Homocystinuria: Advances in metabolic and molecular therapies targeting homocysteine pathways (Review)

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Abstract. Homocystinuria (HCU) is a rare inherited metabolic disorder caused by deficiencies of cystathionine β-synthase (CBS), methylenetetrahydrofolate reductase or methionine synthase, leading to elevated homocysteine and methionine concentrations in blood and urine. If untreated, HCU can result in notable multi-organ complications, including ectopia lentis, thromboembolism, skeletal abnormalities and cognitive impairment. The global prevalence is estimated to be 1 in 300,000, although rates vary regionally with genetic mutation patterns and consanguinity. Current therapies include: i) Vitamin B6, B12 and folate supplementation; ii) methionine-restricted diets; and iii) betaine. These therapies have important limitations, including variable responsiveness and challenges in long-term adherence, and often fail to prevent complications. Novel therapeutic approaches are advancing rapidly. Enzyme replacement therapies such as pegtibatinase, pegtarviliase and CDX-6512 have shown promise in preclinical and early clinical studies, achieving notable homocysteine reduction. Gene therapies using adeno-associated virus serotype rh.10-CBS or minicircle DNA-CBS constructs offer the potential for durable metabolic correction. Pharmacological chaperones, including S-adenosylmethionine and heme arginate, aim to restore CBS activity in mutation-specific contexts, while orthotopic liver transplantation remains the only definitive treatment for severe pyridoxine-non-responsive cases. The present review summarizes these emerging therapeutic strategies, highlighting their potential to correct metabolic imbalances in HCU, improve clinical outcomes, and address the limitations of both conventional and novel treatments. The present review also incorporates novel epidemiological findings, integrates the foundational enzymology of HCU with current genotype-phenotype associations and updates the therapeutic landscape through early 2025 with key developments such as the discontinuation of the pegtarviliase program and the rebranding of CDX-6512 as SYNT-202.

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Abbreviations: HCU, homocystinuria; CBS, cystathionine β-synthase; MTHFR, methylenetetrahydrofolate reductase; DVT, deep vein thrombosis; PE, pulmonary embolism; PLP, pyridoxal 5'-phosphate; CGL, cystathionine γ-lyase; MS, methionine synthase; BHMT, betaine-homocysteine methyltransferase; tHcy, total homocysteine; MMA, methylmalonic acid; MGL, methionine γ-lyase; ERT, enzyme replacement therapy; SAM, S-adenosylmethionine; AAVrh.10, adeno-associated virus serotype Rh.10; PI, proteasome inhibitor; OLT, orthotopic liver transplantation

Key words: HCU, CBS deficiency, ERT, gene therapy, pharmacological chaperones, OLT

Contents

1. Introduction

Homocystinuria (HCU) is a rare genetic metabolic disorder that results from the deficiency of the enzyme cystathionine β-synthase (CBS), which is a key player in the metabolism of the amino acid methionine. As it is an autosomal recessive genetic disease, a child must inherit two defective copies of the gene from the parents to be affected. Other enzymes involved in homocysteine metabolism include methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MS), especially in vitamin B12 or folate deficiency. This CBS enzymatic deficiency leads to the accumulation of homocysteine and methionine in the blood and urine of affected patients (1), eventually leading to a wide range of notable and irreversible multi-organ complications.

HCU imposes a notable and multifaceted healthcare burden, particularly when misdiagnosed in early childhood or

when inadequate metabolic control is achieved. HCU requires lifelong management, strict dietary restrictions, multivitamin supplementation and frequent biochemical monitoring, all of which place financial and logistical burdens on patients, families and healthcare systems. This burden is escalated by the required management and hospitalization of patients due to disease-related complications with often life-threatening consequences. Recent population-based analysis further quantified this burden, documenting substantial rates of thromboembolism, ophthalmic morbidity and healthcare utilization in classical HCU (2).

Foundational syntheses, such as that reported by Schneede *et al* (3), have established the enzymatic basis of HCU, emphasized homocysteine as a pathogenic vascular risk factor and summarized the then-standard treatments: i) Dietary methionine restriction; ii) supplementation with high-dose pyridoxine, folate or B12; and iii) betaine.

Current therapies for HCU such as dietary methionine restriction and supplementation with vitamins B6, B12 and folate often fail to prevent complications, especially in patients who are non-responsive to treatment. With the understanding of disease pathogenesis, patient-focused novel interventions that correct fundamental metabolic imbalances, such as enzyme replacement, gene therapy and RNA-based treatments, have become important (2). The present review revisits the foundational enzymology of HCU in the context of modern genotype-phenotype insights and incorporates novel epidemiological data quantifying the healthcare burden of classical HCU. The present review also integrates developments through early 2025, including the discontinuation of the pegtarviliase program and the rebranding of CDX-6512 as SYNT-202, with corresponding updates from clinical trial data, regulatory announcements and pipeline disclosures.

2. Epidemiology

Consanguinity and gene mutations are the predominant risk factors for HCU, which explains the high incidence of HCU in some populations, such as Irish and Middle Eastern populations (4,5).

Although HCU is a rare genetic enzymatic disorder, with global prevalence estimates of 1 in 300,000 individuals, its prevalence rates vary considerably across different regions due to factors such as genetic mutations and consanguinity (4). Qatar has the highest known incidence at ~1 in 1,800 births, most likely due to the high rates of consanguineous marriages (4), while Kuwait and the eastern area of Saudi Arabia have reported comparably lower rates of 1 in 43,000 (6,7).

In the United States, the estimated reported incidence is 1 in 100,000 (8); however, a previous analysis has suggested that the actual prevalence may be ≥10 times this estimate, most likely because of underdiagnosis (8). In Norway, a study reported a prevalence of 1 in 6,400 (9), whereas surveys in Ireland and Germany have reported incidences of ~1 in 65,000 and 1 in 17,800 births, respectively (5,10).

3. Clinical presentation and complications

The clinical presentation of HCU spans ocular, skeletal, neurologic and thromboembolic complications (11-15) and often differs between pediatric and adult patients, influenced by the severity of enzyme deficiency and responsiveness to therapy (16,17).

