



Regulation of the parental gene *GRM4* by circGrm4 RNA transcript and glutamate-mediated neurovascular toxicity in eyes

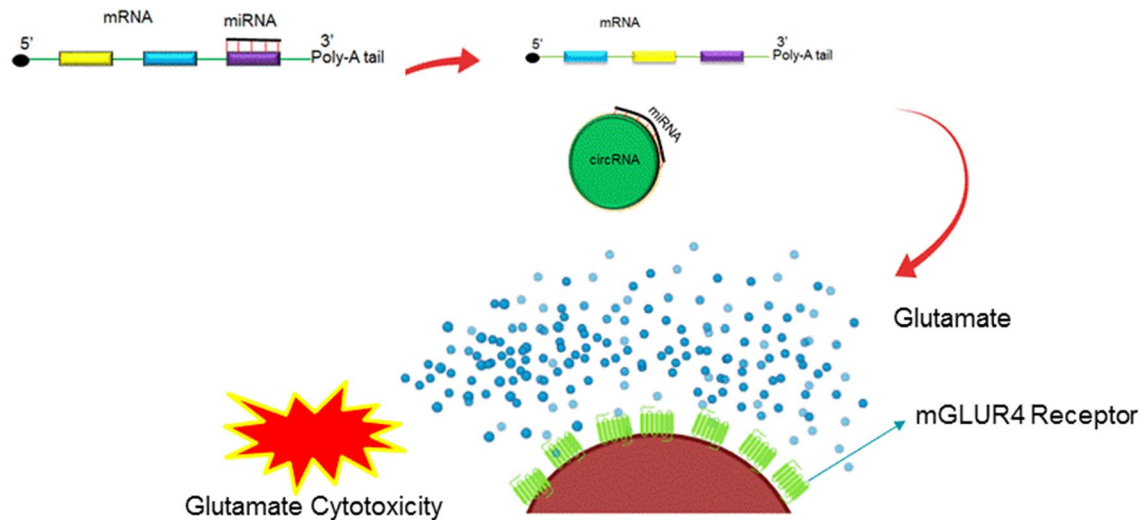
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Abstract

Epigenetic memory plays crucial roles in gene regulation. It not only modulates the expression of specific genes but also has ripple effects on transcription as well as translation of other genes. Very often an alteration in expression occurs either via methylation or demethylation. In this context, “1-carbon metabolism” assumes a special significance since its dysregulation by higher levels of homocysteine; Hcy (known as hyperhomocysteinemia; HHcy), a byproduct of “*I-Carbon Metabolism*” during methionine biosynthesis leads to serious implications in cardiovascular, renal, cerebrovascular systems, and a host of other conditions. Currently, the circular RNAs (circRNAs) generated via non-canonical back-splicing events from the pre-mRNA molecules are at the center stage for their essential roles in diseases via their epigenetic manifestations. We recently identified a circular RNA transcript (circGRM4) that is significantly upregulated in the eye of cystathionine β -synthase-deficient mice. We also discovered a concurrent over-expression of the mGLUR4 receptor in the eyes of these mice. In brief, circGRM4 is selectively transcribed from its parental mGLUR4 receptor gene (*GRM4*) functions as a “molecular-sponge” for the miRNAs and results into excessive turnover of the mGLUR4 receptor in the eye in response to extremely high circulating glutamate concentration. We opine that this epigenetic manifestation potentially predisposes HHcy people to retinovascular malfunctioning.

Graphic abstract



Molecular sponging of microRNAs (miRNAs) by the circular RNA (circRNA) leads to enhanced mGLUR4 expression. Excessive glutamate levels and a concurrent upregulation of the mGLUR4 receptor lead to severe excitotoxic damage to the cell and its functions.

