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REVIEW

Hyperhomocysteinemia in acute hepatic porphyria (AHP) and implications for treatment with givosiran

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ABSTRACT

Introduction: Homocysteine is a sulfur-containing amino acid formed in the intermediary metabolism of methionine. Amino acid metabolism and heme biosynthesis pathways are complexly intertwined. Plasma homocysteine elevation, *hyperhomocysteinemia* (HHcy), has been reported in patients with acute hepatic porphyria (AHP), a family of rare genetic disorders caused by defects in hepatic heme biosynthesis.

Areas covered: This article summarizes published case series in which givosiran, a subcutaneously administered small interfering RNA approved for AHP treatment, appeared to exacerbate dysregulated homocysteine metabolism in patients with AHP. A comprehensive exploratory analysis of ENVISION trial data demonstrated that on a population level, givosiran increased homocysteine but with wide interpatient variations, and there is no proof of correlations between HHcy and changes in efficacy or safety of givosiran.

Expert opinion: The strong correlation and co-increase of homocysteine and methionine suggest that HHcy associated with givosiran is likely attributable to the impaired trans-sulfuration pathway catalyzed by cystathionine β-synthase, which uses vitamin B6 as a cofactor. Data-based consensus supports monitoring total plasma homocysteine and vitamin B6, B12, and folate levels before and during givosiran treatment; supplementing with pyridoxine/vitamin B6 in patients with homocysteine levels >100 µmol/L; and involving patients with homocysteine levels >30 µmol/L in decisions to supplement.

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1. Introduction

Acute hepatic porphyria (AHP) is a family of rare genetic disorders caused by defects in hepatic heme biosynthesis enzymes, with acute intermittent porphyria (AIP) being the most common form [1,2]. The enzyme deficiencies, combined with triggering factors, lead to upregulation of hepatic 5′-aminolevulinic acid (5-ALA) synthase 1 (5-ALAS1), the first and rate-limiting step in heme biosynthesis, and to the resulting accumulation of heme intermediates 5-ALA and porphobilinogen (PBG). Overproduction of neurotoxic 5-ALA and PBG can precipitate acute neurovisceral attacks in patients with AHP [1–3]. Standard of care for acute attacks includes intravenous hemin, which is also used off-label for prophylaxis; long-term and regular use of prophylactic hemin can be associated with chronic complications such as iron overload, tachyphylaxis, and venous obliteration [4].

Givosiran, a subcutaneously administered 5-ALAS1-directed small interfering RNA selectively delivered to the liver [5,6], was recently approved for the treatment of AHP in adults in the United States and in adults and adolescents aged ≥12 years in the European Union [7,8]. In clinical trials, givosiran treatment resulted in sustained lowering of urinary 5-ALA and PBG, and in reduced frequency of acute attacks compared with placebo in AHP patients experiencing recurrent attacks. Givosiran had an acceptable safety profile, with hepatic and renal events and injection-site reactions as the main safety events in the phase 3 ENVISION trial [9–11].

Elevated plasma homocysteine has been previously reported in patients with AHP [12–14]. Homocysteine is a metabolic intermediate of sulfur amino acid metabolism. The clinical significance of homocysteine elevation in plasma is not well understood but potentially associated with increased risk for thromboembolism and vascular disease; cognitive and neurological diseases have also been reported in severely affected patients with genetic defects in the homocysteine metabolic pathway [15–19]. The first observations that treatment with givosiran may induce exacerbation of dysregulated homocysteine metabolism in patients with AHP were reported in 2020 and published in 2021 [20] by the Munich EPNET group and confirmed by several international investigators [14,21–24].

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Article highlights

- Homocysteine Plasma homocysteine elevation, hyperhomocysteinemia (HHcy), has been reported in patients with acute hepatic porphyria (AHP), a family of rare genetic disorders caused by defects in hepatic heme biosynthesis.
- Givosiran, a subcutaneously administered small interfering RNA approved for AHP treatment, appeared to exacerbate dysregulated homocysteine metabolism in patients with AHP in published case series.
- A comprehensive exploratory analysis of data from the givosiran ENVISION trial demonstrated that on a population level, givosiran increased homocysteine but with wide interpatient variations, and there is no proof of correlations between HHcy and changes in efficacy or safety of givosiran.
- The strong correlation and co-increase of homocysteine and methionine suggest that HHcy associated with givosiran is likely attributable to the impaired trans-sulfuration pathway catalyzed by cystathionine β-synthase, which uses vitamin B6 as a cofactor.
- Data-based consensus supports monitoring total plasma homocysteine and vitamin B6, B12, and folate levels before and during givosiran treatment; supplementing with pyridoxine/vitamin B6 in patients with homocysteine levels >100 µmol/L; and involving patients with homocysteine levels >30 µmol/L in decisions regarding use of supplements.
- The management of HHcy in patients with AHP treated with givosiran will require involvement of patients in the decision to treat and careful consideration of potential benefits and risks.

This article reviews the mechanisms and potential clinical implications of homocysteine elevation in patients with AHP and in those treated with givosiran. In addition, it provides an overview of current approaches to managing homocysteine elevation in patients treated with givosiran – an overview informed by expert clinical opinion based on the available findings on this emerging issue.

2. Background on hyperhomocysteinemia

2.1. Homocysteine Metabolism

Homocysteine is a sulfur-containing amino acid not used in protein synthesis; it is a product of methyl-transfer reactions in methionine metabolism (Figure 1) [18,25–27]. Homocysteine can be metabolized via two major pathways: re-methylation and trans-sulfuration (Figure 1).

In the re-methylation pathway, homocysteine is re-methylated into methionine through vitamin B12- and methyl-folate-dependent methionine synthase or through betaine-homocysteine methyltransferase that is highly expressed in the liver. Deficiencies in these enzymes or cofactors (e.g. folate or vitamin B12) are associated with increased plasma

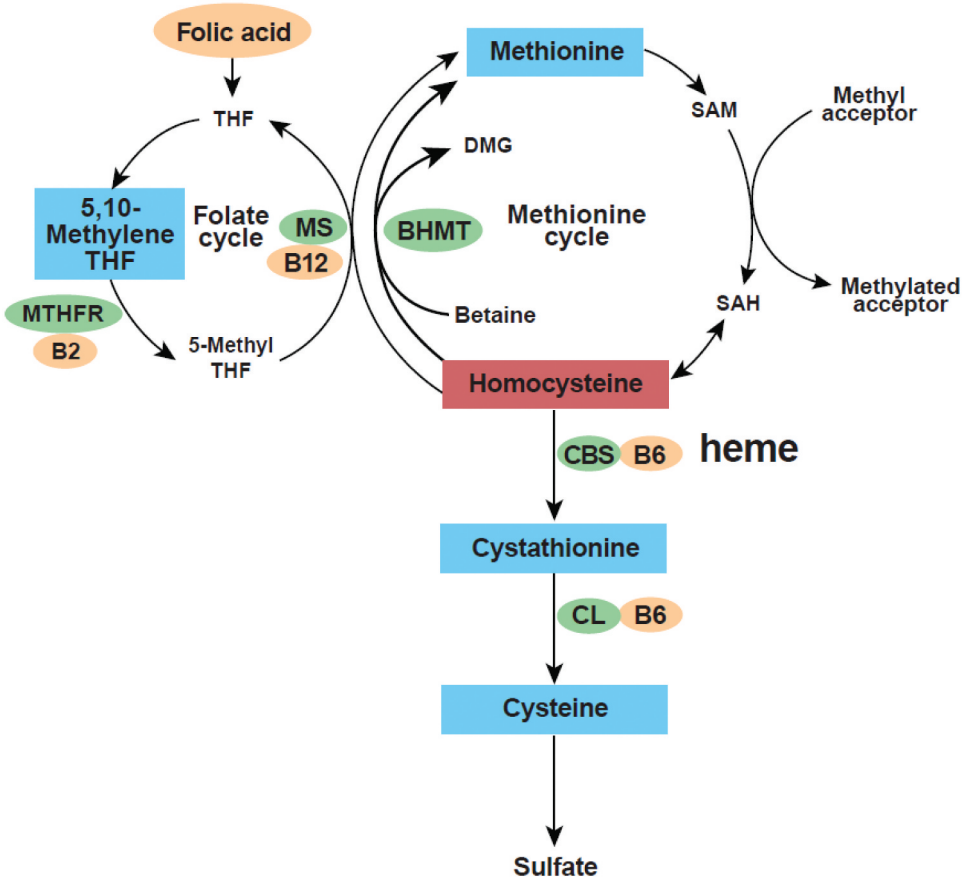

Figure 1. Pathways of homocysteine metabolism [25].
Adapted from *Acta Informatica Medica*, Volume 20, Issue 3, Algherbawe Mushtak, Fahmi Yousef Khan, Baidaa Aldehwe, and Ahmed Abdulrahman Al-Ani, 'Three Different Presentation of Same Pathophysiology,' pp. 190–191, Copyright 2012, with permission from AVICENA. doi:10.5455/aim.2012.20.190–191. License notice: https://creativecommons.org/licenses/by-nc/3.0/.B2, riboflavin; BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β-synthase; CL (or CGL), cystathionine γ-lyase; DMG, dimethylglycine; 5-methyl THF, 5-methyltetrahydrofolate; 5,10-methylene THF, 5,10-methylenetetrahydrofolate; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate.

homocysteine and low methionine levels [18,25–27]. In the folate cycle of the re-methylation pathway, homocysteine receives a methyl group from 5-methyltetrahydrofolate (circulating folate), which is synthesized by methylenetetrahydrofolate reductase (MTHFR). As a result, *MTHFR* gene polymorphisms or defects may also affect homocysteine levels [28].

