The Protein Journal (2021) 40:348–360
https://doi.org/10.1007/s10930-021-09991-8

A Review of Functional Characterization of Single Amino Acid Change Mutations in HNF Transcription Factors in MODY Pathogenesis

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Accepted: 22 April 2021 / Published online: 5 May 2021

Abstract
Mutations in HNF transcription factor genes cause the most common subtypes of maturity-onset of diabetes of youth (MODY), a monogenic form of diabetes mellitus. Mutations in the *HNF1-α*, *HNF4-α*, and *HNF1-β* genes are primarily considered as the cause of MODY3, MODY1, and MODY5 subtypes, respectively. Although patients with different subtypes display similar symptoms, they may develop distinct diabetes-related complications and require different treatments depending on the type of the mutation. Genetic analysis of MODY patients revealed more than 400 missense/nonsense mutations in *HNF1-α*, *HNF4-α*, and *HNF1-β* genes, however only a small portion of them are functionally characterized. Evaluation of nonsense mutations are more direct as they lead to premature stop codons and mostly in mRNA decay or nonfunctional truncated proteins. However, interpretation of the single amino acid change (missense) mutation is not such definite, as effect of the variant may vary depending on the location and also the substituted amino acid. Mutations with benign effect on the protein function may not be the pathologic variant and further genetic testing may be required. Here, we discuss the functional characterization analysis of single amino acid change mutations identified in *HNF1-α*, *HNF4-α*, and *HNF1-β* genes and evaluate their roles in MODY pathogenesis. This review will contribute to comprehend HNF nuclear family-related molecular mechanisms and to develop more accurate diagnosis and treatment based on correct evaluation of pathologic effects of the variants.

Keywords MODY · HNF1A · HNF nuclear proteins · Functional studies · Single amino acid changes

1 Introduction

MODY (maturity-onset diabetes of the young) is a monogenic subtype of non-insulin dependent diabetes mellitus characterized by autosomal dominant inheritance, onset before the age of 25 years and a defect in beta-cell function [1, 2]. The genes related to MODY subtypes first emerged in the 1990s, and up to date 14 different genes (*HNF4-α, GCK, HNF1-α, PDX1, HNF1-β, NEUROD-1, KLF-11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11*, and *APPL1*) have been associated to MODY pathogenesis [3, 4]. However, the most common subtypes are caused by the mutations in three members of Hepatocyte Nuclear Factors; *HNF1-α*, *HNF4-α*, and *HNF1-β*, and Glucokinase gene (*GCK*) [5] Previous studies have demonstrated that mutations in the *HNF1-α*, *HNF4-α*, and *HNF1-β* genes are primarily considered as the main cause of MODY3, MODY1, and MODY5, respectively [6–10]. The prevalence of HNF1-α-MODY (MODY3) is estimated to vary between 30–70% in all MODY cases in different populations while mutations in HNF4-α-MODY (MODY1) and HNF1-β-MODY (MODY5) subtypes constitute almost 10–20% of all MODY cases [11].

Patients with different MODY subtypes have common clinical properties such as reduction in insulin secretion and hyperglycemia, but they are also reported to develop different complications depending on the type of the pathologic mutation [11]. For example, patients with mutations in *HNF1-α* and *HNF4-α* genes carry a high risk for microvascular complications while patients with mutations in *HNF1-β* gene develop renal cysts or urogenital tract problems. The patients diagnosed with different subtypes have also different treatment options, such as HNF1-α and HNF4-α-MODY patients may be treated with diet, oral antidiabetic drugs or insulin depending on the severity of the symptoms while HNF1-β-MODY patients have insulin treatment [12].

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Therefore, a precise interpretation of the pathologic effect of the genetic variations identified in patients is crucial for developing effective diagnostic systems, personalized medicine, genetic counselling for the family and to take precautions for the complications that may develop later [13].

Diagnosis of MODY mainly depends on the clinical criteria and evaluation of the pathologic variants. Functional characterization studies are fundamental to correctly classify the pathologic effects of the mutations identified in MODY patients. However, even more than 400 missense/nonsense mutations have been identified in *HNF1-α*, *HNF4-α* and *HNF1-β* genes, only a small proportion of them were functionally analyzed. The effects of nonsense mutations are more well-defined as they yield nonsense-mediated mRNA decay or non-functional truncated proteins. However, single nucleotide mutations that result in an amino acid change (missense mutations) are more confusing to interpret, as they may have benign effect that does not lead to a disease phenotype or may increase the risk to develop a complex disease. Associations studies in Type II diabetes revealed that rare variants in MODY genes may increase susceptibility but are not sufficient to trigger a disease phenotype alone [14, 15]. In recent years, studies have been reported to re-classify the single amino acid variations identified in MODY genes according to the effects of the mutations on the function of the proteins to clarify the molecular pathogenesis of MODY and to apply correct treatment for the patients [16, 17].

In this review, we aim to discuss the functional characterization of the single amino acid change (missense) mutations identified in *HNF1-α*, *HNF4-α* and *HNF1-β* genes which were discovered in MODY patients and to evaluate their roles in MODY pathogenesis.

2 Functional Characterization Studies for the Identified Variants in HNF Transcription Factors

Hepatocyte nuclear factors (HNF1-α, HNF4-α and HNF1-β) belong to the family of transcription factors that regulate the transcription of a wide range of genes coding for insulin and other proteins that have roles in glucose metabolism and β-cell development [18–21]. HNF-transcription factor family have similar structures which include dimerization, DNA binding and transactivation domains. Functional analysis of the mutations in these genes revealed that they are predominantly loss-of-function mutations that lead to a defect in dimerization ability, DNA binding affinity, transcriptional activity or subcellular localization depending on the location of the mutation [21–25].