Keywords Circular RNA · Epigenetics · Gene regulation · Disease pathology · Visual system

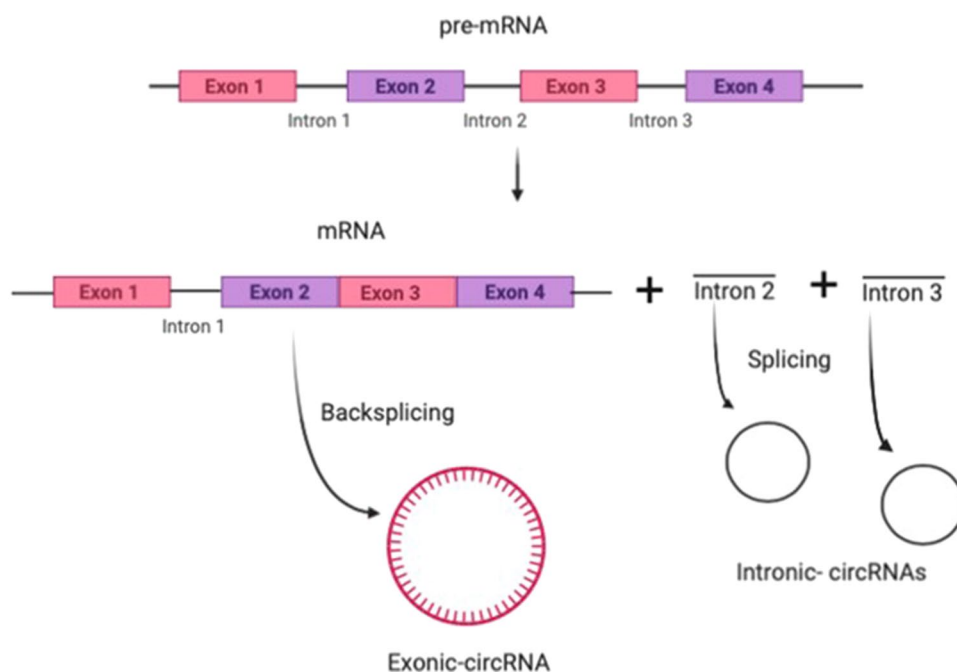
Introduction

The study on how various eye diseases induce blindness has been a low priority for many countries, especially in the developing part of the world [1]. Some examples of retinal diseases affecting vision are diabetic retinopathy (DR) and age-related macular degeneration, AMD [2]. Without a properly functioning retina, phototransduction cannot transpire within the eye. According to the World Health Organization (WHO), more cases of blindness are recognized in the underdeveloped countries. Thus, the examination of this issue in a variety of ways is critical to find solutions for the debilitating, blinding eyes disease such as AMD and DR to negate the increasing cases of blindness. The recent interest in the complexity of gene regulation via epigenetics through a wide range of specific circular RNA (circRNA) transcripts appears to be a missing piece in the link for retinal degradation and vision impairment including blindness. With the recent discovery in the last few years, almost every gene has been shown to produce one or more circular RNA (circRNA) transcript(s) without having any noticeable or significant open-reading frame(s) making it evident that the genomic complexity is far greater complicated than expected, Fig. 1. CircRNAs have been recognized as the major epigenetic regulators in disease pathogenesis via several mechanisms since the production of a circRNA might complete with that of many corresponding linear mRNA molecules. Further, they regulate the gene transcription machinery. Because

the circRNAs contain multiple binding sites for miRNAs that enable them to sequester or buffer effects of various miRNA targets thus modulating the target gene expression by participating in “circRNA-miRNA-mRNA” regulatory network [3–5].

As such, epigenetics is an integral part of gene regulation and its expression since specific genes are conditioned based on the environment or developmental factors [6–11]. Research indicates a strong correlation between hyperhomocysteinemia (HHcy) and the dysregulation of epigenetic mechanisms. Due to its apparent association with “1-carbon metabolism,” Hcy impairs epigenetics through methylation of DNA [12]. This impairment of the DNA can cause improper transcription and translation leading to the over- or under-expression of specific genes and their proteins. Also, during HHcy, an excess concentration of Hcy can increase the amount of glutamate, an excitatory molecule for the neurons. This is because Hcy can act as an agonist to NMDA glutamate receptor, a direct correlation between excitatory neurotransmitter and HHcy [13]. Recent studies have also shown that Hcy complicates the uptake of glutamate in rats and thus potentiates the glutamate cytotoxicity [14]. For this reason, we set out to embark upon the relation between HHcy and glutamate. Since the gene regulation occurs through distinct noncoding RNA species that are required to control the transcriptional and translational aspects. One of the noncoding RNAs (microRNAs or miRNAs) is roughly 20 nucleotides that bind to mRNAs and

