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Genetics of hyperhomocysteinemia and 1-carbon metabolism: implications for retinal structure and eye functions

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**Abstract**

Homocysteine (Hcy); a sulfur-containing non-proteinogenic amino acid is generated as a metabolic intermediate. Hcy constitutes an important part of the “1-carbon metabolism” during methionine turnover. Elevated levels of Hcy known as hyperhomocysteinemia (HHcy) results from vitamin B deficiency, lack of exercise, smoking, excessive alcohol intake, high fat and methionine rich diet, and the underlying genetic defects. These factors directly affect the “1-carbon metabolism (methionine-Hcy-folate)” of a given cell. In fact, the Hcy levels are determined primarily by dietary intake, vitamin status, and the genetic blueprint of the susceptible individual. Although Hcy performs important role in cellular functions, but genetic alterations in any of the key enzymes responsible for the “1-carbon metabolism” could potentially upset the metabolic cycle thus causing HHcy environment in susceptible people. As such HHcy relates to several clinical conditions like atherosclerosis, myocardial infarction, stroke, cognitive impairment, dementia, Parkinson's disease, multiple sclerosis, epilepsy, and the ocular disorders among others. This manuscript sums up the findings from our laboratory and public database regarding genetics of HHcy and its effects on ocular disorders, their respective management during dysregulation of the 1-carbon metabolism.

**Keywords:** genetic polymorphism, hyperhomocysteinemic eye, oxidative stress, vasculopathy, vision loss.

**Introduction**

Homocysteine (Hcy), more precisely known as a thrombogenic non-proteinogenic amino acid is a demethylated derivative of methionine that is produced during the “1-carbon metabolism (methionine-Hcy-folate)” cycle (Ray et al. 2002). It is further converted to S-adenosylmethionine (SAM), the most important methyl group donor in our body (Stipanuk and Ueki 2011). McCully's key findings that linked vascular pathology of homocysteinemia with that of pathogenesis of arteriosclerosis suggested the link between accelerated atherosclerosis and inborn error of homocysteine (McCully 2015). It paved the way in understanding the metabolic aspects of cystathionine β-synthase (CBS) deficiency by attributing the effects of increased concentrations of Hcy, homocysteine, or a derivative of homocysteine (McCully 1969). Cystathionine β-synthase
BS enzyme (EC 4.2.1.22) is a vital regulator of the plasma levels of Hcy and is crucial for the tissue specific synthesis of cysteine. Also, methylenetetrahydrofolate reductase (MTHFR) is involved in the remethylation of Hcy back to methionine. Both CBS and MTHFR enzymes have been shown to be involved in the intracellular metabolism of Hcy, therefore MTHFR or CBS genetic defects are common causes of HHcy in susceptible individuals. HHcy means above normal concentrations of Hcy and it reflects sum of the total thiol-containing Hcy and homocysteinyl moiety of the disulfides Hcy and cysteine-Hcy, either free or bound to proteins (Malinow and Stampfer 1994). These hyperhomocysteinemic individuals are considered with increased risk of medical conditions such as cardiovascular and central nervous system diseases including the neuro-ophthalmological disorders (Ducloux et al. 1999; Martinez-Gutierrez et al. 2011; Mulvihill et al. 2004; Stanger et al. 2005). In total about 5 to 7% of the general population shows HHcy phenotype. The enzyme encoded by CBS gene acts as a homotetramer of 63-kD subunits and requires pyridoxal phosphate (it can be allosterically activated by SAM; AdoMet) and heme for its activity to catalyze the conversion of Hcy to cystathionine, the first step in the transsulfuration pathway. It is important to mention that CBS enzyme catalyzes the first irreversible step of transsulfuration and thus conjugates Hcy and serine to form cystathionine, which is subsequently converted into cysteine and alpha-ketobutyrate. As mentioned above, Hcy undergoes remethylation to form methionine (Kraus et al. 1993; Shan et al. 2001). Interestingly, it was recently discovered that CBS enzyme is a major contributor to the cellular hydrogen sulfide (H$_2$S) production endogenously. Multiple alternatively spliced transcript variants have been found for the CBS therefore enzyme expression may not operate at 100% efficiency in individuals who have one of the single-nucleotide polymorphisms (SNPs) that affects CBS activity. The known CBS variants include A360A, C699T, I278T, N212N, and T42N SNPs. These SNPs, which have varied effects on the effectiveness of the CBS enzyme, can be detected with standard DNA testing methods (de Franchis et al. 2000; Ding et al. 2012; Orendac et al. 1999).