In this regard, to assess the effects of the mutations identified in HNF nuclear factors, several functional characterization assays are carried out to compare the activity of mutant proteins with wild type. For in vitro expression and analysis of mutant proteins, cell lines such as HeLa and Cos-7 are commonly used as they do not express endogenous HNF proteins. Dimerization ability of the mutant proteins is mainly analyzed by means of retardation assay on native gel by using extract of cells which were co-transfected with wild type and mutant HNF genes [26, 27]. DNA binding ability of HNF proteins is evaluated by Electrophoretic Mobility Shift Assay (EMSA). HNF1-α and HNF1-β recognize and bind to same consensus 5′-GTTAATNATTAAC-3′ sequence in the promoter regions of target genes, while the most common binding motif of HNF4-α was reported as direct repeat of AGGTCA with a spacing of 1 nt (DR1, AGG TCAxAGGTCA) [21, 22, 25]. Therefore, in EMSA in vitro expressed mutant or wild type proteins are incubated with labelled double stranded DNA including the recognition sequence of HNF proteins and analyzed on SDS-PAGE. Transactivation ability of HNF proteins is predominantly analyzed by luciferase reporter assay in which the promoter regions of target genes such as *HNF4-α P2* or *GLUT2* for HNF1-α, *CYP2D6* or *HNF1-α* for HNF4-α and *GLUT2* for HNF1-β are cloned into luciferase reporter plasmids. HNF proteins are functional as dimers, therefore whether mutant protein has a dominant negative effect on wild type is also evaluated by transfecting mutant proteins into mouse (Min6) or rat (INS-1) pancreatic cells which express endogenous HNF proteins. Immunolocalization studies are performed in Cos-7 or HeLa cells to investigate the nuclear localization of the mutant proteins as HNF nuclear factors should be transported to the nucleus after they are expressed in the cytoplasm.

2.1 Functional Characterization of Single Amino Acid Mutations in HNF1-α

Hepatocyte nuclear factor 1(HNF1)-α gene codes for a transcription factor which is expressed in different tissues such as liver, kidney, and pancreas [28, 29]. HNF1-α gene, which is located on chromosome 12q24.31, comprises 23,945 bp and consists of 10 exons [30].

HNF1-α is the member of homeodomain-containing protein family, which binds to DNA to regulate the expressions of target genes such as insulin (*INS*) and glucose transporter (*GLUT2*) in mature β -cells particularly [21, 31, 32]. HNF1-α gene codes for a protein with 631 amino acids including three known domains; a dimerization domain (amino acids 1 to 31), a bipartite DNA-binding domain (POU domain: 91-181aa and homeodomain: 198–279aa) and a transactivation domain (amino acids 280 to 631) [33–36]. HNF1-α functions as either homo- and heterodimers with HNF1-β, which are formed through dimerization domain. Functional dimers recognize and bind to DNA via their DNA binding

domains (POU-like and homeodomain motifs) and regulate transcription via their transactivation domains [34, 36–38].

Previous studies have already shown that mutations in the *HNF1-α* gene are the main cause of maturity onset diabetes of the young 3 (MODY3). Patients with HNF1-α mutations were reported to have progressive β-cell dysfunction and hyperglycemia as they have insufficient insulin release in response to increased blood glucose levels [38]. MODY3 patients are treated with sulphonylureas initially to improve glycemic control but insulin therapy is required later when the blood glucose levels cannot be manageable [12, 43].

Up to date, more than 300 nonsense/missense mutations in the coding regions of *HNF1-α* gene have been directly associated to MODY3 phenotype according to "The Human Gene Mutation Database (HGMD)" [44]. As shown in Fig. 1, the majority of these mutations are located on the functional domains which govern dimerization, DNA binding, transactivation ability and nuclear transport of HNF1-α.

In this review, we assessed functional characterization studies of 63 single amino acid changes identified on HNF1-α in MODY patients as shown in Table 1. Nonsense mutations (Q7X, Q170X, Q171X, R229X and Q466X) which lead to premature stop codon and nonfunctional truncated proteins have been included as well. As shown in Fig. 1, the effects of only four mutations (Q7X, L12H, G20R and L27I) were investigated in dimerization domain of HNF1-α (1-31aa). As expected, Q7X mutant protein, which lacks the functional domain, exhibits no binding ability. The role of L12 and G20 residues on the other hand, can be explained by the detailed analysis of the crystal structure of the HNF1-α dimerization [45]. According to this structure, L12 residue is crucial for both stability and functional surface of the dimer and L12H mutation leads to a significant decrease in thermodynamic stability while G20R mutation is associated with severe thermodynamic destabilization and loss of architectural specificity.

Mutations in DNA binding domains of HNF1-α (91-181aa and 198–279aa) may reduce the DNA binding affinity and also transactivation ability of HNF1A. DNA binding assays indicate that the region between K120 and S142 has a critical function as mutations in this region exhibit weak binding ability, less than 20% of wild type HNF1-α. A low transactivation activity along with weak DNA binding is also reasonable in these mutant proteins as HNF1A transcription factor should recognize and bind to consensus sequence in the promoter regions of target genes before