In the trans-sulfuration pathway, homocysteine is irreversibly metabolized to produce endogenous cysteine, a non-essential amino acid; under conditions of excess methionine, this pathway can be over-induced. The first reaction is via cystathionine β-synthase (CBS), using vitamin B6 (metabolized to its active form, pyridoxal-5-phosphate) as a cofactor and also binding heme in its non-catalytic domain. Defects in CBS or its cofactors (e.g. vitamin B6) in the trans-sulfuration pathway are associated with increased plasma homocysteine and high or high–normal methionine levels [18,19,25,26,29].

2.2. Hyperhomocysteinemia

Homocysteine elevation or hyperhomocysteinemia (HHcy) refers to an elevated level of total homocysteine in plasma, defined as the pool of free reduced form, protein-bound forms, and oxidized forms such as the disulfide homocysteine (homocysteine dimer) [30]. The normal levels of total plasma homocysteine in humans range from 5 to 10 μmol/L, not exceeding 15 μmol/L under normal conditions [30]. HHcy is typically classified as mild when plasma total homocysteine is at 16–30 μmol/L, as intermediate or moderate at 31–100 μmol/L, and as severe at >100 μmol/L [30,31]. However, the classification threshold can vary, with mild HHcy defined at 15–25 μmol/L, intermediate at 25–50 μmol/L, and severe at >50 μmol/L [12,13]. Mildly elevated homocysteine (16–30 μmol/L) is common, present in up to 5–7% of the general population. The severe form (>100 μmol/L) is rare [32].

HHcy can be caused by genetic and/or acquired conditions (**Table S1**) [18,26,31,33]. Enzyme defects in homocysteine metabolic pathways are considered to be the most prevalent cause of HHcy [31]. *MTHFR* gene variants affecting homocysteine re-methylation, such as c.C677T or c.A1298C, are found in up to 40% of the US population. These variants may decrease MTHFR enzyme activity to 30–60% of normal, which, combined with other genetic and/or environmental factors, may lead to mild-to-moderate HHcy in some affected individuals [32]. Severe MTHFR deficiency and severe HHcy due to inherited homozygous or compound heterozygous loss-of-function variants in *MTHFR* are very rare [27,34–37]. The most frequent genetic cause of severe HHcy is CBS deficiency in classic homocystinuria due to pathogenic variants in the gene encoding the CBS enzyme affecting the trans-sulfuration pathway [19,27,31] (worldwide prevalence, 1:100,000 to 1:344,000; detailed below). In addition to genetic defects, acquired conditions can cause HHcy, mostly mild to moderate forms [33]. These conditions include nutritional deficiencies of folate, vitamin B12, or vitamin B6 within the context of genetic polymorphisms in *MTHFR* [31,33]; common diseases, such as chronic kidney disease, anemia, hypothyroidism, malignant tumors, etc. [18,31,33]; certain medications, such as lipid-lowering drugs, diabetes drugs (e.g. metformin), anti-rheumatic drugs (e.g. methotrexate), etc. [26,31]; and lifestyle or other conditions (**Table S1**) [26,31]. The mechanisms through which certain medications affect plasma homocysteine levels vary; for example, metformin interferes with vitamin absorption from the gut, while methotrexate interferes with folate and homocysteine metabolism directly [26,31].

2.3. Clinical Relevance of Hyperhomocysteinemia

Severe HHcy as seen in individuals with rare inborn errors of metabolism, such as classic homocystinuria, is usually associated with neurologic, cognitive, skeletal, ocular, or vascular complications and is included in current newborn screening panels for early diagnosis and treatment [18,19,27,38]. Patients with classic homocystinuria have elevated methionine and presence of free homocysteine in the plasma amino acids, and total plasma homocysteine is extremely elevated (usually >100 μmol/L) [18]. In animal models of CBS-deficient homocystinuria, hepatic total homocysteine and hepatic methionine are also significantly increased [39]. Severely affected patients with classic homocystinuria, if not detected during screening, usually present between 3 and 5 years old with intellectual disability and dislocated optic lenses, and may also develop skeletal abnormalities (marfanoid habitus and radiologic evidence of osteoporosis). Life-threatening vascular (coronary, renal, and cerebral arteries) complications may occur during the first decade of life. However, patients vary widely in symptom severity, age at onset, and rate of progression. For mildly affected patients, it could be decades before any apparent clinical manifestation of vascular problems, and patients may also remain asymptomatic throughout life with adequate vitamin B6 supplementation [18,19].

In the general population, the clinical relevance of mild-to-moderate HHcy is less conclusive. Elevated homocysteine may be associated with incidence and progression of vascular calcification, but its role in atherosclerosis is unclear, and results of meta-analyses of observational studies suggest that elevated homocysteine is only a modest predictor of vascular disease, including ischemic heart disease, deep vein thrombosis (with or without pulmonary embolism), and stroke in healthy populations [15–17]. In addition, data on the association between elevated homocysteine and vascular morbidity and mortality have been inconsistent across different observational studies [40–42]. It has even been argued that HHcy could be an effect or marker rather than a cause of cardiovascular disease because elevated homocysteine is also associated with the most standard vascular risk factors [43,44].

The clinical relevance of homocysteine elevation in the general population is further challenged in randomized controlled trials of homocysteine-lowering interventions, which did not prevent or reduce the occurrence of vascular events [45,46]. In response, current guidelines on cardiovascular disease prevention (American College of Cardiology/American Heart Association; European Society of Cardiology and European Association for Cardiovascular Prevention & Rehabilitation) do not include homocysteine as a causal risk factor for cardiovascular disease [47,48]. Most if not all of these

trials, however, were conducted in individuals with mild HHcy (15–30 μmol/L) [15–17,42,45,46]. In a recent consecutive case study, patients who had been hospitalized for thromboembolic and other cardiovascular manifestations and undergone a homocysteine assay were retrospectively evaluated; 81% had moderate HHcy (30–100 μmol/L) and 3% had severe HHcy (>100 μmol/L), raising the issue of whether the clinical relevance of homocysteine elevation should be reevaluated in patients with at least moderate HHcy [49,50].

3. Hyperhomocysteinemia in patients with AHP and in those receiving givosiran

3.1. Hyperhomocysteinemia in Patients with AHP

Elevated plasma homocysteine has been reported in patients with AHP [12–14,24] (Table 1). The original observation of elevated homocysteine was reported by To-Figueras et al. [12] in 24 patients with AIP, all of whom had high excretion of PBG (10–100 nmol/mmol creatinine [normal limit, <0.8 nmol/mmol]). Homocysteine was found to be elevated in 62% of patients, most with mild or intermediate HHcy (Figure 2, Table 1). Treatment of one patient with hemin decreased homocysteine transiently.

In a study of 46 patients with AHP, Ventura et al. [13] found plasma homocysteine elevations in 61% of all patients and in 86% of patients classified as chronic high excreters of 5-ALA/PBG (5-ALA, 8–14 μmol/mmol creatinine [normal limit, <5 μmol/mmol]; PBG, 19–34 μmol/mmol creatinine [normal limit, <1.5 μmol/mmol]) or symptomatic (history of hospitalization for treatment of attacks and/or receiving prophylactic hemin). Homocysteine elevation was less prevalent in another study, by Fontanellas et al. [24], where 34 (37%) of 91 patients with symptomatic AIP (≥1 acute attack during clinical course) had moderately increased plasma homocysteine levels (>15 μmol/L) (Table 1). While To-Figueras et al. [12] did not find an association between HHcy and either urinary excretion of 5-ALA/PBG or clinical recurrence of attacks, in the study by Ventura et al., disease status (symptomatic or high 5-ALA/PBG) was established as the only independent predictor of elevated homocysteine [13]. Among symptomatic patients, those undergoing prophylactic therapy with hemin had significantly higher homocysteine plasma levels than those not undergoing prophylactic therapy [13].

