Review

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The molecular functions of hepatocyte nuclear factors – In and beyond the liver

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Summary

The hepatocyte nuclear factors (HNFs) namely *HNF1α/β*, *FOXA1/2/3*, *HNF4α/γ* and *ONECUT1/2* are expressed in a variety of tissues and organs, including the liver, pancreas and kidney. The spatial and temporal manner of HNF expression regulates embryonic development and subsequently the development of multiple tissues during adulthood. Though the HNFs were initially identified individually based on their roles in the liver, numerous studies have now revealed that the HNFs cross-regulate one another and exhibit synergistic relationships in the regulation of tissue development and function. The complex HNF transcriptional regulatory networks have largely been elucidated in rodent models, but less so in human biological systems. Several heterozygous mutations in these HNFs were found to cause diseases in humans but not in rodents, suggesting clear species-specific differences in mutational mechanisms that remain to be uncovered. In this review, we compare and contrast the expression patterns of the HNFs, the HNF cross-regulatory networks and how these liver-enriched transcription factors serve multiple functions in the liver and beyond, extending our focus to the pancreas and kidney. We also summarise the insights gained from both human and rodent studies of mutations in several HNFs that are known to lead to different disease conditions.

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Introduction

The hepatocyte nuclear factors (HNFs) were first identified as liver-enriched transcription factors.[1] However, HNFs are also expressed in a variety of other tissues and organs, such as the pancreas and the kidney, playing important roles in regulating the development and functions of these tissues. Studies have successfully identified the binding motifs of HNFs from different families and these binding motifs do not appear to be exclusively active in the liver. A variety of hepatic and non-hepatic genes contain the matched HNF binding motifs in their promoter and enhancer regions, suggesting the likely presence of multiple transcriptional regulatory networks involving HNFs that are not restricted to liver development and function. Importantly, many of the HNFs are involved in complex auto-regulatory and cross-regulatory circuits, likely acting in a combinatorial manner to determine the development of various tissues including the developing embryo, pancreas, kidney and intestines. Herein, we comprehensively summarise the diverse expression patterns and functions of HNFs in the developing embryo and in adulthood, as well as the HNF cross-regulatory network and mutations in these HNF transcription factors that give rise to disease conditions that implicate different organs. In this review, particular attention is placed on the liver, pancreas and kidney, where the roles of HNFs are best studied. We aim to highlight the significance of the HNFs in the liver and beyond, to increase the appreciation of the pleiotropic effects that these factors can have and to encourage a more holistic approach to the understanding of their role in development and disease.

Molecular structure of the HNF families

HNFs are classified into four families, namely HNF1, FOXA (or HNF3), HNF4 and ONECUT ([OC] or HNF6), each characterised by distinct regions corresponding to functional domains (summarised in Fig. 1). The HNF1 family comprises of HNF1α and HNF1β, whose DNA-binding domain (DBD) is known to bind to the palindromic consensus sequence GTTAATNATTANC (Fig. 1A). The dimerisation domain at the N-terminus allows both HNF1α and HNF1β to form homodimers or heterodimers.[2,3] The *HNF1α* and *HNF1β* genes each encode three isoforms (A, B and C) that appear to have tissue-specific roles.[4,5]

FOXA (formerly known as HNF3) belongs to the subfamily of the Forkhead box (FOX) proteins, comprising FOXA1, FOXA2 and FOXA3 (formerly HNF3α, HNF3β and HNF3γ).[6,7] The FOXA family of proteins contain a winged helix (WH) structure (also referred to as the forkhead domain) flanked by sequences required for nuclear localisation (Fig. 1B).[8] They also share a highly conserved DBD and bind to the target DNA as a monomer.[9] The N- and C-termini of FOXA proteins are conserved and reported to act as the transactivation domains.[10,11]

HNF4 belongs to the orphan nuclear receptor family and comprises HNF4α and HNF4γ (Fig. 1C). The two transactivation function domains, activation function 1 and 2 (AF-1 and

Key point

The liver-enriched HNFs are not restricted to the liver and are expressed in a variety of tissues and organs, playing critical roles in regulating tissue development and function.

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AF-2), located at the N- and C-terminus respectively, activate transcription in a cell type-independent manner.[12,13] A ligand-binding domain (LBD) is located adjacent to AF-2 and transactivates genes in a ligand-dependent manner, providing an additional point of control for regulating protein activity. Of note, HNF4 proteins also contain a repressor domain, region F, that has an inhibitory function,[12] which has not been characterised in other HNF families. *HNF4α* is encoded by two developmentally-regulated promoters (P1 and P2), and differential promoter usage and alternative splicing are now known to generate up to twelve isoforms (P1-derived *HNF4α1-6* and P2-derived *HNF4α7-12*) that are expressed in a temporal and tissue-specific manner.[14–17]

OC1 (or HNF6) belongs to the one cut homeobox gene family. It consists of a single cut domain (CD) and a divergent homeodomain (HD) (Fig. 1D) that form the bipartite DBD.[18,19] The cut homeodomain contains sequences that mediate nuclear localisation and transcriptional activation, in conjunction with the N-terminal STP box (serine/threonine/proline-enriched region).[9,20] The members of this family include OC1 and its two paralogs, OC2[21] and OC3.[22]

It is clear that while the HNF family members harbour common features such as DNA-binding and transactivation capabilities, they are largely defined by distinct, though related, conserved structural domains that account for their functional diversity. The multiple isoforms within each subfamily lend further complexity to their potential to regulate cellular and tissue functions (Fig. 1). The following sections in this review will illustrate overlapping expression patterns among HNF family members and a complex cross-regulatory network connecting several of these HNF isoforms. Studies on rodent gene perturbations and naturally-occurring mutations in humans further reveal their involvement in disease pathophysiology.

Expression profile of HNFs during mammalian embryonic development and in adulthood

Key point

To-date, the expression patterns of HNFs during development have largely been characterised in rodent tissues, given the limited access to human tissues. These studies have provided important information on the spatial and temporal expression patterns of different HNF families and their isoforms, and thus increased our understanding of the role of these HNFs across multiple tissues.

The expression patterns of HNF family members during mammalian embryonic development and in adulthood have been better characterised in rodents than in human tissues. In this section, we highlight and compare the spatial and temporal expression patterns of the various HNFs, with a particular focus on the liver, pancreas and kidney. These organs are known to exhibit high expression levels of many of the HNF family members, are often implicated in diseases resulting from dysregulation of HNF proteins, and provide the tissues in which the respective roles of HNFs are most well-studied. Discussion of the role of HNF family members in other organs is beyond the scope of this review; nonetheless, their expression patterns across other mouse and human tissues are summarised (Tables 1 and 2).

Rodent embryonic development and adulthood

In the mouse, *Hnf1β* is first detected in the primitive endoderm on embryonic day (E)4.5 and is required for specification of the primitive endoderm lineage.[23,24] The expression of *Hnf1β* precedes *Hnf1α* during embryogenesis, as *Hnf1α* transcripts are first detected in the yolk sac on E8.5.[24] *Hnf4α* is expressed in the visceral endoderm from E4.5,[25–29] and is subsequently detected in the liver bud and hindgut starting from E8.5.[25–28,30] On the other hand, *Foxa1*, *Foxa2* and *Foxa3* have distinct temporal expression patterns and appear in partially overlapping domains of the definitive endoderm and notochord.