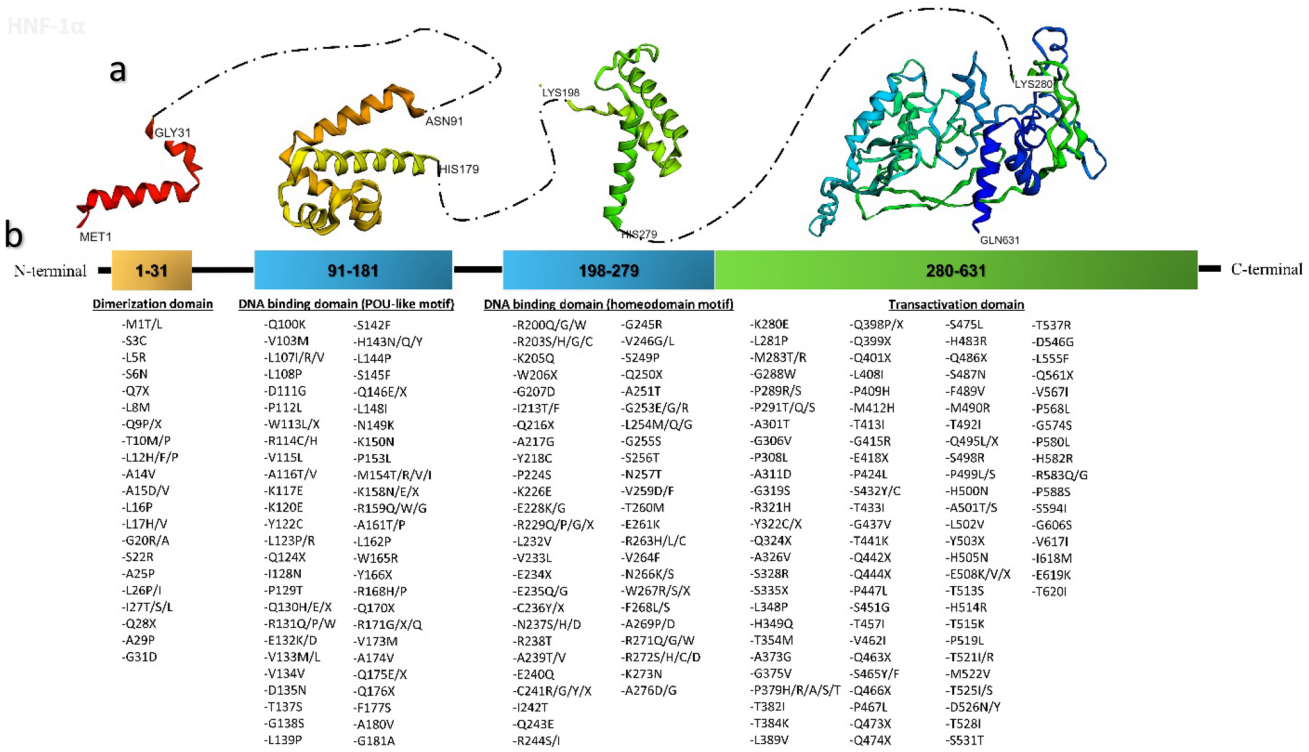

Fig. 1 **a** 3D protein modeling of structural domains of human HNF1α using de novo protein modeling. HNF1α protein model is built through DMPfold 1.0 Fast Mode algorithm on the PSIPRED server [39, 40]. RSCB PDB database; dimerization domain: 2GYP [41], DNA binding domain: 1IC8 [42]. **b** Schematic representation of HNF1-α structure, and distribution of single amino acid mutations identified in MODY patients so far. Three main functional domains; a dimerization domain (amino acids 1 to 31), yellow color, a bipartite DNA-binding domain (POU domain: 91–181 and homeodomain: 198–279), blue color, and a transactivation domain (amino acids 280 to 631), green color. The mutations identified on functional domains are boxed below, and or above (Color figure online)

Table 1 Functional analysis of single amino acid change mutations in the HNF1-α gene

HNF-1α	Mutations	Dimerization ability	DNA binding ability	Transactivation ability	Nuclear localization	References
Dimerization Domain	p. Q7X	-	No	No	-	[2]
	p. L12H	-	-	Reduced	Nucleus	[52]
	p. G20R	-	-	No	-	[53]
	p. I27L	-	-	Reduced	-	[54]
DNA Binding Domain (POU-like motif)	p. V103M	-	Reduced	Reduced	Cytoplasm & nucleus	[34]
	p. L107I	-	Weak	Reduced	Nucleus	[55]
	p. P112L	-	Reduced	Low	Cytoplasm & nucleus	[17, 47, 56]
	p. W113L	-	-	Normal	Nucleus	[16]
	p. R114C	-	Normal	Normal	Cytoplasm & nucleus	[34]
	p. A116V	-	Reduced	Reduced	Cytoplasm & nucleus	[17]
	p. K120E	-	Weak	Reduced	Cytoplasm & nucleus	[16]
	p. Y122C	-	Reduced	-	Cytoplasm & nucleus	[55]
	p. Q130E	-	Weak	Reduced	Cytoplasm & nucleus	[16]
	p. R131W	-	Weak	Reduced	Cytoplasm & nucleus	[47]
	p. R131P	-	Weak	Low	Cytoplasm & nucleus	[16]
	p. R131Q	-	Weak	Reduced	-	[56, 57]
	p. L139P	-	Weak	Low	Cytoplasm & nucleus	[16]
	p. S142F	-	No	No	Nucleus	[57]
	p.M154I	-	Reduced	Low	Nucleus	[16]
	p. K158N	-	-	Reduced	Nucleus	[52]
	p. R159Q	-	-	Reduced	Nucleus	[52]
	p. Q170X	-	-	Low	Cytoplasm & nucleus	[16]
	p. R171X	No	No	No	-	[27, 47]
	p. R171G	-	Normal	Reduced	Nucleus	[34]
	p. R171Q	-	Normal	Reduced	-	[17]
	p. A174V	-	Normal	Reduced	-	[17]
	p. F177S	-	-	Normal	Cytoplasm & nucleus	[16]
	p. G181A	-	Normal	No	-	[17]
DNA Binding Domain (Homeodomain motif)	p. R203H	-	Reduced	Low	Cytoplasm & nucleus	[17]
	p. R203C	-	-	Reduced	Cytoplasm & nucleus	[35, 37, 52]
	p. R229X	-	-	No	Cytoplasm & nucleus	[52]
	p. R229Q	Normal	Weak	Low	Nucleus	[27, 47]
	p. E235Q	-	Normal	Normal	Cytoplasm & nucleus	[34]
	p. G245R	-	Normal	Reduced	Cytoplasm & nucleus	[34]
	p. G253R	-	Reduced	Reduced	Nucleus	[16]
	p. L254G	-	No	Low	-	[58]
	p. T260M	-	-	Low	-	[17]
	p. R263H	-	Reduced	Reduced	Cytoplasm & nucleus	[34]
	p. R263C	-	No	Low	Nucleus	[47]
	p. R271Q	-	Induced	Reduced	Cytoplasm & nucleus	[34]
	p. R271W	-	Reduced	Low	Cytoplasm & nucleus	[47]
	p. R272C	-	No	No	Nucleus	[35, 59]
	p. A276D	-	Reduced	Normal	Cytoplasm & nucleus	[47]

