (parents not tested)
21	Bilateral hyperechogenic kidneys, cortical cysts (MRI), diaphragmatic hernia	Dedifferentiated kidneys (54 and 58 mm) with cysts, acute renal failure at birth, GFR 40 ml/min per 1.73 m^2 at 2 months	*De novo*
22	Bilateral hyperechogenic kidneys, cortical cysts, oligoamnios	TOP; renal histology showed cystic dilation of nearly all glomeruli with collapsed floculus, glomerular cysts were lined by fibrosis, interstitial fibrosis with rarefied tubules	*De novo*
23	ND	Bilateral hyperechogenic kidneys, cortical microcysts, normal GFR at 17 years	*De novo*
24	ND	Bilateral cortical microcysts, bicornuate uterus, diabetes, normal GFR at 20 years	ND (parents not tested)
25	ND	Bilateral hyperechogenic kidneys, cortical microcysts, normal GFR at 3 years	ND (parents not tested)
26	Bilateral hyperechogenic kidneys, pelvic dilation	Bilateral hyperechogenic hypoplastic kidneys, unknown GFR, microcysts in mother	Deletion in the mother
27	ND	Bilateral hyperechogenic kidneys, cortical microcysts, CRF (GFR 65 at 30 years)	ND (parents not tested)
28	Bilateral hyperechogenic kidneys	Bilateral hyperechogenic kidneys CRF (GFR 40 at 3 years)	ND (parents not tested)
29	ND	Bilateral hyperechogenic kidneys cortical microcysts, normal GFR at 35 years; mother with type 2 diabetes	ND (parents not tested)
30	ND	Bilateral hyperechogenic kidneys, cortical microcysts, normal GFR at 6 years; microcystic sole kidney in mother	ND (parents not tested)
31	Bilateral hyperechogenic kidneys	Bilateral hyperechogenic kidneys, CRF (GFR 35 at 1 year)	ND (parents not tested)
32	Bilateral hyperechogenic kidneys	Bilateral hyperechogenic kidneys, diabetes at 17 years, normal GFR at 20 years	ND (parents not tested)
33	Bilateral hyperechogenic kidneys	Bilateral hyperechogenic kidneys, normal GFR at 1 year	*De novo*
34	Unilateral MCD, other kidney hyperechogenic	Unilateral MCD, other kidney hyperechogenic with pelvic dilation, normal GFR at 3 years	*De novo*
35	Bilateral hyperechogenic kidneys	Bilateral hyperechogenic kidneys, normal GFR at 15 years	*De novo*

Table 1. continued

Probands	Prenatal Renal Phenotype	Postnatal Renal Phenotype	Deletion Inheritance
36	Bilateral hyperechogenic kidneys + unilateral macrocysts	Bilateral hyperechogenic kidneys + unilateral macrocysts, CRF (GFR 55 at 3 years)	*De novo*
37	Unilateral MCD, other kidney hyperechogenic	Unilateral MCD, hyperechogenic kidney, normal GFR at 6 years	*De novo*
38	Bilateral hyperechogenic kidneys, cortical microcysts	Bilateral hyperechogenic kidneys, cortical microcysts, normal GFR at 6 years	*De novo*
39	Unilateral agenesis	Single hyperechogenic kidney, cortical microcysts, normal GFR at 10 years	ND (parents not tested)
40	Unilateral agenesis, hyperechogenic kidney with microcysts	Single hyperechogenic kidney, microcysts, CRF (GFR 23 at 1 year); single kidney with cysts in the mother (GFR 75 at 30 years)	Deletion in the mother
41	Bilateral hyperechogenic kidneys	Bilateral hyperechogenic kidneys, unilateral VUR, unknown GFR	ND (parents not tested)
42	ND	Bilateral hyperechogenic kidneys, cortical microcysts, normal GFR at 3 years	ND (parents not tested)

CRF, chronic renal failure; MRI, magnetic resonance imaging; ND, not done; PUJO, pelvi-ureteric junction obstruction; VUR, vesicoureteral reflux.

to end-stage renal failure), and renal function was unknown for one.

We found an *HNF1B* mutation in nine patients who were tested during childhood, two of whom had a family history of renal disease. Eight probands had hyperechogenic kidney and cysts and one hypoplastic kidney and/or uterus anomalies ($n = 2$) and/or pancreatic hypoplasia ($n = 1$). One developed diabetes at the age of 20 years. Renal function was normal in six patients (aged 3 to 20 years) and altered three times (aged 4 to 15 years).

Genotype–Phenotype Correlation

The severity of the renal disease that is associated with *HNF1B* mutation was extremely variable (from prenatal severe renal failure to normal renal function in adulthood). The type of mutation (deletion of the whole gene; missense mutation; or truncating mutation because of nonsense, frameshift, or splice mutation) was analyzed according to the renal phenotype for the 75 probands who carried an *HNF1B* mutation, as well as for other affected family members when their kidney phenotype was known (Figure 2). The percentage of each type of mutation was not statistically different when the group of patients who had prenatal hyperechogenic kidneys was compared with a group that included all other patients. We also looked for a relation between the type of mutation and the severity of the disease in terms of renal failure, independent of the type of renal disease. The patients with severe and early renal failure (six patients with TOP for oligohydramnios and six patients with terminal or preterminal renal failure that occurred before the age of 4 years) were associated either with deletions (seven patients), truncating mutation (three patients), or missense mutations (two patients), a figure that is not different from the proportion of each type of mutation in all patients. Figure 3 shows the number of patients with and without renal failure for each type of mutation. The proportion of patients with renal failure at last follow-up was significantly ($P = 0.012$) higher in patients who carried a truncating mutation than in patients who carried an *HNF1B* deletion; however, for unknown reasons, patients with truncating mutation were older than patients with gene deletion at last follow-up. This age difference may account, at least in part, for the different severity of the renal failure.

Discussion

To our knowledge, we report here the largest series of phenotypic and genetic analysis of patients who harbor renal diseases that are associated with *HNF1B* mutations. We screened 377 unrelated patients and identified an *HNF1B* mutation or deletion in 75 unrelated cases: 10 adults and 65 children or fetuses. This rate of mutation (19.9%) is not significantly different from that (23%) recently reported in a smaller cohort of children with renal malformation (6). Going back to the prenatal ultrasound when available, we report the renal phenotypes before birth in patients with *HNF1B* mutation and analyzed the evolution of their renal function.

We had information regarding prenatal ultrasound for 245 patients, and this study confirms our previous finding that

Table 2. Mutations and phenotypes in patients with *HNF1B* mutations that are not complete deletions

Probands	Nucleotide Change	Protein Change	Exon (Intron)	Reference	Prenatal Renal Phenotype	Postnatal Renal Phenotype	Mutation Inheritance
43	c.3G→A	p.Met1Ile	1	This study	Bilateral cortical cysts	Bilateral cortical microcysts, normal GFR at 7 years; father with renal cysts	Mutation in the father
44	c.3G→A	p.Met1Ile			Bilateral cortical cysts	Bilateral cortical microcysts, normal GFR at 7 years; father with diabetes and renal cysts	Mutation in the father
45	c.211 delAAGGGCC	p.Lys71fs	1	This study	ND	Hypodysplastic kidneys with microcysts, GFR 45 at 28 years; father with hyperuricemic nephropathy (GFR unknown)	Mutation in the father
46	c.232G→T	p.Glu78X	1	This study	Normal ultrasound	Bilateral cortical cysts, ESRF at 3 months	*De novo*
47	c.232G→T	p.Glu78X	1		MCD ×2	TOP; septated uterus	ND
48	c.322 delG	p.Ala108fs	1	This study	ND	Hyperuricemic nephropathy, ESRF at 33 years; father with hyperuricemic nephropathy (with kidney graft) and diabetes	Mutation in the father
49	IVS1 345–1G→A		(1)	This study	Bilateral hyperechogenic kidneys	Bilateral cortical cysts, neonatal renal failure, normal GFR at 8 years	ND
50	IVS1 345–1G→A		(1)		Unilateral agenesis + hyperechogenic kidney	Single hyperechogenic kidney + CRF (GFR 25 at 17 years)	ND (parents not tested)
51	c.452C→G	p.Ser151Cys	2	This study	Unilateral MCD	Unilateral MCD and cortical cysts on the other kidney, normal GFR at 6 years	ND
52	c.476C→T	p.Pro159Leu	2	This study	Bilateral hyperechogenic kidneys	Isolated hyperechogenic kidneys, ultrasound normalized (size and echogenicity) at 10 months, normal GFR at 10 months	ND

Table 2. continued

Probands	Nucleotide Change	Protein Change	Exon (Intron)	Reference	Prenatal Renal Phenotype	Postnatal Renal Phenotype	Mutation Inheritance
53	c.494 G→A	p.Arg165His	2	(20)	ND	Small hyperechogenic kidneys, CRF (GFR 16 at 4 years); father with renal failure and diabetes	ND
54	c.494G→C	p.Arg165Pro	2	This study	Bilateral hyperechogenic kidneys	Bilateral hyperechogenic hypoplastic kidneys CRF (GFR 32 at 10 years) + pancreatic hypoplasia	*De novo*
55	c.513G→A	p.Trp171X	2	This study	Bilateral hyperechogenic kidneys	Bilateral cortical cysts, normal GFR at 11 months	ND
56	IVS2 544 + 3delAAGT		(2)	(6)	ND	Renal cysts and VUR, normal GFR at 6 years; mother with unilateral cysts and gestational diabetes, normal GFR at 30 years	Mutation in the mother
57	IVS2 544 + 3delAAGT		(2)		ND	Single kidney, gout, CRF (GFR 25 at 65 years); mother and maternal cousin with renal failure; daughter with single kidney	ND
58	IVS2 544 + 3delAAGT		(2)		ND	Cysts, CRF (GFR 60 at 33 years), diabetes, hyperuricemia, elevated liver enzymes, hypomagnesaemia; father with ESRF	ND
59	c.544C→T	p.Gln182X	2	(20)	ND	Hyperechogenic kidneys, CRF (but unknown GFR); diabetes in the mother.	Mutation in the mother
60	c.544C→T	p.Gln182X	2		Unilateral MCD, other kidney with cysts	Unilateral MCD, other kidney with cortical cysts, normal GFR at 3 months	ND
61	c.544C→T	p.Gln182X	2		Unilateral agenesis	Single hypoplastic hyperechogenic kidney, CRF (GFR 55 at 7 years)	ND

Table 2. continued

Probands	Nucleotide Change	Protein Change	Exon (Intron)	Reference	Prenatal Renal Phenotype	Postnatal Renal Phenotype	Mutation Inheritance
62	c.758A→C	p.Gln253Pro	3	(3)	Bilateral MCD	TOP cysts in mother (GFR 62 at 25 years) and grandmother	Mutation in the mother
63	c.717delG	p.Ser242fs	3	This study	Bilateral hyperechogenic kidneys	Hyperechogenic kidneys, cortical microcysts, normal GFR at 1.5 years; father with renal cysts and diabetes	Mutation in the father
64	c.766C→T	p.Pro256Ser	3	This study	Bilateral hyperechogenic kidneys	Bilateral cortical cysts, normal-sized kidneys, normal GFR at 8 years; phenotype in the father unknown	Mutation in the father
65	IVS3 809 + 1G→A		(3)	This study	Bilateral hyperechogenic kidneys, bilateral cysts	Bilateral cortical cysts, unilateral UPJ, neonatal renal failure, normal GFR at 3 years; family history of diabetes; phenotype in the father unknown	Mutation in the father
66	c.840delC	p.Pro280fs	4	This study	Enlarged kidneys, large cysts, pyelic dilation, duplicity, pancreas hypoplasia	TOP	ND
67	c.854G→A	p.Gly285Asp		(17)	Bilateral hyperechogenic kidneys	Bilateral hyperechogenic kidneys + unilateral cortical microcysts, normal GFR at 3 years; mother with renal cysts, GFR 55 at 35 years	Mutation in the mother
68	c.883C→T	p.Arg295Cys	4	(17)	ND	Small and cystic kidneys (unknown GFR); mother with renal cysts and CRF (precise GFR unknown)	Mutation in the mother
69	c.883C→T	p.Arg295Cys	4		Bilateral hyperechogenic kidneys, bilateral cortical cysts	Cortical cysts, hyperuricemia, neonatal renal failure, normal GFR at 8 years	*De novo*

Table 2. continued

Probands	Nucleotide Change	Protein Change	Exon (Intron)	Reference	Prenatal Renal Phenotype	Postnatal Renal Phenotype	Mutation Inheritance
70	c.895T→G	pTrp299Gly	4	This study	Bilateral hyperechogenic kidneys, bilateral cortical microcysts	Bilateral hyperechogenic kidneys, cortical microcysts CRF (GFR 51 at 3 years)	ND
71	c.766C→T	p.Asn302Lys	4	This study	Bilateral hyperechogenic kidneys	Bilateral cortical cysts, CRF (GFR 60 at 7 years)	ND
72	Exon 4 deletion c.810_1045 del236	p.Arg270fs	4	(3,13)	Unilateral MCD	Absence of hypertrophy of the contralateral kidney, VUR, CRF (GFR 65 at 4 years)	*De novo*
73	c.1136C→A	p.Ser379X	5	This study	ND	Hyperechogenic kidneys, cortical microcysts, CRF (GFR 61 at 15 years), didelphic uterus + pancreatic hypoplasia	*De novo*
74	c.1360C→T	p.Gln454X	7	This study	Unilateral MCD, other kidney hyperechogenic	Unilateral MCD, other kidney hyperechogenic with cortical microcysts, normal GFR at 2 years	*De novo*
75	c.delAG1363–1364	p.Ser455fs	7	This study	Bilateral hyperechogenic kidneys	Bilateral cortical cysts, left hypoplastic kidney CRF (GFR 80 at 14 years)	ND

CRF, chronic renal failure; ESRF, end-stage renal failure; UPJ, ureteropelvic junction.

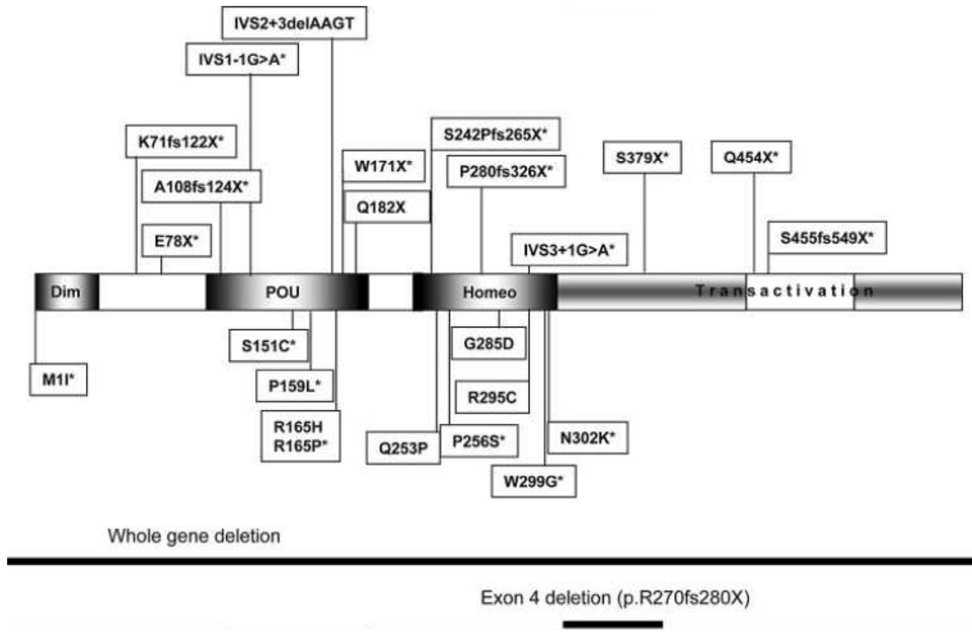

Figure 1. HNF1β protein and localization of the various mutations identified in this study. The N-terminal portion of the protein consists of a short dimerization domain (dim). The DNA-binding domain is characterized by a region distantly related to the POU box-specific domain and an atypical homeodomain structure. The residues required for HNF1β transactivation have been mapped to the carboxy-terminal region. Deletions are indicated by a solid line. *Novel mutation.

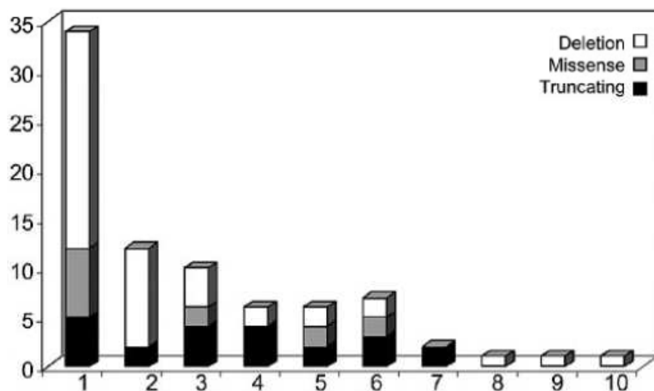

Figure 2. Type of *HNF1B* mutation (□, deletion of the entire gene; ▨, missense mutations; ■, truncating mutations) according to the renal phenotype in patients and affected relatives. 1, prenatal hyperechogenic kidneys; 2, hyperechogenic kidney diagnosed after birth; 3, MCD; 4, unilateral renal agenesis; 5, cystic disease; 6, renal hypoplasia; 7, tubulointerstitial nephritis; 8, pyeloureteral junction; 9, pelvic kidney; 10, lack of renal anomaly.

isolated bilateral hyperechogenic fetal kidneys with normal or slightly enlarged ($\leq$+3 SD) size were the most frequent phenotype observed before birth in patients who carried an *HNF1B* mutation (3); however, one limit of our study is that our population represents patients who had congenital anomalies of the kidney and urinary tract and whose samples were received for *HNF1B* testing in France during a certain period of time. Thus, it will be of interest to perform a prospective study that includes all hyperechogenic kidneys with normal or slightly enlarged size diagnosed before birth and test them for *HNF1B* mutation. Almost all patients with *HNF1B* mutation and moderately enlarged hyperechogenic kidneys before birth displayed normal-sized or small kidneys with hyperechogenicity and/or cortical cysts in the postnatal period, suggesting a slow-down in kidney growth after birth.