Hnf1β transcripts are subsequently detected in the foregut endoderm on E9, from which the liver and pancreas develop. During liver development, both *Hnf1α* and *Hnf1β* transcripts can be detected in the liver primordia by E10.5, and they continue to be present in the liver throughout embryonic life.[31] On E9.5, *Foxa1* and *Foxa2* are also highly expressed during liver bud formation while *Foxa3* is expressed at a lower level. The expression of *Foxa1* and *Foxa2* then falls between E12.5 and 15.5 before increasing again in the adult liver. In contrast, *Foxa3* is initially weakly expressed but its expression increases on E10.5 and remains high throughout liver development.[32] Similar to the expression profile observed for *Foxa1* and *Foxa2*, *Oc1* is detected on E9 when liver differentiation occurs, then disappears transiently between E12.5 and E15, before it continues to be expressed within the extrahepatic biliary system and the liver throughout development.[9,20,33] In contrast to *Foxa* and *Oc1*, high levels of *Hnf4* transcripts are localised to the periphery of the liver where hepatocytes develop from E11.5 to E16, but not in the centre where haematopoietic cells differentiate.[29] In the mouse liver, there is a controlled switch from P2 (distal) to P1 (proximal) promoter-driven transcription of *Hnf4α* from foetal life to birth, and P1-derived transcripts continue to be expressed at significantly higher levels in adult liver.[34] The early activation of *Hnf1β*, *Foxa1*, *Foxa2*, *Hnf4α* and *Oc1* in the developing liver between E8.5–9.5 suggests their importance in the early commitment towards the hepatoblast lineage, alongside other transcription factors and signalling molecules.

In the context of pancreatic development, *Hnf1β* and *Hnf4α* are expressed by most epithelial cells of the pancreatic bud from E9.5, and in $Pdx1^+$ pancreatic progenitors followed by $Ngn3^+$ (Neurog3) endocrine precursors (~E12.5) in the early pancreas.[30] In fact, *Hnf1β* is likely to be a key player in early pancreas morphogenesis as it is expressed in the pre-pancreatic foregut endoderm and in early multipotent pancreatic

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progenitor cells.[35] As the pancreas starts to mature (~E17.5), *Hnf4α* can be detected in the islet, ductal and acinar cells. In contrast, *Hnf1β* gene expression is largely restricted to ductal cells.[30] Hnf4γ protein expression in the pancreas is strongest in the endocrine cells and weakest in the cells lining the ducts.[36] Hnf1α is expressed slightly later in the pancreatic epithelial cells on E10.5, and as pancreatic development continues, Hnf1α expression becomes more confined to both the acinar and developing islet cells, similar to the expression pattern observed for Hnf4α.[37,38] This suggests a role for Hnf1α in the maintenance of the profile of differentiated islet cells. Similar to Hnf1α, *Oc1* is first expressed on E10.5 in the developing pancreas, while *Oc2* exhibits biphasic expression, first decreasing after E12.5 and then increasing after E15.5. Conversely, *Oc1* expression is high until E16.5, after which it decreases.[39] On E18, *Oc1* is absent from islets[33] but is localised to the ductal cells. Unlike *Oc1* and *Oc2*, *Oc3* is not detected in the developing liver or pancreas.[22,39] Overall, it is clear that the transcription factors display dynamic gene expression patterns due to carefully orchestrated mechanisms for the patterning and eventual development of the liver and pancreas from the definitive endoderm.

During kidney development, *Hnf1β* is expressed in the nephrogenic zone of the kidney in newborn rats whereas *Hnf1α* is absent. Subsequently, *Hnf1α* transcripts are restricted to the proximal and distal tubules whereas *Hnf1β* can also be detected in the collecting ducts.[40] From E10.5, mesonephric tubules of the developing kidney express high levels of *Hnf4* transcripts followed by the newly-formed metanephros on E12.5. In the newborn mouse kidney, *Hnf4* is strongly expressed in the proximal and distal convoluted tubules of the cortex but is weakly expressed in the loop of Henle.[29] There is little information on the involvement of members of the Foxa and Onecut families in the development of the kidney.

During adulthood, *Hnf1α* and *Hnf1β* are both expressed in tissues such as the liver, pancreas, kidney and intestine (Tables 1 and 2). *Hnf1β* may have broader regulatory roles as it is expressed in organs that lack *Hnf1α*, such as the thymus, lung, testis and ovary.[1,41] *Foxa1* and *Foxa2* are both highly expressed in the epithelial cells of the developing liver and pancreas but also a number of other tissues in the body (Table 1). *Hnf4* transcripts are most abundantly expressed in the adult liver, kidney and intestines, with relatively weaker expression observed in the pancreas, skin and stomach (Table 1).[42–45] In adult mice, only *Oc1* is detected at high levels in the pancreas and at very low levels in the testis. Both *Oc1* and *Oc2* transcripts are highly expressed in the liver.[22] Members of the Onecut family are also expressed in the neural system, stomach, biliary system and the intestine (Table 1).

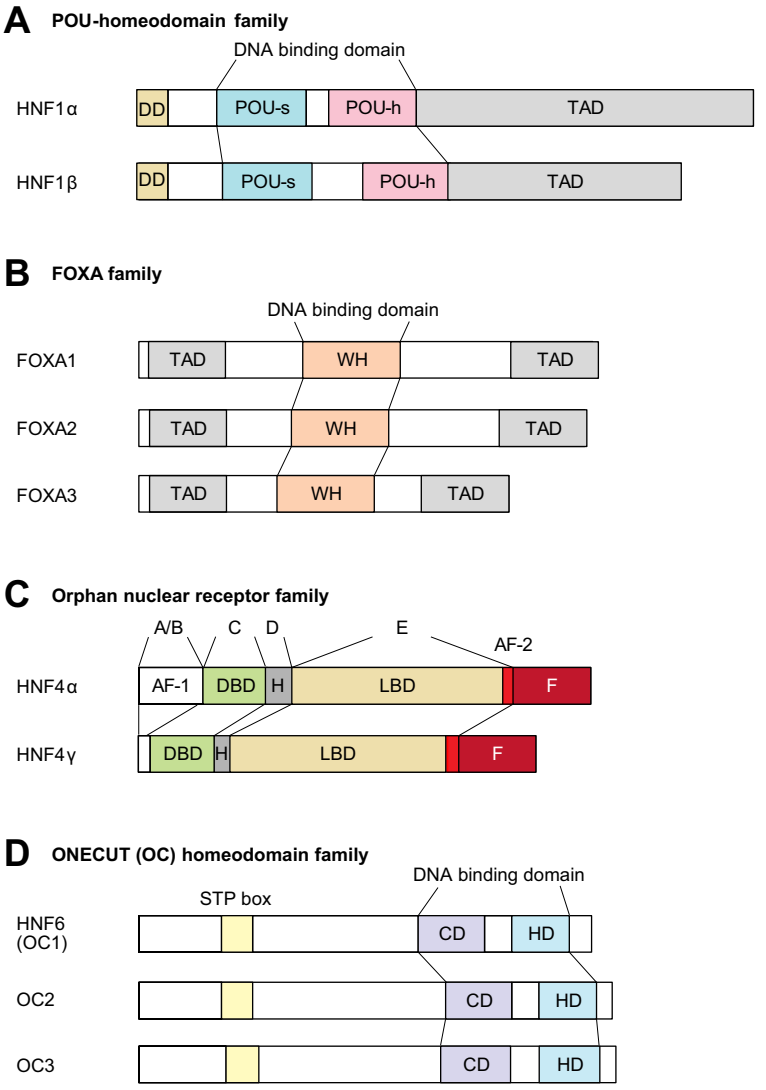


Fig. 1. Structural domains of HNF families. (A) A schematic diagram of HNF1α and HNF1β. The N-terminus consists of the DD. The DBD of the family is made up of a POU-s and a POU-h. The C-terminus contains the TAD which is less conserved between the two members of the HNF1 family. (B) A schematic diagram representing the subfamily members of Forkhead box, namely FOXA1, FOXA2 and FOXA3. The two TADs are flanking at either ends of each of the transcription factors. The DBD of the family consists of a WH structure with sequences for nuclear localisation. (C) A schematic diagram of the orphan nuclear receptor family HNF4 that comprises HNF4α and HNF4γ. Domain A/B contains the N-terminal AF-1, domain C contains a highly conserved DBD, domain D refers to a hinge region (H) that connects domains C and E, while domain E contains a potential LBD with two zinc fingers that recognise and bind to specific DNA motifs. The LBD also contains AF-2. HNF4 proteins also have a repressor region (domain F) at the C-terminus. (D) A schematic diagram of ONECUT gene family members HNF6 (OC1), OC2 and OC3. A serine/threonine/proline-enriched region (STP Box) is located near the N-terminal end of the OC proteins. Together the CD and the HD form the bipartite DBD near the C-terminus. Diagrams are not drawn to scale. AF-1/2, activation function 1/2; CD, cut domain; DBD, DNA-binding domain; DD, dimerization domain; FOXA, forkhead box A; HD, homeodomain; HNF, hepatocyte nuclear factor; LBD, ligand-binding domain; OC, ONECUT; POU-s, POU-specific domain; POU-h, POU-homeodomain; TAD, transactivation domain; WH, winged helix.