Table 1 (continued)

HNF-1α	Mutations	Dimerization ability	DNA binding ability	Transactivation ability	Nuclear localization	References
Transactivation Domain	p. G288W	-	-	Reduced	Nucleus	[16]
	p. G319S	-	Normal	Reduced	-	[50]
	p. Y322C	-	Normal	Reduced	-	[17, 56]
	p. A326V	-	Normal	Low	-	[17]
	p. H349Q	-	Normal	Normal	Nucleus	[34]
	p. P379H	-	-	Reduced	-	[60]
	p. T382I	-	Normal	Reduced	-	[17]
	p. T384K	-	-	Normal	Nucleus	[16]
	p. G415R	-	Reduced	Reduced	Cytoplasm & nucleus	[17, 59]
	p. G437V	-	Reduced	Low	Nucleus	[16]
	p. T441K	-	Normal	-	-	[17]
	p. P447L	Normal	Reduced	Reduced	-	[27]
	p. V462I	-	-	Reduced	-	[17]
	p. Q466X	-	Reduced	Low	Cytoplasm & nucleus	[47, 61]
	p.H483R	-	-	Reduced	Nucleus	[16]
	p. S487N	-	Normal	Normal	Nucleus	[34]
	p. P519L	-	-	Low	-	[17]
	p. T521I	-	-	Reduced	-	[37]
	p. V617I	-	-	Reduced	-	[37]
	p. T620I	Normal	Normal	Induced	-	[27]

Induced represents more than 100% of wild type; Normal, 80% or more; Reduced, 40–80%; Low/Weak, 10–40%; No, 0–10%

inducing transcription. In this region, R131 residue located in POU domain has been reported to substitute with different amino acids (Proline, tryptophan and glutamine) in different MODY patients. Functional analysis of these mutations (R131P, R131W and R131Q) indicated weak DNA binding and reduced transcriptional activity. Molecular docking analysis of two of these mutations (R131W and R131Q) also showed a weak binding affinity, supporting the critical role of this residue [38]. Arginine is a hydrophilic amino acid and was shown to be preferable in interactions with bases [46]. Therefore, substitutions of arginine with other amino acids may abolish this interaction and explain lowered binding affinities. DNA binding assays also indicate strong damaging effects of mutations in the homeodomain such as R229Q, R263C and R272C which also support the essential role of arginine residues in DNA binding [27, 47].

Functional studies also investigate the effects of mutations on transactivation ability associated to the amino acids 280–631 of HNF1-α transcription factor. Here, we classified the effects of mutations on transactivation ability as normal, reduced, low or no transcriptional activity, indicating 80% or more, 40–80%, 10–40% and less than 10%, respectively, by comparing the activity of mutant proteins to the wild type (set as 100%) (Table 1). Unexpectedly, mutations which are directly inside of the transactivation domains have less damaging effect on transcription activity compared to the mutations located in DNA binding domain. Therefore, a reduced transcription activity is mostly associated to lower DNA binding affinity. However, Ban et al. reported that HNF1-α interacts with p300, co-activator of transcription, via a region including amino acids 391–631 in transactivation domain while activating the transcription of the GLUT2 (glucose transporter) gene which is one of the key targets of HNF1-α in pancreas [48]**.** Therefore, the mutations in transactivation domain may impair the interactions with co-activators and lead a reduced transcription activity without any effect on DNA binding affinity.

Nuclear localization of HNF1-α is also crucial for the transcriptional activity, therefore functional studies also investigate the effects of mutations in the import of HNF1-α to the nucleus. Bjørkhaug et al. reported that there are three important regions designated as region A (amino acids

158–171), region B (amino acids 197–205) and region C (amino acids 271–282) which may function as nuclear localization signals on HNF1-α protein [35]. Functional studies indicated that deletion of region B or C has the greatest impact on nuclear localization of HNF1-α. In region B, R203C and R203H mutant proteins have defects in nuclear transport and most of the proteins are trapped in cytoplasm. Arginine to tryptophan (R271W) and glutamine (R271Q) substitutions in region C appeared to be the most damaging mutations, however, arginine to cysteine (R271C) in the same residue do not have an effect on nuclear localization of HNF1-α. In our recent study, we enlighten the interaction of R271W mutant HNF1-α protein with importin receptor KPNA6, which is responsible to recognize and bind to nuclear localization signal on cargo proteins and transport them to the nucleus [49]. The results of in vitro and molecular docking studies indicated that R271W mutation impairs the interaction of HNF1-α with KPNA6 so that mutant HNF1-α proteins are mostly localized in cytoplasm.

