RESEARCH ARTICLE

The role of HCFC1 in syndromic and non-syndromic intellectual disability

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Abstract

Mutations in the HCFC1 gene are associated with cases of syndromic (cblX) and non-syndromic intellectual disability. Syndromic individuals present with severe neurological defects including intractable epilepsy, facial dysmorphia, and intellectual disability. Non-syndromic individuals have also been described and implicate a role for HCFC1 during brain development. The penetrance of phenotypes and the presence of an overall syndrome is associated with the location of the mutation within the HCFC1 protein. Thus, one could hypothesize that the positioning of HCFC1 mutations lead to different neurological phenotypes that include but are not restricted to intellectual disability. The HCFC1 protein is comprised of multiple domains that function in cellular proliferation/metabolism. Several reports of HCFC1 disease variants have been identified, but a comprehensive review of each variant and its associated phenotypes has not yet been compiled. Here we perform a detailed review of HCFC1 function, model systems, variant location, and accompanying phenotypes to highlight current knowledge and the future status of the field.

Keywords: cblX, intellectual disability, HCFC1, functional analysis

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History of Inborn Errors of Cellular Cobalamin Metabolism

Cobalamin, also known as vitamin B12, is an essential molecule that is required for human metabolism. Once ingested from food, cobalamin undergoes various modifications that are required for the function of two enzymes in the body; methylmalonylCoA mutase (EC 5.4.99.2) and methionine synthase (EC2.1.1.13)[1]. The absorption of cobalamin requires multiple steps and is facilitated by many unique cobalamin-binding proteins, which are not the focus of this review. However, ultimately, intracellular cobalamin is reduced into two biologically active forms, 5'-deoxyadenosylcobalamin (AdoCbl) and methyl-cobalamin (MeCbl); these active forms function as co-factors in the mitochondria and cytosol, respectively.

Once in the cytoplasm, cobalamin binds to the enzymatic transport protein, MMACHC[2–4] and subsequently MMADHC.[5] Mutation of either *MMACHC* or *MMADHC* can cause an inborn error of cobalamin metabolism.[6,7] Inborn errors of cobalamin metabolism occur when the processing of cobalamin is defective, thus disrupting the functions of methylmalonylCoA mutase (EC 5.4.99.2) and methionine synthase (EC2.1.1.13). Mutation of *MMACHC* causes methylmalonic aciduria and homocystinuria, cblC type (*cblC*), while mutation of *MMADCH* results in methylmalonic aciduria and homocystinuria, cblD type *(cblD*). Additional sub-types exist and are associated with mutation in either intracellular transport (*cblF* and *cblE*) of cobalamin, the conversion of homocysteine to methionine (*cblG*), or additional enzymes within the mitochondrial pathway that cause similar disorders including *cblA* and *cblB*.[8,9]

Collectively, these inborn errors of cobalamin metabolism present as a multiple congenital anomaly syndrome. Depending on subtype, patients exhibit some form of methylmalonic aciduria or homocystinuria. However, other clinical phenotypes include megablastic anemia, leukopenia, thrombocytopenia, intellectual disabilities, and some degree of seizures.[1] The degree and onset of phenotypes varies with subtype and the mutation spectrum. Recently, clinical phenotypes that mirror specific subgroups have been observed, but these individuals do not have mutations in any of the genes associated with the complementation groups previously described. Such clinical cases fall into disorders of cobalamin metabolism that are of unknown genetic origin. One such example includes the recently described methylmalonic acidemia and homocysteinemia, cblX type (*cblX*).[10] *cblX* is caused by mutations in the *HCFC1* gene, which encodes a transcriptional cofactor with nearly 5000 unique downstream target genes.[11] Interestingly, *HCFC1* has no known function in cobalamin metabolism, yet mutations in this gene cause a *cblC* like disorder with metabolic defects, failure to thrive, craniofacial abnormalities, intractable epilepsy, and intellectual disabilities.

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Table 1: Summary of known *HCFC1* variants with patient phenotype.