Limited information exists on human embryonic development and adulthood

As mentioned previously, *HNF1α* is expressed as three alternatively processed transcripts whose expression is likely to be regulated during development.[4] In humans, *HNF1α(A)* is the major isoform in the adult liver, kidney and foetal

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Table 1. Tissue-specific expression of HNF family members in the adult mouse.

	Brain	Endocrine tissues*	Bone marrow & immune system	Muscle tissues	Lung	Liver and gall bladder	Pancreas	Gastrointestinal tract	Kidney & urinary bladder	Male reproductive tissues	Female reproductive tissues	Adipose & soft tissue	Skin
Hnf1α						■	■	■	■				
Hnf1β		■			■	■	■		■	■	■		
Foxa1					■	■	■	■	■	■			
Foxa2					■	■	■	■					
Foxa3					■	■		■		■			
Hnf4α						■	■	■	■	■			
Hnf4γ	■					■	■			■			
Oc1	■					■		■					
Oc2	■		■	■	■			■			■	■	■
Oc3	■		■	■	■	■	■	■		■	■	■	■

Information consolidated from.[2,7,24,31,32,40,129,163] Coloured squares denote expression. *Endocrine tissues refer to thyroid, parathyroid and adrenal glands. Foxa, forkhead box A; Hnf, hepatocyte nuclear factor; Oc, Onecut.

Table 2. Tissue-specific expression of HNF family members in human embryonic and adult tissues.

		Brain	Endocrine tissues*	Bone marrow & immune system	Muscle tissues	Lung	Liver and gall bladder	Pancreas	Gastrointestinal tract	Kidney & urinary bladder	Male reproductive tissues	Female reproductive tissues	Adipose & soft tissue	Skin
Human embryo	HNF1α						■	■	■	■				
	HNF1β							■	■	■				
	FOXA1					■	■		■					
	FOXA2					■	■	■	■					
	FOXA3						■		■					
	HNF4α						■	■		■				
	HNF4γ						■		■	■				
Adult human	HNF1α			■			■	■	■	■				
	HNF1β			■		■	■	■	■	■	■	■		
	FOXA1			■		■	■		■	■	■	■		
	FOXA2			■		■	■	■	■		■	■		
	FOXA3	■	■	■	■	■	■	■	■	■	■	■	■	■
	HNF4α		■	■	■		■	■	■	■	■		■	■
	HNF4γ	■	■	■	■	■	■	■	■	■	■	■	■	■
	OC1	■				■	■		■	■		■		
	OC2						■		■					

Information on embryonic tissue expression is extracted from "An integrative transcriptomic atlas of organogenesis in human embryos"[52] and consolidated from.[48,51] No information is available for ONECUT family members. Information on adult tissue expression is consolidated from[5,14,48,50] and adapted from the Protein Atlas database (http://www.proteinatlas.org/). Coloured squares denote expression. Endocrine tissues refer to thyroid, parathyroid and adrenal glands. FOXA, forkhead box A; HNF, hepatocyte nuclear factor; OC, ONECUT.

pancreas; whereas *HNF1α(B)* predominates in the adult pancreas.[5] This is in contrast to observations in rodents, where *Hnf1α(A)* predominates in the liver, pancreas and kidney.[46]

P1-driven *HNF4α* isoforms are known to be liver-specific whereas P2-driven *HNF4α* isoforms are pancreas-specific.[17] However, one report had suggested the expression of P1-derived *HNF4α* transcripts in human pancreatic β cells.[47] In a more recent study, the expression of *HNF4α* isoforms in human gestation stage foetal pancreas and adult tissues was more thoroughly examined by isoform-specific real-time PCR.[48] Up to 23% of *HNF4α* expression in 9th–26th week foetal pancreas was found to be of P1 origin, but only P2-driven *HNF4α* isoforms can be detected in the adult pancreas.[48] Immunohistochemistry (IHC) analyses have also shown that P1-driven HNF4α isoforms are expressed in the liver and kidney, whereas P2-driven isoforms are expressed in the pancreas, bile duct and stomach.[49] Northern blot analysis revealed that *HNF4γ* transcripts are weakly expressed in the pancreas, kidney, testis, small intestine and colon, but *HNF4α* is generally expressed at higher levels in all of these tissues, as well as in the liver.[14]

In two other human studies, a total of 18 foetuses were examined for HNF1β expression in the urogenital tract using reverse transcription PCR, ISH (*In situ* hybridisation) or IHC. HNF1β is present in mesonephric duct derivatives such as the efferent duct or epididymis in 12th-, 21st- and 22nd-gestational-week-old foetuses, but not in Müllerian duct derivatives, such as the uterus or fallopian tube after the 18th-gestational week.[50,51] The pattern of expression of HNF family members in human embryonic and various adult tissues is highlighted (Table 2). The former is based on transcriptomic data from various tissues of human embryos at Carnegie Stages 14–22.[52] To date, little has been reported on the expression profiles of FOXA and OC in human development.

Notably, because of the lack of access to human foetal tissues, there is limited information relating to the expression profile of HNFs during human

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embryonic development. There continues to be a heavy reliance on the extrapolation from rodent studies. While it is possible to compare the expression profile of HNFs between rodents and humans in adult tissues, the fact that many of these genes are developmental genes first expressed in the embryo implies that *HNF* gene mutations would have an early impact on tissue development. Given the divergent phenotypes between rodents and humans with heterozygous mutations in several *HNF* genes, further understanding of the expressions and functions of *HNF* genes during human embryonic development is critical. This issue will be further discussed in a later section of this review.

The complex HNF cross-regulatory network in various tissues and organs

The HNF transcription factors operate in a developmental stage- and tissue-specific manner to mediate cellular development and function. In fact, increasing evidence has shown that several of these HNFs are functionally-related and/or part of a shared regulatory network. In this section, we focus on the cross-regulatory interactions between the HNFs and highlight their roles in regulating different tissues during embryonic development, particularly during the development of the liver, pancreas and kidney. The bulk of these studies have been carried out in rodent models, providing valuable insight into the regulatory circuits that involve HNFs.

The role of FOXA, HNF1 and HNF4 transcription factors during embryonic development

FOXA proteins are heavily involved in tissue regionalisation within the definitive endoderm. FOXA1 and FOXA2 regulate genes via different mechanisms although they share similar expression patterns during development[53] (Fig. 2A). Specifically, FOXA2 is likely to act upstream of the HNF cross-regulatory transcriptional network as *Foxa2*-null embryos fail to express *Foxa1* and also express reduced levels of *Hnf4α* and *Hnf1α*.[26] Furthermore, Foxa1 negatively regulates the expression of *Hnf4α* and *Hnf1α* despite sharing identical DNA-binding sites with Foxa2.[53] FOXA1 uniquely achieves its repressor function by competing for binding targets with FOXA2. Insulin purportedly upregulates *Foxa2* while reducing *Foxa1* expression, resulting in a timely regulation of downstream targets.[53]

Foxa3 is involved later during development as its expression is positively regulated by HNF1β and HNF1α proteins.[54] HNF1β likely regulates *Foxa3* expression in early gut endoderm and liver primordium, before HNF1α participates in liver development, consistent with the observed order of their expression patterns in these tissues[31,32,54] (Fig. 2A). As *Hnf4α* precedes *Hnf1α* expression during early embryonic development, *Hnf1α* is not likely to participate in the initial activation of *Hnf4α*. Conversely, HNF1β was found to be a strong inducer of *Hnf4α* when acting together with GATA6 even though it binds to the same motifs as HNF1α.[55]