Most of the mutations display loss of function properties, but exceptions were also reported such as R263H mutant protein with a dominant negative effect on transactivation activity and nuclear localization of HNF1-α [34]**.** The functional evaluation of I27L, G319S and S487N polymorphisms of HNF1-α which are associated to Type II diabetes were also included. I27L variant caused normal DNA binding and mildly reduced transcriptional activity while G319S variant has almost 50% decrease in transcription activity compared to wild type. Benign effect of the I27L variant increases the risk to develop the disease, however does not result in MODY3 phenotype. Type II patients carrying G319S variant on the hand are more prone to develop disease as approximately 40% of affected subjects in the Oji-Cree of northwestern Ontario harbor this variation [50]**.** S487N variant has a normal activity when expressed alone, however coexistence of S487N with another pathogenic mutation such as R271Q on HNF1-α increased the damaging effect of the mutation on protein function [33].

2.2 Functional Characterization of Single Amino Acid Mutations in HNF4-α

Hepatocyte nuclear factor 4 (HNF4)-α gene, which is also known as *NR2A1* resides on the long arm of the chromosome 20 in human and comprises 78,898 bp [62]. HNF4-α gene includes 13 exons in total which produces 12 variant transcripts via alternative splicing or alternative initiation (P1, and P2 promoters), allowing for HNF4-α isoforms [63–65]. Isoforms 1 to 6 are under P1 promoter control which is active in liver and kidney while isoforms 7 to 12 are regulated by the P2 promoter which is activated by HNF1-α in pancreas [8, 64, 66]

The HNF4-α protein is a member of steroid hormone receptor superfamily of transcription factors and expressed in the pancreas, liver, kidney and small intestines [67, 68]. HNF4-α performs its function as dimers, and involved in several processes such as development, metabolism, and epithelial-mesenchymal transition [64, 69]. HNF4-α (isoform 7, UniProt ID: P41235-5) binds to DNA via zinc-finger DNA binding domain which comprises amino acids 51–117 as shown in Fig. 2. HNF4-α involves two transactivation domains, AF1 which is the first 24 amino acids spanning the N-terminal, and AF2, amino acids 128–366 in the C-terminal. Dimerization and ligand binding domains (LBD) are also embedded in the AF2-transactivation domain, from amino acids 175 to 360 that enable the homo- and or heterodimerization with other HNF4-α isoforms [23, 36, 70, 71]. Previous studies by Ko et al. indicated the importance of different HNF4-α isoform dimers in the role of different gene transcriptions and isoform-dependent transactivation activity [64].

Patients harboring mutations in HNF4-α are diagnosed as MODY1. In the first decades of the patients diagnosed with MODY1, blood glucose levels can be regulated by low carbohydrate diet or low-dose sulfonylureas. However, a progressive decrease in insulin secretion due to β-cell failure can be manageable by insulin therapy in later stages of the disease or during pregnancy [12].

Up to date, more than 100 mutations on HNF4-α have been reported according to HGMD, most of which are predominantly nonsense/missense mutations [44]. Single amino acid change mutations have a direct association with MODY1 phenotype while some rare phenotypes such as hyperinsulinemic hypoglycemia, renal Fanconi syndrome and liver disease have been also reported [53, 54]**.** Mostly, these mutations on the HNF4-α are directly targeting to the functional domains as shown in Fig. 2. In this regard, mutations are mainly located on the AF2-transactivation, ligand binding, and dimerization domains while a few of them are on AF1-transactivation and DNA binding domains.

Here, 11 missense/nonsense mutations characterized in HNF4-α were reviewed in Table 2. Mutations in DNA binding domains (G115S, M125I, D126Y/H, and T130I) mainly decrease the DNA binding and also transactivation ability as in the case of HNF1-α. In this domain, further investigation of G115S mutation revealed that serine substitution in DNA binding domain introduced a novel recognition motif for Protein Kinase A (PKA) and phosphorylation at serine residue interferes with DNA binding ability of HNF4-α [77].

Mutations in transactivation domain (AF-2) of HNF4-α, on the other hand, impair transactivation activity as shown in Table 2. In this domain, E276Q shows the most damaging effect and detailed analysis of mutation revealed that E276Q mutant protein is not stable in the cells and degraded into a truncated smaller protein [23]**.** Most of the mutations