Fig. 1 Generation of circular RNAs (circRNAs) via the backsplicing event. A single mRNA molecule can give rise to a variety of circRNAs of different types and sizes such as exonic circRNAs, intronic circRNAs, or even exonic-intronic circRNAs from the same mRNA



thus control the expression of genes. Functionally, miRNAs reduce the levels of their target transcripts, as well as the amount of protein encoded by these transcripts, by direct and imperfect interaction with messenger RNA (mRNA). The microRNAs are mostly intragenic and composed of introns [15]. The circular RNAs (circRNAs) on the other hand have the ability to absorb the miRNAs, and this particular mechanism is referred to as the “molecular-sponging”, causing the deactivation of miRNAs [16]. This is pictorially depicted in the Fig. 2 wherein a circRNA such as circGRM4 recruits miRNAs to avoid impeding the expression of genes. This occurs through specific binding sites on the circRNAs which mobilize specific miRNAs, enabling the mRNA to be translated to form proteins [17].

Glutamate acts as an excitatory neurotransmitter in the retina, but an excessive amount is very toxic to retinal neurons. An accumulation of glutamate within the retina is toxic because it overstimulates the glutamate receptors. The excess glutamate primarily stems from perturbation in the homeostasis of the glutamate metabolism; for example, pathway of metabolism consists of glutamate uptake by glutamate transporters followed by an enzymatic conversion of glutamate to nontoxic glutamine by glutamine synthetase. Since glutamate metabolism requires plenty of energy supply, there is very strong evidence that CBS deficiency creates energy loss thus inhibiting glutamate transporters and glutamine synthetase functions [18–24]. This deficiency in energy is caused by the lack of CBS which is critical because roughly 25% of the glucose in the body helps with the cerebral functions. A lack of glucose can lead to glutamate

being used as another energy source. Glutamate is broken down into carbon dioxide and water through various metabolic pathways. The variety of metabolic pathways include glycogenesis, glycolysis, and pentose phosphate pathway. It is imperative that the energy supply of the brain is continuous because the brain does not have the ability to store energy for later use. The brain requires a lot of energy due to high demand for the maintenance of neurons. Since neurons require significant energy, they rely on high concentrations of neurotransmitters like glutamate which is necessary for the eye as well toward maintaining the functionality of the retina. Without proper regulation of energy metabolism, there can be a rise in eye problems [25].

Significance of circRNAs

The relationship between circRNAs and miRNAs is all dependent on the quality of circRNAs. As illustrated in Fig. 1, circular RNAs (circRNAs) are formed through the back-splicing events of mRNAs. The circRNAs are efficient due to their circularity, allowing them to be resistant to degradation [3]. They also lack both the 5' and 3' ends which increase their survival rate [26]. The circRNAs are 2.5 to 5 times more stable than other linear RNAs [27]. There are various types of circRNA such as exonic circRNAs, intronic circRNAs, and retained intron circRNAs, and their structures vary based on the back splicing of the mRNA [17], (Fig. 1). A majority of circRNAs are found to be in low abundance [28]. This means that high concentrations of circRNAs can



The miRNA Binding Sites with miRNA response elements (MREs) are shown below for the *circGrm4* RNA transcript:

MRE1	MRE2	MRE3	MRE4	MRE5
<u>mmu-miR-7033-3p</u>	<u>mmu-miR-1942</u>	<u>mmu-miR-7659-5p</u>	<u>mmu-miR-6900-3p</u>	<u>mmu-miR-432</u>

Fig. 2 Regulation of genes' activity by the circular RNAs (circRNAs). By recruiting the microRNAs (miRNAs), the circRNAs serve as molecular sponge thereby enhancing the expression of respective

parental genes. The miRNA binding sites with miRNA response elements (MREs) are also shown for the *circGrm4* RNA transcript

lead to the depletion of active miRNAs needed for the regulation of genes (Fig. 2). Also, when there is a downregulation of circRNAs, there appears to be an impairment in the brain functions. Due to these novel qualities, circRNA can effectively participate in the regulation of a host of different forms of miRNAs [29].