Besides hyperechogenic kidneys, *HNF1B* mutations were associated with several other prenatal renal abnormalities but far less frequently: bilateral or unilateral MCD, unilateral renal agenesis, kidney hypoplasia, isolated pyelic dilation, or kidneys with individualized cysts. Because unilateral renal agenesis has been reported in association with *HNF1B* abnormalities only in adults so far (5), it had been suggested that these cases may be due to involution of overlooked MCD (13). Our study shows that genuine renal unilateral agenesis can be associated with *HNF1B* mutation. The absence of cases of bilateral agenesis may be due to the small number of patients tested. In all cases of renal unilateral agenesis associated with *HNF1B* mutation, the single kidney was abnormal. More generally, except for one patient with unilateral hypoplasia and normal contralateral kidney, all probands who carried *HNF1B* mutation displayed bilateral kidney abnormalities. Regarding extrarenal symptoms, no patient with *HNF1B* mutation developed diabetes during early childhood. Only four presented diabetes at 17, 20, 31, and 42 years, respectively, and one developed gestational diabetes. Six other probands had family history of diabetes, but the type of diabetes in relatives was not always known.

Twelve patients with *HNF1B* mutation had early gout and/or hyperuricemia, a feature that has been reported in patients with *HNF1B* mutations (15), but this frequency must be underestimated because the uricemia dosage was not available for many patients in our cohort. Only one adult proband who presented with tubulointerstitial nephropathy and early hyperuricemia that was previously shown not to be associated with *UMOD* mutation was carrying an *HNF1B* mutation. The association of familial hyperuricemic nephropathy with *HNF1B* mutation has been reported previously (5,15), but the mechanisms responsible for the reduced fractional excretion of uric acid are not well understood. HNF1α/HNF1β heterodimers have been shown to bind and positively regulate the proximal promoter region of *SLC22A12*, encoding a transporter that is

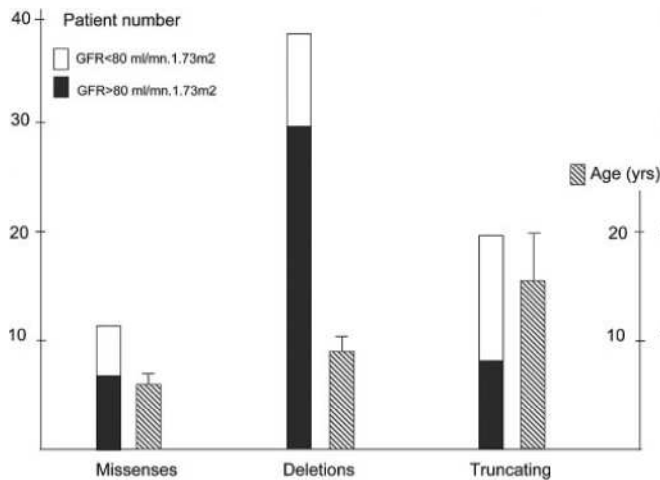

Figure 3. Type of *HNF1B* mutation (deletion, missense mutations, truncating mutations) according to the GFR at last follow-up (■, GFR >80 ml/min per 1.73 m^2; □, GFR <80 ml/min per 1.73 m^2) and age at last follow-up (▨).

responsible for the resorption of urate in the apical membrane of the renal proximal tubule (16); therefore, loss of function would be expected to lead to hypouricemia. The overlap between phenotypes associated with *HNF1B* loss of function and familial *UMOD* hyperuricemic nephropathy may not seem surprising, because *UMOD* was shown to be a target of HNF1β (10); however, familial hyperuricemic nephropathy associated with *UMOD* mutations is thought to be due to a defect in uromodulin transport, associated with a dominant effect, rather than to haploinsufficiency. Thus, the development of the same phenotype associated with *HNF1B* haploinsufficiency is not fully understood. Nevertheless, the finding of hyperuricemia and/or of low uric acid excretion fraction should be an additional argument to screen for *HNF1B* mutation in patients who present with congenital anomalies of the kidney and urinary tract.

Hypomagnesemia was also found in several individuals with *HNF1B* mutation, although blood magnesium dosage was not always performed. Low plasma magnesium level was recently reported by another group and may be related to the transcriptional regulation of *FXYD2* by HNF1β (6). In addition to the frequent and moderate elevation of liver enzymes that was previously reported (17), we observed a severe cholestasis with pruritus in the affected mother of one patient. Cholestasis associated with *HNF1B* mutation was previously reported (18) and is not unexpected given the known role of HNF1β in bile duct morphogenesis (19).

Both the type and the severity of the renal disease were variable in this series, and our data show that *HNF1B* mutations can be associated with very severe prenatal renal failure (in four probands, the pregnancy was terminated because of anamnios, and termination of previous pregnancy for severe renal disease with anamnios was reported in relatives in two additional families) as well as with normal renal function in adulthood. In our series, as in others, there was no obvious correlation between the type of mutation and the type and/or severity of renal disease. We observed both inter- and intrafamilial variability of the phenotype in patients who harbored the same mutation. The lack of genotype–phenotype correlation and the wide variability observed within a given family make the genetic counseling particularly difficult in these families.

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Disclosures

None.

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HNF1B score : a simple tool for both Geneticists and Clinicians

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Abstract

Mutations of *HNF1B* lead to a dominantly-inherited multi-organ disease with a wide inter- and intra-family heterogeneity. The complete phenotype associates diabetes mellitus, pancreas hypoplasia, liver tests abnormalities, genital tract malformations and various kidney disorders (renal cysts, hypoplastic kidneys, hyperechogenicity, urinary tract malformations and/or renal loss of Mg^{2+}). The high rate of *de novo* mutations and the high frequency of incomplete phenotypes mislead both clinicians and geneticists. Consequently, *HNF1B* is frequently tested in patients with isolated renal abnormalities in order to better appreciate the risk of diabetes mellitus in these patients. However, this approach is time and fund consuming and may unnecessarily worry individuals with children in young age. Our purpose was to develop and to validate a composite score for predicting *HNF1B* unmutated patients. After a systematic review of published data, a score was established and validated in two samples of French individuals (n=354 [retrospective cohort] and n=79 (prospective cohort)). In the two cohorts (*HNF1B* mutation in 55/433 patients), HNF1B score discriminated very well mutated and non mutated patients (AUC 0.78 and 0.79, $P < 0.0001$ and $P < 0.001$). The best discriminative score-value was 8 with a negative predictive value higher than 99 %. By applying this score, *HNF1B* would have been screened in only 277 out of the 433 (64%) tested patients In summary, HNF1B score can be particularly useful in dealing with developmental and/or cystic kidney disease, a circumstance that often represents a challenging situation in genetic counseling.

Introduction

HNF1B is a transcription factor that controls the development of kidneys, liver, pancreas and genital tract [1,2]. Mutations of *HNF1B* in humans are associated with a dominantly-inherited multi-organ disease associating kidney malformations, chronic renal failure, electrolytes disorders, maturity-onset diabetes mellitus of the young (MODY) type 5, pancreatic hypoplasia and/or exocrine insufficiency, liver tests abnormalities and various genital tract malformations in both derivatives of the Mullerian and Wolffian ducts [3]. Renal involvement, which is encountered in almost all patients harboring a mutation of *HNF1B* [4,5], varies widely according to the age at presentation: hyperechogenic kidneys with few cysts in fetus [6], hypoplastic kidneys with few cortical cysts in children [7] and slowly progressive chronic renal failure with tubulo-interstitial characteristics and few or no cyst in adulthood [4]. Additional renal findings may also be associated with *HNF1B* mutations (hypomagnesemia, hyperuricemia with early gout, vesico-ureteric reflux, multicystic and dysplastic kidney, solitary kidney) [8,9,4,5]. A small percentage of patients with *HNF1B* mutation may also develop early chromophobe renal cancer [10].

Whole-gene deletion of *HNF1B* as the result of a large recurrent deletion of 1.2 to 1.5 Mb is the most frequent molecular abnormality of *HNF1B* and correspond to about half of the defect identified in this gene [11,6,12]. Point mutations (missense, false-sense, splice site mutation or indel) are mainly located in the DNA binding domain [9,5]. A major consideration for both geneticists and clinicians is the frequency of *de novo* mutations (up to 50 %) [6]. To date no correlation between genotype and phenotype could be demonstrated [12,5].

Many reports have emphasized the wide heterogeneity of the clinical spectrum, even within a family [3,9,4]. Recently, we showed that the phenotype may be limited to kidney involvement in adulthood and that diabetes mellitus may be absent after the fifth decade [4]. These features preclude easy recognition of the disease in adult patients. Lack of familial history of renal or pancreatic disease due to phenotype heterogeneity or *de novo* mutation may also mislead clinicians. Therefore, the detection rate of *HNF1B* mutation varies according to inclusion criteria from 0.6 to 29 % [3,5,13] and additional tools are required to improve both recognition of *HNF1B*-related disease and cost effectiveness of *HNF1B* genetic analyses.

In this study, we designed a composite score (that we named "HNF1B score") based on clinical, radiological and biological data in order to better delineate subsets of patients that should or should not be tested for *HNF1B* mutations. This score was subsequently assessed in two large validation cohorts of 354 and 79 French patients with a known *HNF1B* mutation status.

Patients and Methods

Participants and Genetic analysis

Patients tested for *HNF1B* in the Department of Medical Genetics of the University Hospital of Toulouse (France) were included from January 2008 to June 2011. The first sample of individuals used to validate retrospectively the predictive score comprised 354 individuals with various renal, pancreatic, liver and genital tract disorders tested between January 2008 to February 2011 while the second sample was composed of the 79 subsequent patients (February to June 2011, prospective cohort). According to the French Law, all patients gave written informed consent before DNA collection. The study adhered to the Declaration of Helsinki Principles. As previously recommended, quantitative multiplex PCR of short fragments (QMPSF) allowing the detection of *HNF1B* deletion or duplication was performed first and followed by direct sequencing of the nine exons and intron-exons boundaries if negative [11].

Clinical charts given by the clinicians who managed the patient were reviewed and collected as follows: age at genetic testing, age at the first symptom, renal imaging (cysts, kidney length, echogenicity, urinary tract malformations), electrolytes abnormalities (low serum magnesium and/or potassium, hyperuricemia and gout with early onset (*i.e.* before age 30)), renal biopsy (oligomeganephronia, glomerular cysts), diabetes mellitus, pancreatic hypoplasia or low fecal elastase, liver tests abnormalities and liver cysts, genital tract malformation (uterus malformations, bilateral absence of vas deferens, epididimes cysts, ectopic testis), family history (diabetes mellitus, genital tract malformation or renal disorders of unknown origin in at least one fist-degree relative).

Score definition

We performed an extensive literature review to determine the frequency of *HNF1B* mutations-associated abnormalities by using PubMed database search with the following terms: “HNF1B”, “HNF-1B”, “hepatocyte nuclear factor-1Beta” or “TCF2”. We limited our search to published work in English. Cohort studies as well as case-reports were carefully analyzed.

Renal involvement

Since renal abnormalities are the most heterogeneous involvement in the clinical spectrum of HNF1B-related disease [3,9,5], we divided up the renal phenotype in eight items : ‘hypoplasia’, ‘hyperechogenicity’, ‘cysts’ (cortical and/or medullary), ‘renal absence’, ‘multicystic and dysplastic kidney’, ‘urinary tract malformation’ (vesico-ureteric reflux, caliceal or pyelic non obstructive dilatation, pyélo-ureteric junction syndrome), ‘electrolytes disorders’ (low serum K^+ or Mg^{2+}, hyperuricemia accompanied by gout with early onset) and ‘peculiar histological findings’ (chronic tubulo-interstitial nephritis, oligomeganephronia or glomerular cysts). We considered each kidney as separate, according to the frequent bilateral but asymmetric renal abnormalities [7].

Pancreas involvement

Presentation of diabetes mellitus in HNF1B patients varies (age at presentation, progressive loss of Beta-cells or keto-acidosis, post-transplant diabetes mellitus). Exocrine pancreas insufficiency (low fecal elastase) and pancreas atrophy (mainly bud and tail) are two features highly suggestive for the presence of *HNF1B* mutation when associated with diabetes mellitus and/or renal abnormalities. Thus, we retained the heading 'diabetes mellitus (after exclusion of type 1 diabetes mellitus)' and 'pancreas atrophy / low fecal elastase' in the HNF1B score.

Others

First, fluctuating liver tests abnormalities is frequently observed in patients with *HNF1B* mutations [3,14] while no cystic liver disease has been reported. Second, genital tract involvement (bicornual uterus,…) has emerged as a frequently found condition in HNF1B female patients [15]. In male patients, genital tract abnormalities have been identified [3,4] but with low frequency. Thus, we retained the heading 'liver tests abnormalities' and 'genital tract abnormalities' in the HNF1B score.

Finally, family history with dominant inheritance has been reported but with a wide intra-family phenotype heterogeneity. Hence, the 'Family history' item refers to history of diabetes mellitus, pancreas atrophy, genital tract malformation, renal disorder and/or liver tests abnormalities of unknown origin in at least one first degree relative.

Items value

Value of each item (1, 2 or 4 points) was defined according to the frequency and the specificity of the symptom in HNF1B patients according to literature data.

Statistical analysis

Data are represented as median [minimal - maximal] and percentage. Continuous variables were analyzed with a non parametric test (Mann-Whitney U-test) and categorical variables with the Fisher's exact test. Discrimination between HNF1B positive and negative patients was assessed by determining the area under the curve (AUC) of the receiver operating characteristic curve derived from the score. The discriminative power of the model is thought excellent if the area under the receiver operating characteristic curve (AUC) is greater than 0.80, very good if greater than 0.75, and good if greater than 0.70. A *P*-value < 0.05 was considered as statistically significant. Statistical analyses were performed with the GraphPad_Prism° software (La Jolla, CA, USA).

Results

HNF1B score development

Description of the HNF1B score is represented in Table 1. Three levels of clinical value were pre-specified (1, 2 and 4 points) according to their frequency in HNF1B-related disease. Therefore, according to the significant correlation of renal hyperechogenicity, cystic kidneys and pancreas abnormalities with HNF1B status [3,6,7,16], maximal value (*i.e*. 4 points) was assigned to these symptoms. Because hyperechogenicity and antenatal presentation are closely related [6], the value of this latter was settled at only two points. Two points were assigned to electrolytic disorders (*i.e.* low serum magnesium and potassium, hyperuricemia accompanied by gout with early onset) according to literature data [8,17]. Hypoplastic kidneys are frequent findings in HNF1B patients [7] but are not specific [5,18]. Moreover, distinction between renal atrophy and hypoplastic kidney may be challenging in adult patients in the absence of previous renal imaging. These limitations prompted us to assign a value of two points to this abnormality. Values of other renal disorders were settled at one or two points according to their frequency and specificity.

Diabetes mellitus of MODY type is a frequent finding in HNF1B patients [3,14,9,4] but age at the onset varies greatly [4] and most children are free of diabetes [7]. Among the various MODY, HNF1B mutations are the main cause of pancreas hypoplasia [19]. Thus, we assigned the maximal value (4 points) to the pancreas item.

Genital tract abnormalities identified in patients with *HNF1B* mutations (uterus malformation, bilateral absence of vas deferens, epididimes cysts) reflect a defect during embryogenesis. Their high frequency in HNF1B patients [3,14,4,15] prompted us to assign a value of 4 points to this item. In contrast, causes of liver tests abnormalities are highly heterogeneous in adulthood (metabolic, drug-induced, auto-immune or viral hepatitis) and increased liver enzymes are not frequent in childhood [6,7]. Hence, we settled the value of this item at 2 points.

Study populations

The characteristics of the first validation sample are summarized in Table 2. This sample comprised 354 tested patients with a median age of 9 [0 - 81]). Briefly, 238 patients (67 %) were younger than 16 and first symptoms occurred during the antenatal period in 134 (38 %) patients. Renal, pancreatic, liver tests and genital tract abnormalities were reported by clinicians in 97.5, 8.7, 5.3 and 11 % of tested patients, respectively. Cystic kidneys and renal hypoplasia were reported in 145 (40.9 %) and 115 (32.4 %) of the 354 patients. Hyperechogenic kidneys were more frequently reported in children than in adult patients (32.2 vs. 8.6%, $P < 0.0001$), as well as urinary tract abnormalities (24.8 vs. 8.6 % , $P = 0.0002$). Chronic tubulo-interstitial nephritis without cyst was not a frequent cause of *HNF1B* testing in this cohort (4.8 %), a feature similar to low serum Mg^{2+}/K^{+} and gout with early onset which were reported in only 3.9 and 3.1 % of patients, respectively. Diabetes

mellitus was reported in 25 of the 354 patients (7.1 % ; 22 adult patients (19 %)) and pancreatic hypoplasia in 6 (1.7 % ; .4 adult patients, 3.4 %). No patient had liver cysts. Thirty nine patients had genital tract malformations.

The second validation cohort was composed of 79 patients with a median age of 23 years [0 - 73]. Clinical charts were summarized in Table 3.

In the combined cohorts totalizing 433 individuals, 55 (12.7 %) were found to have a germline mutation of *HNF1B* : 31 gene deletions, 23 point mutations (missense, non-sense, and small deletion), and one gene duplication. Among the different point mutations, twelve were novel (see Table 4). Statistical analyses were performed on the first large validation sample. In univariate analysis, rate of positive testing (13 vs. 11.2%, P = NS) as well as proportion of whole-gene deletion (54.8 vs. 53.8%, P = NS) were similar in both children and adult patients. Patients with *HNF1B* abnormality were younger ($P < 0.05$) and their first symptoms occurred more frequently in antenatal period ($P < 0.05$). Family history of renal, pancreas, liver and/or genital tract disorders compatible with a dominant inheritance was observed in 75/354 (21.2%) patients, but was not significantly associated to the presence of *HNF1B* mutations (P = NS). Bilateral renal cysts and hyperechogenicity were found to be significantly associated with *HNF1B* mutations ($P < 0.001$ and $P < 0.0001$, respectively) but this finding was only confirmed in the pediatric population. Pancreas abnormalities, especially pancreatic hypoplasia and/or low fecal elastase, were significantly associated with HNF1B mutations ($P < 0.05$ and $P < 0.0001$, respectively) while liver tests and genital tract abnormalities were not.

Evaluation of the HNF1B score

We applied the HNF1B score to the first validation sample. Overall, median HNF1B score was 8 [0 – 24] and was significantly higher in patients with *HNF1B* mutation (12 [6 – 22] vs. 8 [0 – 24], $P < 0.001$) irrespective of their age (see Table 2). The receiver operating characteristic (ROC) curve, generated by logistic regression with the HNF1B mutation status as the dependent variable, showed HNF1B score to be a significant predictor of negative HNF1B testing (area under the ROC curve = 0.78 ($P < 0.0001$, 95% CI: 0.72-0.84). On the basis of Youden's index, a cutpoint of 8 would be the optimal cutpoint sensitivity of 0.97 (95% CI: 0.88-0.99), specificity of 0.42 (95% CI: 0.37-0.48), positive predictive value (PPV) of 0.19 and negative predictive value (NPV) of 0.99 (see Fig.1). In children, PPV and NPV were 18.9 and 98.7 compared to 20.6 and 100% in adulthood. Of note, only one *HNF1B* mutated patient had a score below this threshold. This child had bilateral renal hypoplasia diagnosed during antenatal period and genetic analysis disclosed a p.Thr537Asn missense variant not previously described. Genotyping of *HNF1B* in his parents was not available. However, this mutation affects a conserved amino-acid and is predicted to be possibly damaging by *in silico* analysis (Polyphen°)

The good results obtained in this first validation sample prompted us to test prospectively the HNF1B score in the second validation population. Again, a score value of 8 discriminated very well mutated and non mutated patients (AUC 0.79, P = 0.001) and negative predictive value was excellent (*i.e.* 100%). Sensitivity, specificity and positive predictive values were 100, 41.8 and 23.5%, respectively. As shown in Table 5, increase

in cutoff value above 8 was not followed by a significant increase of HNF1B mutations prevalence in the studied population but was associated with a dramatic increase in the rate of false-negative patients.