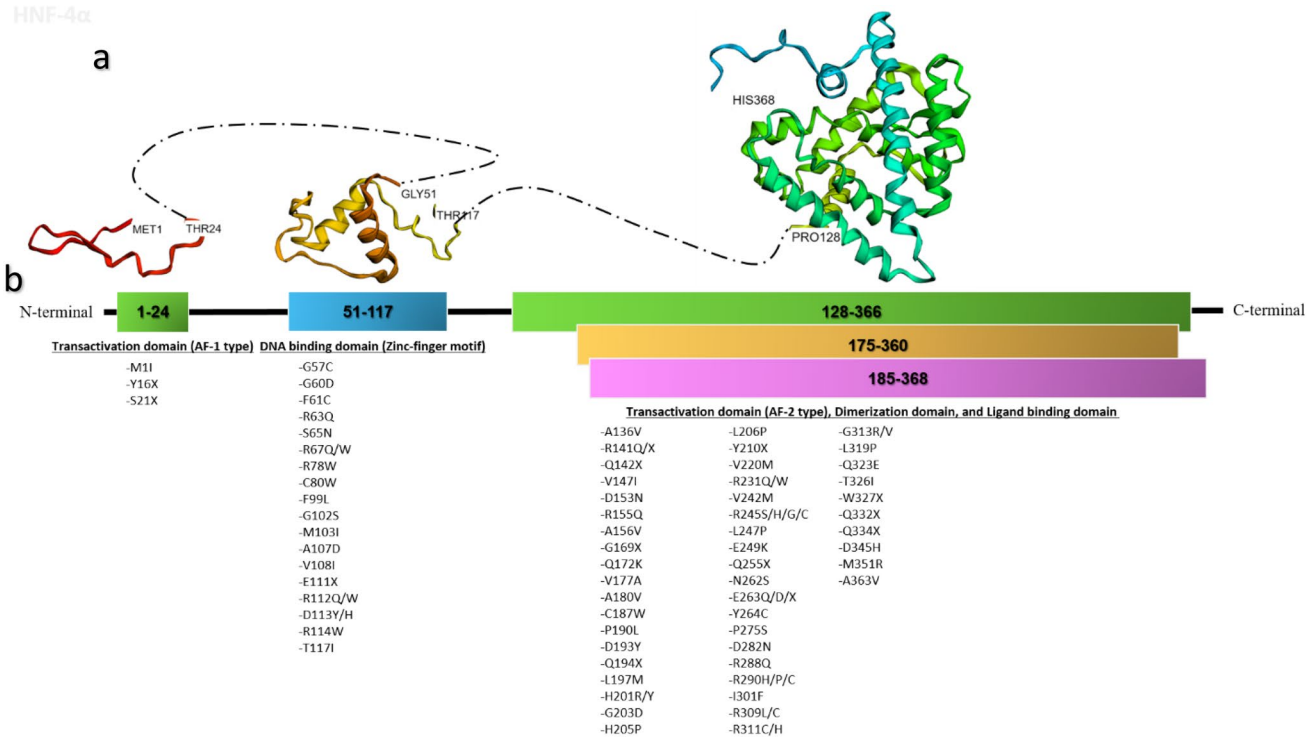

Fig. 2 **a** 3D protein modeling of structural domains of human HNF4α using de novo protein modeling HNF4α protein structure is built through DMPfold 1.0 Fast Mode algorithm on the PSIPRED server. RSCB PDB database; DNA binding domain: 4IQR[72], 3CBB [73] and transactivation domain (AF-1 type), dimerization domain, and ligand binding domain: 6CHT [74]. **b** Schematic representation of HNF4-α structure and distribution of single amino acid mutations identified so far. Three main functional domains; a zinc-finger DNA binding domain which comprises 51–117 amino acids, blue color, a transactivation domain including AF1 which is the first 24 amino acids spanning on the N-terminal, and AF2, 128–366 amino acids at the C-terminal, green color, and a dimerization domain embedded in the AF2-transactivation domain, from 175 to 360. Unlike HNF1-α and HNF1-β, HNF4-α includes a ligand-binding domain also embedded in the AF2-transactivation domain (amino acids 185 to 368), pink color (Color figure online)

characterized in HNF4-α are loss of function mutations and do not have dominant negative effects. Recent studies indicate the involvement of increasing number of co-activators, co-repressors and other transcription factors which cooperate with nuclear receptors like HNF4-α to regulate gene expression [78, 79]. Therefore, the effect of mutations on the interaction of these transcriptional partners may also be evaluated to clarify the molecular pathogenesis of MODY1.

2.3 Functional Characterization of Single Amino Acid Change Mutations in HNF1-β

Hepatocyte nuclear factor 1 (HNF1)-β, encoded by *TCF2* gene, is a transcriptional factor which is closely related to HNF1-α [85]. TCF2 gene is located at chromosome 17q12 which comprises 58,629 bp, coding for nine exons [86, 87]. Similar to HNF1-α, HNF1-β is expressed in such tissues as pancreas, liver, and kidney [88, 89].

Related to HNF1-α, HNF1-β is also a member of homeodomain-containing protein family that binds to the same consensus sequence on DNA as HNF1-α and activates transcription [90, 91]. HNF1-β works as either homo- or heterodimers with the HNF1-α [85, 92, 93]. HNF1-β involves three functional domains including a dimerization domain (amino acids 1 to 32), a DNA binding domain (amino acids 88–319) with POU-like (amino acids 88 to 180) and homeodomain motifs (amino acids 229 to 319), and a transactivation domain (amino acids 320–557) as shown in Fig. 3 [22, 92, 94].

Mutations in HNF1-β gene cause MODY5 phenotype which represent almost 5–10% of all MODY cases [11]. MODY5 patients may also show hepatic insulin resistance thus treatments with sulfonylureas may not manage glycemic control and an intensive insulin therapy may be required. MODY5 phenotype is mostly associated with renal cysts, progressive renal dysfunction, internal genital abnormalities and microvascular complications, therefore patients should be followed up and treated for these phenotypes[12, 43].

According to HGMD, more than 100 mutations have been reported on HNF1-β which are associated to different clinical spectrums such as MODY5, multicystic kidney disease, renal cysts and renal tract malformation. Previous

Table 2 Functional analysis of single amino acid change mutations in the HNF4-α gene

HNF4-α	Mutations	Dimerization Ability	DNA Binding Ability	Transactivation Ability	Nuclear Localization	Reference
	p. S35X	-	-	No	-	[80]
DNA binding domain	p. G115S *(G102S)	-	Reduced	Weak		[77]
	p. M125I *(M103I)	-	Reduced	No	Nucleus	[81]
	p. D126Y *(D113Y)	Normal	Weak	Reduced	-	[82]
	p. D126H *(D113H)	Normal	Weak	Reduced	-	[82]
	p. T130I *(T117I)	-	-	Normal	-	[83]
Transactivation domain, dimerization domain, and ligand binding domain	p. R154X *(R141X)	-	No	No	-	[82]
	p. V255M *(V242M)	-	-	Normal	-	[23, 84]
	p. Q268X *(Q255X)	-	-	No	-	[82]
	p. E276Q *(E263Q)	Normal	No	No	-	[23]
	p. R324H *(R311H)	-	Normal	Normal	-	[82]

Normal represents 80% or more of wild type; Reduced, 40–80%; Low/Weak, 10–40%; No, 0–10%