Hcy in the blood is generally present in four different forms: (1) around 1% as free thiol, (2) 70–80% as a disulfide-bound to plasma proteins and rest (3) 20-30% as a homo-dimer with its self or (4) hetero-dimer with other thiols (Hankey and Eikelboom 1999). Levels of the Hcy are usually controlled by two biochemical processes: (A) roughly ~50% of the Hcy goes to trans-sulfuration pathway for producing the glutathione and the rest (B) ~50% can be re-methylated back to methionine (Cascella et al. 2015; Veeranki and Tyagi 2013). Normally, the synthesis and elimination of Hcy stay pretty much in balance, but in diseased conditions i.e. in
HHcy, the overall plasma Hcy levels tend to increase due to the errors in the Hcy metabolism (Hamelet et al. 2007). There are mainly four different, but related ways individuals develop HHcy (1) consumption of excessive methionine-rich protein diet on a regular basis (2) B12/folate deficiency (3) presence of the CBS \textit{gene} as heterozygous or homozygous and finally (4) poor clearance of Hcy from the kidney. Several other notable factors also such as gender, age smoking, and alcohol intake, certain type of medications and even different disease conditions that can potentially modulate methionine cycle can increase the Hcy levels. Furthermore, there are additional genetic factors that are crucial in promoting the HHcy condition such as substitution of the gene encoding enzymes that are involved in “1-carbon metabolism” (Brustolin et al. 2010; Iqbal et al. 2009; Verhoef et al. 2005). As this cycle is the only pathway which gives methyl group in both biosynthesis of cellular compounds such as creatine, epinephrine, carnitine, phospholipids, proteins, and polyamines and in epigenetic changes (like methylation of DNA, RNA, and histones) (Kamat et al. 2016). Nevertheless, HHcy mediated metabolic malfunctioning because of the higher circulating Hcy levels promote oxidant stress-induced vascular inflammation and vessel dysfunction leading to atherosclerosis, myocardial infarction, stroke, multiple sclerosis, cognitive impairment, epilepsy, dementia, Parkinson's disease, and ocular disorders (Wu C. Y. et al. 2013a; Wu Y. L. et al. 2013b). More often the ocular manifestations of HHcy are serious that can potentially lead to loss of eye sight and blindness. This article sums up the findings concerning roles of genetics in HHcy that are relevant in various ocular disorders and how to effectively manage them (Ding et al. 2012; Eloranta et al. 1990; Finkelstein 1990; Majumder et al. 2018a; Majumder et al. 2018b; Majumder et al. 2018c).

MTHFR is responsible for catalyzing the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for Hcy remethylation to methionine. Defects in MTHFR are the common inborn errors of folate metabolism that result into the phenotypes ranging from asymptomatic to serious neurologic deterioration to early death in the classic form of the MTHFR deficiency encompassing the thermostability of the enzyme variants that have been identified in the past (Rosenblatt et al. 1992). Megaloblastic anemia and homocystinuria are autosomal recessive genetic errors of metabolism resulting from defects in the cobalamin (vitamin B12) dependent pathway that converts Hcy to methionine, and is catalyzed by methionine synthase (MS). Clinical presentation usually includes
delayed psychomotor development, hypotonia, homocystinuria, megaloblastic anemia, and hypomethioninemia, all of which respond to the supplementation of cobalamin. In this context two complementation groups have been described based on studies in the fibroblast: CblE and CblG (Watkins and Rosenblatt 1988, 1989).