Regulation of metabotropic glutamate receptor 4 (mGluR4) by circGrm4 in eyes

A metabotropic receptor is a membrane receptor and acts through a second messenger such as cyclic AMP, cyclic GMP, inositol triphosphate, diacylglycerol, and calcium. On the other hand, primary messengers are extracellular factors, hormones, or neurotransmitters. Based on their functional and structural properties, the receptor for neurotransmitter can be divided into metabotropic receptor and ionotropic receptor. The neurotransmitter focused in this manuscript is glutamate. Briefly, an ionic receptor forms an ion channel, but the metabotropic receptor is indirectly connected with the ion channel on the plasma membrane through signal transduction more often with the G proteins. Therefore, G protein-coupled receptors are metabotropic receptors. Both metabotropic and ionotropic receptors are activated by neurotransmitters. When an ionic receptor is activated, it opens a channel allowing the flow of Na^+ , K^+ , or Cl^- ions into the cell. However; when a metabotropic receptor is activated, a series of intracellular events are triggered that also results in ion channels opening or other intracellular events but would involve a range of second messengers or chemicals. Metabotropic glutamate receptors being the GPCRs can be broken down into 3 different groups. The mGluR4 falls into the group III category of metabotropic glutamate receptors. Like all glutamate receptors, the mGluRs bind with glutamate, an amino acid that functions as an excitatory neurotransmitter (Fig. 3). Thus, studying and managing the mGluR4 are crucial because it controls glutamatergic transmission and acts as a presynaptic heteroreceptor. Activation of the mGluR4 mediates a slow glutamate response through the synapse, and it is usually activated in low concentrations of glutamate, meaning that an excess of glutamate is extremely toxic to the cells [30]. Functionality of activation of the mGluR4 mediates a slow glutamate response through the synapse (Fig. 3). Further, it is critical due to its expression in oligodendrocytes as it helps stabilize the formation of myelin in the central nervous system (CNS) [31]. Glutamate receptors are known to play crucial roles in CNS and are implicated in various brain disorders such as in pathogenesis of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). However, their exact pathophysiological characteristics are still not fully understood in ocular disorders. Since the

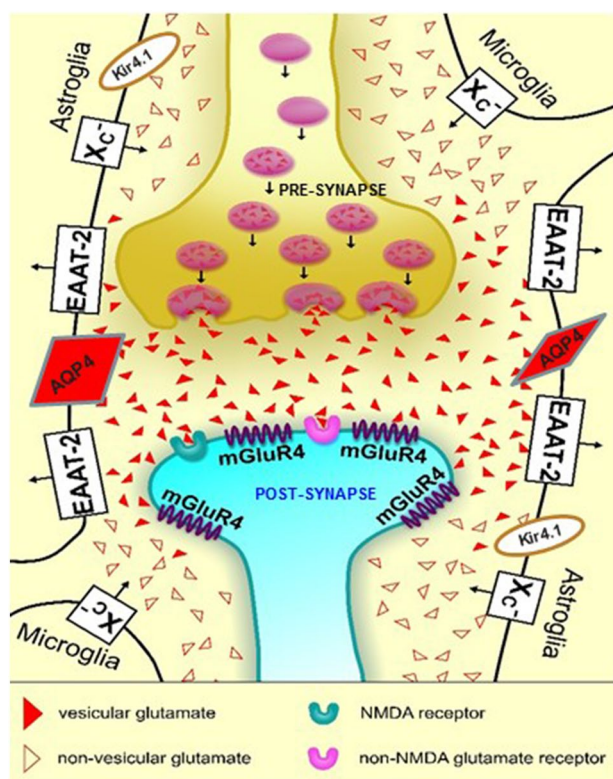


Fig. 3 Glutamate metabolism. Vesicular glutamate is synaptically released and binds to receptors (ionotropic and metabotropic glutamate receptors) at the post-synapse. Glutamate is rapidly taken up by astrocytic glutamate transporters, especially EAAT2. Spillover from the synaptic cleft can activate peri-synaptic receptors. Microglia and astrocytes nonvesicularly release glutamate into the extra-synaptic extracellular space via system x-c(x-c) where it can activate extra-synaptic receptors. It is important to mention that aquaporin-4 (AQP4) and potassium (Kir4.1) channels are the major players in tripartite synapse