Discussion

The poor specificity of most renal malformations (cysts, hypoplasia, vesico-ureteric reflux…), precludes a standardized clinical and molecular approach for both clinicians and geneticists. Most mutations in genes involved in kidney development lead to various renal disorders that can be recognized throughout life including in adulthood [4,20]. Moreover, phenotype of these inherited nephropathies may greatly vary between individuals even in the same family, as exemplified by *HNF1B*-related nephropathy [9,4,5]. In some patients, extra-renal symptoms may help to identify the causative gene. Here, we show that the HNF1B score can be used as a good negative predictive score in patients with various renal, liver, pancreas and/or genital abnormalities and may also be suitable in patients with isolated renal malformations.

HNF1B-related disease has emerged as a one of the most frequent inherited renal disease after autosomal dominant polyscystic kidney disease. However, the easy recognition of *HNF1B*-related disease in patients with highly suggestive phenotype (*i.e.* association of renal abnormalities, diabetes mellitus, pancreas hypoplasia, liver tests abnormalities and genital tract malformation) contrasts with the far more complicated diagnosis of *HNF1B*-related nephropathy in patients with an incomplete phenotype or isolated renal involvement. *HNF1B*-related renal phenotype is highly heterogeneous and is frequently isolated in childhood [7]. In cohorts of patients with various kidney disorders explored in antenatal period, childhood or adulthood, a mutation of *HNF1B* was identified in 0.6 to 29 % of patients, according to their clinical phenotype [6,5,13]. Here, we aimed at better defining the subset of patients that should reasonably be tested for *HNF1B*, in an attempt to (1) avoid useless fear of secondary development of diabetes mellitus [3] or chromophobe renal cancer [10] (and maybe neurological impairment [21]), (2) help clinicians and geneticist during genetic counseling, (3) allow identification of almost all mutated patients and (4) decrease the costs of genetic analyses. Such a score should thus carry a maximal negative predictive value to reach these goals.

HNF1B score aimed to delineate the subset of patients with the highest probability of negative *HNF1B* status. Before assessment of the HNF1B score, other well-known inherited syndromes including the renal-coloboma syndrome (*PAX2* mutations), autosomal dominant or recessive polycystic kidney diseases (*PKD1* / *PKD2* or *PKHD1* mutations, respectively) or branchio-oto-renal syndrome (*SIX1*/ *EYA1* mutations) should be ruled out. Suggestive extra-renal symptoms have to be searched for. Of note, liver cystic disease and/or multiple renal cysts with shrinking parenchyma and increased kidney size were considered as suggestive of *PKD1* or *PKD2* mutations-related polycystic kidneys diseases. In our Genetic Department, pancreas, liver and/or genital tract abnormalities were not a prerequisite for *HNF1B* testing but ophthalmologic and ear examinations were recommended in case of isolated renal hypoplasia.

To develop the HNF1B score we searched for symptoms most frequently associated with *HNF1B* mutations in literature data. The first description of *HNF1B* mutation was reported in a Japanese family with dominantly inherited maturity onset diabetes of the young (MODY) [22]. Subsequently, *HNF1B*-related disease has frequently been reported as the 'renal cysts and diabetes syndrome' [14]. However, some conflicting data have emerged since these early descriptions. First, in different cohorts of patients with MODY, the rate of

HNF1B mutations was very low [23,24]. Second, keto-acidosis may reveal diabetes mellitus in HNF1B patients [4] while progressive deterioration of pancreatic beta-cells function over the years is considered as the hallmark of MODY [25]. Lastly, diabetes mellitus may be absent even in the fifth decade of life [4]. So far, whether mutation of *HNF1B* may lead to isolated MODY is unknown. Thus, in the HNF1B score, diabetes mellitus is defined as a non-type 1 diabetes mellitus, according to WHO guidelines irrespective of its characteristics. Pancreatic hypoplasia was not a frequent feature in our cohort but was significantly associated with *HNF1B* status, a finding consistent with previous studies [3,16,26].

Some limitations related to the studied cohorts could be mentioned. First, median ages of the cohorts are low (9 years). In pediatric cohorts, diabetes mellitus was not a frequent finding [5,7]. As a consequence, renal involvement was identified in almost all patients of our cohort while diabetes mellitus was reported in only 7.5 % of tested patients. Similarly, tested patients had a low frequency of genital tract and liver tests abnormalities as well as low serum magnesium. These findings may represent a bias of inclusion. However, mutations of *HNF1B* are rare in MODY patients [24] or females with isolated uterine malformations [15] while a combination of uterine malformations or MODY and renal abnormalities are far more common in HNF1B patients [3,20,15], a figure similar with fluctuating liver enzymes and hypomagnesemia. As a consequence, a majority of HNF1B patients harbor a renal involvement and could be addressed by the *HNF1B* score. Further work will have to delineate the accuracy of the score in various populations irrespective of their renal phenotype (*i.e.* MODY, chronic hepatitis of unknown origin, genital tract abnormalities…). The lack of systematic screening of organ involvements may also have underestimated the frequency of some disorders (*i.e.* male genital tract malformations, pancreatic hypoplasia) but we do think that our study represents the real life.

No study presenting a large cohort of children and adult patients with HNF1B mutation and a systematic assessment of organs involvements have been reported so far. Thus, the true frequency of each symptom remains therefore currently unknown. We could not accurately measure the link (*i.e.* odd-ratio) between each symptom and the HNF1B molecular status. Cystic kidneys, renal hyperechogenicity and pancreas abnormalities are associated with *HNF1B* status in our study and in previous cohorts, while renal hypoplasia, low serum magnesium and genital tract malformations were not. This discrepancy probably originates from the frequency of missing data for the last two symptoms in our cohort and from the very low specificity of the "renal hypoplasia" symptom. Hence, we arbitrarily assigned a value of two, two and four points to these items according to literature data. Also, atypical familial hyperuricemic and juvenile nephropathy (FHJN) has been associated with *HNF1B* mutations [17] but was not a frequent cause of HNF1B testing in our cohort. Since all patients with atypical FHJN also carried renal malformations (i.e. renal cysts) and since mutations of *HNF1B* have not been previously reported in patients with isolated gout with early onset, we decided not to give the maximal value to the item 'hyperuricemia complicated by gout with early onset (before age 30)'.

In the two cohorts reported here including both child and adult patients with renal involvement, the discriminative power of the HNF1B score was very good with a significant AUC value of 0.78 and 0.79,

respectively. Moreover, predictive negative value was 99.2 – 100% with a score threshold of 8 both in childhood and adulthood. By applying this pre-test scoring, *HNF1B* would have been screened in only 277 out of the 433 (64%) tested patients which represent a gain in both time and financial costs (price per one test 765 euros ; total estimated gain cost 127,000 euros (≈ 176,000 dollars)). With a score cutpoint of eight, positive predictive value and sensitivity were low however the aim of the study was to obtain a score identifying most of the *HNF1B* negative patients without excluding *HNF1B* mutated patients (*i.e.* with the highest negative predictive value).

In summary, we developed a simple composite score (*i.e.* HNF1B score) that can identify with high sensitivity patients that carry HNF1B mutations while reducing the rate of HNF1B test in patients without mutation. Its accuracy was validated in two large cohorts of children and adult patients with various renal phenotype and/or diabetes mellitus, liver tests abnormalities or genital tract abnormalities. Further multicentric prospective studies are needed to confirm these preliminary results, especially in cohorts of patients with MODY, genital tract malformations-related infertility or chronic hepatitis or pancreatitis of unknown origin.

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	Item	Value
Family history*		+ 2
Antenatal disclosure		+ 2
Kidneys and Urinary tract		
Left Kidney	Hyperechogenicity	+ 4
	Cysts	+ 4
	Hypoplasia	+ 2
	Multicystic and dysplastic kidney	+ 2
	Urinary tract malformation	+ 1
	Solitary kidney	+ 1
Right kidney	Hyperechogenicity	+ 4
	Cysts	+ 4
	Hypoplasia	+ 2
	Multicystic and dysplastic kidney	+ 2
	Urinary tract malformation	+ 1
	Solitary kidney	+ 1
Electrolytes disorders	Low serum Mg^{2+}	+ 2
	Gout with early onset (before age 30)	+ 2
	Low serum K^{+}	+ 1
Peculiar histological change	CTIN, oligomeganephrony *or* glomerular cysts	+ 1
Pancreas #	Diabetes mellitus *or* Pancreas hypoplasia *or* low fecal elastase	+ 4
Genital tract	Genital tract abnormalities	+ 4
Liver	Liver tests abnormalities of unknown origin	+ 2

Table 1 : HNF1B score. * Family history of renal malformations suggestive of HNF1B mutation or diabetes mellitus with autosomal dominant inheritance; # Maximal value of the item Pancreas is 4. *CTIN*, chronic tubulo-interstitial nephritis. This score was assessed in patients with various renal, pancreatic, liver and genital tract disorders after exclusion of other recognizable inherited renal diseases or genetic syndromes (*i.e.* autosomal dominant polycystic kidney disease, renal-coloboma syndrome, towns-Brocks syndrome…)

	Total				Children (< 16 years of age)				Adults (> 16 years of age)			
	Total	HNF1B status			Total	HNF1B status			Total	HNF1B status		
	N = 354	Mutation N = 43	Normal N = 311	*P*	N = 238	Mutation N = 31	Normal N = 207	*P*	N = 116	Mutation N = 12	Normal N = 104	*P*
HNF1B score												
Median	8 [0 - 24]	12 [6 - 22]	8 [0 - 24]	#	8 [0 - 24]	15 [6 - 22]	8 [0 - 24]	#	8 [0 - 22]	11 [8 - 16]	7 [0 - 22]	#
Age (years)												
Mean	15.7	11.3	16.2	ǁ	5.2	2.5	5.6	#	37.1	34	37.4	NS
Antenatal disclosure	134 (37.8 %)	24 (55.8 %)	110 (35.5 %)	ǁ	122 (51 %)	23 (74.2 %)	99 (47.8 %)	*	12 (10.3 %)	1 (8.3 %)	11 (10.6 %)	NS
Family history	75 (21.2)	10 (23 %)	65 (20.9 %)	NS	29 (12.2 %)	3 (9.7 %)	26 (12.6 %)	NS	46 (39.6 %)	7 (58.3 %)	39 (37.5 %)	NS
Liver tests abnormalities	19 (5.4 %)	3 (7 %)	16 (5.2 %)	NS	10 (4.2 %)	1 (3.2 %)	9 (4.3 %)	NS	9 (7.7 %)	2 (16.7 %)	7 (6.7 %)	NS
Genital tract malformations	39 (11 %)	5 (11.6 %)	34 (10.9 %)	NS	15 (6.3 %)	2 (6.4 %)	13 (6.3 %)	NS	24 (20.7 %)	3 (25 %)	21 (20.2 %)	NS
Pancreas abnormalities	31 (8.7 %)	7 (16.2 %)	24 (7.7 %)	ǁ	5 (2.1 %)	2 (6.4 %)	3 (1.4 %)	NS	26 (22.4 %)	5 (41.7 %)	21 (20.2 %)	NS
Diabetes mellitus	25 (7.1 %)	2 (4.6 %)	23 (7.4 %)	NS	3 (1.3 %)	0	3 (1.4 %)	NS	22 (19 %)	2 (16.6 %)	20 (19.2 %)	NS
Hypoplasia and/or EI	6 (1.7 %)	5 (11.6 %)	1 (0.3 %)	&	2 (0.8 %)	2 (6.4 %)	0	ǁ	4 (3.4 %)	3 (25 %)	1 (1 %)	*
Kidneys and urinary tract												
Hypoplasia												
Unilateral	37 (10.4 %)	3 (7 %)	34 (10.9 %)	NS	30 (12.6 %)	3 (9.7 %)	27 (13 %)	NS	7 (6 %)	0	7 (6.8 %)	NS
Bilateral	78 (22 %)	7 (16.3 %)	71 (22.4 %)	NS	54 (22.7 %)	4 (12.9 %)	50 (24.2 %)	NS	24 (20.7%)	3 (25%)	21 (20.2 %)	NS
Hyperechogenicity												
Unilateral	22 (6.2 %)	2 (4.7 %)	20 (6.5 %)	NS	22 (9.2 %)	2 (6.5 %)	20 (9.7 %)	NS	0	0	0	NS
Bilateral	92 (26 %)	24 (55.8 %)	68 (21.9 %)	&	82 (34.5 %)	23 (74.2 %)	59 (28.5 %)	&	10 (8.6 %)	1 (8.3 %)	9 (8.7 %)	NS
Cysts												
Unilateral	38 (10.7 %)	6 (13.9 %)	32 (10.3 %)	NS	33 (13.9 %)	4 (12.9 %)	29 (14 %)	NS	5 (4.3 %)	2 (16.6 %)	3 (2.9 %)	NS
Bilateral	107 (30.2 %)	21 (48.8 %)	86 (27.7 %)	*	67 (28.2 %)	15 (48.4 %)	52 (25.1 %)	ǁ	40 (34.5 %)	6 (50 %)	34 (32.7 %)	NS
Renal absence												
Unilateral	40 (11.3 %)	2 (4.7 %)	38 (12.3 %)	NS	22 (9.2 %)	1 (3.3 %)	21 (10.1 %)	NS	18 (15.5 %)	1 (8.3 %)	17 (16.3 %)	NS
Bilateral	1 (0.3 %)	0	1 (0.3 %)	NS	1 (0.4 %)	0	1 (0.5 %)	NS	0	0	0	NS
UT abnormalities												
Unilateral	38 (10.7 %)	4 (9.4 %)	34 (11 %)	NS	34 (14.3 %)	4 (13 %)	30 (15.5 %)	NS	4 (3.5 %)	0	4 (3.8 %)	NS
Bilateral	31 (8.8 %)	3 (7 %)	28 (9 %)	NS	25 (10.5 %)	2 (6.5 %)	23 (11.1 %)	NS	6 (5.2 %)	1 (8.3 %)	5 (4.8 %)	NS
MCDK												
Unilateral	41 (11.6 %)	4 (9.4 %)	37 (11.9 %)	NS	36 (15.1 %)	4 (13 %)	32 (15.4 %)	NS	5 (4.3 %)	0	5 (4.8 %)	NS
Bilateral	18 (5.1 %)	0	18 (5.8 %)	NS	14 (5.9 %)	0	14 (6.8 %)	NS	4 (3.5 %)	0	4 (3.8 %)	NS
Metabolic conditions												
Low serum Mg^{2+}/K^{+}	14 (3.9 %)	2 (4.7%)	12 (3.8 %)	NS	8 (3.4 %)	0	8 (3.9 %)	NS	6 (5.2 %)	2 (16.6 %)	4 (3.9 %)	NS
Gout (early onset)	11 (3.1 %)	0	11 (3.5 %)	NS	0	0	0	NS	11 (9.5 %)	0	11 (10.6 %)	NS
Pathological findings												
Chronic TIN	17 (4.8 %)	0	17 (5.5 %)	NS	2 (0.8 %)	0	2 (1 %)	NS	15 (12.9 %)	0	15 (14.5)	NS
Glomerular cysts or Oligomeganephr.	4 (1.1 %)	1 (2.3 %)	3 (0.9 %)	NS	2 (0.8 %)	1 (3.2 %)	1 (0.5 %)	NS	2 (1.7 %)	0	2 (1.9 %)	NS

Table 2: Clinical characteristics of the first validation sample (n=354). Categorical variable were analyzed with the Fisher's exact test and continuous variable with the Mann-Whitney U-test. &: P < 0.0001; # : *P* < 0.001; * : *P* < 0.01; || : *P* < 0.05; NS : Not Significant. *EI*, exocrine pancreatic insufficiency; *TIN*, tubulo-interstitial nephritis; *Oligomeganephr*, oligomeganephronia; *UT*, urinary tract.

	N = 79
HNF1B score	8 [0 – 20]
Median	
Age (years)	23
Mean	
Antenatal disclosure	19 (22.3%)
Family history	29 (35.8%)
Liver tests abnormalities	6 (7.4%)
Genital tract malformations	5 (6.2%)
Pancreas abnormalities	11 (13.5%)
Diabetes mellitus	10 (12.3%)
Hypoplasia and/or EI	1 (1.2%)
Kidneys and urinary tract	
Hypoplasia	
Unilateral	6 (7.4%)
Bilateral	9 (11.1%)
Hyperechogenicity	
Unilateral	8 (9.9%)
Bilateral	12 (14.8%)
Cysts	
Unilateral	10 (12.3%)
Bilateral	29 (35.8%)
Renal absence	
Unilateral	6 (7.4%)
Bilateral	0
UT abnormalities	
Unilateral	5 (6.2%)
Bilateral	4 (4.9%)
MCDK	
Unilateral	6 (7.4%)
Bilateral	4 (4.9%)
Metabolic conditions	
Low serum Mg^{2+}/K^{+}	3 (3.7%)
Gout (early onset)	5 (6.2%)
Pathological findings	
Chronic TIN	12 (14.8%)
Glomerular cysts or Oligomeganephr	0

Table 3 : Clinical characteristics of the second validation sample (n=79) tested for *HNF1B*. *EI*, exocrine pancreatic insufficiency; *TIN*, tubulo-interstitial nephritis; *MCDK*, multicystic and dysplastic kidney; *Oligomeganephr*, oligomeganephronia; *UT*, urinary tract.