**Genetic determinant of hyperhomocysteinemia**

The total Hcy (tHcy) levels in our body indicate the status of folate and/or vitamin B12 functioning. When vitamins levels fall below the physiological demand of the host then diseases can ensue and affect. Main ailments are cardiovascular in nature along with neural tube defects, central nervous system (CNS) disorders, and the ocular manifestations. Literature indicates that Hcy metabolism, folate, and vitamin B12 are governed by ones’ genetic constitution (Djuric et al. 2018). Since Hcy metabolism is impaired during renal insufficiency, $MTHFR$ 677 C>T, $GCP2$ 1561C>T, $RFC1$ 80G>A, and $TCN2$ 776G>C further aggravate HHcy in susceptible patient populations. It should be emphasized here that the most reliable effect on concentrations of tHcy is observed for 677C>T of $MTHFR$, whereas $GCP2$, $RFC1$, and $TCN2$ SNPs tend to reveal no major effect(s) on tHcy concentrations (Sunder-Plassmann and Fodinger 2003). As briefly mentioned earlier that both MTHFR and CBS are involved in the intracellular metabolism of Hcy, therefore, MTHFR or CBS gene SNPs are the most commonly reported causes for the development of HHcy (Hamelet et al. 2007; Yakub et al. 2012). In fact, metabolism of Hcy depends on several factors and the genetic predisposition to HHcy is categorized by cause, prevalence, and also by severity (Table 1) (Laster et al. 1965) (Fay 2012; Selhub 1999). MTHFR catalyzes the conversion of 5,10-MTHF to 5-MTHF, which is a co-substrate of Hcy in the remethylation reaction (Finkelstein and Martin 1984). Hence, the presence of high SAM prevents Hcy from entering the remethylation pathway and it also acts as an allosteric inhibitor for the MTHFR. Interestingly, higher levels of S-adenosyl methionine (SAM) serve as the allosteric activator for the CBS in the transsulfuration pathway favoring funneling of Hcy into the transsulfuration pathway (Finkelstein et al. 1975; Stabler et al. 2002). As we know that a few organs in our body such as the liver, kidney, pancreas, brain, adipose tissue and small intestine have the capability to synthesize their own CBS enzyme (Finkelstein 1990; Mudd et al. 1965) however in tissues like vasculature, and muscles the CBS is usually not expressed and therefore the higher SAM levels can lead to the transient accumulation of Hcy in the system.
Another factor in Hcy metabolism is the dietary methionine which affects the rate of SAM synthesis that in turn, could determine the pathway Hcy takes up. Either it goes in favor of the remethylation or the transsulfuration pathway (Majumder et al. 2017; Selhub 1999; Selhub et al. 1999; Stolzenberg-Solomon et al. 1999). Hcy cycle through the remethylation pathway when diet contains a basal methionine amount (about 1.5-2.0 times) before it is directed towards the transsulfuration pathway, but when the dietary methionine amount is half the basal one, then cycling of Hcy usually takes place via the remethylation pathway and it increases at least two-fold. Conversely, when methionine level in the diet is high, then Hcy cycles through remethylation and it is reduced by about 1.5-fold (Eloranta et al. 1990). In these circumstances, the high level of intracellular Hcy is then exported out into the circulation (Gupta et al. 1998). SNPs in MTHFR (C677T and A1298C) generally play some minor roles in determining Hcy levels in healthy individuals but a meta-analysis showed that MTHFR 677T genotype is associated with an increased risk of HHcy (Al-Rubeaan et al. 2013; Cai et al. 2014; Castro et al. 2003; Chang W. W. et al. 2013; Heifetz and Birk 2015; Yang K. M. et al. 2014; Yang S. et al. 2013; Zhang D. et al. 2014) (Frosts et al. 1995) (Jacques et al. 1996).

Higher levels of Hcy (i.e. HHcy) have been shown to affect methylation potential and DNA methylation, while in vitro acute treatment of lymphocytes from healthy male donors with HHcy (i.e., >20 µmol/L for 8 h) failed to cause any noticeable change(s) in the methylation or DNA hypo-methylation indicating that HHcy-induced toxicity is most likely the result of a chronic, rather than acute response (Fux et al. 2005). Recent studies have shown that folate supplementation alone does not reduce the risk(s) of coronary artery disease and stroke (Banerjee et al. 2007, Gao et al. 2012, Hsu et al. 2018, Yu et al. 2011). Several clinical studies revealed that Hcy levels to be significantly higher in multiple sclerosis patients than in control individuals without B12 and/or folate deficiency, in the absence of MTHFR mutation (Ho et al. 2002; Kararizou et al. 2013; Kruman et al. 2000; Teunissen et al. 2008). Pregnant mothers with an MTHFR 677 C→T polymorphism are most likely to have significantly elevated levels of Hcy and an increased likelihood of the occurrence of spina-bifida in their offspring (OR, 1.7; 95% CI, 1.1-2.6) (Kapusta et al. 1999). Similarly, those with MTHFR 677C-T or 1298A-C SNPs having decreased MTHFR enzymatic functions, these patients are prone to HHcy development (Chango et al. 2000). There are also other causes of severe HHcy such as homozygous deficiency of MTHFR, methionine synthase (MS), and also the impaired activity of MS due to genetic disorders of the vitamin B12 metabolism (Froese and...
A number of genetically inherited mutations that are responsible for elevated Hcy levels have been described and the most common happens to be the MTHFR C677T that usually leads to HHcy and several associated complications that are serious in nature ((White 2003) (Table 2).