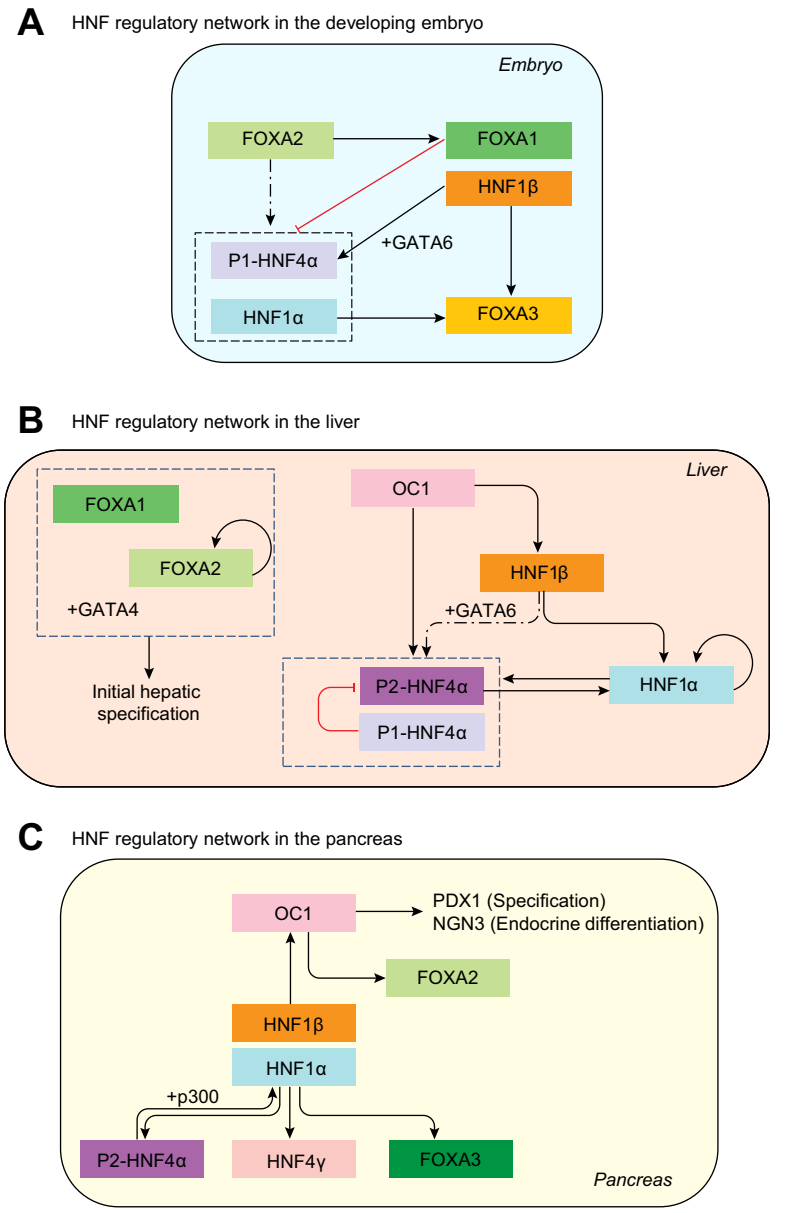


Fig. 2. Schematic outlining key HNF cross-regulatory networks. (A) HNF regulatory network in the developing embryo. FOXA2 acts upstream of FOXA1 to regulate its initial expression in the embryo. FOXA1 negatively regulates the gene expression of P1-driven *HNF4α* and *HNF1α*. FOXA1 achieves its repressor function by competing for binding targets with FOXA2. FOXA3 is positively regulated by HNF1α and HNF1β. HNF1β synergises with GATA6 to strongly regulate expression of P1-driven HNF4α. (B) HNF regulatory network in the liver. FOXA1, FOXA2 and GATA4 are involved in the initial specification of liver progenitors. FOXA2 further self-regulates its own expression. OC1 positively regulates HNF1β and HNF4α. In turn, HNF1β binds to the promoter of *HNF4α* and *HNF1α* to regulate their expression. OC1, HNF1β and HNF1α regulate the expression of HNF4α. P1 promoter-driven HNF4α suppresses the expression of P2 promoter-driven HNF4α in the adult liver. (C) HNF regulatory network in the pancreas: HNF1β activates OC1 expression in pancreatic precursor cells. OC1 is involved in initiating the onset of *PDX1* expression for pancreatic cell fate specification. OC1 regulates pancreatic endocrine differentiation via NGN3 (NEUROG3) expression. OC1 also binds directly to the *FOXA2* promoter. P2 promoter-driven HNF4α and HNF1α form a cross-regulatory loop, forming a complex with p300 to promote downstream gene expression. HNF1α is also involved in regulating the expression of HNF4α, HNF4γ and FOXA3 transcripts in islets. FOXA, forkhead box A; HNF, hepatocyte nuclear factor; OC, ONECUT.

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Complex interplay amongst the HNFs in the stepwise regulation of liver development

In the anterior endoderm, *Foxa1* and *Foxa2* act together with *Gata4* to induce *Albumin* expression, providing the initial specification for liver differentiation.[56] Studies performed in HepG2 cells indicate that FOXA2 binds onto its own promoter to autoregulate its expression. Interestingly, after liver progenitor specification, development of later hepatic differentiation stages is independent of *FOXA*.[57] The liver primordium forms on E8.5–9, beginning with HNF1β–induced liver differentiation.[24,40] HNF1β also cooperates with GATA6 to strongly activate the *HNF4α* promoter[55] (Fig. 2B). As liver differentiation occurs, the expression of *Hnf1β* decreases while that of *Hnf1α* increases. The balance between HNF1β and HNF1α levels may determine the composition of the transcription initiation complex formed on the HNF4α promoter.[55] There appears to be a redundancy between *Hnf1α* and *Hnf1β* in the regulation of *Hnf4α* expression as shown in *Hnf1α*-null mice.[58] However, by adulthood, *Hnf1β* is no longer expressed[59] whereas HNF1α continues to cooperate with OC1 to regulate *HNF4α* gene expression in human liver cells.[55] (Fig. 2B). The mutagenesis of HNF1α or OC1 binding sites within the *HNF4α* promoter reduces promoter activity, while the overexpression of these transcription factors activates *HNF4α* expression.

Key point

Several HNFs are involved in cross-regulatory networks that are functionally-related during embryonic development. The regulation of downstream target genes by specific expression of the HNFs determine cell fate and eventual development of tissues such as the liver or pancreas.

Genome-wide promoter occupancy studies (using chromatin immunoprecipitation microarray; ChIP-on-chip) performed in adult human hepatocytes have provided insight into how the liver-specific HNF cross-regulatory network formed by HNF1α, HNF4α and OC1 regulates hepatocyte function[60] (Fig. 2B). Interestingly, HNF4α was found to bind to more than 40% of the promoters of actively-transcribed genes. It also occupied most of the promoters bound by HNF1α and OC1,[60] suggesting that HNF4α plays a central role in the transcriptional programme in the liver.[61] In addition, other studies have also ascertained that HNF4α binds onto the proximal promoter of the *HNF1α* gene[62] and that *Hnf4α*-null hepatocytes express reduced *Hnf1α* transcripts.[27] *In vitro* studies performed in HepG2 cells show that a point mutation in the HNF4α binding site of the *HNF1α* promoter reduces HNF4α binding, leading to reduced activation of *HNF1α* gene expression.[63] HNF4α is known to bind to the *HNF1α* promoter via an evolutionarily conserved *cis* sequence element, regulating its transcription in hepatocytes.[61] HNF1α also autoregulates its own expression in hepatocytes.[64]