Thromboembolism is a life-threatening complication of homocysteine accumulation. Hyperhomocysteinemia damages the vascular endothelium, causing oxidative stress, inflammation and endothelial dysfunction, which leads to platelet activation and coagulation cascade dysregulation, markedly increasing the risk of arterial and venous thrombosis. Furthermore, homocysteine impairs nitric oxide production, reducing vasodilation and contributing to vascular stiffness, leaving patients at an increased risk of developing deep vein thrombosis (DVT), pulmonary embolism (PE), myocardial infarction and stroke, even at a young age (11,12).

An ocular complication of hyperhomocysteinemia is ectopia lentis (eye lens dislocation). Elevated homocysteine levels interfere with collagen cross-linking, thus disrupting the normal connective tissue integrity of the zonular fibers, which are fibrillin-rich structures that anchor the lens within the eye, leading to ectopia lentis. The displaced lens impairs vision and predisposes the patient to secondary complications, such as myopia, glaucoma, retinal detachment and eventual permanent vision loss. Ectopia lentis typically develops in early childhood and is often bilateral (13).

In the same context, impaired collagen cross-linking and connective tissue integrity lead to impaired bone matrix formation that resembles the features of Marfanoid body habitus, such as long limbs, scoliosis and pectus deformities, as well as osteopenia or osteoporosis, and increases the risk of fractures. These skeletal manifestations are typically more evident during growth spurts in childhood and adolescence (14).

Neurological complications occur because HCU induces oxidative stress, impairs methylation processes that are important for neurotransmitter synthesis and damages the vascular endothelium, thereby increasing the risk of cerebral microvascular injury and stroke. This can result in developmental delays, mental disability, seizures and motor dysfunction during childhood. In adolescents and adults, untreated disease may manifest as behavioral disorders, psychiatric symptoms such as depression, anxiety and psychosis, or cognitive decline (15).

In pediatric patients, developmental delay, failure to thrive and hypotonia are the earliest signs. Intellectual disability is commonly detected in untreated children in combination with behavioral disorders. Ectopia lentis appears within the first 10 years of life and is usually associated with severe myopia or glaucoma, while musculoskeletal anomalies can emerge during growth. Children may also experience seizures or exhibit a clumsy gait due to neurological disorders (16).

In adults, especially those with delayed treatment during childhood, thromboembolic events such as DVT, PE and stroke frequently predominate, even in the absence of known risk factors. Psychiatric symptoms such as depression, anxiety and psychosis can also show delayed manifestations in adults with low treatment responsiveness, who may have impaired intellectual or physical signs (17).

4. Molecular background of HCU

Transsulfuration pathway. The transsulfuration pathway is a hepatic pathway that facilitates the conversion of

homocysteine into cysteine to detoxify excess homocysteine, and contributes to the synthesis of glutathione, taurine and coenzyme A, which are important as antioxidants, for bile acid metabolism and for energy production (18). The transsulfuration pathway starts with the CBS enzyme, which catalyzes the conversion of homocysteine and serine into cystathionine, using pyridoxal 5'-phosphate (PLP), the active form of pyridoxine, as a cofactor. Afterwards, cystathionine γ-lyase (CGL) breaks down cystathionine into cysteine, α-ketobutyrate and ammonia. Absolute deficiency or malfunction of CBS disrupts this pathway, leading to elevated blood and urine levels of homocysteine and methionine, and reduced cysteine levels (18).

CBS mutations show genotype-phenotype variability, influencing the severity of the disease and the responsiveness to pyridoxine therapy. High-dose B6 supplementation may be beneficial in the case of partial CBS activity to enhance residual enzyme function, while total enzyme deficiency requires a methionine-restricted diet, cysteine-supplemented diets and betaine, folate and B12 supplementation to promote alternate remethylation pathways (19,20).

Remethylation pathway. The remethylation pathway works in parallel with the transsulfuration pathway to normalize plasma homocysteine levels, especially in the brain where the transsulfuration pathway is inactive. In this reaction, homocysteine is remethylated to methionine, which is necessary for protein synthesis, through catalysis by MS, which requires B12 as a cofactor and employs 5-methyltetrahydrofolate produced by MTHFR as the methyl group donor (21).

Another hepatic remethylation route involves the enzyme betaine-homocysteine methyltransferase (BHMT), which uses betaine as a methyl donor to remethylate homocysteine into methionine. The BHMT pathway provides an alternative route for homocysteine metabolism in cases of folate or B12 deficiency or genetic disorders that affect the primary route (22).

These interrelated transsulfuration and remethylation pathways are summarized in Fig. 1, which illustrates the metabolic conversion of homocysteine and the key cofactors involved.

5. Genetic variant associations

Genetic variants of HCU serve important roles in determining case severity, response to treatment and clinical outcomes (23,24). The *CBS* gene is located on chromosome 21q22.3 (23). More than 200 pathogenic mutations have been identified in *CBS*, resulting in varying degrees of residual enzyme activity, which markedly influence both phenotype and clinical expression (24).

The *I278T* mutation, which is widely prevalent in Western and Far Eastern populations, has been extensively studied. Patients homozygous for *I278T* often retain residual enzyme activity and respond to B6 therapy, whereas the *G307S* mutation, common in Irish and Australian populations, is associated with a severe phenotype and non-responsiveness to B6 (24-26).

Individuals with partial enzymatic activity tend to show delayed manifestations, milder laboratory abnormalities and more favorable clinical outcomes. This genotype-phenotype association is an important consideration in guiding initial vitamin B6 therapy and in predicting disease prognosis (25).

Variants of other genes involved in homocysteine metabolism, such as *MTHFR*, 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*), MTR reductase (*MTRR*) and metabolism of cobalamin associated C, also contribute to homocysteine toxicity. The MTHFR C677T polymorphism produces a thermolabile enzyme with reduced activity, leading to mild-to-moderate hyperhomocysteinemia, especially in the context of low folate levels (27-29). While not typically causing classical HCU, these variants can worsen biochemical imbalances in genetically predisposed individuals (27-29).

Genetic workups, even in asymptomatic individuals, are valuable for early diagnosis and for predicting vitamin B6 responsiveness (27-29).

Table I summarizes the key genetic variants associated with classical HCU, highlighting their impact on enzymatic activity, B6 responsiveness and clinical severity.