**Hyperhomocysteinemia, vasculopathy and ocular diseases HHcy and ocular diseases**

HHcy is known to inflict vascular changes/disorders because of elevated Hcy levels however the mechanisms by which Hcy damages the blood vessel wall during the prothrombotic effect(s) seems to be complex in nature. The past experience reveal that the metabolically active sulfhydryl-containing non-proteinogenic amino acid Hcy causes thrombosis via increasing the tissue factor expression levels, attenuating the anticoagulant processes, enhancing the platelet reactivity, increasing the generation of thrombin and fibrinogen, augmenting the factor V activity, impairing the fibrinolytic potential, and also injuring the vascular tissue including the endothelial cells lining the vascular walls (de Franchis et al. 2000; Gouveia and Canhao 2010; White 2003). At the molecular levels, underlying prothrombotic actions of Hcy involve oxidative stress response, hypo-methylation of DNA and the proinflammatory effects (Tyagi et al. 2005; Zhang X. et al. 2000). In addition to the above a decreased bioavailability of the nitric oxide (NO), altered expression of various thrombotic factors, mitogenic effect on arterial smooth muscle cells, and expression of the acute stress-related genes (Weiss 2005) (Tsai et al. 1994) (Jakubowski 2006; Postea et al. 2006). Many studies have demonstrated that Hcy causes direct cytotoxic effect(s) by forming disulfide protein derivatives, thereby modifying the vascular cell function(s). It is important to mention here that metabolic conversion of Hcy to a chemically reactive metabolite such as the Hcy-thiolactone has been suggested to be highly toxic to human cells and tissues including the vascular tree. Together, these effects lead to endothelial dysfunction of the affected blood vessels (Selhub 2006). In a population-based study it was observed that mild to moderate increase in the Hcy concentration serves as a significant risk factor for the retinovascular stroke occlusive diseases as it was found to be associated with increased odds ratios for the retinal emboli formation (Undas et al. 2005).

The central retinal artery or vein occlusion is one of the most common retinal vascular diseases in humans. Based on the site of the occlusion and on the type of consequent vascular damage, central retinal vein
Occlusion (CRVO) is the most frequently occurring and remains clinically relevant type of ocular manifestation (McGimpsey et al. 2009). It is usually seen in older adults, and adults and is often associated with other systemic diseases. In many cases, it also occurs in young adults with no other systemic disease or comorbidity. Both local and systemic risk factor(s) have also been associated with CRVO but the cause of CRVO again remains multifactorial in nature. Though hypercoagulability has been reported in the pathogenesis of CRVO in young patients, laboratory tests have not accounted for this cause in most of these patients that have been examined thoroughly. Among the parameters tested, HHcy and circulating antiphospholipid antibodies are reported to be significantly more common in the patients affected with CRVO. It has been reported that there is no significant increase in the factor VIII (von Wilbrand factor), apart from Hcy levels in the CRVO cases compared with that of control subjects. The underlying cause of the dysregulated Hcy metabolism and the molecular mechanism(s) underlying the prothrombotic actions of the Hcy are incompletely understood in cases of CRVO with mild HHcy in affecting the young patients.

Surprisingly, there are not many studies on mild to moderate nature of HHcy associated with young CRVO cases. A 2-year prospective study in young adult CRVO patients was performed to evaluate the relationships between the plasma total Hcy (tHcy) and CRVO in which estimation of some of the key metabolites of the transmethylation and transsulfuration pathways involved in the Hcy metabolism were performed (Narayanasamy et al. 2007). Retinal structural and the corresponding phenotype in HHcy mice that are deficient or lacking the CBS activity exhibited significant disruption of the retinal layers, along with diminished electrophysiological responses and altered retino-vasculature indicating the ischemic nature of the retinopathy in their eyes. These changes were accompanied by increased cellular stress responses via enhanced endoplasmic reticulum (ER) and oxidative stress as well as activation of the N-methyl-D-aspartate (NMDA) receptor which are associated with Hcy-induced blood retinal barrier (BRB) breakdown and leakage (Ibrahim et al. 2016; Mohamed et al. 2017; Srejovic et al. 2015; Tawfik et al. 2013; Tawfik et al. 2014; Tawfik and Smith 2014; Tyagi et al. 2009).