*Corrected amino acid numbers according to NP_000536.6 reference sequence

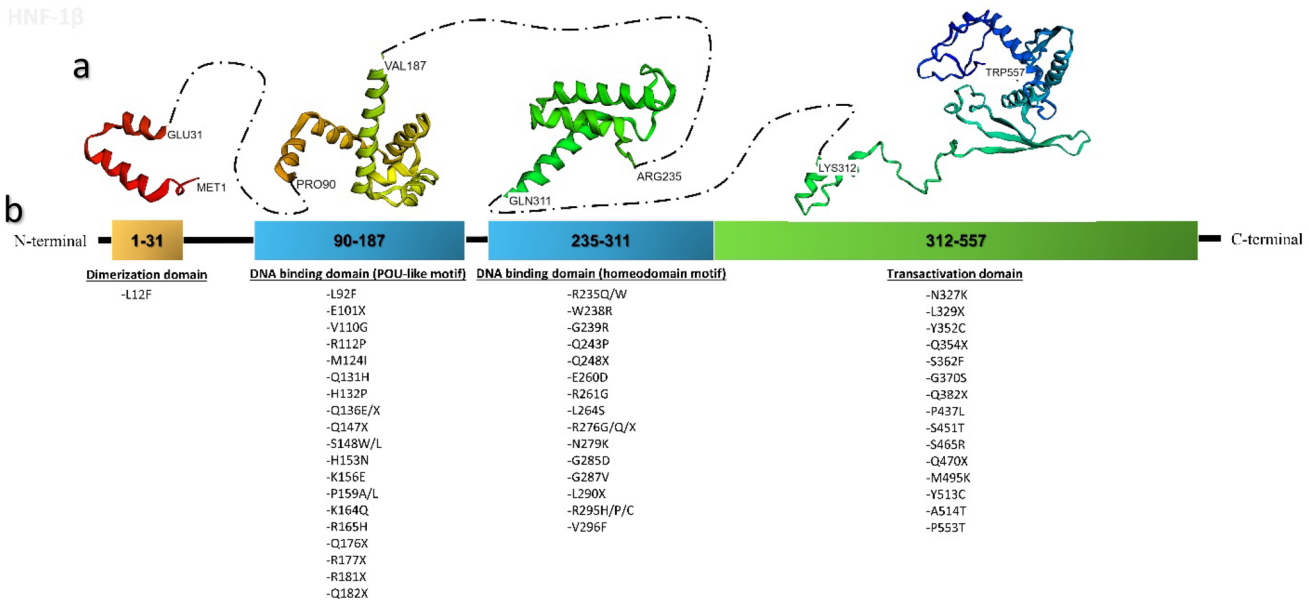

Fig. 3 **a** 3D protein modeling of structural of human HNF1β using de novo protein modeling. HNF1β protein structure is built through DMPfold 1.0 Fast Mode algorithm on the PSIPRED server. RSCB PDB database; DNA binding domain: 2DA6, 2H8R [95], and 5K9S. **b** Schematic representation of HNF1β structure, and distribution of single amino acid mutations identified in MODY patients so far. Three main functional domains; a dimerization domain (amino acids 1 to 32), yellow color, a DNA binding domain (amino acids 106–310) with POU-like from 106 to 178 and homeodomain motifs, and a transactivation domain (amino acids 310–557). The mutations identified on functional domains are boxed below, and or above (Color figure online)

studies have demonstrated that only 43 missense/nonsense mutations in HNF1-β gene are directly related to MODY phenotype (MODY5) as shown in Fig. 3. The majority of single amino acid changes are located on the DNA binding domain while five of them on transactivation domain. No mutation was reported on the dimerization domain directly associated to MODY5.

Functional analysis of HNF1-β mutations were carried out with similar studies for HNF1-α as we mentioned above. In this review, ten missense/nonsense mutations in HNF1-β gene were functionally analyzed as shown in Table 3. Most of the functionally characterized mutations reside in DNA binding domain which result in weak or reduced DNA binding affinity. As expected, nonsense mutations (R147X, R177X and L329X) display a complete loss of transactivation activity as they lack functional domain. MODY5 patients suffer from a progressive decrease in insulin secretion. Kim et al. reported that expression of P159L mutant protein decreased the level of glucose transporter (GLUT2) but not insulin suggesting that a defect in glucose transport levels may explain the impairment in insulin secretion.

3 Conclusion

Precise diagnosis and treatment for MODY patients depends on the clinical diagnosis and accurate evaluation of the mutations. HNF nuclear factor family members, HNF1-α, HNF4-α, and HNF1-β are the most frequently mutated genes in MODY pathogenesis. Mutation analysis of MODY patients revealed missense, nonsense, frameshift and splice site variations in these genes. The consequences of nonsense or frameshift mutations are more comprehensible as they produce predominantly nonfunctional truncated proteins. However, the effects of missense mutations may vary if the mutation occurs in non-conserved residue or results in a substitution of an amino acid with similar properties. Functional characterization studies of missense mutations identified in HNF1-α, HNF4-α, and HNF1-β indicate that most of the pathologic mutations display loss of function properties and do not have dominant negative effects on the wild type although they are functional as homo or heterodimers.

As shown in Fig. 4, multiple sequence alignment of HNF1-α, HNF4-α, and HNF1-β from various species such as mouse, rat, monkey and pig also indicate that all mutated amino acids are evolutionary conserved. However, there are also mutations which do not show any damaging effect on the protein function such as W113L, F177S, H349Q, T384K, T620I mutations on HNF1-α, T130I, V255M and R324H on HNF4-α. These missense variations may not be the causative mutations which result in MODY phenotype and further DNA analysis may be required for these patients. Functional analysis of the mutations may lead to re-classification of the pathogenicity of the variant and also diagnosis of the patient. For example, Malikova et al. identified and characterized G288W mutation in HNF1-α in a MODY patient [16]. The mutation had a benign effect on HNF1-α function; therefore, the DNA of the patient was further analyzed and E265K mutation was detected in GCK gene. The mutations in GCK gene cause MODY2 phenotype which is a milder form of MODY and can be treated with only diet [16]. Therefore, a more comprehensive approach such as next-generation sequencing which aims to screen mutations in all MODY candidate genes could be a more valuable diagnostic tool for MODY patients.