6. Diagnosis of HCU

The diagnosis of HCU involves a combination of clinical evaluation, biochemical testing and molecular genetic analysis. Early and accurate diagnosis is important because prompt treatment can improve the prognosis of HCU and prevent irreversible complications (16,17).

Symptoms and phenotypic features. HCU usually presents with a wide range of clinical symptoms that affect multiple organs. Ocular complications, including ectopia lentis, myopia, astigmatism, glaucoma and blindness, are the earliest and most common manifestations, affecting 90% of poorly managed individuals (13-17).

In the early childhood growth phase, skeletal deformities are common, including marfanoid body habitus, long limbs, scoliosis, pectus deformities, genu valgum and severe osteoporosis, which predispose patients to recurrent bone fractures (14). Neurological deficits and developmental delays commonly occur in the early years of life, are noticed as intellectual disabilities and may progress to repeated seizures (17).

Thromboembolic events, such as DVT, PE and stroke, are the most serious complications that may affect both adolescents and adults. The severity of clinical presentation is markedly dependent on the degree of enzyme deficiency and therapeutic responsiveness (11,12).

Biochemical diagnosis

Plasma homocysteine level. Plasma total homocysteine (tHcy) is a sensitive diagnostic biomarker for the primary diagnosis and therapeutic monitoring of HCU. Impaired metabolism of homocysteine to cystathionine results in elevated tHcy blood levels of >100 μmol/l compared with <15 μmol/l in healthy individuals (30).

Plasma tHcy levels combined with elevated methionine levels can distinguish CBS deficiency from other homocysteine-related disorders, such as remethylation defects, which usually present with high tHcy levels but low methionine levels. Furthermore, tHcy levels also guide clinical decisions during diagnostic B6 challenge tests, in which a notable drop in homocysteine confirms B6-responsive HCU (30).

Plasma methionine levels. Decreased homocysteine metabolism leads to a buildup of methionine, with levels

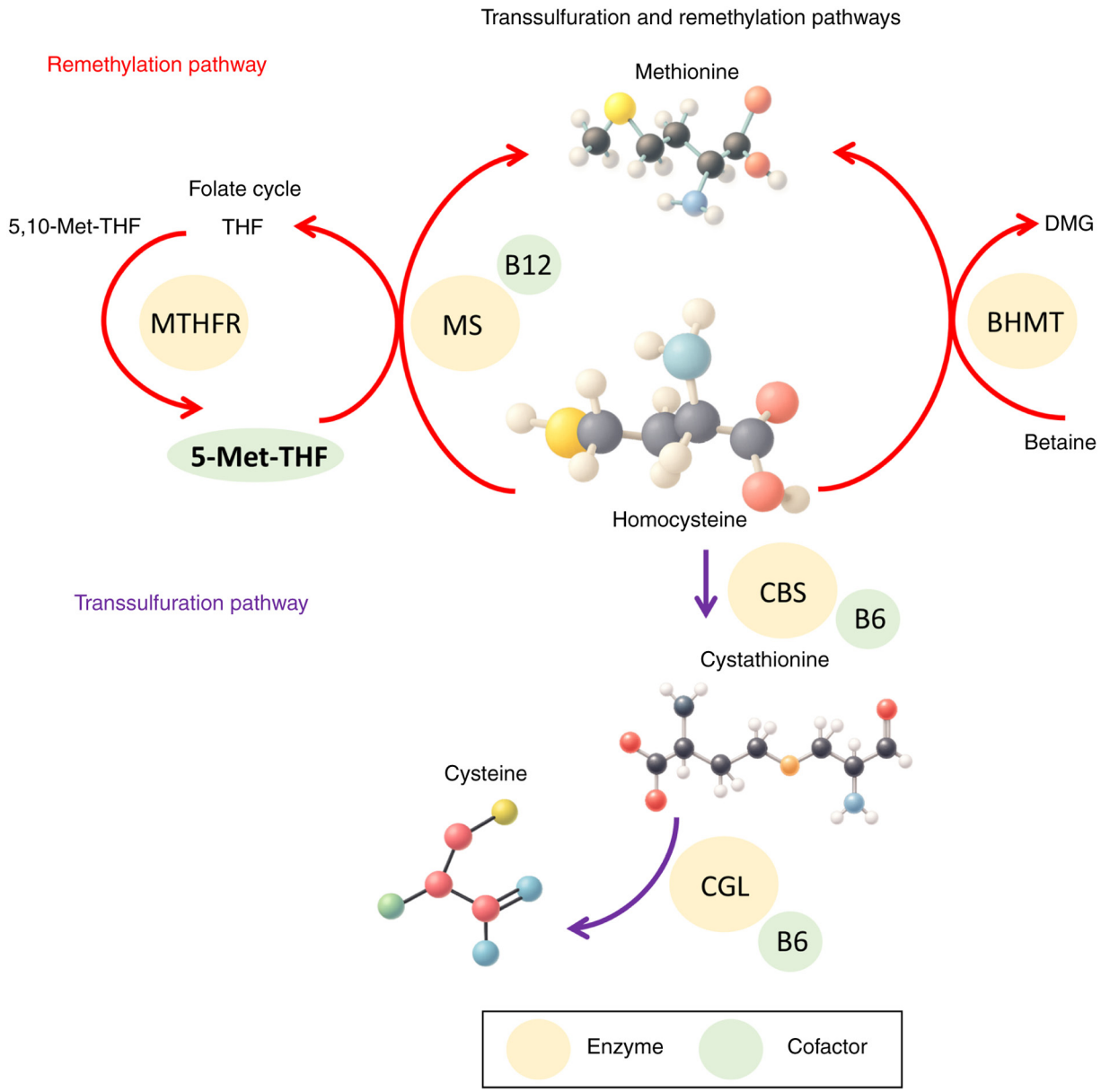

Figure 1. Molecular pathways of homocysteine metabolism. The figure illustrates the normal transsulfuration and remethylation pathways of homocysteine metabolism, as well as the folate cycle. Key enzymes include CBS, MS, MTHFR and BHMT. Important cofactors such as pyridoxal phosphate (vitamin B6), folate (5-Met-THF) and methylcobalamin (vitamin B12) are indicated. Substrates and intermediates shown include homocysteine, methionine, cystathionine, cysteine, 5,10-Met-THF and DMG. THF, tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MS, methionine synthase; DMG, dimethylglycine; BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β-synthase; CGL, cystathionine γ-lyase; 5-Met-THF, 5-methyltetrahydrofolate; 5,10-Met-THF, 5,10-methylenetetrahydrofolate.

ranging from 60 to 100 μmol/l. This concurrent elevation of tHcy and methionine can distinguish classical HCU from hyperhomocysteinemia caused by other remethylation pathway disorders, such as MTHFR deficiency or cobalamin metabolism malfunction, which lead to elevated tHcy levels while maintaining normal methionine levels. In patients on methionine-restricted diets, the normalization of plasma methionine and tHcy levels indicates effective metabolic control. The B6 trial also identifies B6-responsive individuals who show partial improvement in methionine levels (17).