We previously demonstrated that HHcy is responsible for vascular injuries (Castro et al. 2006; McCully 1993), changes in the extracellular matrix (ECM) (Tyagi 1998; 1999) and neuronal cell death (Lipton et al. 1997;
The common enzyme defects associated with increased total Hcy levels are the point mutations such as C-to-T substitutions at nucleotide 677 in the coding region of the gene encoding MTHFR, which is usually associated with a thermolabile MTHFR variant that has about half the normal activity level (Hankey and Eikelboom 1999). However, the most common genetic form of severe HHcy phenotypes and classic homocystinuria (congenital homocystinuria) generally result from a homozygous deficiency of the CBS that increases Hcy levels up to 40-fold during fasting stage. SAM to SAH ratios define the methylation potential of a cell despite the fact that homozygous T/T genotype remains an independent risk factor for the causation of HHcy (Kolling et al. 2004, Malinow et al. 1999). We know that the HHcy metabolic states tend to decrease this ratio that in turn decreases the methylation potential of a given cell. It has been studied in the past that HHcy leads to global DNA methylation changes in the core regions of promoter for the genes including demethylation of the CpG sites thereby eliminating the binding of methyl CpG-binding proteins (George et al. 2018a). These types of changes limit the histone deacetylases (HDAC) binding abilities causing acetylation of the H3 and H4 histones to accumulate and these alterations are responsible for suppression of the expression activities of the respective genes. Further, DNA hypo-methylation and histone acetylation are associated with transcriptional permissive chromatin state of the chromosomes allowing increased access by the repressor proteins, thus leading to the suppression of transcription. For example, to account for the changes in apoA-1 and apoA-IV during HHcy state, similar types of epigenetic regulatory mechanism(s) have been reported in the literature (Handy et al. 2011; Mikael et al. 2012). Interestingly, Hcy-induced DNA hypomethylation of the promoter sequences causes some gene(s) to be upregulated, for example, HHcy increases p66shc expression in endothelial cells thus correlating well with the promoter hypo-methylation and hence contributing to the resultant oxidative stress response (Loscalzo and Handy 2014; McCully 2009).

Despite various mechanism(s) explained for the Hcy mediated ocular changes in eye diseases, the extract mechanism(s) still remains unknown. Among many deleterious effects observed the prominent ones appear to be cytotoxic and vasculopathic in nature causing apoptosis of the retinal ganglionic cells (RGCs), extracellular matrix (ECM) alterations, induction of oxidative stress response, and ischemic vascular dysfunction by the Hcy metabolites such as homocysteine thiolactone (Hcy TL); a metabolite of Hcy that reacts with the amine groups of proteins to form stable amides, homocystamides or N-homocysteinylated version of the proteins.
It was suggested that protein N-homocysteinylation results in the cytotoxicity because of higher Hcy (HHcy) and protein homocysteinylation alterations are responsible for ocular manifestations during HHcy. Further, protein modifications in the endothelial cell result in different pathophysiological consequences including production of the highly reactive anti-Hcy antibodies, enhanced phagocytosis and onset of the harmful inflammatory processes. During HHcy modification of the hemostatic proteins such as antitrypsin, and fibrinogen by the N-homocysteinylation the resultant changes can potentially affect their biological functions (Jakubowski 2000; 2002). Also, the post-translational modifications of proteins in the lens play a crucial role in the formation of age-related cataract in the susceptible individuals over a period of time. It has been estimated that homocysteinylation of lysine residues ~ 33% in MS and ~ 88% of trypsin result in the complete loss of their respective enzymatic activities. Homocysteinylation tends to result in protein damage which impair the functions of the respective target proteins (Karolczak and Olas 2009). A few studies have already demonstrated the causal link between MTHFR polymorphisms, HHcy, 