Exon / Intron	DNA variation	Protein variation	Reference
Exon 1	c.212-217delAAGGGCC	p.Lys71AlafsX42	This study
	c.244 G>A	p.Asp82Asn	This study
	c.356 G>A	p.Trp119X	This study
Exon 2	c.443 C>G	p.Ser148Trp	Yorifuji *et al.*[27]
	c.460 C>T	p.Leu154Phe	This study
	c.494 G>A	p.Arg165His	Heidet *et al.* [5]
	c.494 G>c	p.Arg165Pro	This study
	c.547 C>T	p.Arg181X	Bellanne *et al.* [3]
Exon 3	c.708 C>T	p.Phe236Leu	This study
	c.737 T>C	p.Leu246Ser	This study
	c.883 C>T	p.Arg295Cys	Heidet *et al.* [5]
Exon 4	c.895 T>G	p.Trp296Gly	This study
	c.904 A>G	p.Asn302Asp	This study
	c.967-968 insA	p.Thr323AsnfsX36	This study
Exon 5	c.1046 delG	p.Gly349GlufsX26	This study
	c.1136 c>A	p.Ser379X	Heidet *et al.* [5]
Exon 8	c.1610 c>A	p.Thr537Asn	This study
Intron 1	c.345-1 G>A	Splice site mutation	Heidet *et al.* [5]
Intron 2	c.544+1 G>T	Splice site mutation	Bingham *et al.* [17]
	c.544+1 G>C	Splice site mutation	Ulinski *et al.* [7]

Table 4 : Point mutations (n=19) identified in 23 out of 56 HNF1B patients .

HNF1B score	Population studied N	HNF1B mutated patients N (%)
0-24	433	55 (12.7)
≥ 6	337	55 (16.3)
≥ 8	277	54 (19.7)
≥ 10	197	45 (22.8)
≥ 12	127	36 (28.3)
≥ 14	80	23 (28.7)
≥ 16	56	18 (32.1)

Table 5: Prevalence of HNF1B mutations in the overall studied population according to HNF1B score value.

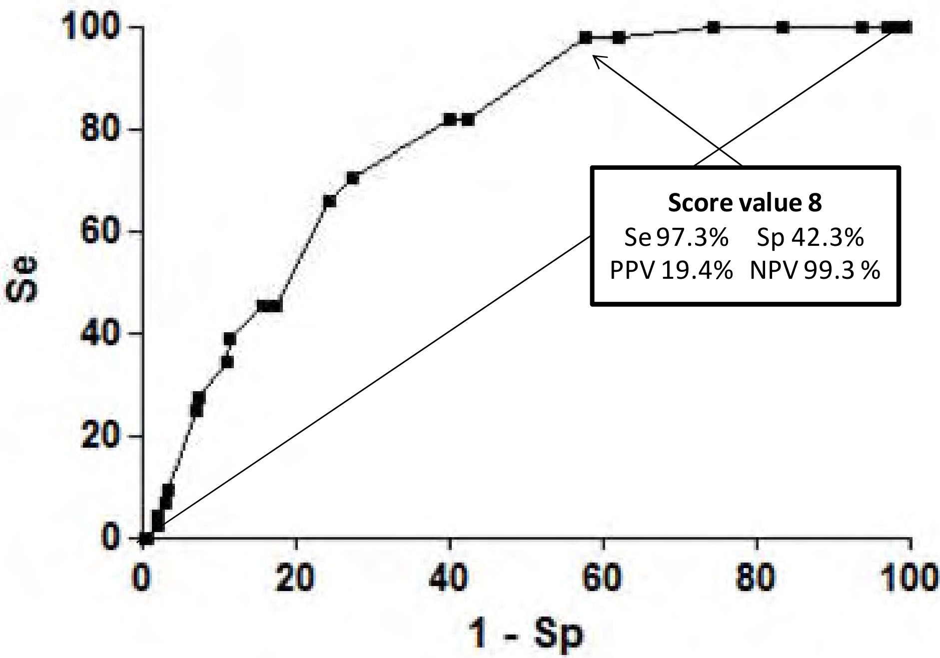

Figure 1 : Receiving operating characteristics curve established from the first validation sample describing the ability of the HNF1B score to discriminate patients with and without *HNF1B* mutation

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Nephrology Dialysis Transplantation

Letters

Advance Access publication 17 April 2009

Mutations in the RARE and MARE regulatory sequences of *HNF1*β are not a frequent cause of kidney/urinary tract malformation

Sir,

Mutations in several genes have been identified in congenital abnormalities of kidneys and urinary tract (CAKUT): heterozygous mutations in *HNF1*β account for 8%, while other genes (*PAX2*, *EYA1, SALL1, SIX1*) have been identified as causing isolated renal hypo/dysplasia to a lesser extent in humans [1].

Despite extensive *HNF1*β molecular analysis, including direct sequencing of the minimal promoter and of the nine exons associated with screening for exonic rearrangement, *HNF1*β genetic testing failed to identify mutations in a majority of patients with CAKUT, even in individuals with very evocative renal phenotype [2,3]. Recently, specifically conserved *cis*-acting RARE (retinoic acid responsive element) and T-MARE (MafB responsive element) regulatory sequences have been identified [4]. RA signalling cascade and MafB bind RARE and T-MARE regulatory DNA sequences, respectively, and promote the expression of *Hnf1*β in both the caudal hindbrain (r4/r5 boundary) and pancreas. These sequences are both located in intron 4 of *HNF1*β and are not actually studied for the purpose of diagnosis. Finally, the roles of RA and MAFB in kidney development suggest that the RA-MAFB-HNF1β pathway may also be functional in human kidney.

In this study, we hypothesized that mutations in the highly conserved (see Figure 1) RARE and T-MARE regulatory sequences could disturb the early embryologic process of intermediate mesoderm, from which kidneys and urinary tract both derived, and consequently lead to renal phenotype mimicking *HNF1*β-related nephropathy.

Among the 63 individuals we tested for *HNF1*β, a mutation was identified in 12 (19%). We therefore performed molecular analysis of these regulatory sequences in the 51 remaining patients with isolated or syndromic CAKUT of unknown origin for whom previous molecular analysis of *HNF1*β (QMPSF and direct sequencing) had failed to identify any causative mutation. All patients gave informed consent according to French law. Renal and extra-renal features of the 51 tested patients [31 males, 20 females, median age 9 years (0–53)] are summarized in Tables 1 and 2, respectively. RARE and T-MARE regulatory sequences were amplified by PCR using a set of primers deduced from the genomic sequence (forward: TC CCCAGAACCCTCTTCCTA, reverse: TGGTTCAAAGC CCCAACTTA), corresponding to amplification of a 344 bp fragment (G1266–17138 to G1266–16794). Among the 51 tested patients, no sequence variation of the RARE

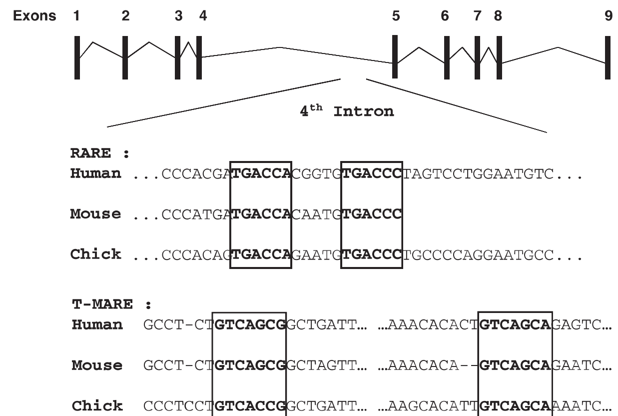

Fig. 1. Alignment of human, mouse and chick nucleotide sequences from the enhancer of *HNF1*β (within intron 4): RARE and T-MARE regulatory sequences (boxes). Dashes indicate conserved residues.

Table 1. Renal characteristics of 51 patients with CAKUT tested for RARE and T-MARE regulatory sequences of *HNF1*β

Renal features	*N*	
Hyperechogenicity	16	31%
Renal cysts	33	65%
Kidney size		
Normal	27	52%
Enlarged	4	8%
Unilateral hypoplasia	8	16%
Bilateral hypoplasia	12	24%
Urinary tract malformation		
Bilateral VUR	6	12%
Solitary kidney	5	10%
Ectopic kidney	3	6%
UPJO	1	2%
Oligomeganephronia	1	2%
Chronic renal failure	17	33%

CAKUT: congenital abnormalities of kidneys and urinary tract, VUR: vesico-ureteric reflux, UPJO: uretero-pyelic junction obstruction.

Table 2. Extra-renal characteristics of 51 patients with CAKUT tested for RARE and T-MARE regulatory sequences of *HNF1*β

Extra-renal features	*N*	
Genital tract		
Cryptorchidism	2	4%
Bicornual uterus	1	2%
MRKH syndrome	1	2%
Vas deferens absence	2	4%
Testis + semen vesicle hypoplasia	1	2%
Liver		
Abnormal liver tests	1	2%
Choledochal cyst and DM	1	2%
Pancreas		
Chronic calcified pancreatitis and DM	2	4%
Mental retardation	1	2%
Retinal oedema	1	2%

MRKH: Mayer-Rokitansky-Küster-Hauser, DM: diabetes mellitus.

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and T-MARE regulatory sequences of *HNF1*β was evidenced.

In conclusion, although mutations in RARE and T-MARE regulatory sequences of *HNF1*β may nevertheless be implicated in some renal congenital disorders, we show here that mutations in these sequences are not a frequent cause of CAKUT. More experiments are required to assess the role of the RA-MAFB-HNF1β pathway in kidney development. Moreover, the molecular basis of these renal malformations is still poorly understood, and further works remain to be done to identify new CAKUT genes. Delineation of transcriptional networks involved in early human metanephros development may be a way to identify these new genes.

Conflict of interest statement. None declared.

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Conductivity pulses needed for Diascan® measurements: does it cause sodium burden?

Sir,

Recent, New techniques based on conductivity measurement enable physicians to evaluate the adequacy of haemodialysis dose (HDD) on all haemodialysis treatments by on-line monitoring of Kt/V. Ionic dialysance (Diascan® Hospal-Gambro, Mirandola, Italy) is a parameter calculated from the dialysate conductivity at the dialyser inlet and outlet. Every half hour, the inlet dialysate conductivity increases by 1 ms/cm during 5 min, while the outlet dialysate conductivity-cell measures the effect of this increase. The calculated ion transfer across the membrane (largely sodium) is almost equivalent to urea transfer and therefore ionic dialysance reflects the urea clearance. This method has been shown to have a good correlation with Kt/V measured by the mathematical urea equations in several studies [1–4].

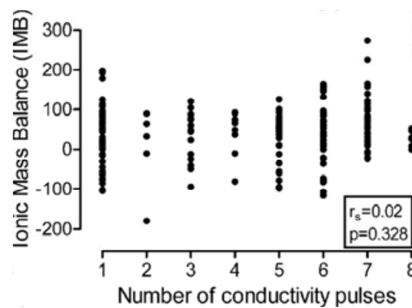

Fig. 1. Correlation between IMB during isovolaemic haemodialysis and number of conductivity pulses.

However, some investigators suggest that during the conductivity pulse of 5 min, significant amounts of sodium may be transferred into the patient [5]. In our centre, we studied that effect. Diascan® also measures ionic mass balance (IMB) and plasma conductivity (PC) that are likely to represent sodium balance and plasma sodium, respectively (plasma sodium in mmol/l ≈ plasma conductivity in ms/cm × 10). This is performed by constant measurements of the conductivity in the dialysate inlet and outlet, according to the formulas

$$PC = [Cd_{out} - (1 - D/Qd_{in}) \times Cd_{in}]/(D/Qd_{in}),$$

where Qd_{in} and Qd_{out} are dialysate flow at, respectively, inlet and outlet, Cd_{in} and Cd_{out} are dialysate conductivity at, respectively, inlet and outlet and D is ionic dialysance, and

$$IMB = (Qd_{in} \times Cd_{in} - Qd_{out} \times Cd_{out}) \times 10 \times \text{time(min)}.$$

A positive IMB means sodium removal from the patient. A negative IMB means sodium transport to the patient.

In patients with zero inter-diaytic weight gain, IMB was measured in 200 isovolaemic haemodialysis sessions by Diascan®, 137 sessions were performed with four to eight conductivity pulses. A total of 63 sessions was performed with only one conductivity pulse.

The results showed a highly significant correlation between pre-dialytic plasma conductivity and IMB (Spearman rank $r_s = 0.902$, $P < 0.005$), in agreement with previous studies [6]. There was no correlation between IMB and the number of conductivity pulses (Spearman rank $r_s = 0.02$, $P = 0.328$) (Figure 1).

Hnf-1β transcription factor is an early Hif-1α-independent marker of epithelial hypoxia and controls renal repair

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Abstract

Epithelial repair following acute kidney injury (AKI) requires epithelial-mesenchyme-epithelial cycling associated with transient re-expression of genes normally expressed during kidney development as well as activation of growth factors and cytokine-induced signaling. In normal kidney, the Hnf-1β transcription factor drive nephrogenesis, tubulogenesis and epithelial homeostasis through the regulation of epithelial planar cell polarity and expression of developmental or tubular segment-specific genes. In a mouse model of ischemic AKI induced by a 2-hours hemorrhagic shock, we show that expression of this factor is tightly regulated in the early phase of renal repair with a biphasic expression profile (early down-regulation followed by transient over-expression). These changes are associated to tubular epithelial differentiation as assessed by KSP-cadherin and megalin-cubilin endocytic complex expression analysis. In addition, early decrease in *Hnf1b* expression is associated with the transient over-expression of one of its main target genes, the suppressor of cytokine signaling *Socs3*, which has been shown essential for renal repair. *In vitro*, hypoxia induced early up-regulation of Hnf-1β from 1 to 24 hours. When prolonged, hypoxia induced Hnf-1β down-regulation while normoxia led to Hnf-1β normalization. However induction of the hypoxia-inducible factor Hif-1α in normoxic condition was not associated with Hnf-1β up-regulation suggesting a more complex mechanism than a sole direct Hif-1α regulation. Taken together, we showed that Hnf-1β may drive recovery from ischemic AKI by regulating both the expression of genes important for homeostasis control during organ repair and the state of epithelial cell differentiation.

Introduction

In the general population, acute renal failure (ARF) is noted in 1% of all hospital admissions. Hospital-acquired ARF occurs in up to 7% of all hospitalizations. Ischemic acute kidney injury (AKI) is the major cause of ARF and is associated with increased mortality in hospitalized populations.[1] Renal pathological changes of ischemic AKI are characterized by changes in proximal tubule epithelium. Disruption of the cytoskeleton and the loss of cell polarity result in the loss of the proximal tubule brush border, patchy loss of tubule cells, focal areas of proximal tubular dilatation and distal tubular casts. and areas of cellular regeneration characterized by cell proliferation.[2] Following AKI, replacement of lethally injured tubular cells is achieved by a complex reparative process leading to cell proliferation. Epithelial-mesenchyme-epithelial cycling allows almost complete recovery of renal architecture and renal function in most of the cases.[3] Tight regulation of this complex process involves cell cycle control,[4] transient re-expression of genes normally expressed during kidney development, including *Pax2*, *Notch-2*, *Wnt4* and *Ets-1*,[5-8] and transient activation of growth factors- or cytokine-induced signaling, including the hepatocyte and epidermal growth factor (HGF/EGF) and the interleukine-6 receptor pathways.[9,10]

During early kidney development, hepatocyte nuclear factor-1β (Hnf-1β) drives nephrogenesis, tubulogenesis and epithelial maturation through the regulation of epithelial planar cell polarity and expression of developmental or tubular segment-specific genes.[11-13] *Hnf1b* is expressed during early mouse embryogenesis, especially in the Wolffian duct, the ureteric bud and the metanephric kidney.[14] During later stages of kidney development, *Hnf1b* is expressed in all segments of the nephron, from the proximal tubule (PT) to the collecting duct. Hnf-1β is a transcription factor that controls the expression of a number of genes including *Pkhd1*, *Umod* and *Socs3*.[15, 16] Hnf-1β acts as a homo- or heterodimer with the closely related transcription factor Hnf-1α. The latter is mostly expressed in PT cells where it drives cells differentiation.[17] Antenatal kidney-specific conditional inactivation of *Hnf1b* in mice induces polycystic kidney disease with lethal renal failure around three weeks after birth.[15, 18] Some recent studies suggested that Hnf-1β may have a role in epithelial kidney and liver repair.[19, 20]

Data concerning the role of Hnf-1β in renal repair following AKI are scarce. Interestingly, invalidation of *Hnf1b* after the end of renal development (*i.e.* after P10 in mouse) is not followed by renal changes, except when cells are forced to enter the cell cycle.[19] In mice with renal specific *Hnf1b* invalidation after P10, ischemic AKI promotes tubular dilatation and cystic kidney disease. Among Hnf-1β target genes *Socs3* is a key player in epithelial repair following ischemic AKI. Within the first hours following ischemic injury, a dramatic increase in the intra-renal expression of *Socs3*, a suppressor of cytokine signaling, induced through an interleukine-6-mediated feedback, has been identified.[21] Reduced *Socs3* expression in proximal tubular cells accelerates acute renal failure[22]. In addition, it has been demonstrated that *Socs3* negatively regulates signaling of various growth factors

and cytokines, including EGF, leukemia inhibitor factor, fibroblast growth factor, angiotensin-II and insulin-like growth factor-1, all involved in renal repair.[23-26]

Surprisingly, expression of *Hnf1b* during early steps of renal repair has not been studied. We thus investigated the expression of *Hnf1b* in parallel with some target genes in an ischemic AKI model. We found that Hnf-1β drive recovery from ischemic AKI by regulating both the expression of important genes for homeostasis control during PT repair, and the state of epithelial cell differentiation. In addition, we deciphered the respective roles of the hypoxia-inducible factor Hif-1α up-regulation and low oxygen pressure per se in the regulation of the *Hnf1b* expression.

Results

Assessment of AKI in a mouse model of hemorrhagic shock

We used a recently developed mouse model of AKI induced by a 120-minutes hemorrhagic shock-related hypotension, as previously described.[27] Renal damages in this model were confirmed by determining functional, histological and mRNA expression changes of key AKI genes. At day 2 and 6, a significant decrease of the glomerular filtration rate was observed in shocked mice (Fig.1a). Periodic acid-Schiff and Masson's trichrome staining of kidney sections from shocked mice showed typical features of AKI, including disruption of the epithelial brush border, flattening of the epithelia and tubular casts, while these histological changes were not observed in sham mice (Fig.1c-f). Consistent with previous mouse models using an ischemia/reperfusion (I/R) model to mimic AKI[21,28], assessment of cell proliferation by *Pcna* mRNA expression showed a significant increase within the first 10 hours (Fig. 1b).

Thus a 120-minutes hemorrhagic shock resulted in significant AKI with dramatic functional, histological and mRNA expression changes of key AKI genes, and may be a valuable tool to decipher the mechanisms of renal repair.

Renal expression of Hnf-1β and some of its target genes after ischemic AKI in mouse

In this mouse model of hemorrhagic shock-induced AKI, , we now show a significant ~ 50 % decrease in the expression of *Hnf1b* within the first 10 hours post-shock followed by a transient over-expression at 24 hours (Fig.2a). The kinetics of Hnf-1β expression was confirmed at protein level (Fig.2b).