Urinary homocysteine. Measurement of urinary homocysteine levels provides evidence for the diagnosis of classical HCU, particularly in limited-resource settings where plasma tHcy testing is not available. In CBS deficiency, elevated plasma homocysteine levels exceed the renal reabsorption capacity, leading to increased renal excretion of both free and tHcy. Although the urinary homocysteine level is less precise than the plasma tHcy test, it is used as an initial screening test, especially in symptomatic children. However, elevated urinary homocysteine levels can also be detected in other homocysteine metabolism disorders, and may vary with hydration status and renal function (31).

Vitamin B12, folate and methylmalonic acid (MMA). Being cofactors in the remethylation pathway that converts homocysteine to methionine, deficiencies in vitamin B12 or folate can lead to elevated plasma tHcy levels but are not indicative of CBS deficiency. Thus, measuring serum B12 and folate levels rules out nutritional or acquired causes of hyperhomocysteinemia (32,33).

Table I. Summary of key genetic variants of CBS and related genes, their geographic prevalence, enzyme activity, vitamin B6 responsiveness and associated clinical severity of HCU.

Mutation	Geographic prevalence	B6 responsiveness	Residual CBS activity	Clinical severity (phenotypic severity of homocystinuria)
I278T	Western (Europe and North America) and Far Eastern (China)	Responsive	Partial	Mild to moderate
G307S	Ireland and Australia	Non-responsive	Minimal to none	Severe, early onset
Other CBS variants (*p.R125Q*, *p.R266K*, *p.R336C* and *c.1224-2A>C*)	Global	Variable	Variable	Variable
MTHFR *C677T*	Global (especially Europe and Asia)	No (not classical HCU)	Thermolabile MTHFR	Mild to moderate hyperhomocysteinemia

CBS, cystathionine β-synthase; HCU, homocystinuria; MTHFR, methylenetetrahydrofolate reductase.

Elevated MMA levels suggest functional B12 deficiency or disorders of cobalamin metabolism, which may be associated with elevated tHcy levels. However, in classical HCU, MMA levels are typically normal, which helps to exclude remethylation disorders from the potential diagnosis and indicates that the elevated homocysteine level is due to transsulfuration pathway impairment rather than defective remethylation (34).

Molecular genetic testing. Genetic testing using next-generation sequencing that utilizes the *CBS* gene, or whole-exome sequencing in asymptomatic cases, is a decisive tool for confirming the diagnosis of classical HCU, uncovering the underlying *CBS* gene mutations responsible for CBS deficiency, as well as predicting B6 responsiveness and guiding treatment decisions (35,36).

Over 190 *CBS* gene mutations with clinically significant genotype-phenotype associations have been isolated. The common *p.I278T* mutation is often associated with partial or full responsiveness to B6 therapy, whereas *p.R125Q, p.G307S* and *p.R266K* mutations are associated with non-responsiveness and severe symptoms (37).

Enzyme activity assays. CBS activity assays performed on cultured skin fibroblasts or fresh liver biopsy samples evaluate the functional capacity of CBS, which is typically absent or markedly reduced in HCU. Enzymatic activity testing assists in distinguishing between the B6-responsive and non-responsive forms of CBS, as residual activity may be enhanced by the addition of PLP *in vitro*. The use of enzyme assays has lost its applicability in clinical practice as it requires an invasive tissue sampling technique, a long turnaround time and challenging interpretation in cases with residual activity (36,38).

7. Treatment

Conventional therapies

Pyridoxine (vitamin B6) supplementation. High-dose B6 therapy is the first line of treatment for patients with residual CBS enzyme activity. B6 is converted into PLP, an important cofactor for the enzymatic activity of CBS. Genotypes such as *I278T* typically retain fractional enzyme functions and respond satisfactorily to B6 therapy (39,40). Notably, a study involving European and sub-Saharan populations has identified multiple haplotypes of the *CBS* gene, suggesting that an ongoing mutational process may contribute to the diminished long-term effectiveness of pyridoxine therapy in sustaining optimal clinical outcomes (39). By contrast, null mutations in *G307S* result in no functional CBS enzymes and are usually pyridoxine-non-responsive; the *CBS c.1224-2A>C* mutation is a null, splice-site variant associated with vitamin B6 non-responsiveness (41). Other mutations such as *p.R336C* alter the molecular properties of CBS, leading to a severe HCU phenotype unresponsive to B6 therapy (42).

Comprehensive genotyping and *in vitro* enzyme activity assays are not available in numerous clinical settings. Therefore, clinicians rely on empirical pyridoxine trials without accurately predicting the outcomes. A pyridoxine trial is performed in all newly diagnosed cases, typically starting at 100-500 mg/day, followed by therapeutic monitoring of plasma tHcy and methionine levels. Patients able to achieve tHcy levels <50 μmol/l with B6 therapy alone are classified as responsive and usually exhibit milder clinical presentations (17).

B6 therapy faces some challenges, as not all pyridoxine-responsive patients demonstrate clinical improvement with B6 supplementation. This may be due to additional confounders such as epigenetics, coexisting polymorphisms and nutrient status. Furthermore, some patients exhibit only a partial response and may continue to develop further complications. These patients often require combination therapy, and their classifications can be ambiguous (17). Additionally, long-term adherence to B6 therapy may be challenging because of rising concerns regarding peripheral neuropathy caused by chronic high-dose use (43).