Corel and colleagues also demonstrated that elevated vitreal Hcy levels in the eyes were associated with decreased lysyl oxidase activity in cases suffering from proliferative diabetic retinopathy condition (Coral et al. 2009). Both, Hcy and its Hcy-TL can inhibit the activity of lysyl oxidase in the vascular endothelial cells of the vessels, as well (Raposo et al. 2004). An elevated Hcy level also causes changes in the optic nerve head (ONH) microvasculature and impairing the blood flow via a vasoconstrictive effect, endothelial injury, smooth muscle proliferation, platelet activation, thrombogenesis, and via the apoptotic cell death of RGCs. Loss of RGCs were demonstrated in mouse model with endogenously elevated Hcy levels due to deletion of CBS gene (Ganapathy et al. 2009). Studies using cell culture as well as following the Hcy intravitreal injection revealed that Hcy induces apoptosis of the RGCs (Ganapathy et al. 2010; Martin et al. 2004). Important changes in the glaucomatous optic neuropathy were also associated with the Hcy-induced vascular injury, alterations in ECM remodeling, and neuronal toxicity followed by death of the cells (Fingert et al. 2006; Turgut et al. 2010). The increased risk of vascular disease among patients with pseudoexfoliation glaucoma (PEXG) can be explained to the impaired
endothelium dependent vasodilatation by elevated Hcy levels (Fingert et al. 2006; Hankey and Eikelboom 1999). Similarly, a direct Hcy mediated effects were also reported on the retina since high levels of Hcy were found in various retinal layers, particularly in the RGCs and also in the vitreous fluid, suggesting the direct toxic effect of the Hcy. It was reported that short-term HHcy-induced oxidative stress response can activate RGCs in rat leading to the increased vascular endothelial growth factor expression in the retina (Lee et al. 2007). Homocysteinylation of the outer and inner segments of the photoreceptor cells led to their degeneration and eventually resulted in retinopathy (Chang H. H. et al. 2011). The reactive oxygen species (ROS) acting synergistically with impaired ONH blood supply can further induce the vasoconstriction and may thereby further favor the disease progression in the eyes. The impaired ONH blood supply as well as oxidative stress response has been implicated as a potential risk factor for the development of glaucoma and its subsequent progression (Ferreira et al. 2004; Wagenfeld et al. 2013). In proliferative diabetic retinopathy (PDR), and nephropathy there is extra-cellular membrane (ECM) remodeling component with neovascularization and the basement membrane changes. The diabetic neovascular membrane showed increased immunostaining of the lysyl oxidase. As such, proteins in the retina are liable to the attack by the homocysteinylation during HHcy and besides that Hcy-TL had been reported to possess more pro-inflammatory properties than the Hcy itself which could potentially lead to more damages in the ocular compartment of the affected individuals (Kerkeni et al. 2006).

Diabetic retinopathy (DR) leads to vasculopathic damage because of neovascularization and the diabetic macular edema (DME). Increasing evidence demonstrate that diabetes can in fact induce direct damage including damage to neurons, RGCs, and photoreceptors and such damages can be ascribed to the visual dysfunction and loss of vision (Kern and Barber 2008). Hcy also increases DNA damage (Kruman et al. 2000) and as mentioned earlier hypo-methylation of DNA and subsequent altered gene expression are two important mechanisms leading to the neuronal damage as caused by elevated Hcy levels. Deficiency of the folate or B12 causes low SAM and DNA hypo-methylation in cultured neuroblastoma cells (Fuso et al. 2005). Epidemiological data in adults paralleled these observations that elevated levels of Hcy are associated with premature DR phenotype (Brazionis et al. 2008). As previously mentioned, retinal and lens proteins are liable to be attacked by homocysteinylation and oxidative stress response including the influence of HHcy on dislocation of the optic lens (Jhee and Kruger 2005). Since Hcy-TL possesses stronger cytotoxicity and pro-inflammatory properties than
Hcy it eventually leads to more retinal damage and pathological changes (Ajith and Ranimenon 2015). CBS mutation mediated ocular homeostatic disturbance leads to the dislocation of the lens in the affected eyes of the hyperhomocysteinemic patients and that is due to the deterioration in the zonular fibers that suspend the lens from the ciliary body (Mulvihill et al. 2001). So, it is clear that presence of the risk alleles either in the MTHFR or in the CBS could be indicative of the severe HHcy conditions.

Management of HHcy-mediated pathologies

Many studies have provided ample evidence to support that HHcy mediates and is responsible for the various systems dysfunction in our body (Coral et al. 2006; Gopinath et al. 2013; Handy et al. 2005). Vitamins’ status is a primary determinant of mild-to-moderate HHcy and accounts for approximately two thirds of all such cases. Thus, supplementation of vitamins at appropriate dosages can result in the normalization of plasma Hcy in most of the cases if not all. Hcy metabolism requires the participation of folate as well as vitamin B12 (cobalamin) and vitamin B6 (pyridoxal phosphate) coenzymes and therefore reduction of Hcy levels in plasma requires that all three of these vitamins are supplemented appropriately (Selhub 1999). Hydrogen sulfide (H2S) administration as a therapeutic approach (Behera et al. 2018) or targeting of the circular RNA (circRNA) molecules as a therapeutic tool might turn out to be the future therapeutic interventions for the management of chronic HHcy mediated ocular pathologies (Singh et al. 2018). Findings suggest that the changes in the concentration of cysteine, folate and flavin mononucleotide seem to be predictors of changes in the total Hcy concentrations in the body. Since circRNAs are capable of fine-tuning the post transcriptional expression dynamics of the metabolically important parent-genes via their interactions with micro-RNAs (miRNAs) or with single or multiple RNA binding proteins (RBPs), they can prove as outstanding tools in regulating the down-stream pathogenetic machinery that is responsible for the deregulation of the homeostatic environment in the eyes. This option might in fact prove as a potential strategy for the effective management of the HHcy (Singh et al. 2018). Also, H2S treatment might also become as an intervention tool for chronic conditions such as HHcy (George et al. 2018b). Similarly, there are other options to augment the endogenous levels of H2S such as by practicing endurance building exercise regimen for the better management of HHcy mediated pathologies (George et al. 2018a).
**Conclusion**