Renal expression of *Cdh16* (KSP-cadherin*), Pkhd1* (Polyductin) *and Socs3*, three genes directly regulated by Hnf-1β, were also assessed. *Cdh16* and *Pkhd1* are known to be positively regulated, while *Socs3* is negatively regulated by Hnf-1β.[15, 16] A significant decrease of *Cdh16* and *Pkhd1* expression was observed 10 hours after the hemorrhagic shock followed by progressive normalization until day 21 (Fig. 3a-b). Conversely, the expression of *Socs3* (a gene negatively regulated by Hnf-1β) displayed a mirror expression profile with Hnf-1β in this model (Fig.3c). These results suggest that the expression of Hnf-1β and three of its target genes is tightly regulated during the regeneration phase following ischemic AKI in mice.

Renal expression of Hnf1a and proximal tubule markers after ischemic AKI in mouse

Hnf-1α is involved in epithelial repair in the liver[28]. In addition, Hnf-1α is required for proper differentiation of renal proximal tubule[17] and acts in homo- or heterodimers with Hnf-1β, but respective target genes of Hnf-1β - Hnf-1β and Hnf-1β - Hnf-1α dimers have not been fully characterized. We have therefore studied *Hnf1a* renal expression after ischemic AKI in parallel with *Hnf1b*. A significant ~ 50 % decrease in the expression of *Hnf1a* was observed at 10 hours followed by a transient rebound at 48 hours (Fig. 4a). Thus, temporally changes in *Hnf1a* expression occur later than the change in *Hnf1b* expression. We also studied the renal expression of proximal tubule markers *Lrp2* and *Cubln*, which encode for the endocytosis complex megalin-cubilin, in this ischemic AKI mouse model. Expression of both genes was significantly decreased from 3 up to 24 hours after hemorrhagic shock. Expression of *Cubln* normalized at 48 hours while *Lrp2* remained down regulated until 21 days after the insult (Fig.4b-c). Persistent down-regulation of *Lrp2* at day 21 suggested permanent proximal tubule dysfunction escaping clinical and pathological detection.

In vitro studies of Hnf1b and Hif-1α expression under hypoxic conditions

To decipher the molecular mechanisms that controls *Hnf1b* expression in kidney during and after ischemic injury, we assessed the consequences of hypoxia on Hnf-1β *in vitro.* HK-2 cells (a cell line issued from human proximal tubule) were grown under normoxic (21% oxygen) and hypoxic (1% oxygen) conditions, and Hnf-1β levels were measured at various time of hypoxia. Western blot analyses showed a significant increase of Hnf-1β expression as early as one hour after hypoxia and up to 24 hours (Fig.5a), followed by a progressive decline reaching control values after 48 hours hypoxia and a down regulation at 72 hours. Restoring normoxia after 2 hours of hypoxia leads to the normalization of Hnf-1β expression at 24 hours (Fig. 5c). Contrasting with changes of Hnf-1β protein, *Hnf1b* mRNA was significantly decrease during hypoxia (Fig. 5b). Hence, these data suggest that Hnf-1β amount in epithelial cells submitted to hypoxia is controlled at both protein (early effect) and mRNA (late effect) level.

The transcriptome adaptive response to hypoxia has been shown to be primarily controlled by hypoxia-inducible factor Hif-1α [29] To determine whether *Hnf1b* expression is dependant of Hif-1α we analyzed its expression in HK-2 cells submitted to hypoxia (see raw data of western blot in supplementary material online). As shown in figure 5d, Hif-1α was early and significantly up-regulated when HK-2 cells were submitted to 1%-hypoxia reaching a maximal value after 2 hours of hypoxia. This increase was transient since persistent hypoxia lead to down-regulation of Hif-1α after 24 hours of hypoxia. This down regulation became highly significant at 72 hours of hypoxia. As Hnf-1β and Hif-1α expression followed a similar but not overlapping expression profile with time in

hypoxic HK-2 cells, we hypothesized that the increase in Hnf-1β could be related to Hif-1α overexpression. To test it, we cultivated HK-2 cells in normoxic conditions and added to the culture medium an inhibitor of Hif-1α specific prolylhydroxylases (PHD) to inhibit Hif-1α degradation. As expected, PHD inhibition was accompanied by a significant over-expression of Hif-1α (Fig. 6a), while Hnf-1β expression was not modified (Fig. 6b), a finding consistent with a differentially targeted regulation for each of the transcription factor under hypoxia. According to this result, we failed to find HIF-1α binding region ([A/G]CGTG motif ; *i.e.* hypoxia responsive element), conserved in at least four species[30] (Homo Sapiens, Mus Musculus, Bos Taurus and Equus Caballus), in the 4000 pb upstream of the transcription start site of *Hnf1b*.

Discussion

Hnf-1β is a transcription factor that controls planar cell polarity, tubulogenesis and tubular segment differentiation,[11-13, 15] which are all events required for proper renal repair. To date, the role of *Hnf1b* in epithelial regeneration remains highly elusive. The main finding in this study is that *Hnf1b* expression is tightly regulated during epithelial repair following ischemic AKI induced by a 120-minutes hemorrhagic shock-related hypotension. The biphasic kinetics suggests that *Hnf1b* may control expression of molecular actors required for proper epithelial repair (e.g. *Socs3*). However, injury of tubules in ischemic AKI happen in both ischemic and reperfusion periods. In our model of hemorrhagic shock, tubular injury were identified up to 6 days after the insult. Hence, further studies will have to decipher the respective roles of Hnf-1β in injury and regeneration.

Verdeguer *et al.* showed that ischemic AKI in mice with conditional renal-specific invalidation of *Hnf1b* in adulthood induces a severe tubular disorder characterized by the lost of planar cell polarity and abnormal epithelial cell proliferation with subsequent cystic dilatation.[31] Hnf-1β may drive the tubular regeneration by controlling the network of epithelial genes that have to be early re-expressed after mitosis. This finding was identified *in vitro* with conditions mimicking physiological process of cell renewal. The consequences of (ischemic) AKI on *Hnf1b* expression and role were not reported in this article. In a rat model of cisplatin- or gentamicin-induced renal failure, Xu et al. showed that *Hnf1b* expression is reduced three days after nephrotoxicants injection[32]. However, the characteristics of this model (i.e. induction of complete proximal tubule dysfunction leading to a renal Fanconi syndrome phenotype) precluded any analogy concerning the role of the Hnf-1β transcription factor during renal repair following ischemic insult.

In our study, we identified a more complex role of *Hnf1b* after ischemic AKI. First, during the early steps of epithelial repair, its expression is significantly decreased which is consistent with the concomitant dramatic up-regulation of *Socs3*, a gene negatively regulated by Hnf-1β.[16] *Socs3* acts as an inhibitor of signaling pathways downstream of the interleukine-6-receptor and the EGF/HGF receptors, two pathways actively involved in renal repair.[10, 33-35] Thus, one of the roles of Hnf-1β during renal epithelial repair could be to control a number of cytokines or growth factor signaling pathways by allowing the transient up-regulation of *Socs3*. Second, the subsequent transient over-expression of *Hnf1b* was accompanied by the progressive normalization of the expression of its target genes *Cdh16* and *Pkhd1*. Hence, together with the report from Verdeguer *et al,*[31] our findings also confirm the role of Hnf-1β in the epithelial renal repair following ischemic AKI through the tight regulation of a number of cystic disease-associated genes that control renal tubular morphogenesis.

The molecular mechanisms that underlie the kinetics of Hnf-1β expression after AKI remain unknown. The sharp decrease suggests a direct post-transcriptional effect involving an as yet unknown pathway. We hypothesized that hypoxia, inflammatory response or growth factors may trigger the

expression of *Hnf1b in vivo.* Relevant to the first possibility, we assessed the expression of Hnf-1β in a proximal tubule cell line (HK-2 cells) grown in various conditions of hypoxia. *In vitro*, hypoxia was followed by an early up-regulation of Hnf-1β (one hour), while Hnf-1β down-regulation was observed only if hypoxia was maintained for 48 to 72 hours. This effect of hypoxia was reversible as Hnf-1β expression returned to a basal value after 24 hours of normoxia. Interestingly, we demonstrated that Hnf-1β is controlled at both mRNA and protein level by hypoxia. The timing of Hnf-1β up-regulation was similar to that of Hif-1α over-expression. Hif-1α is a hypoxia-inducible transcription factor dramatically up-regulated during cell hypoxia (as soon as 0.5 to 1 hour after the start of hypoxia). Under normoxic condition, hydroxylation of Hif-1α by specific prolylhydroxylase 1-3 (PHD) leads to its degradation through the proteasomal pathway. Hypoxia inhibits Hif-1α specific PHD and abolishes the degradation of Hif-1α which is thus translocated to the nucleus where it acts as a transcription factor. During hypoxia, Hif-1α directly regulates the expression of specific target genes, like the vascular endothelial growth factor (VEGF),[36] and induces a dedifferentiation of epithelial cells submitted with loss of epithelial markers like E-cadherin.[37] Herein, we showed that up-regulation of Hnf-1β is a very early event during hypoxia but is not dependent on Hif-1α expression. Further studies will have to better delineate the molecular pathways that regulate *Hnf1b* expression during epithelial hypoxia.

Interestingly, our *in vivo* study showed that epithelial repair is accompanied by modulation of *Hnf1b* expression potentially allowing specific transcriptional changes like transient *Socs3* over-expression. However, contrasting with *in vitro* data, first Hnf-1β change observed after ischemic insult was a dramatic fall despite the concomitant low intra-renal oxygen partial pressure observed in this model, as demonstrated by pimonidazole staining.[38] These conflicting results suggest that the expression of *Hnf1b* during epithelial regeneration is dependent of various pathways and that hypoxia is not the main regulator of *Hnf1b* in this context.

Altogether, these results suggest that (1) hypoxia may have short and long term effect on *Hnf1b* expression, (2) molecular mechanisms underlying *Hnf1b* changes observed during epithelial renal repair are more complex than a sole response to hypoxia/normoxia condition and (3) *Hnf1b* may drive an adaptative response to epithelial injury by regulating growth factors / cytokine signaling (*Socs3* expression) and epithelial differentiation (*Cdh16*, *Pkhd1* expression). Whether these findings may be extended to other cause of acute kidney injury (*i.e.* septic or toxic insult) and to other ischemic epithelial insults (*i.e.* liver or gut injury) needs to be addressed.

Materials and Methods

Hemorrhagic shock protocol

Validation of this model of ischemic AKI was recently reported.[38] Animal experimentations were performed according to national and institutional animal care and ethical guidelines, and were approved by local board (Comités régionaux d'éthique en matière d'expérimentation animale, INSERM, Toulouse, France). Briefly, a 2 hours-hypotension (35 mmHg mean arterial blood pressure) induced by temporary blood removal was applied to C57/Bl6 female mice (Harlan France, Gannat, France), further called shocked mice. Anesthesia was based on ketamine and xylazine (250 mg/kg and 10 mg/kg, respectively). Mice were intubated and left jugular vein and femoral artery were catheterized. Mechanical ventilation through intratracheal canula was realized with a specific ventilator Minivent 845© (Hugo Sachs Electronik, Germany). At the end of that period, shed blood and lactated Ringer’s solution (twice the shed blood volume) were infused in order to provide adequate fluid resuscitation. Sham-operated mice (further called sham mice) underwent the same anesthetic and surgical procedures, but neither hemorrhage nor fluid resuscitation were performed. Shocked (n=5-6) and sham mice (n=4) were sacrificed at different times: 3 hours, 10 hours, 24 hours, 2 days, 6 days and 21 days after the beginning of the blood removal.

Cell culture

The human proximal tubule epithelial cell-line HK-2 was purchased from ATCC° (Molsheim, France) and were grown in DMEM/F12 + Glutamax° (1/1) medium supplemented with 50 U/mL Penicillin, 50 mg/mL Streptomycin, 400 µg/mL of hydrocortisone, 10 µg/mL of EGF, 1.4 µg/mL of triiodothyroxine, 5 mg/mL of insulin and 10% fetal calf serum. The cells were grown in 6-wells plates. All experiments were conducted with confluent cultures. For hypoxic culture, cells were placed in a hypoxic (1% O2, 5% CO2, 37°C) incubator (Sanyo 02/C02 incubator, Ontario) for 0, ½, 1, 2, 24, 48 or 72 h. Control cells were incubated for equivalent periods under normoxic conditions (21% O2, 5% CO2, 37°C). Last, normoxic up-regulation of Hif-1α was obtained with the use of specific prolylhydroxylase inhibitor (Calbiochem-Merck°, Fontenay-sous-bois, France).

Molecular analyses

The left kidney of mice was excised, washed with 1X PBS and subsequently used for molecular studies.

Total RNA was collected with the help of the RNeasy© Mini Kit (Qiagen, Courtaboeuf, France) and 1 µg of total RNA was engaged in a reaction of RT-PCR (Superscript II Rnase H Reverse

Transcriptase© (Invitrogen, Villebon sur Yvette, France)). cDNA were subsequently quantified using the LightCycler Mix (Roche, Meylan, France) using the following conditions : 95°C for 5 min followed by 40 cycles (95°C for 15 secondes, 60°C for 10 secondes and 72°C for 15 secondes) and denaturation. Quantitative PCR was carried out on a LightCycler© 480 sequence detection system. The primers were designed using Primer3 and GeneQant softwares. The efficiency of each set of primers was assessed by dilution curves and only set with efficiency higher than 95% were used. Ct differences between the reference (*Gapdh* and *18S*) and target genes were calculated for each sample. The formula used to quantify the relative changes in target over references mRNAs between the two groups is derived from the $2^{-\Delta\Delta Ct}$ formula as recommended. Primers used for quantitative PCR were the following : *Cdh16* (D) CCAGCCTGGAGACACATACA (R) GGATCACAACAGTGGCAGAA ; *Pcna* (D) AAGTGGAGAGCTTGGCAATG (R) CAGTGGAGTGGCTTTTGTGA ; *Socs3* (D) GAGATTTCGCTTCGGGACTA (R) AACTTGCTGTGGGTGACCAT ; *Lrp2* (D) TGGGTGTGTGACCAGGATAA (R) ACACACTGACCATTGGAGCA ; *Hnf1a* (D) GAACCTCAGCCCAGAGGAG (R) GATGTTGTGCTGCTGCAAGT ; *Hnf1b* (D) GCGGTGACTCAGCTACAGAA (R) CACCATTGCAGATGGGAAC ; *Pkhd1* (D) AAGTCAAGGGCCATCACATC (R) ATGTTTCTGGTCAACAGCCC ; *Cubln* (D) CCCTTTGGACCGTTCTGTGGCA (R) ACGGTTGCATACTCCGCATGGA.

For protein extraction, kidney samples were homogenized in lysis buffer (10 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA and 0.1% SDS) with a protease inhibitor cocktail (Complete Mini kit (Roche, Meylan, France)) and separated on nitrocellulose membranes. Membranes were blocked with TBS-0.1% Tween + 5% BSA at room temperature for 2 hours and probed with the monoclonal antibodies anti-*Hnf1b* (sc-22840X, SantaCruz Biotechnology, Santa Cruz CA, USA; 1:1000) overnight at 4°C. The membranes were then incubated for 1 hour with the appropriate secondary antibody (1:7500) conjugated with peroxidase (ECL™ anti-rabbit, GE Healthcare-little Chalfont-GB). Detection was performed with SuperSignal@ West Pico Chemiluminescent Substrate (ThermoScientific, Rockford, USA) and analyzed by the photon detector Gene Gnome@ (Syngene Bio Imaging, Cambridge, UK). Membranes were reprobed with polyclonal goat anti-B-actine (1:10 000). Protein bands were quantified by densitometry using ImageJ public open source software and results are expressed as Hnf-1β/Beta-actin ratio.

Statistical analysis

Quantitative parameters are reported as median and limits, and qualitative parameters as numbers and percentage. Continuous variables were compared using one-way ANOVA test followed by unpaired t-test. A *P*-value < 0.05 was considered as statistically significant. Analyses were performed using GraphPad Prism 4 (Graphpad software Inc, San Diego, USA).

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Figure 1* : *Renal functional, histological and mRNA expression changes after a 2 hours-hemorrhagic shock in mouse. *A*. GFR assessed by inulin clearance at 2 days and 6 days after a 2-hours hemorrhagic shock. Significant decrease of GFR was detected at day 2 and day 6 in shock compared to sham mice ; *B*, Sequential whole-kidney expression of *Pcna* normalized to *Gapdh* mRNA amount at 3 hrs, 10 hrs, 24 hrs, 48 hrs, 6 days and 21 days (H3, H10, H24, H48, D6 and D21, respectively) after hemorrhagic shock. Data are shown as ratio of mRNA expression between shock and sham mice. * P < 0.05, ** P < 0.01 shock (n=5) vs. sham (n=4) ; *C-F*, Renal injury after hemorrhagic shock (sham mice (*E-F*); shock mice (*C-D*)) at day 2 and day 6 (PAS coloration, x400). *GFR*, glomerular filtration rate; *NS*, not significant.

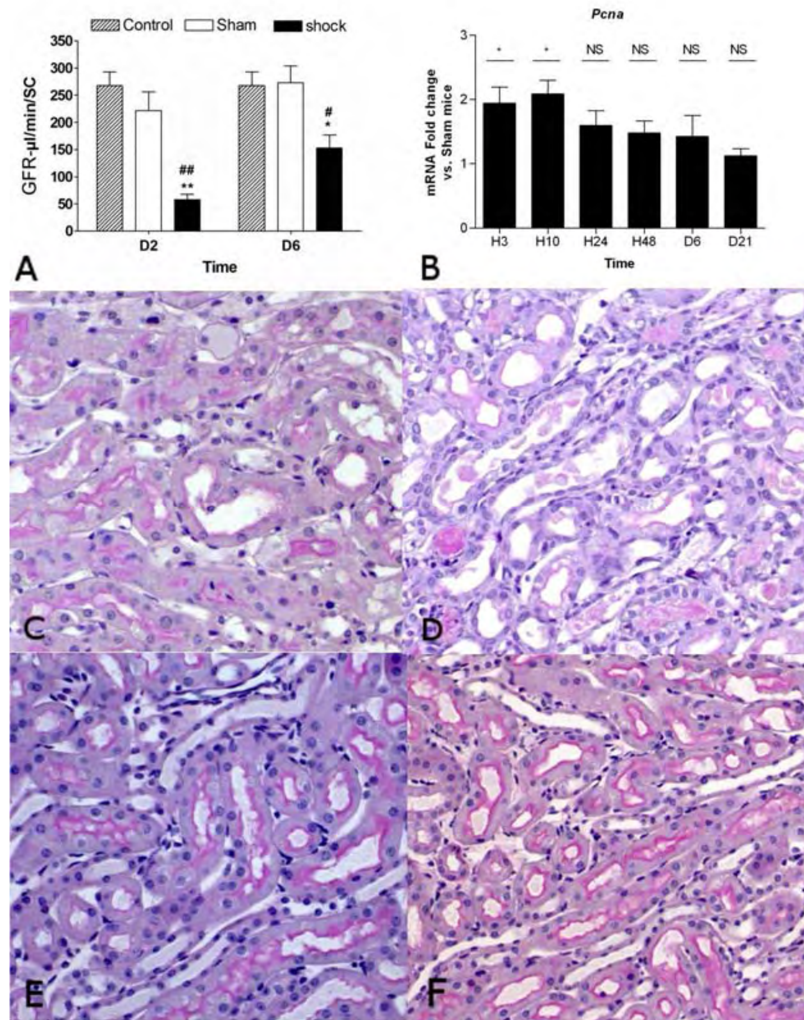
***Figure 2*: Sequential whole-kidney expression of *Hnf1b, and Pcna* after hemorrhagic shock.** *A, C,* Sequential whole-kidney expression of *Hnf1b* and *Pcna* normalized to *Gapdh* mRNA amount at 3 hrs, 10 hrs, 24 hrs, 48 hrs, 6 days and 21 days (H3, H10, H24, H48, D6 and D21, respectively) after hemorrhagic shock. B, whole-kidney expression of Hnf-1β (protein) normalized to Beta-Actin amount at 10 hrs, 24 hrs and 21 days after hemorrhagic shock. Data are shown as ratio of mRNA or protein expression between shock and sham mice. * P < 0.05, ** P < 0.01; shock (n=5) vs. sham (n=4); *NS*, not significant.