Although vitamin B12 and folate levels were mostly normal, vitamin B6 deficiency has been reported in almost half of the patients with AHP [12,13,51–53], which is substantially higher than the incidence in the general population (9–24%) and similar to that in elderly hospitalized patients (51%) [54,55]. Vitamin B6 deficiency could be due to poor nutrition in patients with AHP [56], but it may also be caused by chronic hyperactivity of 5-ALAS1 (first step in heme biosynthetic pathway, a rate-limiting enzyme for 5-ALA formation) in recurrent AHP [57]. In a preclinical study, deficiency in vitamin B6, a cofactor for 5-ALAS1, did not seem to significantly impair its activity [58], suggesting that 5-ALAS1 may have a high affinity for vitamin B6 and that hyperactive 5-ALAS1 may sequestrate most cytoplasmic B6 and deprive other B6-dependent metabolic pathways [13]. Because CBS is dependent on heme and also vitamin B6 as cofactors [29], investigators [12–14,23] postulated reduced hepatic CBS activity as the mechanism for homocysteine accumulation in patients with AHP, either from low heme availability or consumptive intrahepatic vitamin B6 depletion caused by increased 5-ALAS1 activity.

3.2. Hyperhomocysteinemia in AHP Patients Receiving Givosiran: Patient Case Series

Several patient case series have reported elevated homocysteine in plasma in AHP patients receiving givosiran treatment. The first cases were reported by Petrides et al. [20] and involved 2 patients with AIP treated with givosiran in the phase 3 ENVISION study. These patients responded well to givosiran during ~2 years of therapy but experienced severe adverse events, generalized skin reactions in one and pancreatitis in the other. Both patients discontinued givosiran therapy [20]. After HHcy was reported in patients with AHP [12,13], the investigators measured plasma homocysteine in these 2 patients and found it elevated in both (100–400 μmol/L) [20]. However, no pre-givosiran measurements were available, and the extent to which homocysteine elevation may have caused or contributed to the adverse reactions in these two patients could not be determined [20]. Plasma methionine was determined in one patient and shown to be slightly elevated [20]. Except when extremely high, such as >~1000 μmol/L (normal, <42 μmol/L), elevated methionine has not been reported to cause serious toxicity in healthy populations [59,60]. Based on its mechanism of action, givosiran inhibition of 5-ALAS1 can cause a drop in free heme in the liver that may lead to acquired inhibition of heme-dependent CBS. The investigators therefore treated the two patients with hemin. Homocysteine levels rapidly declined during hemin treatment and then increased again after hemin discontinuation [20].

Subsequent to the case report, To-Figueras et al. conducted a long-term follow-up analysis of HHcy in the aforementioned small group of patients with AIP [12] and also expanded the study to include 9 recurrent AIP patients receiving givosiran treatment either through ENVISION or an early access program [14]. Long-term (up to 9 years) follow-up analysis in 28 patients (18 nonrecurrent AIP and 10 recurrent AIP) with serial measurement of plasma total homocysteine available showed a large between-day intrapatient variation in homocysteine (range, normal to >100 μmol/L) [14]. Of the nine patients with recurrent AIP treated with givosiran, six experienced increased total plasma homocysteine levels (>100 μmol/L in five patients) after 6–16 months of givosiran treatment, compared with pre-givosiran homocysteine levels. There were co-increases in plasma homocysteine and methionine in all six patients [14].

Givosiran-induced exacerbation of the homocysteine metabolic phenotype in AIP patients receiving prophylactic hemin was later confirmed by four other case series [21–24] (Table 1). In patients with homocysteine measurements available before and after givosiran treatment, homocysteine levels were further increased, compared with pre-givosiran levels, in 4/4 AHP patients (>100 μmol/L in 2 patients) after 2 months of givosiran treatment in the study by Vassiliou

Table 1. Summary of published studies with homocysteine measurements in patients with AHP and in those receiving givosiran treatment.

Study	Patients	Homocysteine	Methionine	MTHFR	5-ALA/PBG and/or attack	Factors associated with HHcy	Vitamin levels	Serious adverse event	Treatment for HHcy and outcome
Patients with AHP									
To-Figueras et al., 2010 [12]	AIP $N = 24$	Chemiluminescence immunoassay and confirmed by HPLC Elevated in 63%: 60% with mild HHcy (15–25 µmol/L), 20% with intermediate HHcy (25–50 µmol/L), 20% with severe HHcy (>50 µmol/L)	NR	NR	All were high excreters; some had intermittent exacerbations; 4 had recurrent attacks requiring regular hemin	No association between HHcy and either urinary excretion of 5-ALA/PBG or clinical recurrence of attacks	All had normal vitamin B12 and folate; 54% had low vitamin B6	NR	Treatment of one patient with hemin decreased homocysteine transiently
Ventura et al., 2020 [13]	AHP $N = 46$ (31 AIP, 15 variegate porphyria)	HPLC Elevated in 61% of all patients and 86% of patients classified as chronic high excreters of 5-ALA/PBG or symptomatic[a]	NR	20 patients had c.C677T polymorphism: 5 homozygous, 15 heterozygous	10 were chronic high excreters (no history of attacks); 18 were symptomatic[a]	Disease status (symptomatic or high 5-ALA/PBG) was an independent predictor of HHcy; symptomatic patients with prophylactic hemin had higher homocysteine	Vitamin B6, B12, and folate were deficient in 46%, 28%, and 48% of patients, respectively; symptomatic patients or high 5-ALA/PBG excreters had higher prevalence of vitamin B6 deficiency (61%)	NR	NR
To-Figueras et al., 2021 [14]	AIP $N = 37$[b] (26 nonrecurrent, 11 recurrent[c])	Electrochemiluminescent immunoassay Elevated in 62% of patients with nonrecurrent AIP and 82% of patients with recurrent AIP[c]	NR	5 patients had c.C677T polymorphism: 1 homozygous, 4 heterozygous	11 had recurrent AIP requiring prophylactic hemin	HHcy was more frequent and concentrations higher in patients with recurrent attacks on prophylactic hemin (vs. nonrecurrent)	All had normal vitamin B12; HHcy was associated with low levels of folates and vitamin B6, which were more frequent in patients with recurrent AIP (low folate, 22%; low vitamin B6, 33%)	NR	NR
Fontanellas et al., 2021 [24]	AIP $N = 91$	Assay NR Elevated in 37% of all patients[d]	NR	NR[d]	All were symptomatic (≥1 acute attack during clinical course)	NR	NR[d]	NR	NR[d]
Patients with AHP who received givosiran treatment									
Petrides et al., 2021 [20]	AIP $N = 2$ Recurrent attacks; prophylactic hemin discontinued	LC-MS/MS Elevated in both patients, who received ~2 years of givosiran treatment (100–400 µmol/L); no pre-givosiran measurements	Determined in one patient and found elevated	Both patients had c.C677T polymorphism: one homozygous, one heterozygous	Clinical improvement during givosiran treatment	NR	Vitamin B12, B6, and folic acid levels were normal	Generalized skin reactions in one patient and pancreatitis in other patient; givosiran discontinued	Hemin rapidly reduced homocysteine levels, which increased again after hemin discontinuation

(*Continued*)

Table 1. (Continued).