HHcy has been linked with several eye diseases including retinopathy, optic atrophy, pseudoexfoliative glaucoma maculopathy, cataract, and retinal vessel atherosclerosis. The underlying molecular mechanisms for these diseases have also been reported as impaired ECM, alterations in vascular endothelial functions, apoptosis of RGCs, reduction in the lysyl oxidase activity, inflammation, and oxidative stress. The metabolism of methionine and Hcy requires enzymes with vitamins such as folic acid, vitamins B12 and B6. Elder patients may supplement with these vitamins to attenuate the ocular damages (Ajith and Ranimenon 2015). In our body the Hcy concentration refers to the sum of the thiol-containing amino acid Hcy and the homocysteinyl moiety of the disulfides Hcy and cysteine-Hcy, whether free or as bound to the proteins (Malinow and Stampfer 1994). All the circulating Hcy is primarily derived from the dietary methionine that serves as the methyl group donor in the form of S-adenosyl methionine or SAM. After donating the methyl group, it forms the S-adenosyl Hcy or SAH and then to Hcy. As mentioned earlier Hcy is a sulfur-containing non-proteinogenic amino acid that is either metabolized to cystathionine by the transsulfuration pathway, requiring B6 vitamin or it is converted back to the methionine by B12 vitamin and folate, requiring transmethylation (Fig. 1). The main causes of HHcy are multi-factorial but severe form of the HHcy occurs mainly due to the genetic defects resulting from the deficiencies of the CBS or MTHFR enzymes (Fig. 2). Mild HHcy can arise due to the impairment in either of these enzymes via the transmethylation pathway associated with or without nutritional deficiencies involving B12 vitamin and folate. In the past several studies have been conducted to uncover the direct or indirect influence of increased levels of Hcy in several medical conditions and it was revealed that polymorphisms in the genes that are part of the methionine metabolism can result in HHcy, suggesting that these gene variants play important roles in disorders of high prevalence. Vitamins’ supplementation is a cost-effective way to decrease HHcy concentrations and the broad vitamin supplementation could prevent at least some of these disorders in susceptible individuals. Despite recent developments the role of HHcy as an etiological factor has been associated with several ocular disorders, however the beneficial role of exercise and that of H2S in mitigating the HHcy metabolic effects remain a matter of ongoing discussion in the scientific community.

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Declaration of interest

The authors report no conflicts of interest and are responsible for the content and writing of this manuscript.

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Figure legends

Fig. 1. Schematic representation of homocysteine (Hcy) metabolism showing principal metabolic pathways (A). Dietary methionine is converted to Hcy through S-adenosyl methionine (SAM) and S-adenosyl homocysteine (SAH) and then back to methionine. During transmethylation pathway Hcy is produced from methionine wherein a methyl group is transferred to DNA, RNA or histone resulting into SAH formation which releases adenosine and produces Hcy. Hcy bifurcates to transsulfuration pathway where it is converted to cysteine in the presence of rate-limiting enzymes cystathionine-β synthase (CBS) and cystathionine-γ lyase (CSE). Cysteine thus generated through transsulfuration is converted to glutathione (GSH). In remethylation the recycling of Hcy to methionine includes the folate cycle which acts as methyl donor and provides carboxyl group necessary to convert Hcy into methionine, (B) Conversion of cobalamin (Vitamin B12) to methyl-B12 in the presence of methionine synthase reductase (MTR) is necessary for remethylation of 5-methyl-tetrahydrofolate (THF) to THF, (C) Dietary folic acid (Vitamin B9) enters folate cycle after its conversion first to dihydrofolate (DHF) and then to THF. The 5, 10-methyltetrahydrofolate reductase; MTHFR is a key enzyme that converts 5, 10-methylene-THF to 5-methyl-THF. DNMT; DNA methyltransferase; MS; methionine synthase; ATP; adenosine triphosphate; BHMT; betaine-Hcy methyltransferase; B 6; vitamin B 6; B 9; vitamin B 9; B 12; vitamin B 12.