Dietary methionine restriction. A lifelong methionine-restricted diet remains the basic strategy for managing HCU due to *CBS* gene mutations. The diet aims to reduce methionine accumulation while ensuring adequate nutrition using methionine-free nutritional formulas. This approach requires careful follow-up of plasma amino acid levels to maintain

metabolic control, prevent protein malnutrition and ensure the fulfillment of demands for normal growth, especially in pediatric patients (44).

Although a methionine-restricted diet is effective in lowering plasma tHcy levels, it poses a long-term compliance challenge because of the poor palatability of dietary formulas (45). In addition, dietary restrictions alone may not be sufficient to prevent disease complications and may require adjunctive therapies (44,45).

Numerous preclinical trials in animal models have attempted to overcome the challenges related to dietary restrictions. Using a mouse model, CDX-6512, an engineered orally-stable methionine γ-lyase (MGL), was administered following a high-protein meal and led to a dose-dependent ability to locally degrade methionine in the gastrointestinal tract (GIT), suppressing plasma methionine and homocysteine (46). Another experimental model by Perreault *et al* (47) used a bolus dose of an engineered probiotic *E. coli* Nissle strain to degrade methionine in the GIT. The resulting SYNB1353 strain metabolized methionine in mice, non-human primates and humans, resulting in lower plasma methionine levels.

Betaine supplementation. Betaine, also known as trimethylglycine, is a commonly used adjunctive therapy for B6-unresponsive patients with HCU that acts as a methyl donor in the hepatic BHMT remethylation pathway (48). In a study enrolling patients with B6-unresponsive HCU, betaine administration resulted in individual mean reductions in plasma tHcy levels ranging from 47.4 to 105.0 μmol/l (49). Another study involving healthy subjects showed that betaine supplementation of 6 g/day for 3 weeks significantly reduced homocysteine levels (P=0.030) (50). A study by Lu *et al* (51) reported a 10% reduction in the plasma homocysteine concentration when using a combination of betaine and low-dose B vitamins: 400 μg folic acid, 8 mg vitamin B6, 6.4 μg vitamin B12 and 1 g betaine.

However, prolonged betaine therapy of >100 mg/kg/day in patients with inadequate dietary methionine restriction led to notable hypermethioninemia-related adverse events (52), while another study reported marked elevations in total cholesterol levels with betaine supplementation, necessitating close monitoring in patients at risk of cardiovascular disease (53).

Folate and B12 supplementation. Folate and B12 are important drugs in HCU management protocols, particularly in patients with *MTHFR*, *MTR* and *MTRR* mutations. These vitamins serve important roles in the remethylation of homocysteine to methionine (54). The active form of folate, 5-methyltetrahydrofolate, donates a methyl group to homocysteine, converting it to methionine. This reaction is catalyzed by MS with methylcobalamin, the active form of vitamin B12, as a cofactor. Insufficient folate and B12 supplies impair this pathway, leading to elevated tHcy levels (55). A randomized clinical trial by Kok *et al* (56) demonstrated marked reductions in tHcy levels in elderly participants consuming 400 g folic acid and 500 g vitamin B12 daily over 2 years.

Limitations and challenges of conventional therapies. Despite being the basis of HCU management, conventional therapies have non-negligible limitations, including variability in patient responsiveness to B6. Adherence to low-methionine diets is often difficult, affecting metabolic control, even with strict diet control and vitamin supplements. Numerous patients fail to meet the desired tHcy levels, leaving them at risk of complications. Furthermore, the long-term outcomes of current interventions are inconsistent and there is limited capacity to reverse established complications (17,43-45).

Novel treatment approaches. Mechanistic and structural studies on CBS have provided the basis for emerging therapeutic concepts (57-62). Building on these and on prior reviews, such as that by Majtan *et al* (63), this section emphasizes treatment developments since 2023 and practical translational considerations across enzyme replacement, gene- and proteostasis-directed strategies.

Fig. 2 provides an integrated overview of these molecular pathways with annotated therapeutic targets and intervention points.

Enzyme replacement therapy (ERT). Human CBS is a complex multidomain enzyme that requires PLP for its activity and binds to a heme group that serves a regulatory role in its enzyme activity. The CBS protein is composed of four identical subunits comprising 551 amino acids. Each subunit consists of three domains, with the N-terminal domain containing the heme-binding site. In this region, the heme is linked by cysteine at position 52 and histidine at position 65 (57).

ERT is being developed as a mutation-agnostic approach for CBS deficiency that delivers functional enzymes directly, bypassing reliance on residual endogenous activity. While ERT can normalize biochemistry quickly, the need for chronic administration and the typical costs and immunogenicity risks of biologics remain practical challenges for widespread adoption (58).

i) Pegtibatinase. Pegtibatinase, also known as OT-58 or TVT-058, is a recombinant human CBS catalytic core (amino acids 1-413) that has been modified with the addition of polyethylene glycol (PEG) and lacks the CBS autoinhibitory regulatory domain, yielding a constitutively active, predominantly dimeric enzyme. This modification stimulates the catalytic activity of the enzyme, rendering it predominantly in a dimeric state (58). To boost pegtibatinase stability and minimize unwanted protein aggregation, a cysteine-to-serine substitution at position 15 is introduced to prevent the formation of interdimer disulfide bonds. The enzyme is also chemically modified by PEGylation, in which five PEG chains of 20 kDa each are attached to each CBS subunit. This extends the circulation half-life by 10-fold, making the PEGylated form more suitable for therapeutic use in HCU than the unmodified enzyme (59).

In the phase 1/2 COMPOSE study, pegtibatinase demonstrated promising tolerability and a safety profile with only two incidents of mild injection site urticaria that resolved upon temporary dose interruption and subsequent dose titration. Using a subcutaneous dose of 1.5 mg/kg twice weekly of pegtibatinase achieved a 67.1% mean relative reduction in tHcy levels from baseline (97 to 32 μM) at follow-up intervals of 6, 8, 10 and 12 weeks, maintaining tHcy levels below the clinically meaningful threshold of ≥100 μM (60). A preclinical study in CBS-deficient animal models has demonstrated that pegtibatinase ameliorated related complications such as ocular and skeletal manifestations, decreased facial alopecia, and enhanced liver metabolism of glucose and lipids (61).