Study	Patients	Homocysteine	Methionine	MTHFR	5-ALA/PBG and/or attack	Factors associated with HHcy	Vitamin levels	Serious adverse event	Treatment for HHcy and outcome
To-Figueras et al., 2021 [14]	AIP *N* = 9 Recurrent attacks; prophylactic hemin discontinued	Electrochemiluminescent immunoassay Homocysteine increased vs. pre-givosiran levels in 6/9 patients (>100 µmol/L in 5) after 6–16 months of givosiran treatment	Plasma methionine increased in 7/9 patients (co-increased in all 6)	Five patients had c.C677T polymorphism: one homozygous, four heterozygous	Normalized urinary 5-ALA/PBG and reduced attacks during givosiran treatment	No correlation between HHcy and MTHFR c. C667T polymorphisms	All had normal vitamin B12; some patients had low levels of vitamin B6 and folate	NR	NR
Vassiliou & Sardh, 2021 [21]	AHP *N* = 15 Recurrent attacks	LC-MS/MS Homocysteine increased vs. pre-givosiran levels in four patients (>100 µmol/L in 2) after 2 months of givosiran treatment; among the 11 patients without pre-givosiran homocysteine measurements, two had severe HHcy (>100 µmol/L) and nine had mild-to-moderate HHcy with 8–43 months of givosiran treatment	Methionine elevated in 6/15 patients	NR	NR	NR	13/15 patients had normal levels of folate and cobalamin or methylmalonic acid (vitamin B6 was not measured)	NR	Oral pyridoxine for 8 weeks normalized or significantly decreased homocysteine levels in nine patients with homocysteine >50 µmol/L; methionine levels decreased in most patients
Ricci et al., 2021 [22]	AHP *N* = 9	HPLC Homocysteine increased vs. pre-givosiran levels in 9/9 patients (>100 µmol/L in 5) after 28 months of givosiran treatment	NR	Eight patients had c.C677T polymorphism: four homozygous, four heterozygous	NR	NR	Folic acid and vitamin B12 deficiencies in most patients (vitamin B6 was not measured)	NR	Supplementation with folate, vitamin B6, B12, B2, betaine, and zinc for 30–45 days restored homocysteine levels to normal or near normal in seven patients with homocysteine >30 µmol/L
Fontanellas et al., 2021 [24]	AIP *N* = 4 Unstable porphyria; prophylactic hemin discontinued	Assay NR Homocysteine increased vs. pre-givosiran levels in 3/4 patients (>100 µmol/L in 2) during 4–12 months of givosiran treatment	NR	One patient with very high homocysteine had homozygous c.C665T variant	Significantly decreased urinary 5-ALA/PBG in 3/4 patients and remission of attacks in 4/4 patients during givosiran treatment	NR	Folic acid was low in one patient with very high homocysteine and normal in other patients; vitamin B6 was low in two patients; all had normal vitamin B12	NR	Folic acid replenishment decreased (but did not normalize) homocysteine levels in one patient with homocysteine >100 µmol/L

(*Continued*)

Table 1. (Continued).

Study	Patients	Homocysteine	Methionine	MTHFR	5-ALA/PBG and/or attack	Factors associated with HHcy	Vitamin levels	Serious adverse event	Treatment for HHcy and outcome
Poli et al., 2022 [23]	AIP *N* = 25 Recurrent attacks[e]	Assay NR Patients received givosiran for 4–36 months; homocysteine elevated in all 23 patients with homocysteine data; homocysteine increased vs. pre-givosiran levels in all 14 patients (>100 µmol/L in 7) with homocysteine data before and after givosiran treatment	NR	NR	Sustained decrease in urinary 5-ALA and 96% of patients attack-free during givosiran treatment	NR	Folate and vitamin B12 status were not evaluated	Acute pancreatitis in one patient (homocysteine NR), leading to givosiran discontinuation; pulmonary embolism in another patient (homocysteine, 187 µmol/L)	Four patients with homocysteine >100 µmol/L were treated with vitamin B6; homocysteine levels normalized in one patient and greatly decreased in three patients

AHP: acute hepatic porphyria; AIP: acute intermittent porphyria; 5-ALA: 5′-aminolevulinic acid; HHcy: hyperhomocysteinemia; HPLC: high-performance liquid chromatography; LC-MS/MS: liquid chromatography/tandem mass spectroscopy; NR: not reported; PBG: porphobilinogen.

Normal levels: homocysteine <15 µmol/L for all studies except Poli et al. [23], where homocysteine <10.7 µmol/L; methionine <40 µmol/L [14,21] or 6 mg/L [20]; normal ranges of vitamin B6, vitamin B12, and folate levels varied by studies.

[a]Patients were considered symptomatic if they had a history of hospitalization for treatment of attacks and/or were receiving prophylactic hemin.

[b]To-Figueras et al. conducted a long-term (up to 9 years) follow-up analysis of HHcy in the aforementioned small group of patients with AIP [12] and expanded the study to include more patients, including nine recurrent patients receiving givosiran treatment [14]. Some of the 37 patients with nonrecurrent (n = 26) or recurrent (n = 11) AIP were already described in the previous analysis [12].

[c]Among the 11 patients with recurrent AIP, 9 patients received givosiran through either ENVISION or an early access program. Data from these nine patients are summarized in table's next section, 'Patients with AHP who received givosiran treatment' [14].

[d]Among the 91 patients with symptomatic AIP, 4 patients received givosiran. Data from these four patients are summarized in table's next section, 'Patients with AHP who received givosiran treatment,' along with changes in 5-ALA/PBG, attacks, vitamin levels, *MTHFR* polymorphism, and treatments for HHcy [24].

[e]Recurrent attacks defined as four or more attacks per year and/or under prophylactic hemin therapy.

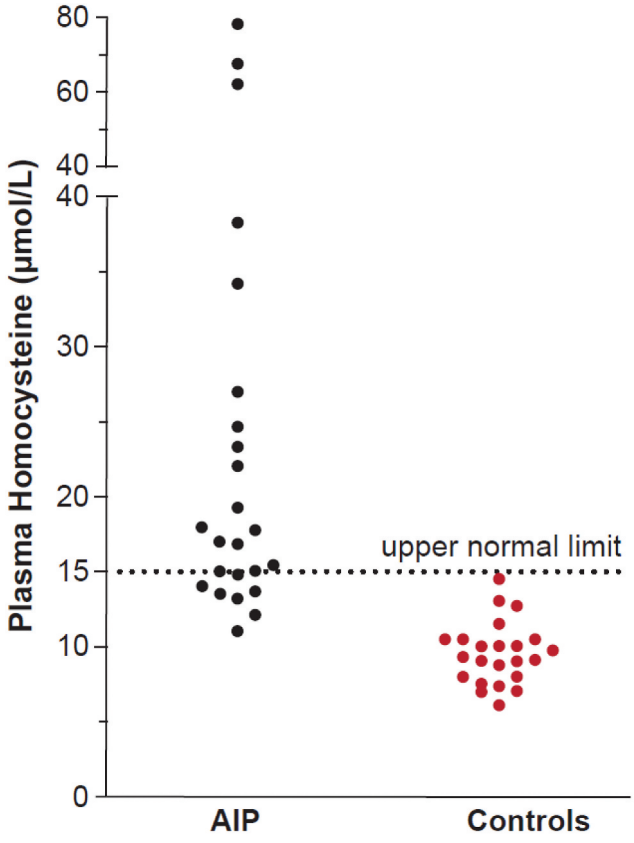

Figure 2. Plasma homocysteine levels in patients with AIP [12].

and Sardh [21], in 9/9 AHP patients (>100 µmol/L in 5 patients) after 28 months of givosiran treatment in the study by Ricci et al. [22], in 3/4 AIP patients (>100 µmol/L in 2 patients) during 4–12 months of givosiran treatment in the case report by Fontanellas et al. [24], and in 14/14 AIP patients (>100 µmol/L in 7 patients) during 4–36 months of givosiran treatment in the study by Poli et al. [23]. Vassiliou and Sardh [21] also measured methionine in 15 patients with AHP and recurrent acute attacks who received givosiran treatment for 2–43 months, and, again, methionine levels were found to be elevated in 6/15 patients (**Table S2**).

In these published case series [14,20,23,24], givosiran treatment normalized or significantly decreased 5-ALA and PBG urinary excretion and effectively reduced acute attacks, despite elevated plasma homocysteine levels. Analysis of genes important in homocysteine metabolism revealed only *MTHFR* polymorphisms (c.C677T homozygous or heterozygous except one case of c.C665T homozygous) [14,20,22,24], which are usually associated with mild-to-moderate HHcy [41], and there was no correlation between HHcy and *MTHFR* c.C667T polymorphisms [14]. Vitamins B12, B6, and folic acid were deficient in some of the evaluated patients [14,22,24] (Table 1).

Because of the important role of vitamins in homocysteine metabolism (Section 2.1), the effectiveness of vitamin supplementation in treating HHcy was explored in AHP patients receiving givosiran (Table 1). Vassiliou and Sardh [21] and Poli et al. [23] showed that oral pyridoxine 80 mg/day supplementation in 9 patients with homocysteine levels >50 µmol/L and vitamin B6 250 mg/day supplementation in 4 patients with homocysteine levels >100 µmol/L significantly decreased or normalized homocysteine levels during time of observation (up to 5 months); methionine levels were also decreased in the majority of patients [21]. Fontanellas et al. [24] found the use of folic acid decreased plasma homocysteine levels in 1 patient. Ricci et al. [22] reported on the use of other B-vitamin supplementation for the treatment of HHcy in 7 AHP patients with homocysteine >30 µmol/L. A daily pill containing folate (as 5-methyl-tetrahydrofolic acid) 400 µg, vitamin B6 (as pyridoxine) 3 mg, vitamin B12 (as cyanocobalamin) 5 µg, vitamin B2 (as riboflavin) 2.4 mg, betaine 250 mg, and zinc 12.5 mg restored homocysteine levels to normal or near normal in all patients after 30–45 days of supplementation.