***Figure 3*: Sequential whole-kidney expression of *Cdh16*, *Pkhd1* and *Socs3* after hemorrhagic shock.** Relative mRNA expression of *Cdh16* (A)*, Pkhd1* (B) and *Socs3* (C) at 3 hrs, 10 hrs, 24 hrs, 48 hrs, 6 days and 21 days (H3, H10, H24, H48, D6 and D21, respectively) normalized to *Gapdh* mRNA. Data are shown as ratio of mRNA (or protein) expression between shock and sham mice * P < 0.05, ** P < 0.01; shock (n=5) vs. sham (n=4); *NS*, not significant.

***Figure 4*: Sequential whole-kidney expression of *Hnf1a* and the *Lrp2* and *Cubln* megalin-cubilin endocytic complex after hemorrhagic shock.** Relative mRNA expression of *Hnf1a*, *Lrp2* and *Cubln* at 3 hrs, 10 hrs, 24 hrs, 48 hrs, 6 days and 21 days (H3, H10, H24, H48, D6 and D21, respectively) normalized to *Gapdh* mRNA. Data are shown as ratio of mRNA expression between shock and sham mice. * P < 0.05, ** P < 0.01; shock (n=5) vs. sham (n=4); *NS*, not significant.

***Figure 5*: Expression of Hnf-1β and Hif-1α in HK-2 cells under hypoxic conditions.** Relative protein expression of Hnf-1β protein (A) and mRNA (B), and Hif-1α (D) after 30 min, 1hr, 2 hrs, 24 hrs, 48 hrs or 72 hrs of hypoxia. (C) Relative expression of Hnf-1β after 2 hours of hypoxia followed by 24 hours of normoxia. Relative expression of Protein expression was normalized to Beta-actin. Data are shown as ratio of protein expression between cells grown under normoxic (n=6) or hypoxic condition (n=6). * P < 0.05, ** P < 0.01; *NS*, not significant.

***Figure 6*: Expression of Hnf-1β and Hif-1α in HK-2 cells incubated with Hif-1α-specific prolylhydroxylase inhibitor**. Relative protein expression of Hif-1α (A) and Hnf-1β (B) after 3 and 4 hours of exposure to Hif-1α specific prolyl hydroxylase inhibitor. Protein expressions were normalized to Beta-actin (n=6). ** P < 0.01; *NS*, not significant.



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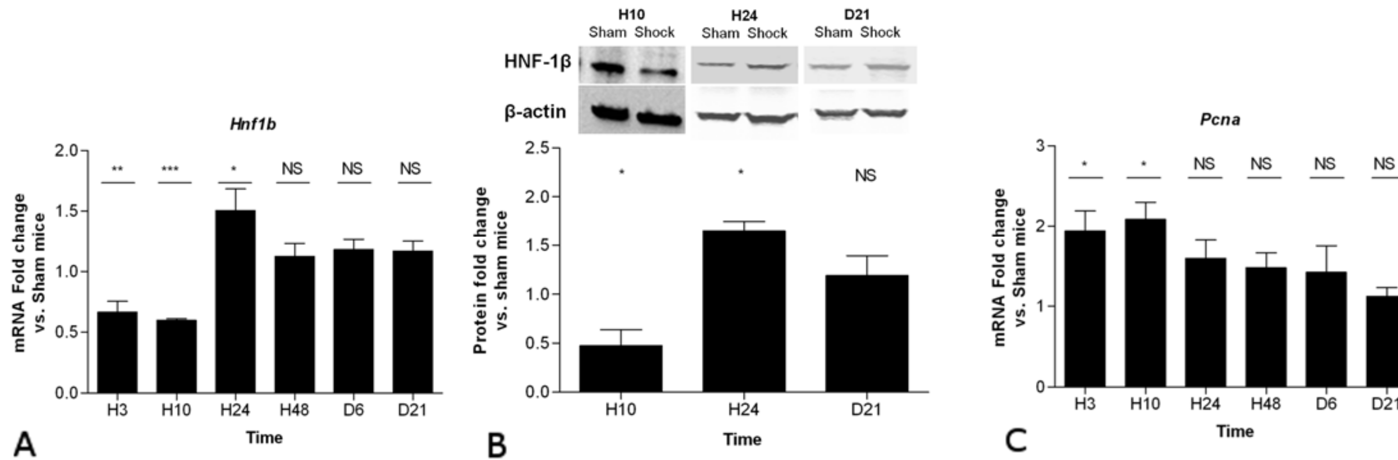

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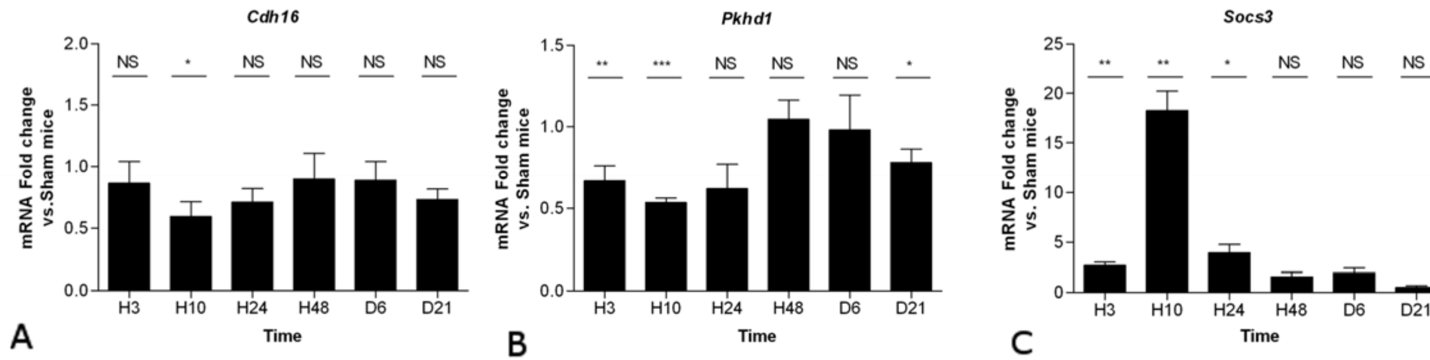

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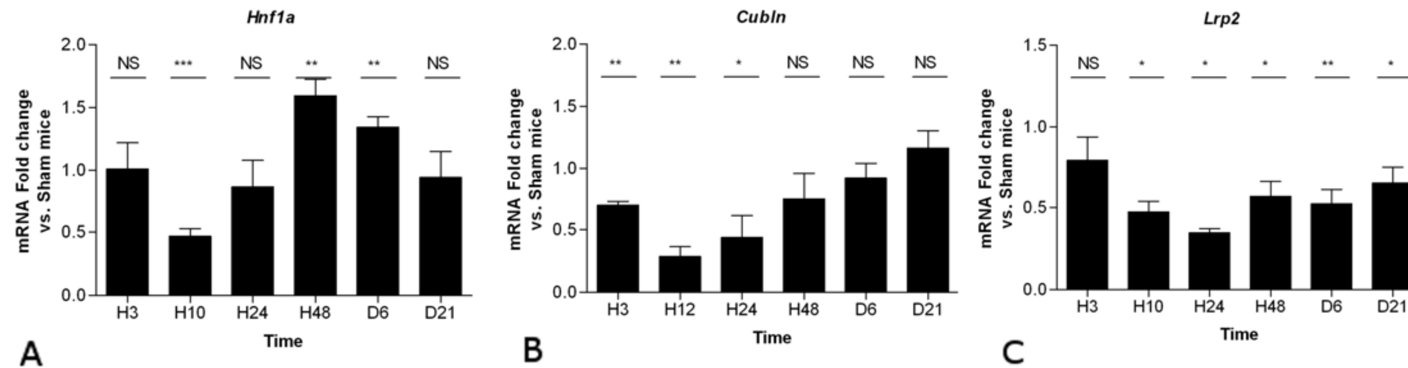

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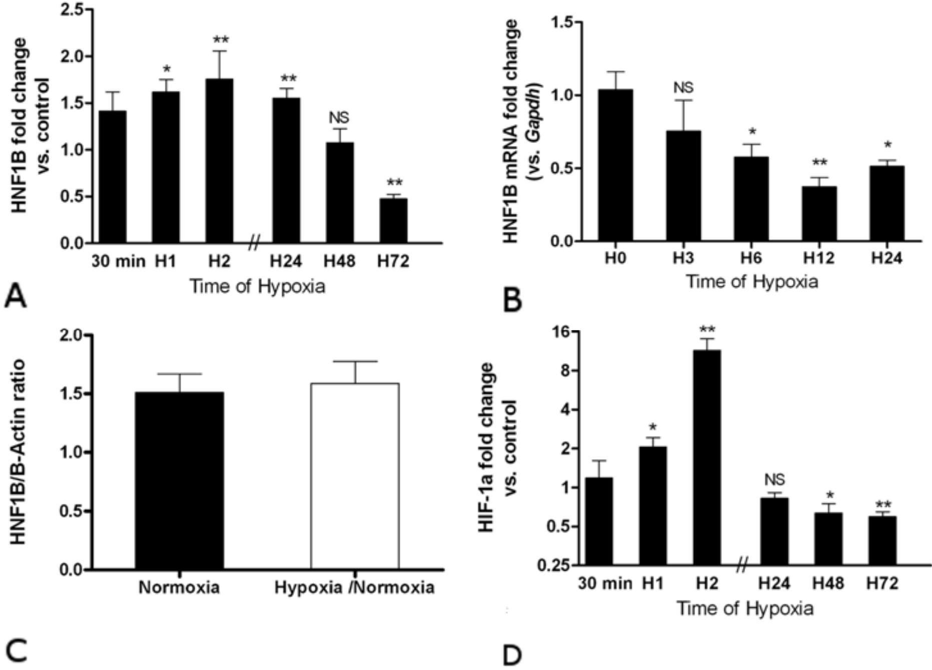

***Figure 5*: Expression of Hnf-1β and Hif-1α in HK-2 cells under hypoxic conditions.** Relative protein expression of Hnf-1β protein (A) and mRNA (B), and Hif-1α (D) after 30 min, 1hr, 2 hrs, 24 hrs, 48 hrs or 72 hrs of hypoxia. (C) Relative expression of Hnf-1β after 2 hours of hypoxia followed by 24 hours of normoxia. Relative expression of Protein expression was normalized to Beta-actin. Data are shown as ratio of protein expression between cells grown under normoxic (n=6) or hypoxic condition (n=6). * $P < 0.05$, ** $P < 0.01$; *NS*, not significant.

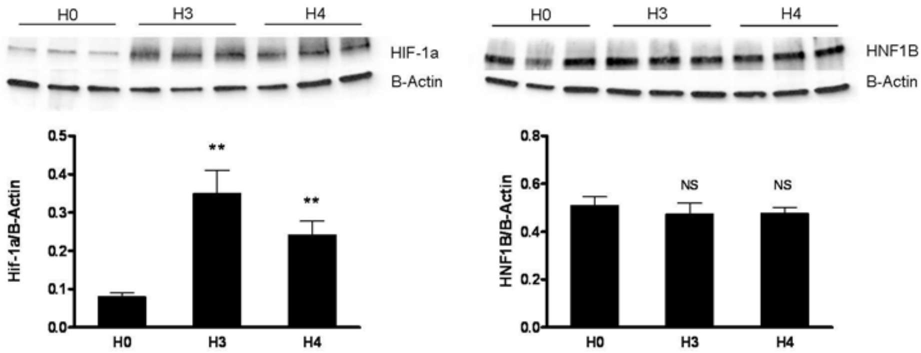

***Figure 6*: Expression of Hnf-1β and Hif-1α in HK-2 cells incubated with Hif-1α-specific prolylhydroxylase inhibitor**. Relative protein expression of Hif-1α (A) and Hnf-1β (B) after 3 and 4 hours of exposure to Hif-1α specific prolyl hydroxylase inhibitor. Protein expressions were normalized to Beta-actin (n=6). ** $P < 0.01$; *NS*, not significant.

Renal cystic genes expression in patients with *HNF1B* mutations

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Abstract

Background/Aims: *HNF1B* nephropathy is characterized by dominantly inherited renal hypodysplasia with few cysts, slow renal decline and hypomagnesemia. Mouse with antenatal inactivation of *HNF1B* is characterized by polycystic kidneys, renal failure and a profound decrease in cystic genes (*Pkhd1*, *Umod*, *Pkd2*) expression. Mice with inactivation after post-natal day 10 have no renal phenotype.

Methods: Quantification of mRNA expression of *HNF1B,* six of its potential target genes (*PKHD1*, *PKD1*, *PKD2*, *IFT88*, *TMEM27* and *UMOD*) and 3 genes involved in the Mg^{2+} renal homeostasis (*ATP1A1*, *FXYD2* and *CLDN16*) in the urinary sediment of 11 individuals with mutation of *HNF1B* and in 9 controls (non-invasive assessment of the renal transcriptome).

Results: As compared to controls, no difference was observed in the urinary mRNA amount of *HNF1B* and the renal cystic genes. A significant increase in the expression of *ATP1A1*, which encodes the α1 subunit of the Na^{+}-K^{+}-ATPase, was identified in *HNF1B* patients consistent with its role in Mg^{2+} homeostasis.

Conclusion: Assessment of mRNA expression in urinary sediment is a non-invasive method applicable to gain insights into the pathophysiology of inherited nephropathies in human. *HNF1B* nephropathy is generally not associated with postnatal down-expression of renal cystic genes in human, a finding consistent with mouse models.

Introduction

In humans, *HNF1B* (Hepatocyte Nuclear Factor-1Beta) mutations result in a multisystem disease with autosomal dominant inheritance. In accordance with the role of *HNF1B* during development, pancreas, genital tract, liver and kidneys may all be affected, resulting in maturity onset diabetes of the young type 5, genital malformations, liver tests abnormalities and nephropathy [1-3]. In mouse kidney, *Hnf1b* is expressed in all the nephron segments, from proximal tubule to collecting duct [4]. Renal phenotype in *HNF1B*-related disease is highly heterogeneous. It mostly encompasses 1) bilateral renal hypodysplasia most often translating into hyperechogenic kidneys or renal cortical cysts from antenatal period through childhood; 2) in adulthood, renal cysts are frequently lacking or few; 3) at any age, the nephropathy harbours a profile of chronic tubulointerstitial nephritis with slowly progressive renal decline, low-range proteinuria without hematuria, and a distinctive renal loss of magnesium and potassium [1,5,6]. In addition, we and others have emphasized a wide inter- and intra-family variability [5,7]. Beside whole-gene deletions, which account for more than half of all mutations [8], private heterozygous point mutations of *HNF1B* have been reported, including missense, nonsense, frameshift and splicing mutations [7]. Molecular mechanisms underlying the clinical heterogeneity are currently unknown and may involve stochastic effects or modifying genes.

While *Hnf1b*$^{+/-}$ mice are fertile and do not exhibit renal involvement, *Hnf1b*$^{-/-}$ die early in embryogenesis due to the lack of visceral endoderm differentiation [9]. In mice, bi-allelic renal-specific *Hnf1b* invalidation leads to polycystic kidney disease and early renal failure [10]. Gresh *et al* showed that *Hnf1b* controls a large transcriptional network including genes coding for proteins that co-localize to the primary cilium, a structure involved in epithelial cells proliferation and planar cell polarity [10,11]. Hence, a significant down-regulation of the renal expression of *Tmem27*, *Ift88*, *Umod*, *Pkd2* and *Pkhd1* was observed in this model [10,12]. Given their implication in renal cystic diseases, these genes were collectively referred to as “renal cystic genes”. More recently, Verdeguer *et al*. showed that renal-specific invalidation of *Hnf1b* after post-natal day 10 was not followed by a down-regulation of these renal cystic genes, except for the *Umod* gene, while transient increase in epithelial cell proliferation (induced by ischemic acute kidney injury) promoted cystic kidney disease in this model [13].

In an attempt to confirm these findings in humans and to decipher the molecular mechanisms of magnesium renal loss, we used a direct approach relying on mRNA expression analysis in urinary sediment. This method has been established as a non-invasive tool to diagnose renal-allograft rejection [14,15] and more recently, to determine gene expression in podocytes and tubular epithelial cells in the mouse [16,17] and in humans [3,18]. We assessed the expression of *HNF1B* and of six cystic genes *PKHD1*, *PKD1*, *PKD2*, *IFT88*, *TMEM27* and *UMOD* in urinary cells of 11 *HNF1B*-mutation carriers (HNF1B patients) and 9 controls. We extended the analysis to urinary expression of *ATP1A1*, *FXYD2* and *CLDN16*, which respectively encode for the α1 and the γ-subunit of the Na^+/K^+-ATPase and claudin-16. These genes are involved in magnesium homeostasis and the two latter have been found mutated in familial hypomagnesaemia [19,20]. Limited availability of mRNA did not allow to assess the mRNA of *TRPM6*, *EGFR* and *KV1.1*, three additional genes involved in the fine tuning of Mg^{2+} in the distal convoluted tubule (DCT) [21-23].

Material and Methods

Patients and controls

Patients were recruited in the Renal paediatric unit at University hospital of Toulouse, France. Genotyping of *HNF1B* was performed in all patients as previously recommended [8] : quantitative multiplex PCR of short fragment (QMPSF) was applied to identify gene deletion, followed by direct sequencing of the nine exons and all exon-intron boundaries in patients with normal QMPSF.