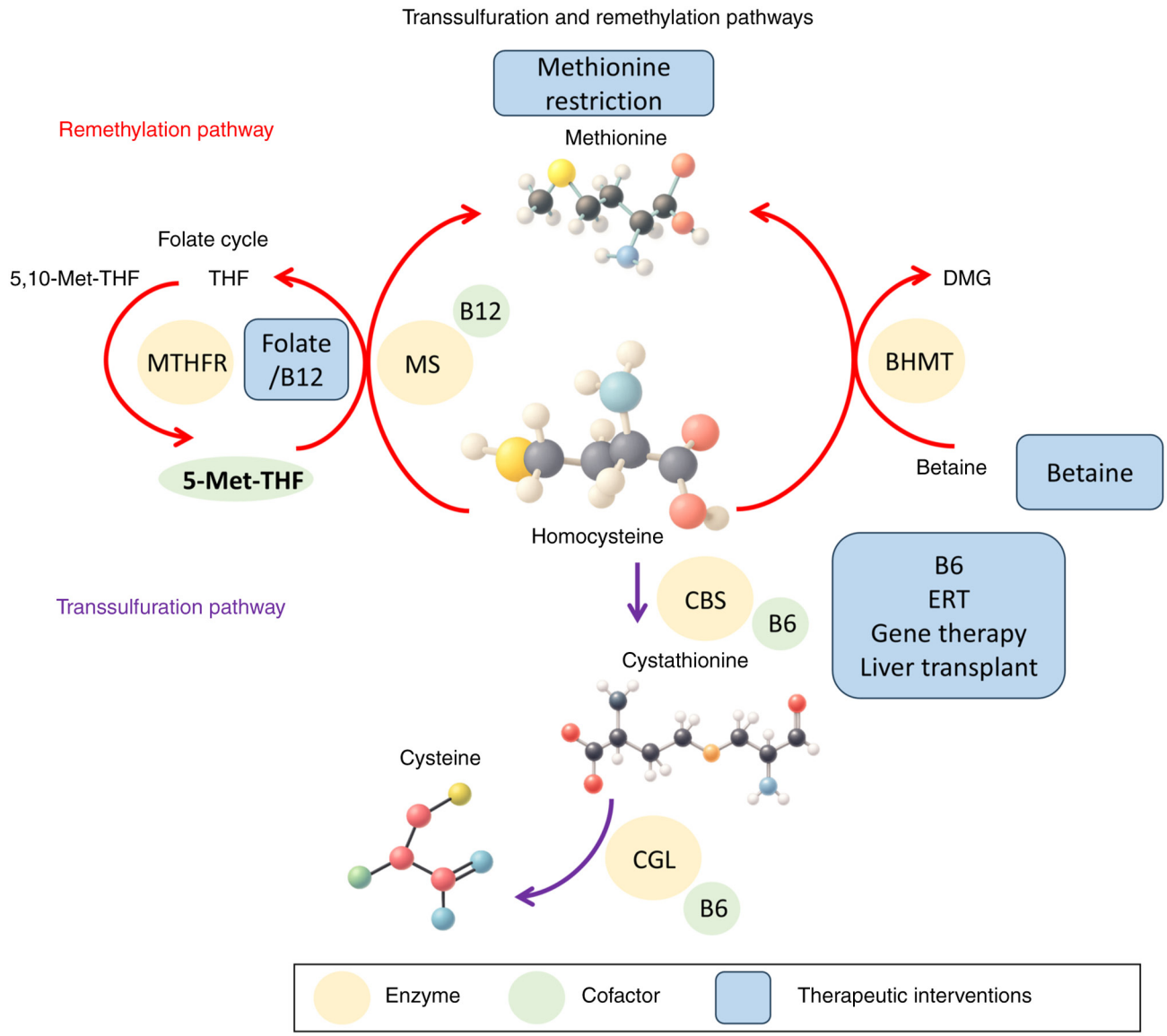

Figure 2. Therapeutic interventions and intervention points in homocysteine metabolism. The figure presents the molecular pathway of homocysteine metabolism with annotated sites of action for conventional and novel therapies, including dietary modification, vitamin supplementation, betaine therapy, ERT, gene therapy and orthotopic liver transplantation. THF, tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MS, methionine synthase; DMG, dimethylglycine; BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β-synthase; ERT, enzyme replacement therapy; CGL, cystathionine γ-lyase; 5-Met-THF, 5-methyltetrahydrofolate; 5,10-Met-THF, 5,10-methylenetetrahydrofolate.

ii) Pegtarviliase. Pegtarviliase, also known as AGLE-177, is an engineered CGL variant (CGL-ILMDRGVS) with markedly increased affinity for homocysteine compared with wild-type CGL. It contains eight missense mutations, referred to as the CGL-ILMDRGVS construct, and exhibits a 60-fold increased affinity for homocysteine compared with the wild-type human CGL enzyme (62). Furthermore, pegtarviliase shows an enhanced capability for degrading multiple forms of homocysteine compared with wild-type CGL, along with improved stability and circulation time, potentially resulting in a more comprehensive reduction in tHcy levels (62).

A preclinical study of pegtarviliase in mice using subcutaneous doses of 1, 3 and 10 mg/kg twice weekly between postnatal days 10 and 70 reported a marked improvement in 30-day survival rates, at >75 vs. <20% in the treated and control groups, respectively. The treated mice also showed resolution of liver steatosis and alopecia. Furthermore, a dose of 10 mg/kg led to an ~43% reduction in plasma tHcy levels and an 87% reduction in brain tHcy levels (63).

In a phase 1/2 dose-escalation trial aiming to assess the safety, tolerability, pharmacokinetics and efficacy of pegtarviliase in patients with classical HCU, three cohorts of participants received weekly subcutaneous injections of pegtarviliase at doses of 0.15, 0.45 and 1.35 mg/kg, respectively over the course of 4 weeks. A 3-day post-treatment dose-dependent reduction in tHcy levels of 26.3 and 33.0% was observed in cohorts 1 and 2, respectively. However, some participants in cohort 3 experienced injection site reactions and increased tHcy levels, which can be explained by the development of anti-drug antibodies. The study highlighted the promising tolerability and efficacy of pegtarviliase at low doses, with a clear need for modified clinical strategies to resolve the immunogenicity issues (64). As of 2023, Aeglea BioTherapeutics, Inc., announced the exploration of

strategic alternatives for the pegtarviliase program following interim phase 1/2 results (65), which ultimately led to the discontinuation of its clinical development in 2024.

iii) CDX-6512. CDX-6512 is an investigational modified MGL enzyme that is specifically engineered to maintain stability and activity in the GIT. In 2022, the U.S. Food and Drug Administration granted CDX-6512 the 'orphan drug designation' for the treatment of HCU. Using artificial intelligence and machine learning, 12 iterative rounds of enzyme evolution were performed, and >27,000 variants were screened for activity under simulated gastric and intestinal conditions. The resulting enzyme exhibited high resistance to deactivation by gastric pH and other enzymes (46).