The clinical implications of homocysteine elevation in givosiran-treated AHP patients are unclear. It has been previously shown that homocysteine elevation may impair kidney function in patients with hypertension [61]. A transient moderate increase in serum creatinine under givosiran treatment was reported in a small case study of AIP patients by Lazareth et al. [62]. Although there were no signs of kidney injury associated with givosiran, a long-term deleterious impact of ALAS1 inhibition on renal function in some patients could not be excluded [62]. It will be interesting to evaluate plasma homocysteine levels in givosiran-treated AIP patients with transiently decreased kidney function. Acute pancreatitis has been reported in givosiran-treated patients, one in the study by Poli et al. [23] (homocysteine levels not reported) and one in the case report by Petrides et al. [20] (homocysteine >100 µmol/L), both leading to discontinuation of givosiran treatment. A fatal event of hemorrhagic pancreatitis occurred in a givosiran phase 1 study (homocysteine not measured); the investigator considered the event unlikely related to givosiran because there was no clear temporal relationship between the event and givosiran injections, and gallbladder sludge was identified as a plausible cause [9]. Pulmonary embolism occurred in one patient with homocysteine levels of 187 µmol/L in the study by Poli et al [23]. It has been shown that thromboembolism and vascular disease (Section 2.3) and pancreatitis may occur in patients with inborn homocystinuria [63–65] and may be associated with HHcy [66–68]. However, whether homocysteine elevation was related to the adverse events reported in these givosiran-treated AHP patients requires further investigation.

3.3. Hyperhomocysteinemia in AHP Patients Receiving Givosiran: ENVISION Trial

Based on the two cases of HHcy reported in patients treated with givosiran [20], the sponsor of the ENVISION trial initiated a comprehensive analysis of patients' plasma homocysteine levels in late 2020. By then, all patients had completed at least 24 months on the ENVISION study. Because plasma homocysteine measurement was not included in the original study plan, only archived serum samples from patients who consented to exploratory biomarker assessment and had baseline samples (N = 92) were analyzed. On a population level, treatment with givosiran led to an increase in plasma homocysteine levels, measured by a two-reagent enzymatic assay, during the 6-month double-blind period and open-label

extension period up to Month 36 (Figure 3). Median (range) plasma homocysteine levels were 17 (6–158) µmol/L before givosiran treatment and increased to 85 (7–400) µmol/L, 83 (8–400) µmol/L, 98 (13–400) µmol/L, 47 (14–345) µmol/L, 44 (9–269) µmol/L, and 22 (10–207) µmol/L after 6, 12, 18, 24, 30, and 36 months of givosiran treatment, respectively. There was no accumulation of plasma homocysteine over time. The percentage of patients with normal plasma homocysteine levels (≤15 µmol/L), mild HHcy (>15 to ≤30 µmol/L), intermediate HHcy (>30 to ≤100 µmol/L), and severe HHcy (>100 µmol/L) was 36%, 52%, 9%, and 1%, respectively, before givosiran treatment, and 2%, 9%, 22%, and 62%, respectively, during givosiran treatment (worst post-baseline category). Consistent with the reported linear correlation between kidney dysfunction and increased plasma homocysteine levels [69], patients with a baseline estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 showed a trend toward higher mean plasma homocysteine levels, compared with patients with a baseline eGFR ≥60 mL/min/1.73 m^2. At the individual level, not all patients had elevated homocysteine, and those who did had wide interpatient variations (Figure 3). Confounding factors (e.g., kidney function impairment at baseline) may have contributed to these individual variations. Givosiran dose appeared not to have a substantial impact, as homocysteine levels were similarly increased in both dose groups (1.25 and 2.5 mg/kg).

Homocysteine elevation was not associated with changes in efficacy or safety of givosiran. There was no correlation between changes in homocysteine levels from baseline and changes in 5-ALA or PBG levels from baseline at Month 6 of givosiran treatment (for both correlations: Pearson correlation coefficient, – 0.068; P = 0.54; **Figure S1**). No correlation between changes in homocysteine and average number of attacks was observed during givosiran treatment in patients with and without significant homocysteine elevations (arbitrarily defined as either >2 × upper limit of normal or >2 × baseline; or >100 µmol/L for >12 months) (Figure 4). Similarly, no correlation or trend of adverse events was observed with homocysteine status during givosiran treatment.

Folate deficiency was detected in 44/79 patients with ≥1 nonfasting folate measurement during givosiran treatment (Table 2); 42/67 patients had a *MTHFR* c.C677T polymorphism (14 homozygous, 28 heterozygous; Table 2). Folate status did not correlate with homocysteine elevations in patients treated with givosiran (Table 2); the *MTHFR* genotype was not associated with homocysteine elevations at baseline or with givosiran treatment (Table 2). Serum vitamin B6 was not prospectively measured in the ENVISION study. Testing of vitamin B6 using exploratory serum samples could not be done because of the specific collection and processing procedures (eg, protection from light) required for assaying vitamin B6. A strong positive correlation, however, was found between change in plasma homocysteine and change in plasma methionine during givosiran treatment (Pearson correlation coefficient, 0.8; P < 0.0001; Figure 5), consistent with the co-increase of homocysteine and methionine observed in case series [14,20,21].

3.4. Hypotheses for Mechanism of Hyperhomocysteinemia in AHP Patients Receiving Givosiran

Current evidence supports the likelihood that an association between homocysteine increase and givosiran treatment is attributable to an impaired trans-sulfuration pathway catalyzed by CBS [14,20–22]. An altered re-methylation pathway seems unlikely based on the lack of correlation between folate status or *MTHFR* genotype and homocysteine elevations in patients treated with givosiran in the ENVISION study and the lack of reduction in methionine levels (methionine levels

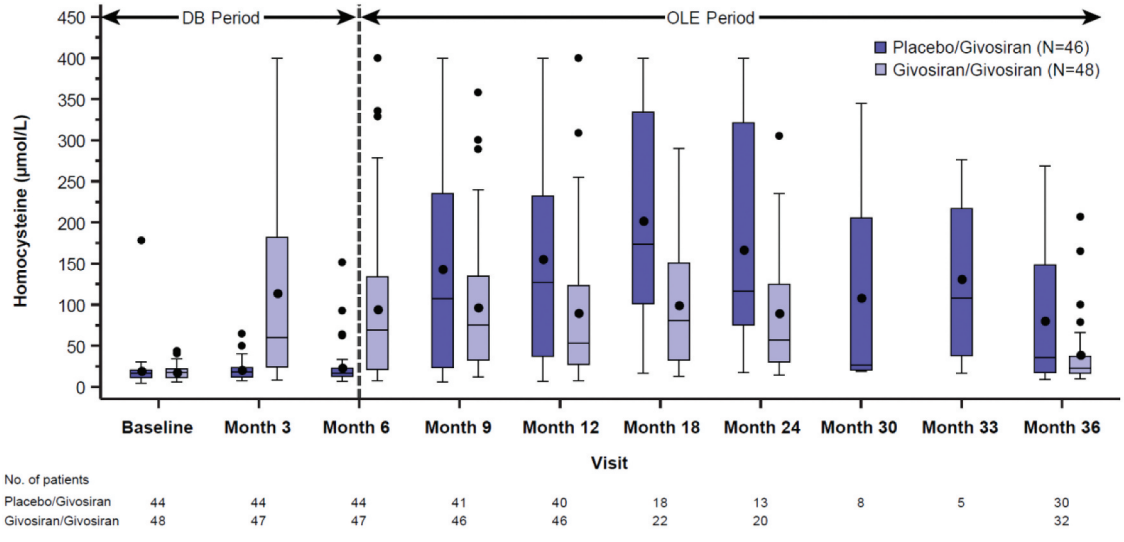

Figure 3. Plasma homocysteine levels in ENVISION patients with AHP treated with givosiran.
Exploratory analyses of total plasma homocysteine levels were conducted using archived serum samples from 92 AHP patients who consented to exploratory biomarker assessment and had baseline samples. Blood samples were processed to serum within 120 minutes of collection. Homocysteine levels were measured by Medpace Reference Labs (Cincinnati, Ohio, USA) using Diazyme's Dual Reagent Enzymatic Homocysteine Assay on a Beckman Coulter chemistry analyzer. The assay was validated for serum samples with an analytical range of 2.00–400.00 µmol/L. On a population level, treatment with givosiran led to an increase in plasma homocysteine levels during the 6-month double-blind period and open-label extension period up to Month 36. AHP, acute hepatic porphyria; DB, double-blind; OLE, open-label extension.