Position of Mutation	Domain	Patient Phenotype	Citation
p.Gln68Glu	K1	Cobalamin Disorder, Intellectual disability	10
p.Ala73Thr	K1	Cobalamin Disorder, Intellectual disability	10
p.Ala73Val	K1	Cobalamin Disorder, Intellectual disability	10
p.Tyr103His	K2	Intellectual Disability, Cobalamin Disorder, Craniofacial dysmorphia, Microcephalus, Intractable epilepsy, Profound neurocognitive impairment	15
p.Ala115Val	K2	Cobalamin Disorder, Intellectual disability	10
p.Ala115Thr	K2	Cobalamin Disorder, Intellectual disability	10
p.Ser225Asn	K4	Cobalamin Disorder (12), Intellectual Disability (13)	12 and 13
p.Thr239Met *(p.Thr142Met)	K4	Intellectual Disability	12 *(22)
p.Ala477Asn	FN3	Severe ID, absent speech and displayed frequent febrile seizures during infancy, elongated face, large ears, arachnodactyly and a lean body habitus.	13
p.Ala864Thr	Basic	No metabolic disorder, Intellectual Disability	23
p.Gly876Ser *(p.Gly779Ser)	Basic	No metabolic disorder, Autistic MMACHC expression changes	12 *(22)
p.Ala897Val	Basic	No metabolic disorder, Intellectual Disability	14
p.Ala1756Val	Acidic	Neural Development, Intellectual disability	12 and 13
p.Arg2016Trp	FN3	Neural Development	12 and 13

*Originally annotated in Reference #22 and Corrected in reference #12.

The mechanisms by which mutations in *HCFC1* cause such a myriad of clinical phenotypes are not completely understood. However, HCFC1 is known to regulate genes that are essential for cell proliferation and metabolism.[17,18] For example, the metabolic deficits in *cblX* syndrome are the result of defects in the expression of *MMACHC*, the gene associated with *cblC* syndrome. The expression of *MMACHC* in patient derived fibroblasts is severely blunted and siRNA knockdown of *HCFC1* reduces *MMACHC* expression.[10] Subsequent reports have demonstrated that each mutation can have varying effects on *MMACHC* expression.[13] Some mutations including pSer225Asn and pGly876Ser (Table 1) moderately reduce the expression of *MMACHC*, while others have

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no effect on the level of *MMACHC* expression [(pAla1756Val and pArg2016Trp) (Table 1)].[13] These data strongly suggest that individual missense mutations disproportionately affect protein function. In support of this hypothesis, there are reports of individuals with mutations in *HCFC1* associated with intellectual disability, microcephaly, and neural development defects in the absence[12,14] or presence[15,16] of cobalamin phenotypes.

HCFC1 regulates gene expression as part of a complex of other proteins including THAP11[19] and ZNF143.[11] Interaction with THAP11 and ZNF143 are essential to modulate the expression of *MMACHC*.[17] In accordance, a *cblX*-like syndrome has been reported upon mutation of *THAP11* [20] and *ZNF143*. [21(p143)] Each of these disorders is associated with defects in *MMACHC* expression. These defects are likely the result of abnormal protein interactions as both THAP11 and ZNF143 have been shown to interact with the HCFC1 kelch domain (Figure 1), the site of mutations in HCFC1 that cause *cblX* syndrome. However, the phenotypes of these syndromes are not limited to cobalamin metabolism and these individuals suffer from complex syndromes with hallmark neurological and neurodevelopmental defects.