In a preclinical study using the Tg-I278T $CBS^{-/-}$ mouse model of HCU, an oral dose of 148 mg/kg CDX-6512 administered after a high-protein meal led to an almost 50% reduction in plasma tHcy levels after 4 h. Plasma methionine levels also declined in a non-significant dose-dependent manner, with up to a one-third decrease at the highest dose (66).

A study in healthy non-human primates that received a high-protein meal followed by an oral dose of 370 mg/kg CDX-6512 treatment led to a statistically significant, dose-dependent reduction in plasma methionine levels, demonstrating the effectiveness of the enzyme in breaking down dietary methionine in a physiologically-similar model to humans (46). In the same preclinical program using Tg-I278T $CBS^{-/-}$ mice, daily oral administration of CDX-6512 with a high-protein meal over 2 weeks effectively maintained baseline plasma tHcy levels. By contrast, untreated controls showed a 39% increase in homocysteine levels, suggesting that homocysteine levels can be sustained with long-term CDX-6512 treatment (46). In 2024, the program was acquired and rebranded as SYNT-202 by Syntis Bio, with continued preclinical optimization focused on gut-restricted methionine degradation; no human data under the new designation have been disclosed at present (67).

Limitations and challenges of ERT. Although ERT offers mutation-independent metabolic control, injectable agents such as pegtibatinase face lifetime adherence and immunogenicity hurdles, and oral agents such as SYNT-202 still require durable efficacy and safety data; cost-of-goods and access considerations further suggest that ERT will likely complement, rather than fully replace, diet and vitamin-based care (58-61).

Gene therapy. Gene therapy offers a potential one-time treatment for CBS deficiency by restoring intrinsic CBS function at the genetic level. The strategy delivers a functional CBS gene to the liver, via viral or non-viral vectors, to achieve sustained metabolic correction.

Adeno-associated virus serotype rh.10 (AAVrh.10)-CBS-based gene therapy. AAVrh.10-CBS uses an AAVrh.10 vector to deliver a functional human CBS gene under a cytomegalovirus early enhancer/chicken β-actin promoter, targeting hepatocytes involved in homocysteine metabolism. In the CBS-deficient mouse model I278T, a single intravenous dose reduced plasma tHcy levels by ~97% within 1 week and sustained an ~81% reduction at 1 year from administration, with observed reversal of alopecia, skeletal defects and abnormal fat distribution (68). Pre-existing neutralizing antibodies to AAVrh.10 appear to be less prevalent than those to AAV2 or AAV8, supporting the suitability of AAVrh.10 for liver-directed applications (69). Furthermore, the durability of liver-directed AAV expression observed in a human hemophilia B gene therapy trial (70) suggests the potential for long-term transgene persistence in clinical applications.

Minicircle DNA-CBS gene therapy. Minicircular DNA vectors, which lack bacterial backbone sequences, can more efficiently enhance transgene expression and reduce innate immune activation compared with plasmids. Foundational work on vector design and dosing has also highlighted safety principles to minimize hepatic genotoxicity in gene-delivery programs (71).

In CBS-deficient mice, a single liver-targeted injection of the minicircle CBS construct MC.P3-hCBS reduced plasma homocysteine levels by ~50% within 1 week, restored hepatic CBS activity ~34-fold and sustained metabolic correction for >6 months (72).

Advantages, limitations and challenges of gene therapy. Gene therapy directly addresses the genetic cause of HCU, offering the possibility of durable metabolic correction. However, immune responses to viral vectors, the dilution of treatment effects with hepatic growth in children and complex manufacturing remain key challenges (68-72).

Pharmacological chaperones. Pharmacological chaperones are small molecules that bind to misfolded CBS proteins, stabilizing their structure and enhancing catalytic activity. Examples include glycerol, trimethylamine-N-oxide and dimethyl sulfoxide, which have been shown to restore activity in certain CBS mutants in cell and animal models (73,74). Yeast expression systems for human CBS mutants have also demonstrated that chaperones can promote proper assembly and tetramerization, supporting mutation-specific therapeutic potential (75).

S-adenosylmethionine (SAM). SAM, an allosteric activator of CBS, binds to the regulatory domain of the enzyme to boost activity (76,77). A study by Mendes *et al* (77) demonstrated that ~50% of tested patients with HCU showed defective SAM activation, often linked to specific CBS mutations.

Heme arginate. CBS is a heme-dependent enzyme; some *CBS* gene mutations impair heme binding. Heme arginate can stabilize these variants and restore heme-binding activity in cell models (78). A study by Melenovská *et al* (78) showed that administration of heme arginate restored heme binding, improved tetramer formation and enhanced catalytic activity of the CBS enzyme in several B6-resistant mutants.

Advantages and limitations of pharmacological chaperones. Chaperones may complement diet and gene therapy, but most remain unvalidated in clinical trials. Mutation-specific responsiveness suggests that genotype-guided therapy may be required (73-78).

Proteasome inhibitors (PIs). Proteasome inhibition can stabilize partially active but misfolded CBS proteins. Bortezomib, an oncology drug, increased hepatic CBS activity >20-fold and reduced plasma tHcy levels by 97% in a p.R266K mouse model (79-81). Carfilzomib has shown similar

Table II. Comparison of treatment modalities in homocystinuria.