Clinical history of renal and extra-renal involvement in the HNF1B patients was recorded through a standardized assessment of the patient's hospital records. According to age, estimated glomerular filtration rate (eGFR) was calculated using the simplified MDRD formula or Schwarz formula in individuals below 16. Hypomagnesemia was defined by serum magnesium level < 0.75 mmol/L. Imaging studies of the kidney consisting of ultrasonography and/or computed tomography (CT) were recorded. Diabetes was diagnosed on the basis of receiving either insulin or oral agents, or biochemical evidence of diabetes in accordance with WHO guidelines. Pancreas imaging (ultrasonography before 15 years of age and CT-scan thereafter) was performed in all patients.

Controls consisted of nine healthy young individuals (5 males and 4 females, median age 38 years [25-47]). Seven were first- degree relatives of the *HNF1B* cohort, for whom genetic testing ruled out a mutation of *HNF1B*.

According to the French Law, informed written consent was obtained from all subjects and tested relatives and the study was conducted in agreement with the Declaration of Helsinki Principles.

Immunostaining of urinary cells

Freshly voided urine (100 mL) of a control individual was centrifuged at 700 g for 10 minutes. Supernatant was carefully aspirated and the sediment pellets was washed two times with phosphate-buffered saline (PBS). The pellet was then resuspended in 500µL of PBS. The resuspended sediment was then spun onto slides, whichwere air-dried and then fixed with 3 % paraformaldehyde at room temperature for 15 min. After washing with PBS, the slides were incubated with anti-human cadherin-16 antibody (Abcam ab64868) at a dilution of 1:100 for 60 min. After being washed with PBS, a biotin-conjugated secondary antibody and subsequent streptavidin-biotin complex formation was

performed according to manufacturer's instructions. The sediment was counterstained with DAPI nuclear stain to distinguish whole cells from cells fragments.

Urinary cells RNA isolation

To avoid technical bias related to urinary sampling, two to five (median 3) diurnal micturitions (median volume 80 mL [25-150]) were collected from each patient. Isolation and lysis of urinary cells and RNA extractions were performed using the *ZR Urine RNA isolation kit* ™ *(ZymoResearch,* Orange, CA, USA) according to the manufacturer's protocol. RNA quality was assessed by measurement of the optical density (OD) 260/280 ratio with a target OD ratio higher than 1.7, as previously recommended [14].

Real-time quantitative PCR (q-PCR)

cDNA were generated from urinary cell RNAs and quantified using the *Verso*™ *SYBR Green 2-Step qRT-PCR Fluorescein* reagent (ThermoScientific-Fisher, Illkirch, France). The primers were designed using Primer3 software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). Their sequence is provided in Supplementary Table 1. The efficiency of each set of primers was assessed by dilution curves and the Ct differences between the reference (*GAPDH*) and target genes calculated for each sample of each patient. The formula used to quantify the relative changes in target over *GAPDH* mRNAs between the two groups is derived from the $2^{-\Delta\Delta Ct}$ formula recommended by Pfaffl [24]. The RT-PCR conditions were as follows: 51°C for 60 min, 95°C for 2 min. Quantitative PCR was carried out on an ABI PRISM® 7600 sequence detection system using SYBR® green with the following conditions: 95 °C for 15 min followed by 40 cycles (95 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min).

Statistical analysis

Data are presented as absolute medians and limits. Significant differences between *HNF1B* patients and control were tested by the Mann-Whitney test. *P*-values less than 0.05 were considered significant.

Results

Clinical, genetic and biological characteristics of HNF1B patients

We studied eleven *HNF1B* patients (7 children and 4 individuals $\geq$ 16 years-old ; 7 males and 4 females from) at a median age of 4 (range 2 – 40), belonging to nine unrelated families (Figure 1). Familial screening indicated that the mutation occurred *de novo* in seven families. Molecular analysis of *HNF1B* identified a whole-gene deletion in seven patients and point mutations in four (c.185-1A>G, p.Gln253Pro, p.Asn289Asp and p.Gly456X), a distribution observed in previous studies [3,8].

Phenotypes of the *HNF1B* patients are summarized in Table 1. Renal disorder was the first symptom identified in all patients except in patient 6.1 who presented with infertility and genital tract malformation. Briefly, 4 out of the 11 patients were in chronic renal failure (CRF; CKD stage 1 to 3) according to their estimated creatinine clearance (23, 38, 45 and 64 mL/mn at 16, 18, 21 and 40 years of age, respectively). Low serum magnesium (0.61-0.70 mmol/L) was found in four patients. All of them had increased urinary magnesium excretion (as defined by the ratio of magnesium to creatinine clearances) from 5 to 9%.

Renal imaging detected a solitary kidney in six individuals, including two with hypoplastic kidney (patients F1.1 and F2.1); in four, yearly abdominal imaging demonstrated slow involution of their multicystic dysplastic kidney. Among the remaining patients, imaging showed unilateral hypoplastic kidney (n=1), bilateral hypoplastic kidneys (n=1) or bilateral normal sized kidneys (n=3). Renal cysts were observed in eight individuals (multicystic dysplastic kidney in one and few cysts in seven). Renal hyperechogenicity was identified in eight individuals (mainly of paediatric age). Patient 6.1 had a solitary ectopic kidney with few cysts diagnosed at age 36 while his two children had hyperechogenic kidneys with few cysts diagnosed antenatally. Last, imaging was not predictive of renal function in this study.

mRNA expression in urinary sediment

Using immunostaining of urinary cells with an antibody directed against the kidney-specific cadherin, cadherin-16, we first confirmed in a control individual that renal tubular cells may be collected and analyzed from urine sample (Supplementary data). Renal/urothelial cells ratio was

almost 1/10. After urinary cell isolation and lysis, mRNA was extracted and reverse transcribed. Thereafter, we confirmed that renal cells collected in urine come from the different tubular segments, as demonstrated by RT-PCR using specific primers of *AQP1*, *UMOD*, *CLDN16*, *AQP2* and *AVPR2* (Data not shown). Leucocyturia was ruled out in all patients with urinary dipstick.

Relative quantification of the expression of *HNF1B* mRNA in urinary cells showed no difference in transcript levels between *HNF1B* patients and controls (Figure 2). In the subgroup of seven patients with *HNF1B* whole-gene deletions in whom a decrease of expression levels of 50 % could be anticipated from haploinsufficiency, no significant difference in *HNF1B* expression was detected.

We next examined the expression of potential target genes of *HNF1B* in humans and observed that the expression of *UMOD*, *PKHD1*, *PKD1*, *PKD2*, *TMEM27* and *IFT88* mRNAs were not statistically different between patients and controls (see Figure 3). Neither the age at enrolment into the study nor the type of *HNF1B* mutation affected relative expressions of these genes (data not shown).

Last, we assessed the expression of three additional transcripts involved in magnesium homeostasis, *ATP1A1* (encoding for the α1-subunit of the Na^+/K^+-ATPase), *FXYD2* (encoding for the γ-subunit of the Na^+/K^+-ATPase) and *CLDN16* (which encodes for claudin-16). The normalized expression of *ATP1A1* mRNA was significantly higher in *HNF1B* patients as compared to controls (6-fold, $p = 0.009$) while no difference was detected in expression of *FXYD2* and *CLDN16* (Figure 4). *ATP1A1, FXYD2* and *CLDN16* mRNA expression was not correlated with serum magnesium level (data not shown).

Discussion

To investigate whether *HNF1B*-related nephropathy is associated with a peculiar postnatal profile of renal cystic genes in human, we have performed a non-invasive expression analysis following collection of urinary cells in 11 patients aged between 2 and 40 years. We also used this method to investigate whether hypomagnesaemia observed in patients with *HNF1B*-related nephropathy is associated with differences in expression of genes involved in Mg^{2+} metabolism. The main limit of this study is the high degree of variability of the tubular to urothelial cells ratio that may be collected in urine samples. Subsequently, high variability of expression levels was observed when GAPDH was used as reference gene. Because renal transcriptomic changes are currently unknown in patients with *HNF1B* mutations and *Hnf1b* may modify expression of segment specific tubular markers in mouse , we could not use them as reference gene. Note, previous studies have shown that GAPDH may be used as reference gene to identify intra-renal mRNA changes (ref). Last, we did not use *HNF1B* itself as reference gene because of its expression in urothelial cells (ref) and the lack of data concerning its expression in each tubular segment in adult normal and *HNF1B* patients.

In these 11 patients with heterogeneous renal phenotype, including 7 with a whole-gene deletion, we did not observe any change in expression of *HNF1B* mRNA as compared to controls, suggesting that after antenatal period, one copy of *HNF1B* could be sufficient to reach normal expression of this gene in post-natal kidney.

The lack of down-regulation of renal cystic genes may account for the low cystic involvement in most patients of this series diagnosed with *HNF1B*-related nephropathy in adulthood, as exemplified in patients F3.1, F5.1, F6.1 and F9, in whom imaging studies exhibited no or few renal cyst. This is reminiscent of pathological findings from our early experience: rare cysts of glomerular or tubular origin were observed on kidney specimen from 6 unrelated individuals [1].

The lack of postnatal detectable difference in expression level of *HNF1B* and related cystic genes in *HNF1B*-mutated individuals is not entirely unexpected according to previous findings. First, genetic discrepancies between mouse models and human disease suggest that the underlying molecular mechanisms are different. In contrast to rodent models, which share recessive inheritance at tissue level [9,10,12], *HNF1B*-related human disease is dominantly inherited [25]. While a significant down-

regulation of the expression of renal cystic genes *Tmem27*, *Ift88*, *Umod*, *Pkd2* and *Pkhd1* was observed postnatal in mice with antenatal renal-specific conditional inactivation of *Hnf1B* [10,12], a sustained expression of *PKD1*, *PKD2* and *PKHD1* was observed in epithelial cells layering both cystic and non-cystic renal structures of two *HNF1B*-mutated human foetuses [26]. This could be related to the persistent expression of the non-mutated allele. Moreover, there is no evidence for a two-hit process within renal tissue in *HNF1B* individuals, with the exception of somatic deletion of the second allele in neoplastic tissue of the rare individuals who developed chromophobe renal carcinoma superimposed on *HNF1B* nephropathy [27]. Second, target genes of transcription factors may widely diverge between humans and mice. This applies to the HNF family, with recent recognition of a 40-80% divergence among HNF1A and HNF4A target genes between mouse and human [28]. Of note, the over-expression of *HNF1B* in a human embryonic kidney cell line (HEK293) was not followed by an up-regulation of the target genes of *Hnf1b* previously identified in mouse model [29]. Last, rodent models have recently shed light on the key role of *Hnf1b* in the transcriptional switch occurring at the end of the kidney maturation process, around postnatal day 10 (P10) [30]. While conditional renal-specific inactivation of *Hnf1b* in embryos and neonates until P3 results in severe polycystic kidney disease, no cystic lesions develop in mice induced at P10 or later and renal transcriptome analysis demonstrated a sustained expression of cystic genes (*Pkd2*, *Pkhd1*, *Kif12*, *Tmem27*). This finding is consistent with the lack of difference of urinary mRNA amounts of *PKHD1*, *PKD2* and *TMEM27* between patients and controls investigated in this study. Our study highly suggests that the expression of most cystic genes in human quiescent renal cells is not dependent of *HNF1B*. Exact role of *HNF1B* in adult human kidney remains thus to be defined.

In adults, mutations of *HNF1B* may be associated with low serum magnesium or low serum potassium level due to renal loss of either ion [5,31]. These peculiar findings have not been reported so far in animal models, which hampers molecular analysis. Among the 11 patients tested in this series, urinary loss of magnesium and potassium were identified in four and two individuals, respectively. Renal magnesium homeostasis is a complex physiological process that involves many transporters and receptors (for instance claudin-16, claudin-19, Na^+/K^+-ATPase) all along the different tubular segments (review by Naderi *et al* [32]). We tested here the expression of *CLDN16* (claudin-

16), *FXYD2* and *ATP1A1*(encoding for the γ-subunit and the α_1-subunit of the Na^+/K^+-ATPase) in renal tubular cells of *HNF1B* patients. We found a six-fold increase in mRNA of *ATP1A1* of *HNF1B* patients while mRNA expression of *CLDN16* and *FXYD2* were not different in patients and controls (Figure 5). At first glance, the up-regulation of the Na^+/K^+-ATPase seems to be consistent with its role for maintenance of the trans-epithelial electrical gradient required for renal magnesium paracellular reabsorption. However, whether *ATP1A1* is a direct target of HNF1B in the human kidney remains unknown. Using the Consite program (http://asp.ii.uib.no:8090/cgi-bin/CONSITE/consite), we failed to identify any putative *HNF1B* binding site in the 4.000 bp preceding the first *ATP1A1* exon. Changes in the level of *ATP1A1* expression may be a functional consequence of hypomagnesaemia rather than a direct effect of *HNF1B*, since mutations of *ATP1A1* have not yet been described in humans. Recently, In our study performed before a report showing that *HNF1B* increased expression of a specific isoform of *FXYD2 in vitro,*[31] we failed to show a decrease in urinary amounts of *FXYD2* mRNA in *HNF1B*-mutated patients but we only assessed the overall expression of *FXYD2* in urinary cells regardless of various isoforms specific expression. Limited availability of mRNA did not allow us to assess the relative expression of the various isoforms of *FXYD2*.

In summary, we show here that measurement of mRNA expression in urinary sediment is a valuable approach to assess the renal epithelial transcriptome in inherited renal diseases. We demonstrate that the expression of cystic renal disease genes, previously recognized as target genes of *Hnf1b* in mouse, are not dramatically reduced in postnatal (quiescent) renal epithelial cells of human patients carrying a mutation of *HNF1B*, thus confirming distinct functions of the *HNF1B* transcription factor during nephrogenesis and in the mature kidney. Further studies will have to confirm and expand these preliminary results obtained in a small cohort of patients in order to decipher the transcriptional network involving *HNF1B* in post-natal human kidney.

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Figure 1: **Summary of phenotype and genotype changes in nine French families with *HNF1B* mutation.** Individuals recruited for urinary transcriptome analysis in the study are identified by a number.

Figure 2 : **mRNA levels of *HNF1B* in urinary cells of 11 patients with *HNF1B* mutation and 9 control individuals**. Dots represent the quantification levels of *HNF1B* normalized mRNA ($2^{-\Delta\Delta Ct}$) relative to the quantity of *GAPDH* transcripts. ● whole-gene deletion, ○ point mutation.

Figure 3 : **mRNA levels of *UMOD*, *PKD1*, *PKD2*, *PKHD1*, *TMEM27* and *IFT88* in urinary cells of 11 patients carrying a mutation of *HNF1B* and 9 control individuals**. The box-plot representation shows the medians and the percentile values 10, 25, 75, and 90 for the quantification levels of normalized mRNA ($2^{-\Delta\Delta Ct}$) relative to the quantity of *GAPDH* transcripts.

Figure 4 : **mRNA levels of *FXYD2, CLDN16* and *ATP1A1* in urinary cells of 11 patients carrying a mutation of *HNF1B* and 9 control individuals**. The box-plot representation shows the medians and the percentile values 10, 25, 75, and 90 for the quantification levels of normalized mRNA ($2^{-\Delta\Delta Ct}$) relative to the quantity of *GAPDH* transcripts.

Table 1: **Clinical and genetic characteristics of 11 individuals from 9 unrelated families with *HNF1B* mutation.** *Pt* patient, *CRF* chronic renal failure (eGFR < 60 ml/mn/1.73m², according to simplified MDRD formula, or Schwarz formula in individuals below 16 years), *M* male, *F* female, *HE* hyperechogenicity, *MCDK* multicystic dysplastic kidney, *BAVD* bilateral absence of vas deferens, *NA* not available.

Pt	Age	Gender	Mutation	Kidney malformations	CRF	Serum Mg^{2+} level (N : 0,75-1 mM)	Pancreas abnormalities	Liver test abnormalities	Genital tract abnormalities
F1.1	3	M	p.Glu253Pro	Hypoplastic horseshoe kidney, few cysts, HE	No	Normal	No	Yes	NA
F2.1	4	F	Deletion	Right : involution Left : hypoplastic, one cyst, HE	No	Normal	No	Yes	NA
F3.1	16	M	c.185-1G>A	Right : normal Left : involution	Yes	0.7 mmol/L	Hypoplastic Low fecal elastase	No	NA
F4.1	4	M	Deletion	Right : MCDK, HE Left : hypoplastic	No	0.61 mmol/L	No	Yes	NA
F5.1	18	M	Deletion	Right hypoplastic, HE Left : hypoplastic, HE	Yes	Normal	No	No	NA
F6.1	40	M	Deletion	Right : few cysts, pyelic dilation Left : absent	Yes	0.65 mmol/L	Hypoplastic	No	BAVD Ectopic testis
F6.2	4	F	Deletion	Right few cysts, HE Left : few cysts, HE	No	Normal	No	Yes	Ectopic testis
F6.3	4	M	Deletion	Right : few cysts, HE Left : few cysts, HE	No	0.7 mmol/L	No	Yes	NA
F7	4	M	Deletion	Right : few cysts, HE Left : few cysts, HE	No	Normal	No	Yes	NA
F8	2	F	p.Gly456X	Right : involution Left : few cysts, HE	No	Normal	No	No	NA
F9	21	F	p.Arg289Asp	Right : absent Left: normal	Yes	Normal	No	No	No

Table 1: **Clinical and genetic characteristics of 11 individuals from 9 unrelated families with *HNF1β* mutation.** *Pt* patient, *CRF* chronic renal failure (eGFR < 60 ml/mn/1.73m², according to simplified MDRD formula, or Schwarz formula in individuals below 16 years), *M* male, *F* female, *HE* hyperechogenicity, *MCDK* multicystic dysplastic kidney, *BAVD* bilateral absence of vas deferens, *NA* not available.