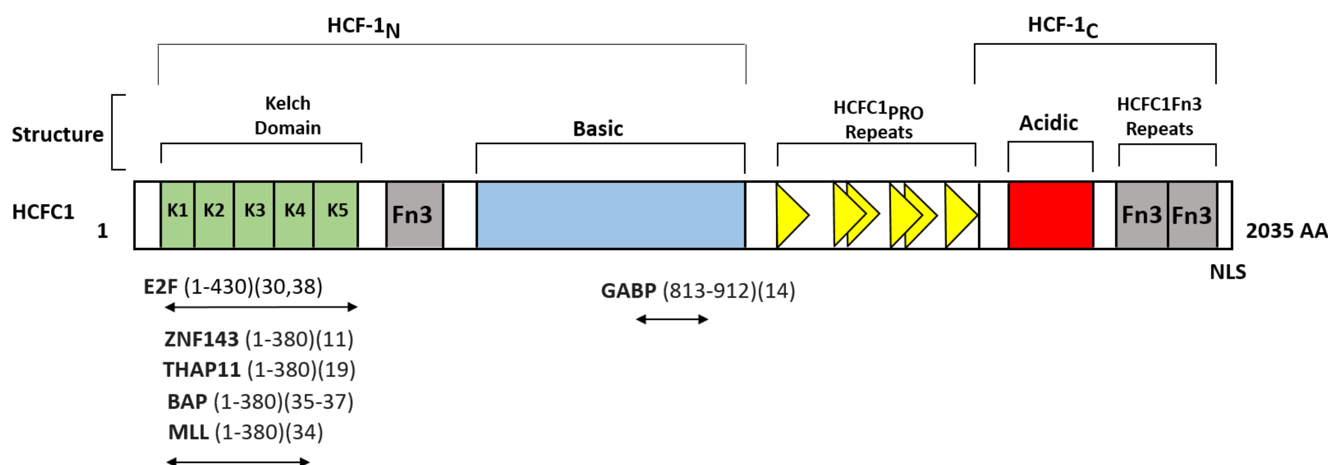

Figure 1: HCFC1 Interacting Partners by domain. The HCFC1 protein structure is depicted from N to C terminus. The uncleaved protein is 2,035 amino acids in length. The primary conserved domains include the kelch domain (K1-5), the basic region (blue), multiple fibronectin domains (Fn3), an acidic domain (red), and the HCFC1 repeats that signal cleavage events (yellow). Of the interacting proteins discussed here, the vast majority bind to the kelch domain via conserved HBM domains. The binding domain of YY1 is not depicted and has not been characterized to our knowledge. Relative binding regions are shown based on literature review with citations associated. These represent the minimal region required for binding and are not drawn to scale.

Analysis of known *HCFC1* variants and corresponding phenotypes

Previous studies have established that mutations in *HCFC1* are associated with a complex disorder that can include heterogeneous phenotypes spanning multiple body systems. *cblX* syndrome is primarily associated with mutation of the kelch protein

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interaction domain of HCFC1.[10(p1)] These mutations appear to be unique because there are several other reports of mutations in other domains of HCFC1 that do not cause cobalamin deficiency.[12(p1),14(p1),16(p1)] For example, mutation in the promoter region of the *HCFC1* gene is associated with intellectual disability, but without cobalamin phenotypes.[12] Kelch domain mutations exclusively cause syndromic intellectual disability with cobalamin phenotypes,[10(p1),15(p1)] with the exception of p.Ser225Asn reported by Huang and colleagues[12] (Table 1). However, the p.Ser225Asn was later shown to regulate *MMACHC* expression[13] and was a presumed loss of function allele. However, whether this mutation is a loss of function allele has not been completely tested. There is one reported mutation within the fibronectin domain, the p.Ala477Asn variant, which is associated with intellectual disability and facial dysmorphia, but not cobalamin defects.[13] Mutations in the basic region have been described, which are generally associated with intellectual disability.[13,14,22,23] Of these, the p.Gly876Ser variant has been associated with mild regulation of the *MMACHC* promoter. Interestingly, other C-terminal variants including the p.Ala1756Val and p.Arg2016Trp variants are associated with intellectual disability and abnormal brain development, but not other phenotypes.

Known Functions of HCFC1

The HCFC1 protein has several conserved protein domains including a kelch protein interaction domain, a fibronectin domain, and an HCF domain, which contains cleavage sites that facilitate protein processing and function[24–26] (Figure 1). The HCFC1 protein is cleaved into N and C-terminal fragments before functionality is unleashed.[25,27] The two cleavage products seemingly have different functions, but both individually regulate cell proliferation[26,28] and interact with one another.[29] HCFC1 lacks a true DNA binding domain, but regulates gene expression through interactions with transcription factors such as YY1, GABP, THAP11, ZNF143, and E2F[11,30] (Figure 1). Many of these interacting partners have a conserved HCFC1 binding motif (HBM) and this domain has been shown to interact with the kelch domain of HCFC1 (Figure 1). Of the interacting partners found in Figure 1, only GABP interacts in a protein domain outside of the kelch domain (Figure 1) and the specific binding site of YY1 has not been well established.

HCFC1 is located on the X chromosome and has long been associated with Herpes Simplex Viral (HSV) infection where it is needed for the activation of early genes through its interactions with the VP16 transcription factor.[31] HCFC1 interacts with VP16 via its kelch domain and mutation of the kelch domain *in vitro* causes defects in cell proliferation[32] and disrupts HCFC1 binding with VP16. These data support the notion that cellular proteins behave in a similar fashion as VP16. Notably, we identified at least five proteins in the literature that all interact with HCFC1 via the kelch domain (Figure 1). Thus, mutations within the kelch domain are likely to interfere with protein: protein interactions and downstream functions. Such downstream functions include the regulation of *MMACHC* expression resulting in cobalamin

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deficiencies. Yet *cblX* is complex and it is unclear whether other phenotypes are regulated via this same mechanism.