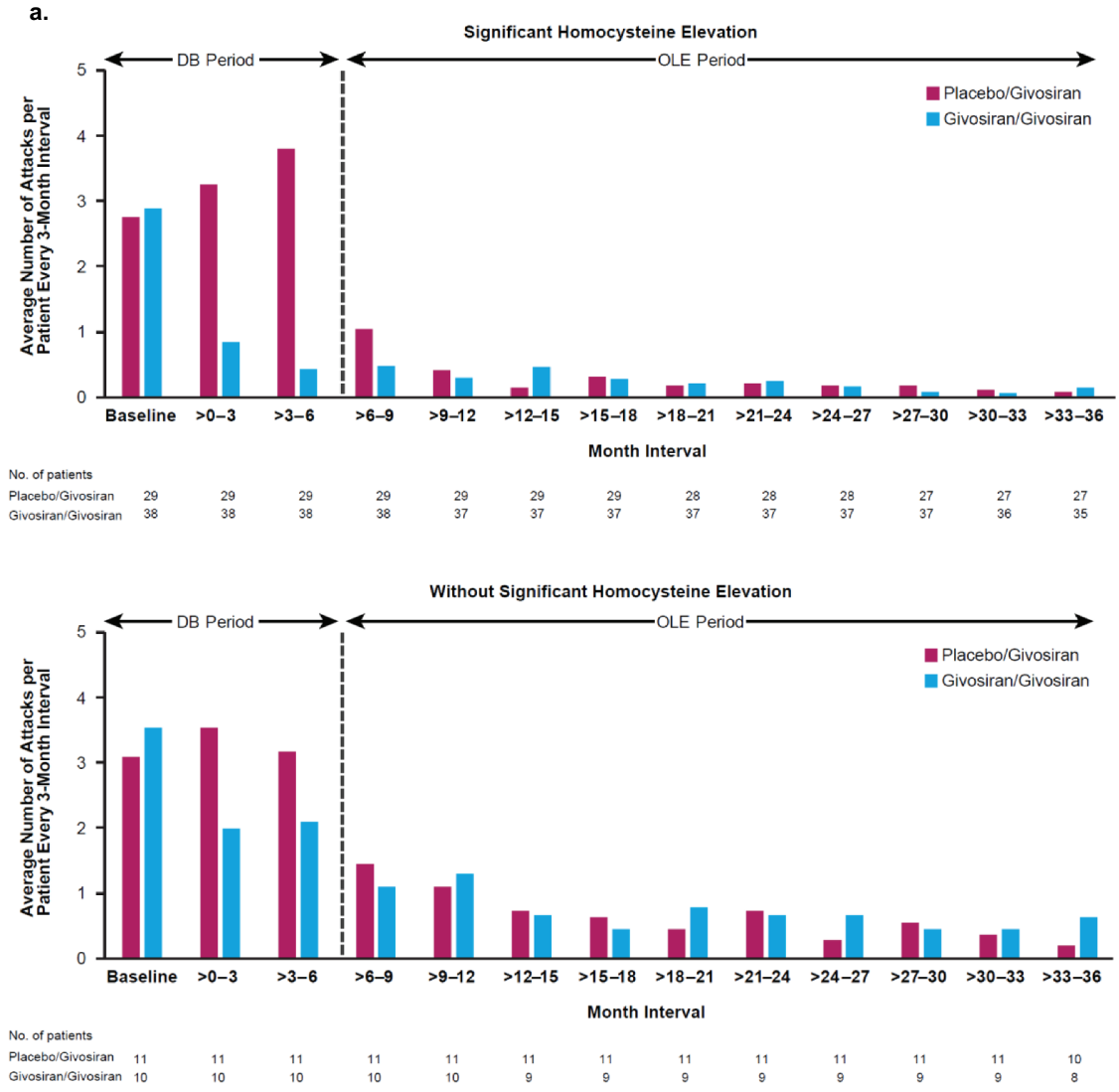

Figure 4. Average number of attacks per patient by every 3-month interval during givosiran treatment in ENVISION patients with and without significant homocysteine elevation.

(a) Significant homocysteine elevation defined as 2 × threshold. Average number of attacks per patient by every 3-month interval is shown separately for the subgroup of patients with significant homocysteine elevation (upper panel) and the subgroup of patients without significant homocysteine elevation (lower panel). Significant homocysteine elevation was defined as 2 × threshold: when baseline homocysteine was <ULN, a post-givosiran value of >2 × ULN was significant; when baseline homocysteine was ≥ULN, a post-givosiran value of >2 × baseline was significant. No correlation was found between changes in homocysteine and average number of attacks during givosiran treatment based on this definition of significant homocysteine elevations. (b) Significant homocysteine elevation defined as >100 µmol/L > 12 months. Average number of attacks per patient by every 3-month interval is shown separately for the subgroup of patients with significant homocysteine elevation (upper panel) and the subgroup of patients without significant homocysteine elevation (lower panel). Significant homocysteine elevation was defined as >100 µmol/L for more than 12 months. No correlation was found between changes in homocysteine and average number of attacks during givosiran treatment based on this definition of significant homocysteine elevations. DB, double-blind; OLE, open-label extension; ULN, upper limit of normal.

should become lower, not higher, in homocysteine re-methylation defects). Case reports have also shown that not all patients with homocysteine elevations had folate or vitamin B12 deficiency or *MTHFR* polymorphisms [14,20,21]. On the other hand, the strong correlation and co-increase of homocysteine and methionine observed in the ENVISION study and case reports support attributing givosiran-related homocysteine increases to an alteration in the trans-sulfuration pathway [14,20,21]. An effective reduction in homocysteine levels via supplementation with a CBS cofactor, vitamin B6 [21] or hemin [20], lends additional support to a potential causative connection between a reduction in CBS activity and HHcy in patients treated with givosiran.

Theoretically, the reduced CBS enzymatic activity could be caused by a reduction in the hepatic pool of heme resulting from givosiran targeting 5-ALAS1 messenger RNA. Administered as consecutive monthly injections, givosiran in principle could interfere with a compensative mechanism of heme production in patients with AHP, causing a relative heme deficiency. Temporary correction of homocysteine levels by hemin injection appears to support this argument [14,20–22]. However, the effect of givosiran on heme status in the

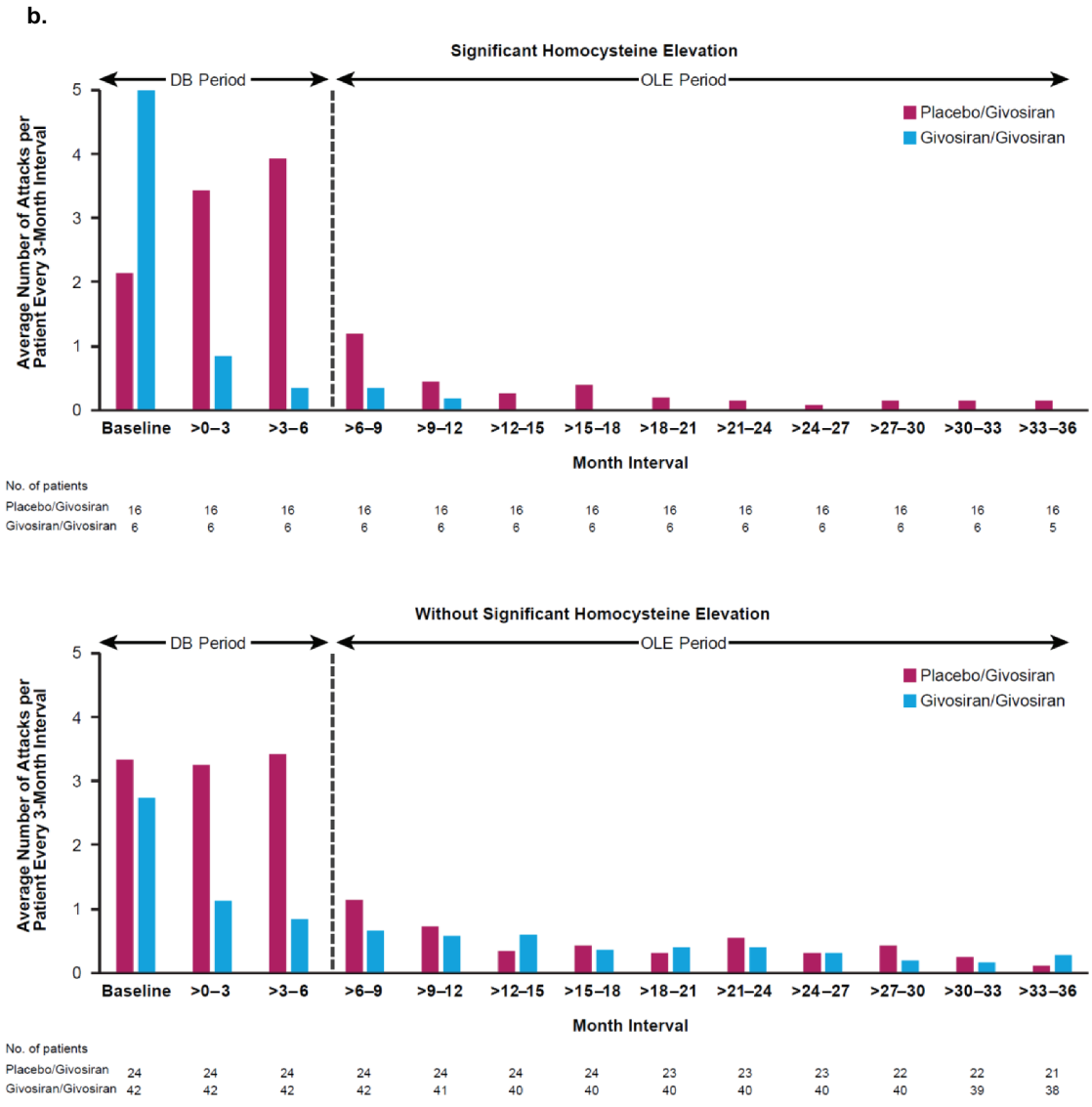

Figure 4. (Continued).