Treatment modality	Mechanism of action	Genotype association	Effectiveness	Limitations/challenges
Pyridoxine (vitamin B6)	Cofactor for CBS enzyme; enhances residual CBS activity.	Effective primarily in CBS mutations with residual enzymatic activity such as *p.Ile278Thr*.	Highly effective in pyridoxine-responsive genotypes; reduces homocysteine and improves prognosis.	Not effective in pyridoxine-non-responsive genotypes; responsiveness must be tested.
Betaine	Remethylates homocysteine to methionine via betaine-homocysteine methyltransferase, bypassing the CBS pathway.	Effective in both responsive and non-responsive CBS genotypes.	Reduces plasma homocysteine levels in non-responsive patients.	Can increase methionine to toxic levels; requires careful monitoring.
Methionine-restricted diet	Limits precursor amino acid (methionine) to reduce homocysteine production	Universally applied, regardless of genotype.	Reduces total homocysteine load; supportive in both responsive and non-responsive cases.	Difficult compliance, especially in older children and adults; risk of nutritional deficiency.
Folic acid and B12	Cofactors in remethylation of homocysteine to methionine.	Useful in remethylation defects and cases with mild elevation in homocysteine.	Beneficial in methylenetetrahydrofolate reductase and cobalamin defects; used adjunctively in patients with CBS mutations.	Not curative for CBS deficiency; requires a combined approach
Experimental enzyme replacement therapy	Supplements defective or absent CBS enzyme.	Under investigation for CBS-deficient genotypes.	Potential for correcting enzymatic deficiency directly.	Not yet clinically available; challenges in enzyme delivery and immunogenicity.
Experimental gene therapy	Introduces functional CBS gene into host cells.	Targeted at CBS-null or severe mutation genotypes.	Preclinical studies show promise; long-term correction potential.	Experimental; delivery vectors and sustained expression are hurdles.
Liver transplantation	Provides normal CBS activity from the liver of the donor.	Effective in CBS-deficient patients with severe mutations.	Can normalize homocysteine metabolism; curative in some cases.	Invasive; limited to severe, refractory cases; lifelong immunosuppression required.

CBS, cystathionine β-synthase.

biochemical benefits in preclinical HCU models, although its long-term safety remains yet to be fully elucidated (82).

Advantages and limitations of PIs. Potential risks of PIs include systemic proteasome suppression. Liver-targeted delivery strategies and dose minimization are being explored to mitigate toxicity (80-82).

Probiotic treatment (SYNB1353). SYNB1353 is an engineered *E. coli* Nissle strain expressing methionine γ-lyase to degrade dietary methionine. In preclinical models, it reduces plasma homocysteine and methionine levels without adverse events (83). A phase 1 trial in healthy volunteers showed promising tolerability and dose-dependent methionine reduction (84). A phase 2 study in classical HCU (ClinicalTrials.gov identifier, NCT05651054) is ongoing, with efficacy data pending.

Orthotopic liver transplantation (OLT). OLT has emerged as the only definitive treatment for severe pyridoxine-non-responsive HCU. Multiple case reports have demonstrated complete metabolic normalization following OLT. A case report by Lin *et al* (85) described a 24-year-old patient with HCU with notable complications, including cerebellar infarctions and hypertension, who underwent a liver transplant, after which their metabolic control normalized without dietary restrictions. Another case report described a patient with HCU who demonstrated post-transplant normalized homocysteine and methionine levels at the age of 4 years and remained stable for 6 years, indicating the resolution of HCU. The patient had no complications before the transplantation, highlighting the potential of early liver transplantation as a curative strategy for HCU (86).

A retrospective genetic analysis uncovered 18 CBS variants in 13 patients diagnosed with classic HCU between 10 days of age and 14 years of age, all of whom exhibited elevated methionine and tHcy levels. Three B6 non-responders underwent liver transplantation at the ages of 3, 8 and 8 years, and achieved normalized methionine and homocysteine levels within a week post-transplant (87).

Although liver transplantation offers a curative approach for B6 non-respondents, it carries notable challenges, including surgical risks, the need for prolonged immunosuppression and limited donor availability. Additionally, OLT does not reverse existing complications, and early diagnosis is important to optimize outcomes (85-87).

8. Personalized medicine and biomarkers in HCU management

The integration of genetic and metabolic profiling allows physicians to tailor treatment according to the molecular profile of a patient (25,40). Patients with p.I278T mutations may exhibit variable responses to B6, whereas those with p.R125Q or p.R266K mutation variants require different therapeutic approaches (25,40,81). Early identification of these mutations through genetic workups not only supports accurate diagnosis but also allows for prompt and tailored treatments, as summarized in Table II, which compares treatment modalities, their mechanisms, genotype associations, effectiveness and limitations (17,38).

Metabolic biomarkers are important tools for therapeutic monitoring and follow-up of disease progression (17,30). The plasma tHcy level is the primary biochemical marker for assessing metabolic control in HCU (17,30). However, novel biomarkers such as cystathionine, methionine and SAM are being investigated for their potential to provide a more accurate view of disease status (30,55). Stratifying patients according to their residual CBS activity or proteostatic disparities may help guide the use of emerging therapies (73,74,81,82).

9. Limitations of the present review

The present narrative review synthesizes peer-reviewed literature, clinical trial data, and select pipeline updates on metabolic and molecular therapies for HCU. As a narrative rather than systematic review, study selection and emphasis in the present review may reflect some degree of author judgment. Furthermore, regional differences in prevalence, genotype distribution and patterns of consanguinity, together with heterogeneity in CBS mutations and pyridoxine responsiveness, may limit the generalizability of certain therapeutic approaches or outcome expectations across diverse patient populations. Several discussed interventions are supported mainly by early-phase or preclinical data, limiting conclusions on long-term safety and efficacy. Additionally, some pipeline updates are based on press releases or conference reports, which may change as further evidence emerges. Despite these constraints, the present review provides a timely, integrated synthesis of current and emerging therapeutic strategies, contextualized with the latest clinical and translational developments through early 2025.

10. Conclusion

HCU is an underrecognized metabolic disorder associated with severe multi-organ complications. Timely diagnosis and individualized therapy are important to prevent long-term morbidity and improve patient outcomes. Advances in molecular genetics have deepened the current understanding of disease pathophysiology and provided the basis for innovative therapeutic strategies.

By integrating updates from therapeutic pipelines with clinical considerations and emphasizing how regional and genetic variability shape the applicability of emerging treatments, the present review offers a novel and clinically-oriented synthesis. It underscores both the implications for current practice and the priorities for future research aimed at transforming care for individuals with HCU.

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Competing interests

The author declares that they have no competing interests.

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