Fig. 2. Diagram highlighting the putative roles played by exogenous and endogenous factors affecting Hcy metabolism that can lead to dysfunctional 1-carbon metabolism ultimately resulting into HHcy environment in the eyes. HHcy leads to epigenetics alterations including hypermethylation of regulatory elements (gene promoters), RNAs, homocysteinylation of proteins, glutamate excitotoxicity, oxidative, endoplasmic reticulum and mitochondrial stress responses. The overall collapse of the homeostatic system in the eyes results in the progressive loss of visual functions and the blindness in susceptible people.

Fig. 2. Diagram highlighting the dynamic roles played by exogenous and endogenous factors affecting Hcy metabolism that can lead to dysfunctional 1-carbon metabolism ultimately resulting into HHcy environment in the eyes. HHcy leads to epigenetics alterations including hypermethylation of regulatory elements (gene promoters), RNAs, homocysteinylation of proteins, glutamate excitotoxicity, oxidative, endoplasmic reticulum and
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**Fig. 3.** A cartoon highlighting the beneficial effects of H\(_2\)S, exercise, vitamin B supplementation, inhibition of harmful miRNAs, circRNAs, LncRNAs and gene correction for alleviating the HHcy-induced metabolic disturbances in the ocular cavity that lead to structural, physiological, and functional changes in the retina leading to progressive visual impairment and blindness in the susceptible individuals.
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Treatment with H₂S, exercise, vitamins B complex, inhibition of harmful circRNAs, miRNA, LncRNAs, and gene correction.

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Draft

Genetic polymorphism in CBS and MTHF, epigenetically governed process by miRNAs, circular RNAs, LncRNAs

Vitamin B deficiency, lack of exercise, smoking, excessive alcohol, high fat and methionine rich diet

HHcy

Homocysteinylation of proteins, glutamate toxicity, endoplasmic, mitochondrial, and oxidative stress responses

Vision impairment and blindness

Exogenous Factors
- Vitamin B deficiency, lack of exercise, smoking, excessive alcohol, high fat and methionine rich diet

Endogenous Factors
- Genetic polymorphism in CBS and MTHF, epigenetically governed process by miRNAs, circular RNAs, LncRNAs

Molecular Level
- Epigenetic changes (like methylation of DNA, RNA and histones)

Cellular Level
- Homocysteinylation of proteins, glutamate toxicity, endoplasmic, mitochondrial, and oxidative stress responses

Dysregulated 1 carbon metabolism
Retinal cell

HHcy

ROS

Redox imbalance

Mitochondrial dysfunction

DNA damage

ER stress

Neuronal damage

Pyroptosis

Macular degeneration

Optic atrophy

Macular edema

Vascular permeability

Macular atrophy

Lens opacification

Inflammation

ECM alterations

Visual function impairment and blindness

Treatment with H₂S, exercise, vitamins B complex, inhibition of harmful circRNAs, miRNA, LncRNAs, and gene correction
Table 1. Types and causes of hyperhomocysteinemia (HHcy).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hcy levels</th>
<th>Main causes</th>
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</thead>
<tbody>
<tr>
<td>Severe HHcy</td>
<td>31- &gt;100 mmol/L</td>
<td>Mutations in the CBS, and MTHFR genetic sequences.</td>
</tr>
<tr>
<td>Mild HHcy</td>
<td>15-30 mmol/L</td>
<td>Mild impairment in the methylation pathway (i.e. folate or B12 deficiencies or MTHFR thermolability).</td>
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<tr>
<td>Post-methionine load</td>
<td>&gt;15 mmol/L</td>
<td>Impaired Hcy transsulfuration (heterozygous CBS defects, and B6 deficiency).</td>
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<tr>
<td>Genes</td>
<td>SNPs</td>
<td>Associated complications</td>
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<tr>
<td>CBS</td>
<td>844INS68</td>
<td>Peripheral artery occlusive disease</td>
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<tr>
<td></td>
<td>T833C</td>
<td>Stroke</td>
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<tr>
<td></td>
<td>844INS68</td>
<td>Thrombosis</td>
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<tr>
<td>MTFHR</td>
<td>C677T</td>
<td>Retinal vein occlusion</td>
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<td>Venous thromboembolism</td>
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