liver is not clear. In a preclinical pharmacokinetic study, single-dose givosiran did not significantly interfere with five major heme-dependent hepatic cytochrome functions [22,70], although potential cumulative heme depletion with continuous givosiran treatment cannot be ruled out. Assessment of other hepatic heme proteins (e.g. kynurenine pathway enzymes) in givosiran-treated patients showed that the kynurenine-to-tryptophan ratio in plasma was normal, indicating normal metabolism of tryptophan by these heme-dependent enzymes [14]. There is as yet no published evidence of a link between reduced hepatic heme in humans and CBS activity or HHcy [29,71]. Although the CBS enzyme contains a heme-binding motif [29], how this motif affects CBS activity is not fully understood, as it is not directly involved in the catalysis but may play a role in stabilizing the structure of the protein [29,71]. The complex intertwining of amino acid metabolism and heme biosynthesis suggests that heme might also affect CBS activity indirectly through other pathways [22]. Fontanellas et al. [24] proposed that decreased heme availability might lead to inflammation or oxidative stress, which has been shown to inactivate methionine-adenosyltransferase I/III, an enzyme that converts methionine to S-adenosylmethionine (SAM) [72]. Because SAM is an allosteric activator of CBS, reduction or depletion of SAM might contribute, at least partially, to reduced CBS activity. However, this idea remains hypothetical, and dietary SAM supplementation does not appear to significantly affect plasma homocysteine levels in healthy humans [73]. To potentially clarify these underlying mechanisms, researchers need to determine if givosiran treatment impacts hepatic heme status and CBS enzymatic activity in either liver biopsies or animal models.

4. Conclusion

On a population level, givosiran treatment increased homocysteine, but with wide interpatient variations. Severe HHcy with homocysteine levels >100 µmol/L has been reported [14,20–24]. There is no proof of correlations between HHcy

Table 2. Folate status or *MTHFR* genotype and homocysteine elevation in ENVISION patients with AHP during givosiran treatment.

Folate status	**≥10.0 nmol/L (normal)**	**<LLN of 10.0 nmol/L (deficient)[a]**	
Significant homocysteine elevation based on 2 × threshold,[b] *n* (%)			
Significant homocysteine elevation (*N* = 61)	25 (41.0%)[c]	36 (59.0%)	
Without significant homocysteine elevation (*N* = 18)	10 (55.6%)	8 (44.4%)[d]	
Significant homocysteine elevation ≥100 µmol/L for 12 months, *n* (%)			
Significant homocysteine elevation (*N* = 21)	5 (23.8%)[c]	16 (76.2%)	
Without significant homocysteine elevation (*N* = 58)	30 (51.7%)	28 (48.3%)[d]	
***MTHFR* genotype**	**677CC (wild type)**	**677CT (heterozygous)**	**677 TT (homozygous)**
Number of patients with *MTHFR* genotype[e]	25	28	14
Homocysteine at baseline (µmol/L)[f]	17.1 (6.1)	22.0 (27.5)	18.9 (12.2)
Any post-givosiran homocysteine >100 µmol/L, *n* (%)[g]	14 (56.0)	19 (67.9)	8 (57.1)
Homocysteine >100 µmol/L for >6 months, *n* (%)[h]	9 (36.0)	11 (39.3)	3 (21.4)
Homocysteine >100 µmol/L for >12 months, *n* (%)[h]	5 (20.0)	9 (32.1)	2 (14.3)
n/N (%) with folate <LLN of 10.0 nmol/L[i]	11/23 (47.8)	16/27 (59.3)	6/11 (54.5)

AHP: acute hepatic porphyria; LLN,: lower limit of normal; ULN: upper limit of normal.
[a]Among ENVISION patients with at least one non-fasting folate measurement during givosiran treatment (*n* = 79), 44 patients (55.7%) were folate deficient (<10.0 nmol/L of lower reference range).
[b]2 × threshold: when baseline homocysteine was <ULN, a post-givosiran value of >2 × ULN was significant; when baseline homocysteine was ≥ULN, a post-givosiran value of >2 × baseline was significant.
[c]Notable percentage of patients with significant homocysteine elevation did not have folate deficiency.
[d]Almost half of patients without significant homocysteine elevation were folate deficient.
[e]42/67 patients had a *MTHFR* C677T polymorphism (14 homozygous and 28 heterozygous).
[f]Homocysteine levels were elevated at baseline but not higher for TT genotype (homozygous variant).
[g]Percentage of patients with homocysteine levels >100 µmol/L were comparable among different genotypes.
[h]Lower percentage of 677 TT individuals with post-givosiran homocysteine elevations >100 µmol/L for more than 6 or 12 months may be due to chance with small numbers of individuals evaluated.
[i]Not all patients had folate level measurements.

and changes in efficacy or safety of givosiran treatment. The strong correlation and co-increase of homocysteine and methionine suggest that the homocysteine elevation associated with givosiran treatment is likely attributable to an impaired trans-sulfuration pathway catalyzed by CBS [14,20–22]. Larger and long-term studies or registries are needed to confirm the risk of HHcy in AHP patients treated with givosiran and to determine if there are any potential clinical consequences.

Because givosiran is a long-term therapy for AHP and the potential risks of long-term exposure to elevated homocysteine cannot be neglected, we recommend monitoring total plasma homocysteine and homocysteine-related vitamin (B6, B12, and folate) levels before and during givosiran treatment. Likewise, we recommend pyridoxine/vitamin B6 supplementation to reduce homocysteine levels in patients with severe HHcy (homocysteine >100 µmol/L) and initiating this supplementation in patients with homocysteine >30 µmol/L. Collaborative efforts should be undertaken to better understand the mechanism for this specific metabolic issue, which could be best addressed with the use of metabolic profiling of patients with AHP [74].

5. Expert opinion on clinical management of hyperhomocysteinemia in Ahp patients receiving givosiran

5.1. Monitoring Before and During Givosiran Treatment

We recommend that all AHP patients being considered for givosiran treatment be screened for basal total plasma homocysteine levels and closely monitored during givosiran therapy [20–22]. The givosiran prescribing information specifies measuring blood homocysteine levels prior to initiating AHP patients on givosiran treatment and monitoring for changes during treatment [8]. Total plasma homocysteine is a better marker than free homocysteine, which is measured by plasma amino acids and is an insensitive indicator of total plasma homocysteine [19]. In addition, because of the large between-day intrapatient variations in total plasma homocysteine [14], it may be useful to obtain serial measurements (e.g. ≥2 different measurements). In patients with elevated homocysteine levels, methionine levels (measured by plasma amino acids) can help determine the etiology of HHcy (**Table S3**).

All AHP patients being evaluated for givosiran treatment were screened for homocysteine-related vitamin (B6, B12, and folate) status before starting givosiran treatment and checked periodically while on treatment [20,22]. If a vitamin deficiency is detected before or during givosiran treatment, we implemented supplementation therapy promptly [20,22] (**Table S3**).