As a conclusion, for precise diagnosis and treatment, interpretation of the identified variants and establishing correct genotype–phenotype correlation are crucial. If the functional analysis does not support the pathogenicity of the variant, further genetic testing should be carried out to improve the diagnosis and choice of treatment.

Table 3 Functional analysis of single amino acid change mutations in HNF1-β gene

HNF1-β	Mutations	Dimerization Ability	DNA Binding Ability	Transactivation Ability	Nuclear Localization	Reference
DNA binding domain	p. R112P	-	Reduced	Reduced	Nucleus	[96]
	p. Q136E	-	No	No	Nucleus	[96]
	p. Q147X	-	No	No	Cytoplasm & nucleus	[96]
	p. H153N	-	No	Reduced	-	[97, 98]
	p. P159L	-	Reduced	Reduced	-	[22]
	p. K164Q	-	No	-	Nucleus	[96]
	p. R165H	-	Reduced	Reduced	Nucleus	[96]
	p. R177X	-	No	No	Cytoplasm & nucleus	[96]
	p. R295H	Normal	Reduced	Reduced	-	[96]
Transactivation domain	p. L329X	Normal	Normal	No	Nucleus	[96]

Normal represent 80% or more of wild type; Reduced, 40–80%; Low/Weak, 10–40%; No, 0–10%

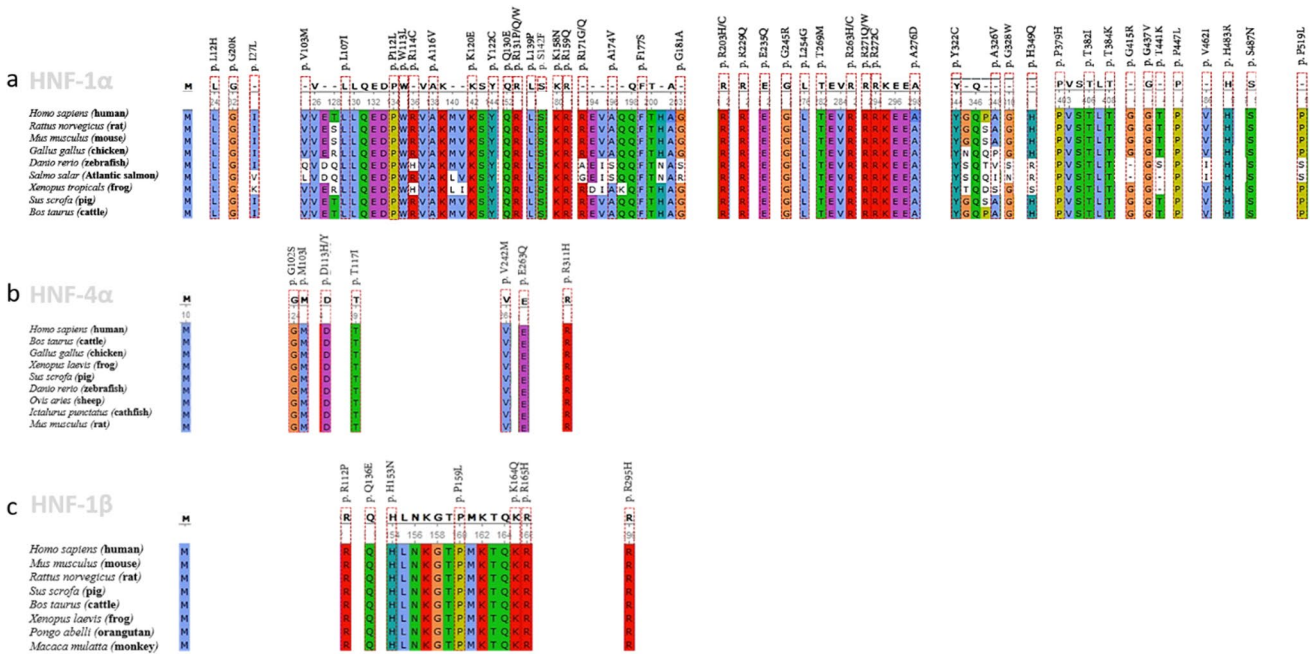

Fig. 4 Evolutionary conservation analysis for mutations in HNF1-α, HNF4-α and HNF1-β identified in MODY patients. Multiple alignment is performed using UGENE software (Kalign algorithm) [99]. **a** The alignment of HNF1-α sequences of human (NP_000536.6), rat (NP_036801.1), mouse (NP_033353.2), chicken (NP_001025839.2), zebrafish (NP_739570.1), atlantic salmon (NP_001117146.1), frog (NP_001116920.1), pig (NP_001027560.1), and cattle (NP_001179453.1). **b** The alignment of HNF4-α sequences of human (NP_787110.2), cattle (NP_001015557.1), chicken (NP_001026026.1), frog (NP_001080070.1), pig (NP_001038036.1), zebrafish (NP_919349.1), sheep (NP_001192082.1), catfish (NP_001187505.1), and rat (NP_032287.2). **c** The alignment of HNF1-β sequences of human (NP_000449.1), mouse (NP_033356.2), rat (NP_001295077.1), pig (NP_999121.1), cattle (NP_001179784.1), frog (NP_001080685.1), orangutan (NP_001124797.1), and monkey (NP_001181477.1)

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