In the liver, *Oc1* and *Oc2* have redundant roles in the differentiation of both hepatocytes and biliary cells.[65] Oc1 stimulates *Hnf1β* expression in the liver for intrahepatic bile duct morphogenesis.[66] This is consistent with the observation that *Oc1*-null mice exhibit diminished *Hnf1β* expression, failed development of the gallbladder, and severe abnormalities in both extra- and intrahepatic bile ducts[66,67] (Fig. 2B). Studies in *Oc1*-null/*Oc2*-null mice further revealed that Oc1 and Hnf1 regulation of *Hnf4α7* expression (P2 promoter-driven form) is suppressed by Hnf4α1 (P1 promoter-driven form) later in liver maturation.[68]

The HNF1α-HNF4α and OC1-HNF1β cross-regulatory network in the pancreas

In the pancreas, Hnf1α is known to regulate the expression of *Hnf4α*. *Hnf1α*-null mice display reduced expression of *Hnf4α*, *Hnf4γ* and *Foxa3* transcripts in the islets.[69,70] Specifically, HNF1α regulates the P2 promoter of the *HNF4α* gene in the pancreas.[17,69] A naturally-occurring mutation in the human *HNF4α* P2 promoter, which abolishes binding by HNF1α, is known to result in maturity onset diabetes of the young 1 (MODY1). The significance of this HNF1α-*HNF4α* gene regulation is reflected by similarities in phenotypes caused by the disruption of *Hnf4α* transcription by Hnf1α and loss-of-function *Hnf1α* mutations.[71] Conversely, in the liver, expression of the P1 promoter-driven form of *Hnf4α* is not activated by Hnf1α.[17,69,72] These studies reflect tissue-specific differences in the HNF1α-HNF4α-mediated transcriptional regulatory network (Fig. 2B, C).

The cross-regulatory loop between the two transcription factors is made even more complex as HNF4α is also an essential activator of *HNF1α* gene expression (Fig. 2B). The AF-2 module and LBD of HNF4α collectively mediate the regulation of *HNF1α* gene expression 73. HNF4α can facilitate the recruitment of transcriptional coactivators such as p300 to enhance the activation of *HNF1α* expression. In fact, direct protein-protein interaction also occurs as HNF4α can bind cooperatively with HNF1α, thereby improving the docking of p300 onto the HNF4α-HNF1α complex, regulating downstream target gene expression in a synergistic manner.[73] Moreover, studies have shown that HNF4α and HNF1α share common transcriptomic signatures in the pancreatic islets and that their functional activities are interdependent, though this is likely to be target-specific.[74] This is important for our understanding of how defects in either HNF4α or HNF1α can lead to islet dysfunction and diabetes (discussed in a later section).

Oc1 and *Oc2* exert partially redundant functions in dorsal and ventral pancreas organogenesis.[75] The Onecut family members are also involved in initiating expression of the key pancreatic transcription factor *Pdx1*. In contrast to observations in liver cells, the expression of Oc1 in the pancreatic precursor cells is activated by Hnf1β, leading to the expression of *Pdx1*, which is critical for the specification of pancreatic cell fate.[76] At later stages of development, *Oc1* regulates pancreatic endocrine differentiation via *Ngn3* expression.[67,77] OC1 also binds to the proximal promoter region of *FOXA2* to regulate its expression in the gut endoderm[78] (Fig. 2C). Evidently,

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OC1 and *FOXA2* are uniquely co-expressed to regulate cellular identity in hepatocytes, pancreatic and intestinal cells.[9]

Complex differential regulation of HNF4α isoforms in the liver and pancreas

The multiple isoforms of *HNF4α* generated via alternative splicing, highlight the regulatory complexity of *HNF4* expression and function. The consensus is that isoforms HNF4α1-3 derived from the *HNF4A* P1 promoter are predominantly expressed in adult liver (also in kidney) while isoforms HNF4α7-9 derived from the P2 promoter are active in both embryonic liver and in pancreatic β cells[17] (Fig. 3). Binding sites for HNF1, OC1 and GATA6 have all been reported on the P1 promoter.[79] In the embryonic liver, it is suggested that HNF1β cooperates with GATA6 to activate *HNF4α1* expression 55 (Figs. 2A, 3). However, in the adult liver, synergism between FOXA2 and OC1 drives *HNF4α1* expression 79 (Figs. 2B, 3).

The alternative P2 promoter, which gives rise to *HNF4α7,* can be activated by HNF1β and OC1 in the embryonic liver.[79] Whilst, in the pancreas, the P2 promoter is activated by pancreatic transcription factors HNF1α, OC1 and PDX1 (Fig. 3). The striking developmental and tissue-specific variation in the expression of HNF4α isoforms can play a role in moderating disease outcome depending on the precise location of the disease-causing mutation and its consequential effects on isoform expression and activity.[48]

HNF1β and HNF1α function in the kidney

Hnf1β is expressed in the kidney and controls the expression of cystic genes such as *Pkhd1*, *Pkd2* and *Umod.*[80,81] Hnf1β directly regulates the expression of *Pkhd1* in tubular epithelial cells that are involved in tubulogenesis in the kidney,[82,83] and *Socs3* which is essential for renal repair.[84]

Although *Hnf1α* is expressed in the developing kidney and plays a role in kidney development, it is also a key regulator of numerous essential metabolic processes during adulthood. These include maintaining glucose homeostasis through regulation of the glucose-6-phosphatase system and the glucose transporter, as well as regulating other transporters that impact on the metabolism of amino acids because of impaired renal reabsorption.[58,85–87] For instance, *Hnf1α*-null mice express lower levels of *Npt1* and *Npt4* genes, members of the sodium/phosphate cotransporter family. *Npt1* is likely to be a direct target of HNF1α.[88] As these observations have been obtained from knockout mouse models, the physiological impacts of *HNF1A* mutations on kidney function in humans will require further in-depth investigation.

Overall, it is clear that the HNFs contribute to a complex cross-regulatory network exhibiting both specificity and redundancy across different tissue types. Nevertheless, most of these insights were gained from studies in rodents, and it is important to understand if the same applies to humans. This is important for the understanding of the roles of HNFs in disease pathophysiology, as heterozygous mutations in humans result in diseases that are not recapitulated in rodent models.

The role of mutations in the HNF family members in health and disease

Mutations in several HNF transcription factors are well-known to cause MODY, a rare autosomal dominantly inherited form of diabetes that often occurs before 25 years of age, typically leading to dysfunctional pancreatic β cells with accompanying liver and/or kidney defects. These MODY genes include *HNF1α* (MODY3), *HNF1β* (MODY5) and *HNF4α* (MODY1)[79,89] (Tables 3 and 4). In the previous section, we discussed the complex HNF cross-regulatory transcriptional network that is activated in a developmental stage- and tissue-specific manner, focusing on the liver, pancreas and kidney. The multifactorial roles of these HNFs imply that a single inactivating mutation is likely to affect the function of more than one tissue (liver and beyond). Much of the existing knowledge on the functional mechanisms underlying the HNFs has been obtained from rodent studies because of the inaccessibility of human tissues. However, rare genetic mutations identified in HNF genes that result in pathophysiological conditions have provided a 'natural' human disease model for the study of gene function. Here, we compare the phenotypes that arise from the perturbations in HNF genes in mice and humans. We also highlight the mechanistic insights gained from rodent studies and the limitations of rodent models, which may not necessarily recapitulate human HNF physiology (Tables 3 and 4).