5.2. Watch-and-Wait vs. Initiate Treatment

Not all AHP patients on givosiran with elevated homocysteine may need treatment. Watch-and-wait may be a reasonable strategy in patients with mildly or moderately elevated homocysteine, given the uncertainty as to its clinical relevance [40–42]. Patients with classic homocystinuria develop severe HHcy (>100 µmol/L) [18] at a very young age and experience serious clinical consequences – an indication of the detrimental effects of exposure to very high homocysteine levels (>100 µmol/L) over a long period. In published case studies, investigators have started treatment in patients with AHP and homocysteine levels >30 µmol/L, >50 µmol/L, or >100 µmol/L [21–24]. Based on the information available, we recommend the following strategies: for patients with homocysteine >100 µmol/L (severe HHcy), implement treatment to reduce homocysteine levels; for patients with homocysteine >30 µmol/L, discuss treatment options with them and involve them in the decision to either watch-and-wait

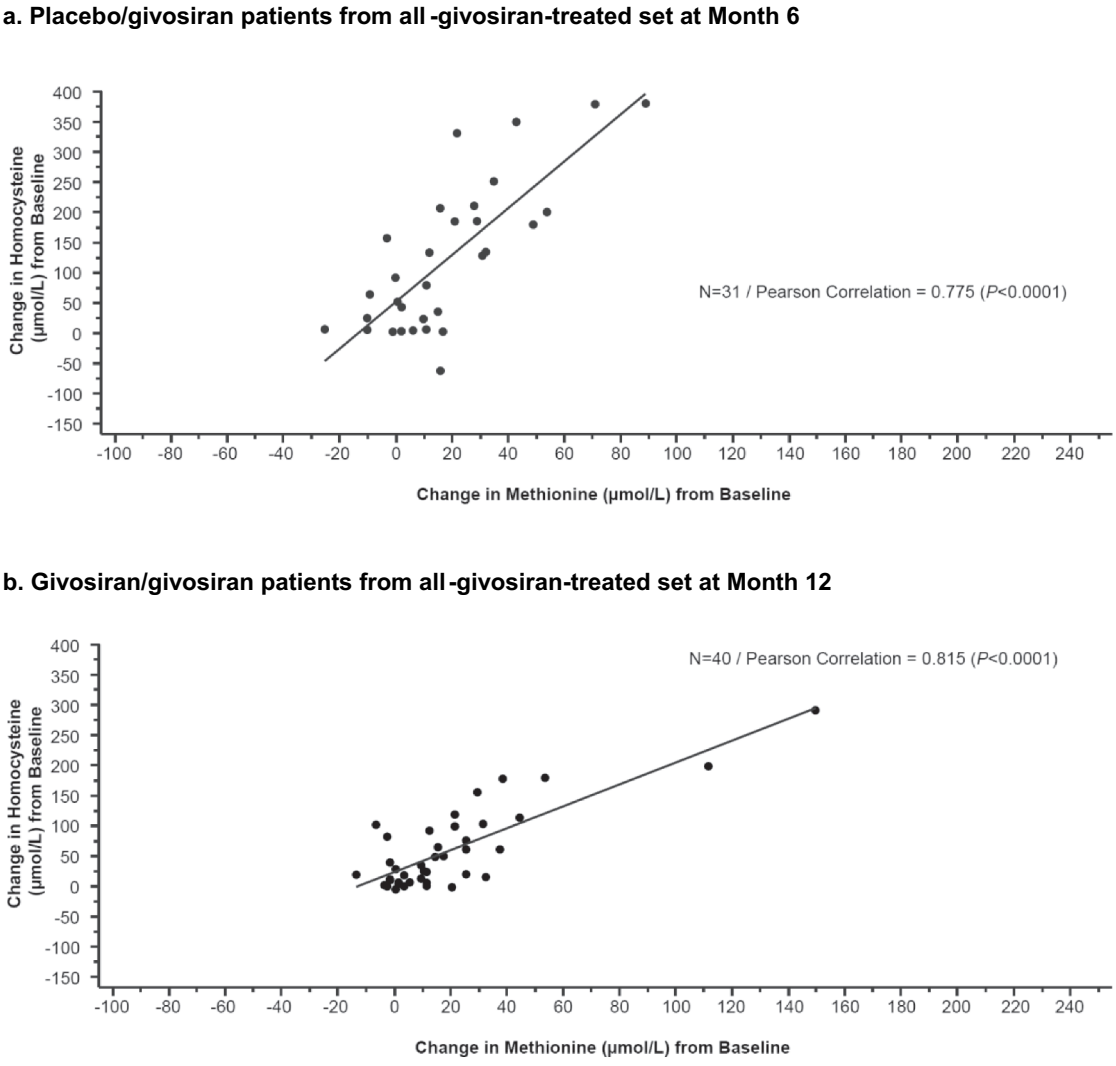

Figure 5. Correlation between change in plasma homocysteine and change in methionine from baseline during givosiran treatment in ENVISION.
Amino acid profiles were obtained from blood samples of 31 givosiran-treated patients in the placebo/givosiran group after 6 months of treatment and 40 givosiran-treated patients in the givosiran/givosiran group after 12 months of treatment. The profiles were derived using a validated liquid chromatography tandem mass spectrometry assay. A strong positive correlation was observed between the change in plasma homocysteine levels from baseline and the change in plasma methionine levels from baseline during givosiran treatment in both groups. (a) Placebo/givosiran patients from all-givosiran-treated set at Month 6. (b) Givosiran/givosiran patients from all-givosiran-treated set at Month 12. Linear regression analysis is shown.

or initiate treatment (**Table S3**). We recommend also referring to the local label for additional guidance.

5.3. Treatment Approaches

Given the potential mechanism of HHcy with givosiran (impaired trans-sulfuration pathway catalyzed by CBS) and significant reduction in homocysteine by vitamin B6 in case reports of patients with AHP [14,20–22], we recommend vitamin B6 supplementation to normalize homocysteine levels in AHP patients receiving givosiran. Several different dosages have been used by the authors of case reports in clinical practice and shown to be effective in reducing plasma homocysteine levels in givosiran-treated patients: 80 mg daily [21], 250 mg daily [23], or 3 mg daily along with other vitamins or trace elements (folate, vitamin B12, vitamin B2, betaine, and zinc) [22] (Section 3.2).

It is important to acknowledge that there is no evidence that vitamin supplementation as a means to reduce homocysteine levels may decrease the occurrence of vascular events. Nevertheless, vitamin supplementation, particularly with B6, should be used in patients on givosiran with severe homocysteine elevation because such treatment is simple, generally safe [75], and not costly, which means there is a positive benefit–risk ratio for this supplementation therapy. Most importantly, the potential vascular harm caused by long-term exposure to high homocysteine levels cannot be ignored, while further clinical study data are being generated, which could take years. Interestingly enough, although oral pyridoxine is a standard regimen in patients with an inborn CBS deficiency, the exact mechanism by which pyridoxine lowers homocysteine in humans is still unknown [76].

To summarize, currently available clinical evidence indicates that givosiran may exacerbate dysregulated

homocysteine metabolism in patients with AHP. We have offered a hypothesis for the mechanism of HHcy associated with givosiran and recommended monitoring and treatment approaches for HHcy in patients with AHP before and/or during givosiran therapy. The management of this specific metabolic issue will require the involvement of patients in the decision to treat and careful benefit-risk considerations.

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Declaration of interest

P Ventura reported receiving advisory board fees and lecture fees from Alnylam Pharmaceuticals and advisory board fees from Recordati Rare Diseases. E Sardh reported receiving grant support and personal fees, paid to Karolinska Institute, from Alnylam Pharmaceuticals. N Longo reported receiving clinical trial support, consulting fees, or advisory board fees from Aeglea, Alnylam, Amicus Therapeutics, ACI Clinical trials, Audentes/Astellas, AvroBio, BioMarin, BridgeBio/CoA Ther, Censa/PTC Ther., Chiesi/Protalix, CTI-Clinical Trial, Genzyme/Sanofi, Hemoshear, Homology, Horizon Pharma, Jaguar Gene Therapy, Leadiant Biosciences, Moderna, Nestle' Pharma, Pfizer, Recordati, Reneo, Retrophin, Shire/Takeda, Stealth BioTherapeutics, Synlogic, Ultragenix. M Balwani reported receiving grant support, consulting fees, advisory board fees, and lecture fees from Alnylam Pharmaceuticals, advisory board fees from Recordati Rare Diseases, grant support and advisory board fees from Mitsubishi Tanabe, and advisory board fees from Alexion, Genzyme/Sanofi, and Takeda. In addition, Mount Sinai faculty are named co-inventors with Alnylam on a patent related to the development of givosiran, the study drug. The Icahn School of Medicine at Mount Sinai receives payments related to this patent from Alnylam, and a portion of these payments are also distributed to faculty and other co-inventors. L Gouya reported receiving travel support and financial support from Alnylam Pharmaceuticals. J Phillips reported receiving consulting fees, advisory board fees, and lecture fees from Alnylam Pharmaceuticals, advisory board fees from Recordati Rare Diseases, and advisory board fees from Mitsubishi Tanabe. S Rhyee, MJ Fanelli, and MT Sweetser reported being employed by and owning stock and stock options in Alnylam Pharmaceuticals.

PE Petrides reported receiving advisory board fees and lecture fees from Alnylam Pharmaceuticals and advisory board fees from Recordati Rare Diseases. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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