HCFC1 is known to interact with various proteins, but the interaction with E2F and MLL (Figure 1) proteins drives cell proliferation.[30,33,34] Coordination of HCFC1 interactions with the members of both protein families helps to facilitate the activation of S-phase promoter elements. For example, HCFC1 can recruit MLL family members to E2F responsive promoters inducing transcriptional activation. However, some protein interactions that mediate cell proliferation are quite complex. HCFC1 interacts with BAP1, a tumor suppressor that inhibits cell growth.[35] BAP is a deubiquitinating enzyme, thereby affecting protein stability. BAP1 interacts with the N-terminal domain of HCFC1 resulting in deubiquitination (Figure 1), which promotes cell growth.[36,37] There are likely various unknown mechanisms by which the cell regulates HCFC1 activity. Many of these are presumably via protein: protein interactions. Interestingly, a majority of these protein interactions seem to regulate cell survival and DNA damage.[38] Based on these studies it is clear that HCFC1 function is complex, cell type specific, and highly reliant on the protein milieu. Consequently, missense mutations are highly likely to have distinct effects on protein function as they might interfere distinct protein interactions.

Brain Development and HCFC1

Mutation of *HCFC1*, either in *cblX* or related syndromes, is associated with severe neurological defects, intractable epilepsy, and neural development defects.[10(p1),12,13,20] *HCFC1* is expressed in fetal tissue[31], but the function of this gene in brain development is not completely understood and complex. Early *in vitro* assays performed by Huang and colleagues demonstrated that over expression of *Hcfc1* in mouse derived neurospheres reduced cell proliferation and biased differentiation of neural precursor cells (NPCs) towards an astrocyte lineage.[12] Supporting evidence for these observations was observed upon knockdown of *Hcfc1* in neurospheres, whereby decreased *Hcfc1* expression increased the numbers of NPCs, increased proliferation, and decreased differentiation.[13] Interestingly, disrupting the expression of *Hcfc1* does not recapitulate the effects of missense mutations. For example, over expression of wild type Hcfc1 reduces proliferation, but the over expression of the p.Arg2016Trp variant (Table 1), a patient derived variant does not.[13] The function of each individual variant during brain development has not been described. However, it is highly likely that unique variants have specialized functions; consequently causing heterogeneous syndromes of varied severity.

Murine models of Hcfc1 function

In early 2016, a Cre-recombinase floxed allele of *Hcfc1* was created.[39] *Hcfc1* is required for embryonic development and therefore, deletion is lethal for hemizygous and homozygous carriers in mice.[40] Interestingly, female heterozygous carriers utilize compensatory mechanisms and X-linked inactivation to select for cells carrying the wild type allele, thus tolerating the mutation with only a temporary growth retardation. However, owing to the early

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lethality, this model was not appropriate for analysis of brain development. To circumvent this approach, a cell type specific conditional allele was created.[41] *Hcfc1* was conditionally deleted in a subpopulation of NPCs (Nkx2.1+) in the ventral telencephalon resulting in decreased numbers of GABAergic interneurons and glia, likely caused by increased cell death. Structural defects in brain development were also observed, which is consistent with the phenotypes reported in a subset of patients with *cblX* syndrome.[10] These data are the first to implicate deletion of *Hcfc1* in the developing brain or in NPCs with increased apoptosis. Interestingly, similar strategies in murine hepatocytes demonstrated an increase in cell proliferation.[42] These data suggest a cell type specific function for *Hcfc1*. Moreover, deletion of *Hcfc1* in Nkx2.1+ cells contrasts with *in vitro* assays because knockdown *in vitro* causes increased NPCs, whereas cell type specific deletion causes a decrease in Nkx2.1+ cells. But these differences could be the result of the degree of knockdown, the brain microenvironment, and the influence of cell-type specific transcription factors that interact with HCFC1. In addition, the marker used to track and follow NPCs is different across studies, which could affect overall interpretations.