HNF1α mutations in mice and men

Heterozygous *HNF1α* mutations in humans give rise to MODY3, the most common form of MODY. Patients with MODY3 exhibit progressive failure in glycaemic control with impaired β cell function as the primary cause of diabetes.[89,90] A considerable number of patients also develop microvascular and macrovascular complications often seen in patients with type 1 and 2 diabetes (T1D and T2D).[91] In MODY3, the progressive loss of the insulin secretory capacity can be treated with sulfonylureas with excellent results.[92,93] In fact, MODY is often misdiagnosed as T1D or T2D. In contrast, heterozygous *Hnf1α* knockout mice do not exhibit a disease phenotype, whereas homozygous *Hnf1α* knockout mice exhibit stunted growth, reduced size and only weigh ~50–60% of their wild-type counterparts by five weeks.[94] *Hnf1α*-null mice also exhibit abnormal glucose-stimulated insulin secretion and develop diabetes two weeks after birth.[94,95] These mice have reduced β cell mass and express low levels of insulin and insulin-like growth factor-1 (Igf1).[96]

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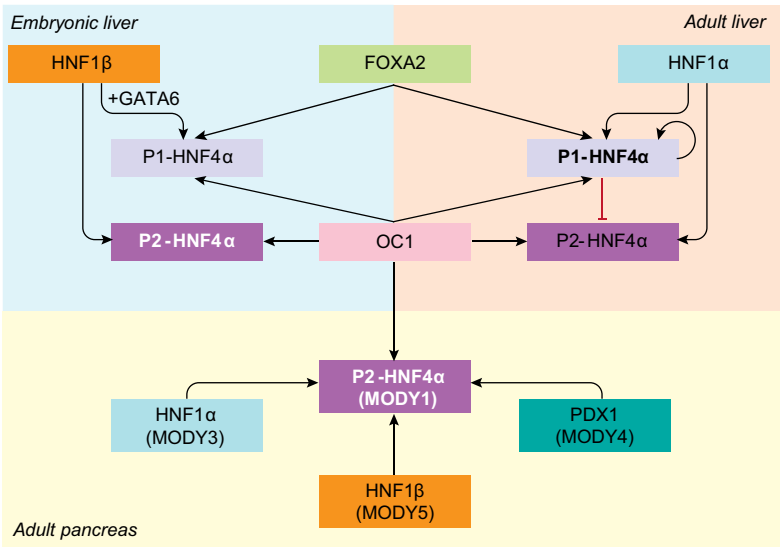


Fig. 3. Complex differential regulation of HNF4α isoforms in the liver and pancreas. HNF1, OC1, GATA6 and FOXA2 are involved in activating P1 promoter-driven *HNF4α* expression in the embryonic liver. HNF1β cooperates with GATA6 to activate P1-*HNF4α* while in the adult liver, synergism between FOXA2 and OC1 drives P1-*HNF4α* expression. Similarly, P2-*HNF4α* expression is driven by OC1, HNF1β (embryonic liver) and HNF1α (adult liver). The *HNF4α* P2 promoter contains binding sites for transcription factors in which their mutations lead to MODY, namely HNF1α (MODY3), HNF1β (MODY5) and PDX1 (MODY4). FOXA, forkhead box A; HNF, hepatocyte nuclear factor; MODY, maturity onset diabetes of the young; OC, ONECUT.

Some patients with MODY3 also suffer from renal dysplasia, growth hormone deficiency and hypothyroidism. Other abnormalities include infantile uterus and unidentifiable ovaries, leading to infertility – a phenotype also observed in *Hnf1α*-null mice.[94,97] *Hnf1α*-null mice also develop abnormalities in the reproductive system, rendering them sterile.[94,95]

One reason for the observed glycosuria (excretion of glucose into the urine) could be a low renal threshold for glucose, possibly because of decreased expression of *SGLT2* (*SLC5A2*) and decreased glucose reabsorption in the proximal tubules.[87] In the kidney, HNF1α binds to the *SGLT2* promoter.[87] *Hnf1α*-null mice express reduced *Sglt2* transcripts in the tubular cells.[87] Complete ablation of *HNF1α* results in severe renal proximal tubular dysfunction in the kidney, which can be likened to the clinical phenotype observed in human patients with Fanconi syndrome.[58] However, Lee *et al.* (1998) reported that the *Hnf1α*-null mice show no signs of renal dysfunction, which is quite surprising as it is in contrast with previous results showing that HNF1α inactivation leads to hepatic dysfunction, phenylketonuria and renal Fanconi syndrome.[58]

HNF1α mutations have also been reported in 35–40% of patients with hepatocellular adenoma (HCA).[98] In contrast to MODY3, bi-allelic inactivation of *HNF1α* has been identified in tumour tissue and this is often a result of two inactivating somatic mutations, and on rare occasions one germline mutation with a second inactivating mutation. These results suggest that *HNF1α* could play a role as a tumour suppressor gene in the liver.[99,100] Individuals with germline *HNF1α* mutations develop HCA at a younger age and usually have a family history of liver adenomatosis.[99,101] Furthermore, *HNF1α* mutations result in increased fatty acid synthesis and perturb fatty acid transportation, leading to lipid accumulation.[102] Hence, it is not surprising that intratumoural steatosis has been observed in *HNF1α* mutation-induced HCA.[101,103]

HNF1β mutations in mice and men

Individuals with a heterozygous *HNF1β* inactivating mutation develop MODY5, a relatively severe condition. It is characterised by disorders in multiple organs, from lower birth weight (by ~800 g) resulting from reduced insulin, to partial pancreatic agenesis, exocrine dysfunction, renal cystic disease, occasional genital tract abnormalities and abnormal liver function.[104–108] It is clear that HNF1β plays a critical role in pancreas organogenesis although gene dosage differs between humans and mice.[109] *Hnf1β-null* embryos die at the blastocyst stage (E3.5) because of abnormal or absent extraembryonic endoderm.[23] *Hnf1β*-null embryos rescued via tetraploid complementation do not have a detectable ventral pancreas, form only a small dorsal pancreas, fail to express *Ngn3* and do not successfully form endocrine cells.[110] In contrast to humans, mice that are heterozygous for the *Hnf1β* mutation exhibit no apparent phenotype.

Patients with MODY5 typically develop kidney abnormalities, such as bilateral renal cysts, renal dysplasia and/or familial hypoplasia glomerulocystic kidney disease.[111,112] This can ultimately lead to end-stage renal failure. Conditional knockout of *Hnf1β* in young mice (at postnatal P1-P10 stages) also results in the development of polycystic kidneys, whereas knockout of *Hnf1β* at P10 or later results in significantly delayed cyst formation.[81,113] Rare cases of genital tract abnormalities, such as rudimentary uterus, vaginal aplasia, bicornuate uterus and double vagina have also been observed in patients with MODY5.[114–116] *Hnf1β*-null mice also show defects in Wolffian duct tube development and as a result, a lack of Müllerian duct formation.[117]

Patients with MODY5 also present with liver deficiencies, such as neonatal cholestasis, adult-onset cholestasis,[118] hypercholesterolaemia, neonatal jaundice, lack of intrahepatic bile ducts and liver dysfunction.[119,120] In mice, targeted inactivation of *Hnf1β* in hepatocytes and bile duct cells results in the development of severe neonatal cholestasis and jaundice because of abnormal development of the gall bladder and intrahepatic bile ducts, affecting bile acid transport and metabolism.[121]

FOXA mutations in mice and men

The few studies that reported on *FOXA* mutations in humans were typically linked to cancer, but

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Table 3. Summary of HNF mutations and the respective phenotypes observed in the affected organ(s) in mice.