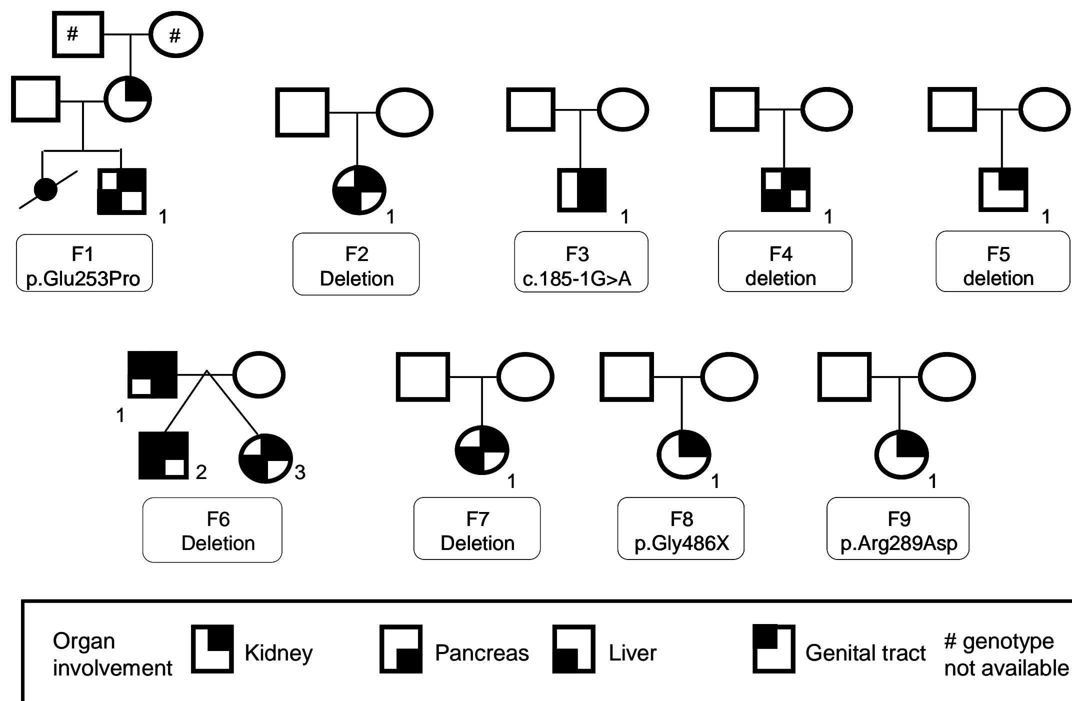

Figure 1: **Summary of phenotype and genotype changes in nine French families with *HNF1β* mutation.** Individuals recruited for urinary transcriptome analysis in the study are identified by a number (from 1 to 3).

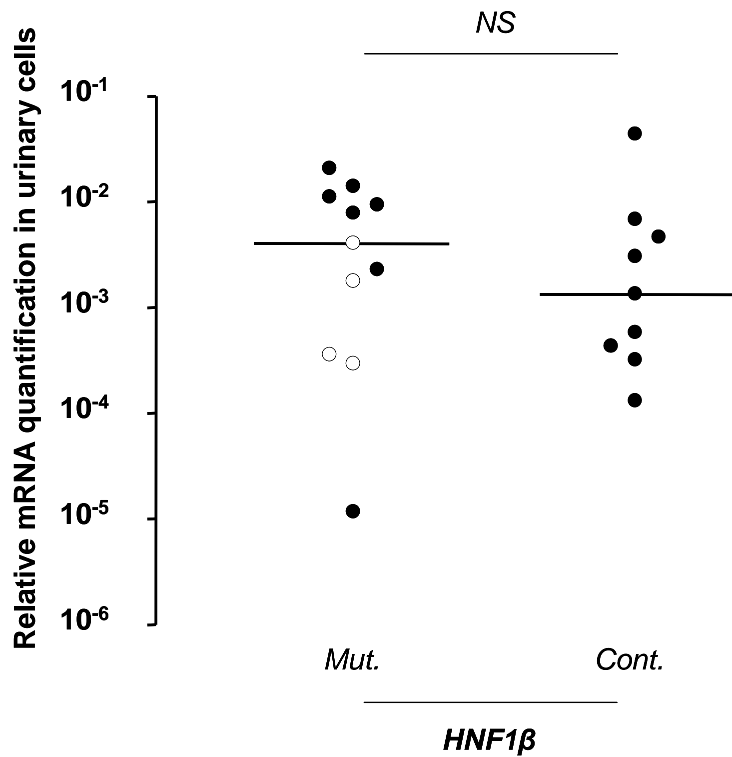

***Figure 2 :* mRNA levels of *HNF1β* in urinary cells of 11 patients with *HNF1β* mutation and 9 control individuals**. Dots represent the quantification levels of *HNF1β* normalized mRNA ($2^{-\Delta\Delta Ct}$) relative to the quantity of *GAPDH* transcripts. There was no significant difference in *HNF1β* quantification in urinary cells between *HNF1β*-mutated and control individuals ($p > 0.05$). ● whole-gene deletion, ○ point mutation.

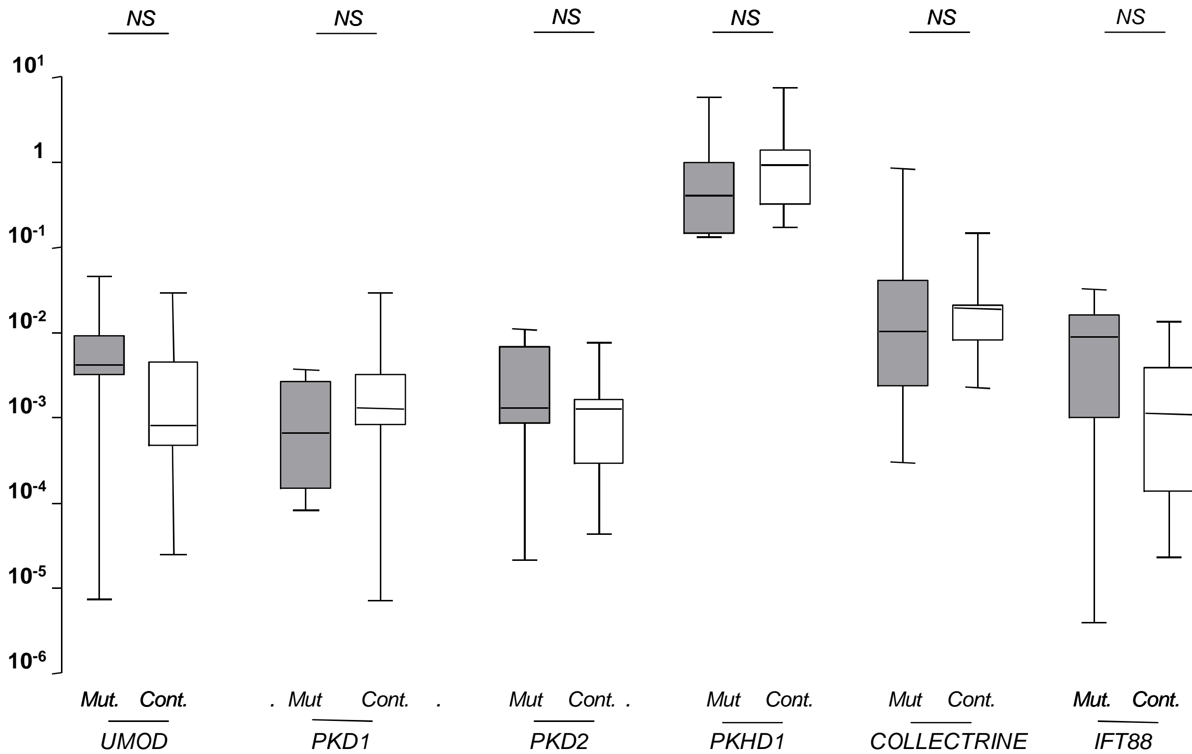

***Figure 3 :* mRNA levels of *UMOD*, *PKD1*, *PKD2*, *PKHD1*, *TMEM27* and *IFT88* in urinary cells of 11 patients carrying a mutation of *HNF1β* and 9 control individuals**. We excluded from this analysis one patient with dramatic down-expression of *HNF1β* (patient F2.1) that was not observed in the 10 remaining patients. The box-plot representation shows the medians and the percentile values 10, 25, 75, and 90 for the quantification levels of normalized mRNA ($2^{-\Delta\Delta Ct}$) relative to the quantity of *GAPDH* transcripts. No significant difference in *UMOD*, *PKD1*, *PKD2*, *PKHD*1, *TMEM27* (Collectrine) and *IFT88* genes quantification was identified in urinary cells ($p > 0.05$).

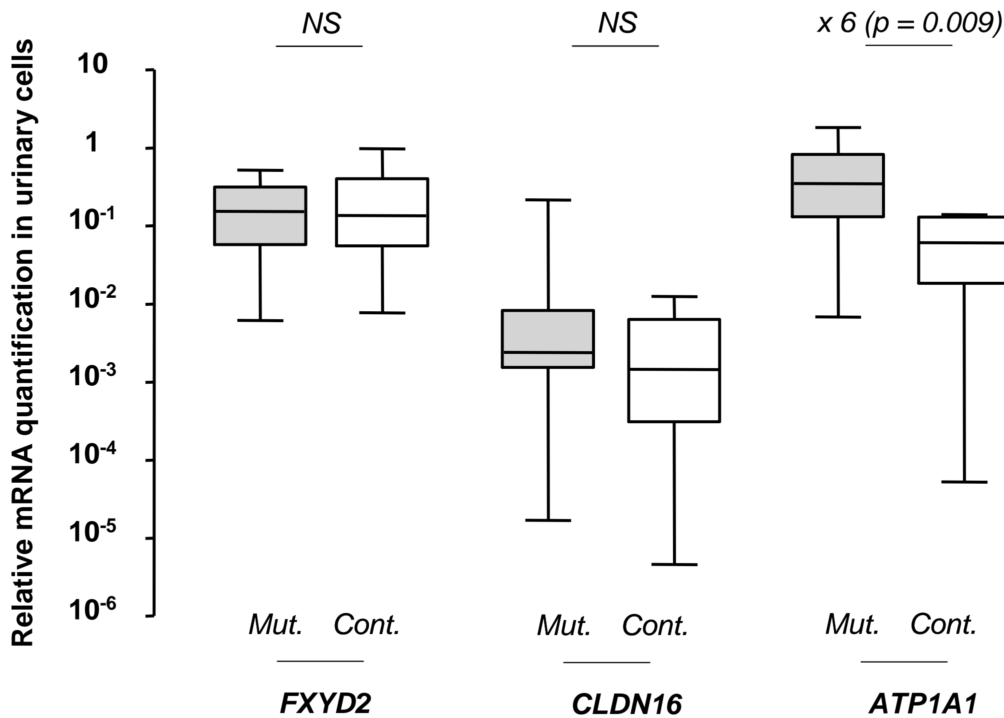

Figure 4 : **mRNA levels of *FXYD2, CLDN16* and *ATP1A1* in urinary cells of 11 patients carrying a mutation of *HNF1β* and 9 control individuals**. We excluded from this analysis a patient with dramatic down-expression of *HNF1β* (patient F2.1) that has not been observed in the 10 remaining patients. The box-plot representation shows the medians and the percentile values 10, 25, 75, and 90 for the quantification levels of normalized mRNA ($2^{-\Delta\Delta Ct}$) relative to the quantity of *GAPDH* transcripts. There is a significant increase in *ATP1A1* expression (6-fold, p = 0.009) in urinary cells when comparing *HNF1β*-mutated and control individuals, while no significant difference in *FXYD2* and *CLDN16* genes quantification was identified (p > 0.05).

Phenotypic and genotypic characterization of the HNF-1β-related nephropathy

-

Role of HNF-1β in experimental acute kidney injury

The *HNF1B* gene encodes for the HNF-1β transcription factor (hepatocyte nuclear factor-1β). In human, *HNF1B*-related disease is a highly heterogeneous dominantly inherited multi-organ disease, which encompasses renal, pancreas, liver and genital tract abnormalities. In the kidney, HNF-1β controls all the steps of the nephrogenesis (tubulogenesis, planar cell polarity (oriented mitotic division) and tubular segment specification and maintenance.

Aims of this thesis were to better detail the phenotype associated with HNF1B mutations (clinical part), to better define the genotype of the HNF1B patients (genetic part) and to assess the role of HNF-1β in acute kidney injury (scientific part).

1. Clinical part. First, we reported two atypical presentations of *HNF1B* mutation (cystic kidney disease mimicking an autosomal polycystic kidney disease ; esophageal atresia and urinary tract/renal abnormalities). We also reported the clinical charts of 27 adult patients with HNF1B mutation and highlighted the following data : in adult patients, HNF1B nephropathy has the characteristics of a chronic tubulo-interstitial nephritis with frequent hypomagnesemia and hypokaliemia. Last, we could perform a genotype-phenotype analysis in a large cohort of HNF1B individuals (mostly lower than 18 years of age).
2. Genetic part. In addition to the genotype-phenotype correlation study, we tried to better define the individuals that could beneficiate from *HNF1B* analysis. Studying a large cohort of 433 patients tested in the Genetic department of the University hospital of Toulouse, we could establish a predictive score of *HNF1B* mutation in order to help both clinicians and geneticist.
3. Scientific part. First, role of HNF-1 β in post-natal human kidney was assessed by studying the expression of its target genes in the urinary cells pellets of HNF1B patients and their first-degree relatives free of mutation. With this indirect approach, we could confirm preliminary data observed in mouse models : in post-natal kidney, expression of HNF-1 β target cystogenes was similar in individuals with and without *HNF1B* mutation.
   Then, we aimed at better delineate the role of HNF-1β in acute kidney injury. Studying a mouse model of hemorrhagic shock, we could characterize the kinetic of HNF-1β expression in injured kidney and to correlate it to the expression of its target genes, like *Socs3*, a key actor of epithelial repair which control an adaptative response of the epithelium to extra-cellular signals (IL-6, EGF, HGF…). *In vitro*, respective roles of the hypoxia-inducible factor HIF-1α and hypoxia *per se* were detailed. Given the expression of HNF-1β in many epithelial cells, we hypothesized that this transcription factor may also be involved in the reparation of other organ (liver, gut…). Last, in order to better decipher the consequences of an epithelial injury on HNF-1β expression, experiments in a mouse model of endotoxinic shock (lipopolysaccharide infusion) are currently on going.

In summary, this work aimed at better delineate the genotype and phenotype of *HNF1B* mutations and decipher the role of this transcription factor in acute (ischemic and septic) kidney injury. Characterization of the role of HNF-1β in epithelial repair and identification of its regulatory factors could open a new field of research : may HNF-1β prevent epithelial dedifferentiation, a well-known trigger of renal fibrosis.

AUTEUR : Dr Stanislas FAGUER
TITRE DE LA THESE : Caractérisation phénotypique et génotypique de la néphropathie liée aux mutations du facteur de transcription HNF-1β – Rôle d'HNF-1β dans l'insuffisance rénale aiguë expérimentale
DIRECTEUR DE THESE : Pr Dominique CHAUVEAU
LIEU ET DATE DE SOUTENANCE : le 25 février 2013 à Toulouse

RESUME

Le gène *HNF1B* code pour le facteur de transcription HNF-1β (hepatocyte nuclear factor-1β). Chez l'homme, les mutations d'*HNF1B* sont associées à une maladie à transmission autosomique dominante touchant à des degrés divers le rein, le pancréas, le foie et l'appareil génital. Dans le rein, HNF-1β régule la néphrogenèse précoce et tardive, la tubulogenèse, la polarisation planaire des cellules tubulaires (division orientée dans l'espace) et plus tardivement l'homéostasie des cellules tubulaires.

Les objectifs de ce travail de thèse étaient de trois ordres : mieux caractériser le phénotype associé aux mutations d'*HNF1B* en particulier à l'âge adulte (partie clinique), mieux définir le génotype des patients *HNF1B* (partie génétique) et caractériser le rôle d'HNF-1β au cours de la régénération suivant une agression épithéliale aiguë (partie fondamentale).

(1) Caractérisation phénotypique. Outre la publication de deux phénotypes originaux (atrésie de l'œsophage – anomalies rénales ; néphropathie kystique mimant une polykystose autosomique dominante), nous avons pu colliger les données d'une cohorte de 27 patients adultes porteurs d'une mutation d'*HNF1B* et rapporter les faits suivants : à l'âge adulte, la néphropathie est de type tubulo-interstitielle chronique et associe fréquemment une hypomagnésémie et une hypokaliémie. Nous avons également pu réaliser une analyse génotype-phénotype précise d'une large cohorte d'individus mutés (majoritairement d'âge pédiatrique).

(2) Caractérisation Génétique. En plus de participer à une étude de corrélation génotype – phénotype, nous avons entrepris de mieux définir la population pouvant bénéficier d'une exploration du gène *HNF1B*. L'étude d'une large cohorte de 433 patients testés dans le laboratoire de Génétique du CHU de Toulouse nous a permis de définir un score prédictif de mutation d'*HNF1B* utile à la fois aux cliniciens et aux généticiens.

(3) Caractérisation du rôle d'HNF-1β chez l'homme et en pathologie. Dans un premier temps, le rôle d' HNF-1β dans le rein humain post-natal a été appréhendé par l'analyse de l'expression de ses gènes cibles dans le sédiment urinaire de patients HNF1B (succédané du tissu rénal habituellement non disponible dans cette pathologie) et de leurs apparentés au 1er degré indemnes de la mutation. Nous avons pu confirmer par cette approche indirecte les données parcellaires disponibles chez la souris : l'expression des cystogènes cibles d'HNF-1β après la phase du développement rénal n'est pas différente de celle des patients témoins. Dans un second temps, nous avons tenté d'appréhender le rôle d'HNF-1β au cours des phases de régénération épithéliale suivant une agression rénale aiguë. L'étude d'un modèle murin de choc hémorragique contrôlé nous a permis de définir la cinétique d'expression d'HNF-1β et de la rapporter à celle de ses cibles géniques, dont *SOCS3*, acteur clé de la réparation de l'épithélium rénal et de la réponse épithéliale aux stimuli extra-cellulaires (IL-6, HGF, EGF…). *In vitro*, l'impact de l'hypoxie et du facteur induit par l'hypoxie Hif-1α sur l'expression d'HNF-1β a également pu être détaillée. L'expression d'HNF-1β n'étant pas limitée au rein, nous faisons l'hypothèse que ce facteur pourrait également être impliqué dans la régénération d'autres épithélia, comme le foie ou le tube digestif. Enfin, afin de mieux caractériser l'impact d'une agression épithéliale sur l'expression d'HNF-1β et en déduire les mécanismes moléculaires de sa régulation, nous détaillons actuellement l'impact d'un choc endotoxinique (injection de lipopolysaccharide) sur l'expression d'HNF-1β et de ses gènes cibles dans les différents organes sus-cités. L'étude in vitro de cellules rénales soumises au même stress nous a également permis de définir une nouvelle voie de régulation d'HNF-1β.

En résumé, ce travail aborde un ensemble de problématiques attenant au facteur de transcription HNF-1β (manifestions phénotypiques et génotypiques de ses mutations, caractérisation du rôle d'HNF-1β en situation d'agression épithéliale aiguë). Par extension, la caractérisation du rôle d'HNF-1β dans la réparation épithéliale et de ses modes de régulation pourrait ouvrir un nouveau champ de recherche sur son éventuelle capacité à prévenir la dédifférenciation tubulaire, prémisse à l'apparition d'une fibrose rénale.

MOTS CLES : facteur de transcription, HNF-1β, néphropathie héréditaire, régénération de l'épithélium rénal
DISCIPLINE ADMINISTRATIVE : Génétique moléculaire

Cette Thèse a été réalisée dans l'unité INSERM U563 (CPTP, Hôpital Purpan) et dans l'unité 1048 (I2MC, équipe 12, Hôpital Rangueil) à Toulouse