Zebrafish models of *hcfc1* function

While studies in mice have provided some understanding of *Hcfc1* function, the early lethality and *in utero* gestation times have limited the effectiveness of these models. Consequently, additional model systems have been developed. Zebrafish are an alternative system for *in vivo* functional analyses.[43] They are externally fertilized, have a high fecundity, and easy to modify genetically by either transient knockdown or germline mutation. Zebrafish genome duplication has resulted in two *hcfc1* paralogs, *hcfc1a* and *hcfc1b*. These paralogs share approximately 70% identity and are conserved with both human and mouse *HCFC1*. Zebrafish do not have sex chromosomes and consequently the two genes are found on chromosomes 11 (*hcfc1a*) and 8 (*hcfc1b*). Knockdown of *hcfc1b* using anti-sense morpholinos is associated with reduced expression of the zebrafish *mmachc* gene,[44] suggesting this interaction is conserved across vertebrates. In addition, morphants present with craniofacial abnormalities owing to defects in the proliferation of neural crest cells (NCCs), a transient multi-potent progenitor cell population that gives rise to cartilage and bone of the head, neck, and face. More recently, additional analysis has demonstrated that knockdown of *hcfc1b* is associated with increased numbers of NPCs, consistent with *in vitro* assays, but contrasting with cell type specific murine models.[20] Similar facial and brain phenotypes were observed upon knockdown of the zebrafish *thap11* gene. Interestingly, these facial phenotypes can be restored by supplementing morphants with exogenous human MMACHC protein. In humans, THAP11, ZNF143, and HCFC1 interact to regulate *MMACHC* expression. Given the positive restoration of *hcfc1b* facial phenotypes by MMACHC, the presence of facial phenotypes upon deletion of *thap11*,[20] and the decreased *mmachc* expression in *hcfc1b* morphants[44]; it is plausible that the

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THAP11/ZNF143/HCFC1 axis regulates facial development and NCCs in an MMACHC dependent manner.

Interestingly the effects of *hcfc1b* knockdown on NPCs is distinct from the effects on NCCs.[20,44] These differences are intriguing as both NPCs and NCCs are progenitor cell populations, albeit with different functions, microenvironments, and fate. Decreased *hcfc1b* expression causes increased numbers of NPCs and increased NPC proliferation; a phenotype that is consistent with knockdown of *thap11*. Interestingly, mutation of zebrafish *znf143* genes causes defects in brain development.[45] Recent *mmachc* mutants were created, but not characterized for NPC deficits; however, the presence of these germline mutants[46] would rapidly enable the characterization of NPCs in this system. While knockdown of *hcfc1b* results in increased NPCs, these data are not consistent with the observations by Minocha and colleagues, which suggest that deletion of *Hcfc1* in Nkx2.1+ precursors causes reduced cell proliferation.[41] However, these studies were performed by deleting *Hcfc1* (mouse) in a subset of precursors. In zebrafish, NPCs have primarily been analyzed according to the expression of Sox2, an NPC marker.[47(p2)] These cells are present across the entire brain and therefore are likely to include some Nkx2.1+ cells, which are restricted to the forebrain, but other subpopulations of precursors as well. During brain development, NPCs are likely to represent a heterogeneous cell population.

As discussed previously, zebrafish have two *hcfc1* paralogs. Thus far, most of the published literature has focused on *hcfc1b*. Knockdown of *hcfc1a* was performed in 2014, but the phenotypes associated with decreased *hcfc1a* were moderate relative to those observed in *hcfc1b* morphants. Both genes are ubiquitously expressed, but appear to have divergent functions. For example, *hcfc1b* is essential craniofacial development, but *hcfc1a* appears to be dispensable for NCC function. In addition, *mmachc* expression is not significantly decreased in *hcfc1a* morphants, but is dramatically reduced in *hcfc1b* morphants. Whether *hcfc1a* and *hcfc1b* have an overlapping function in brain development is still unclear and has not been characterized to date.

Conclusions and Future Directions

Human genetics has revealed approximately 13 variants of *HCFC1* associated with either non-syndromic or syndromic neurological impairment. Two other sequencing reports have demonstrated additional variants, including p.Thr239Met and pAla864Thr[22,23] associated with intellectual disability. These data collectively suggest an essential role for HCFC1 during brain development. While N-terminal variants are primarily associated with syndromic versions of the disease, C-terminal variants appear to specifically disrupt brain development. Cobalamin deficits are likely the result of disrupted protein interactions with ZNF143 and THAP11, as mutation of these genes causes a similar syndromic disease. Ultimately, these findings also suggest that each missense variant of HCFC1 has a unique function, which based on current animal studies could be cell type specific. Thus, in order to comprehensively understand *cblX* and related

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disorders, individual models of each variant are necessary for future therapeutic development and patient specific treatment options.

Conflicts of Interest

Authors report no conflicts of interest

Author Contributions

AMQ wrote and edited the manuscript, VLC designed and developed figures and tables and edited final manuscript.

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