Hnf mutations in mice (genotype)	Phenotype(s)
Hnf1	
Hnf1α (+/−)	-
Hnf1α (−/−)	Develop severe diabetes 2 weeks after birth; develop liver and reproductive system dysfunction, rendering the mice sterile.[94,95] Reduced β cell mass and develop renal Fanconi syndrome.[164] Establish Laron-type dwarfism; the mice showed no signs of renal dysfunction.[94] Display reduced expression of *Glut2 (Slc2a2)* glucose transporter and L-type pyruvate kinase (*pklr*) genes specifically in pancreatic insulin-producing cells.[165]
Hnf1β (+/−)	-
Hnf1β (−/−)	Die at E5.5-E6 due to abnormal or absent extraembryonic endoderm.[23]
Hnf1β (Tetraploid complementation)	Fail to develop ventral pancreas and do not form endocrine cells.[110] No hepatic bud formation, defective hepatic specification of the entire ventral endoderm,[166] affected organs include lung, gall bladder and ventral pancreas. Ureter bud branching morphogenesis disruption and absence of nephrogenesis induction.[167]
Hnf1β (Targeted deletion)	Neonatal cholestasis and jaundice due to an abnormal development of gall bladder and intrahepatic bile ducts.[121]
Foxa	
Foxa1 (+/−)	-
Foxa1 (−/−)	Hypoglycaemic and die between postnatal day 2 to 12.[8,134] Develop nephrogenic diabetes insipidus shortly after birth.[131] Develop HCC in a sexual dimorphic manner.[127] No mature luminal epithelial cells observed in the prostate.[168]
Foxa2 (+/−)	-
Foxa2 (−/−)	Embryonic lethal after gastrulation, mice established absence of organized node and notochord formation and subsequent defects in dorsal-ventral patterning in the neural tube.[129] Develop HCC in a sexual dimorphic manner.[127]
Foxa2 (Targeted deletion)	Targeted deletion of *Foxa2* in β cells results in hypoglycaemia and relative hyperinsulinemia condition.[135]
Foxa3 (+/− or −/−)	Display reduced male fertility and increased germ cell apoptosis.[131]
Hnf4	
Hnf4α (+/−)	-
Hnf4α (−/−)	Embryonic lethal.[25]
Hnf4α (Tetraploid complementation)	*Hnf4α*-null foetal liver failed to express a large array of genes that are associated with mature hepatocyte function[27]
Hnf4α (Targeted deletion)	Deletion of *Hnf4α* in the liver results in steatosis, severe disruption of gluconeogenesis and death at 6–8 weeks of age.[25,139] Leads to HCC. Knockdown of *Hnf4α* in the kidney results in apoptosis in the condensed mesenchyme.[169]
Hnf4γ (−/−)	Appears to have neurological defects, with reduced locomotor activity at night.[170]
Onecut	
Oc1 (−/−) *Oc2* (−/−)	Severe defects in the liver and die before postnatal day 10.[66,67,159] Perturbed islet morphology[67,77] and severe diabetes reported.[66,67] Pancreatic hypoplasia.[77] Hindlimb paresis at birth.[171]

(-) indicates no difference observed in mutants compared to the healthy wild-type littermates. Foxa, forkhead box A; HCC, hepatocellular carcinoma; Hnf, hepatocyte nuclear factor; Oc, Onecut.

Table 4. Summary of the HNF mutations and the respective phenotypes reported in the affected organ(s) in humans.

HNF mutations in humans (genotype)	Phenotype(s)
HNF1	
*HNF1α** (+/−)	MODY3.[89] Some MODY3 patients also suffer from renal dysplasia, growth hormone deficiency and hypothyroidism. Patients display infantile uterus and unidentifiable ovary phenotypes, leading to infertility.[97] Patients develop hepatocellular adenoma.[99,101]
*HNF1β** (+/−)	Patients establish partial pancreatic agenesis-mediated MODY5, renal cystic disease, occasional genital tract abnormalities and abnormal liver function.[104–107]
FOXA	
FOXA1 (+/−)	Associated with breast and prostate cancer in humans.[126]
FOXA1 or *FOXA2* (+/−)	Patients develop hepatocellular carcinoma in a sexual dimorphic manner, with higher occurrence in males.[127]
FOXA3 (−)	No reported cases of mutation leading to human disease yet.
HNF4	
*HNF4α** (+/−)	MODY1.[146] Patients develop typical dominant Fanconi syndrome in addition to a β cell phenotype.[172]
HNF4γ (−)	No reported cases of HNF4γ polymorphisms leading to human disease yet.
HNF6	
HNF6 (−)	No reported cases of HNF6 polymorphisms leading to human disease yet.

* There is no reported homozygous mutation in HNF1α, HNF1β and HNF4α. FOXA, forkhead box A; HNF, hepatocyte nuclear factor; MODY, maturity onset diabetes of the young; OC, ONECUT.

not the development or function of the liver, pancreas or kidney. *FOXA1* mutations have been linked to human cancers, such as acute myeloid leukaemia, oesophageal, lung, thyroid, pancreatic, breast and prostate cancer.[122–126] Single nucleotide polymorphisms in *FOXA1* and *FOXA2* are associated with hepatocellular carcinoma (HCC) in a sexual dimorphic manner, with a significantly higher incidence in males. This phenomenon is replicated in *Foxa1*- and *Foxa2*-deficient mice, which suggested that ERα (oestrogen receptor)-dependent gene regulation in female mice

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prevents cancer formation, while AR (androgen receptor)-mediated regulation promotes it. This is in part linked to the regulation of downstream targets of ER and AR by FOXA1 and FOXA2.[127] In lung cancer, inactivation of the anti-proliferative protein C/EBPα has resulted in the inhibition of *FOXA2* expression. Restoration of *FOXA2* expression causes proliferative arrest and apoptosis of the tumour cells.[128] These expression patterns are suggestive of *FOXA1* and *FOXA2* acting as pro- or anti-tumourigenic genes. We are not aware of any studies linking mutations in *FOXA3* to human disease.

In contrast to the naturally-occurring *FOXA* mutations linked to cancer in humans, the phenotypes in mice are quite different. *Foxa1*-null embryos are phenotypically indistinguishable from wild-type embryos while *Foxa2*-null embryos are embryonic lethal.[129,130] *Foxa1*-null mice exhibit electrolyte imbalance, become dehydrated, and develop nephrogenic diabetes insipidus shortly after birth.[131] *Foxa1*- and *Foxa2*-null embryos fail to form the liver bud in the foregut endoderm.[132] However, the ablation of *Foxa1* or *Foxa2* after liver specification does not affect hepatocyte development.[101] While *Foxa1* and *Foxa2* play critical roles in liver specification and metabolism, *Foxa3* is important for hepatic glucose homeostasis as *Foxa3-null* mice exhibit reduced expression of the glucose transporter *Glut2,* resulting in hypoglycaemia during prolonged fasting.[8,101,132,133]

In the context of the pancreas, *Foxa1*-null mice have a 70% reduction in proglucagon expression, making them severely hypoglycaemic and resulting in death between postnatal day 2–12.[8,134] Targeted deletion of *Foxa2* in pancreatic β cells results in hypoglycaemia and relative hyperinsulinemia. These β cells not only fail to secrete insulin upon glucose challenge but secrete insulin inappropriately in response to amino acids.[135] It remains to be determined whether perturbations in *FOXA* genes in humans will affect foregut, liver and pancreas development or function.

HNF4α mutations in mice and men

Inactivating mutations in *HNF4α* result in MODY1, which gives rise to insulin secretory defects similar to those in MODY3.[89,136,137] Patients with MODY1 also exhibit liver disorders, such as reduced HDL cholesterol, apolipoprotein A1 and A2, and triglyceride levels, as well as increased LDL cholesterol levels.[138] In studies of liver-specific *Hnf4α*-null mice, *Hnf4α* was found to be dispensable for the early development of the liver, but important for regulating many genes involved in hepatic function, such as the apolipoproteins and metabolic proteins (*APOA1*, *APOB*, *PAH*, *FABP1*).[27] The loss of *Hnf4α* also affects the development of the hepatic epithelium,[28] leading to steatosis, severe disruption of gluconeogenesis and death at six to eight weeks of age.[25,139]

Since *Hnf4α* plays a critical role in rodent liver homeostasis, it is not surprising that mutations in the human *HNF4α* gene can also contribute to HCC. In many cases of HCC, *HNF4α* gene expression has been shown to be downregulated.[140,141] Concordantly, overexpression of *Hnf4α* in mouse HCC models confers protection against carcinogenesis and metastasis. Therefore, *Hnf4α* possibly acts as a tumour suppressor gene.[141,142]

While β cell-specific *Hnf4α*-null mice appear to be indistinguishable from their healthy littermates and do not develop diabetes, they exhibit altered cholesterol and triglyceride profiles,[139] dysregulation of insulin secretion,[143] and are hyperglycaemic during an intraperitoneal glucose tolerance test with no change in insulin sensitivity.[144] In contrast, patients with MODY1 and heterozygous *HNF4α* mutations typically exhibit an increase in birth weight (macrosomia), transient hypoglycaemia and/or diazoxide-responsive hyperinsulinemia at birth.[145] They subsequently display progressive hyperglycaemia associated with impaired insulin secretion that worsens with time.[146] Since HNF1α regulates the expression of the *HNF4α* gene, this could explain the similarity in clinical phenotype between MODY1 and MODY3.[137]

In contrast to *HNF4α* mutations, *HNF4γ* gene mutations or polymorphisms have yet to be linked to any human diseases.

Genetic variants in HNF1α, HNF1β and HNF4α are associated with T2D risk

Genetic variants in *HNF1α*, *HNF1β* and *HNF4α* that are likely to be less penetrant have also been associated with the risk of polygenic forms of diabetes (T2D) in genome-wide association studies (GWAS). Variants in *HNF1α* that have been associated with T2D risk include common missense variants I27L and A98V and low-frequency variant E508K, that may result in the impairment of insulin secretion because of changes in *HNF1α* transcriptional activity.[147–149] Further detailed studies of non-diabetic cohorts have also shown that associated variants in *HNF1α* are likely to play a role in the regulation of insulinogenic index.[150,151] In *HNF1β*, a number of non-coding variants have previously been associated with T2D.[152,153] One of these variants in the 3′UTR was located in a miRNA-binding site in *HNF1β* and was found to impact on the action of two miRNAs (hsa-miR-214-5p/has-miR-550a-5p) to influence T2D susceptibility.[154] *HNF1β* variation could impact on T2D risk by affecting insulin sensitivity.[150] In *HNF4α*, a low-frequency missense variant T139I has been reported to be associated with T2D in multiple large-scale studies and is distinct from the common non-coding GWAS signal.[155–157] Many studies reporting an association between *HNF1α*, *HNF1β*, *HNF4α* and T2D in multiple ancestry groups have not been described here because they are out of scope. However, the

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evidence implies that disruption of the function of these factors not only impairs pancreatic islet development and function (in the case of MODY) but also contributes to the susceptibility to T2D.

ONECUT mutations in mice and men

There is little information describing *OC1* mutations in humans that cause developmental defects in tissues or organs. The expression level of *OC1* appears to be decreased concomitantly with its target genes in pancreatic intraepithelial neoplasms and invasive pancreatic ductal adenocarcinoma.[158] This implicates the involvement of *OC1* as a tumour suppressor gene in pancreatic cancer. However, more definitive studies are required.

Oc1- and Oc2-null mice develop severe defects in the liver and biliary system, leading to liver failure and death before postnatal day 10.[66,67,159] The migration of hepatoblasts in the septum transversum and differentiation of hepatoblasts into hepatocytes and biliary lineages is affected, leading to hybrid cells that display characteristics of both cell types.[65,159] The loss of *Oc1* in hepatoblasts results in cholestatic liver injury characterised by extensive hepatic necrosis and fibrosis.[160] These liver defects result in a high incidence of early postnatal lethality.[67]

Oc1- and Oc2-null mice that survive have pancreatic developmental defects and exhibit dysfunctional islets, complete absence of a gallbladder and abnormal extrahepatic bile ducts, leading to severe diabetes.[66,67] As *Oc1* regulates *Pdx1* expression, it is not surprising that pancreatic hypoplasia occurs in *Oc1*-null mice because of defects in early pancreas specification.[77] *Oc1*-null embryos also lack the expression of *Ngn3*, a key pancreatic endocrine progenitor marker essential for endocrine specification. The lack of *Ngn3* expression results in the impairment of pancreatic endocrine cell differentiation.[67] Although there are few endocrine cells at birth in *Oc1*-null murine pancreas, islets eventually develop. However, both islet morphology and duct morphogenesis are perturbed.[67,77] It is currently unclear why *OC1* mutations in humans have not been reported to affect human liver or pancreas development.

Conclusion

The HNF family members have diverse spatial and temporal expression patterns that are made even more complex by the different isoforms present in multiple tissues and organs, from the earliest stages of embryonic development through to adulthood. Our comparisons between the expression profiles of HNFs in rodents and humans reflect the value of rodent studies in providing biological insight into gene expression and function, given the relative inaccessibility of human tissue. Though rodent models serve as a critical foundation upon which we can start to build our knowledge of HNF biology, the differences in disease phenotypes caused by heterozygous inactivating mutations in humans and rodents reveal the limitations of rodent studies, which do not always translate to the human context. In this respect, human induced pluripotent stem cell models, especially those that are derived from patients, are a promising tool for the analysis of human-specific disease mechanisms during the development of different tissues.[161,162]

Though many HNF proteins and their isoforms share a high degree of homology, they may play both interdependent as well as distinct roles depending on their expression levels and the tissue(s) involved. The complexity is reflected in the cross-regulatory circuits formed by the HNF proteins that specify tissue identity and function. While studies have now begun to tease out the molecular roles of each of these factors, future work may be aimed at comparing the genome-wide binding targets and downstream effectors of the different HNF isoforms at different developmental stages and in different tissues, to achieve better resolution.

While HNFs are highly enriched in the liver and play critical regulatory roles in liver development and function, it is evident that mutations in HNFs also give rise to disorders related to the pancreas and kidney (Table 4, Fig. 4). In many instances, the precise molecular mechanisms by which these mutations affect the transcriptional networks in specific tissues or organs remain to be clarified in humans. It is still unclear if the high expression levels of HNFs in the liver result in redundancy and compensation that protect patients from severe liver disorders, despite heterozygous inactivating mutations. Further investigations into the molecular functions of HNFs within the liver and beyond will not only shed light on the variability of clinical phenotypes arising from the different

Key point

Mutations in HNFs contribute to disorders that implicate not just the liver but also the pancreas and kidney. While rodent models provide a strong basis for understanding disease development caused by perturbation of HNF gene function, there are key differences in phenotypes arising from heterozygous mutations in humans and rodents. In this respect, human stem cell models could provide a more suitable tool for analysing human-specific disease mechanisms.

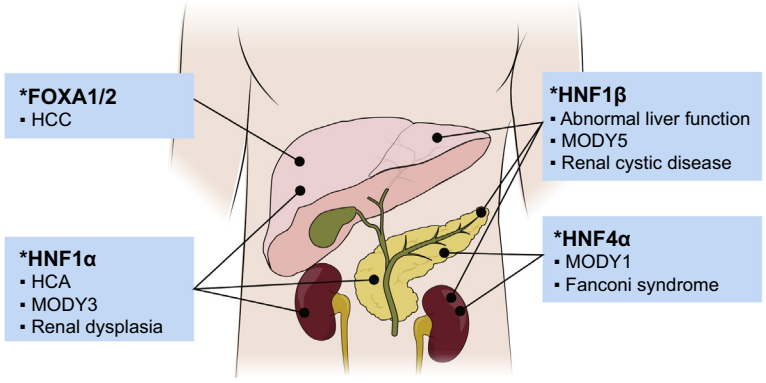


Fig. 4. Overview of key implications in the liver, pancreas and kidney due to dysregulation of HNF proteins. Mutations in *HNF1α* have been associated with HCA, MODY3 and renal dysplasia. Patients with *HNF1β* mutation display abnormal liver function, partial pancreatic agenesis-mediated MODY5 and renal cystic disease. *FOXA1* and *FOXA2* mutations have been reported to be associated with HCC in a sexual dimorphic manner. Patients with *HNF4α* mutation develop MODY1 and Fanconi syndrome. FOXA, forkhead box A; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; HNF, hepatocyte nuclear factor; MODY, maturity onset diabetes of the young; OC, ONECUT.

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mutations (within and between gene families) but may also uncover novel pathways to tackle human diseases that implicate HNF dysregulation.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

HHL and JBJ prepared the figures and tables. HHL, NHJN, LSWL, JBJ and AKKT wrote the manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhep.2017.11.026.

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Author names in bold designate shared co-first authorship

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