circRNAs are critical components of the gene regulation machinery [32], therefore, the effect of glutamate toxicity for the functionality of the eye appears to be detrimental.

Effect of elevation of circGrm4 on intraocular pressure (IOP) and retinal vasculature

Deficiency of CBS (CBS^{+/-}) enzyme coupled with dysregulation of 1-carbon metabolism leads to high circulating concentrations of glutamate as reflected by increased Hcy and s-adenosylhomocysteine hydrolase (SAHH) levels. Furthermore, enhanced oxidative and endoplasmic reticulum (ER) stress responses disrupt the ocular homeostasis in these CBS mutant mice which have been shown to exhibit higher IOP. Also, the animals show impairment in vision-guided behavior as revealed by novel object recognition test (NORT) and the light dark box test (LDBT) findings. This corroborated

well with the results for diminished amounts of the occludin protein—an integral plasma membrane protein of the cellular tight junctions. The blood-retinal barrier (BRB) was also compromised, in that the vessel leakage became evident [33]. It appears that elevation of *circGrm4* leads to enhanced expression of mGLUR4 receptor in order to cope-up with the glutamate neurotoxicity as a compensatory mechanism in association with extracellular matrix metalloproteinase inducer (EMMPRIN), also known as basigin or CD147 which is located on chromosome 19. It is a glycoprotein that is enriched on the surface of cells and stimulates production of several matrix metalloproteinases. There have been studies showing that mice deficient in CD147/basigin (*Bsg*) have malfunctioning retinae because of the plasma membranes' failure to integrate through multiple chain triglycerides [34]. The transmembrane domain of the CD147 also contains a glutamate residue. It is worth mentioning that CD147 can be harmed when there is a potent concentration of glutamate [35, 36].

The Wnt/ β -catenin signaling pathway is critical for the development of the eye, and therefore, it is tightly controlled. When this pathway is activated, β -catenin is transported into the mitochondria. It also binds to transcription co-factors leading to transcription of Wnt genes. The Wnt/ β -catenin signaling controls development of eye because of its ability to regulate cell proliferation and fate of the cell. Its presence has been depicted in the lens and retinal pigment epithelium (RPE) development. Whenever there is a depletion of the β -catenin, it can lead to an abnormal lens morphogenesis [37]. In addition, β -catenin has also been seen as a regulator in the glutamate/glutamine cycle wherein it regulates glutamine synthetase positively. Glutamine synthetase has the responsibility of catalyzing glutamate to glutamine, and in that context, if there is an excess of glutamate, then there will be a decrease in the amount of glutamine synthetase available for catalyzation. Since there is a positive correlation between the expression of β -catenin and glutamine synthetase, β -catenin could be exhausted and thus degraded if glutamate toxicity continues for an extended period [38].

In fact, the Wnt/ β -catenin pathway controls the energy metabolism in the brain, and that appears via the indispensable astrocytes. During hypoxic conditions, there can be an upregulation of astrocytes caused by the Wnt/ β -catenin pathway. Due to the abundance of astrocytes in the brain, it is imperative to understand what affects astrocytes. When astrocytes enter a state of hypoxia, the main pathway that affects the astrocytes is the Wnt pathways. Functionality of astrocytes is crucial in maintaining proper neural regulation and in providing the metabolic assistance. Under hypoxic conditions, there is an imbalance within astrocytes which disrupts metabolic homeostasis. Hypoxia can also trigger the Wnt pathway. Also, when astrocytes were placed in hypothermic conditions, then inhibition of the Wnt pathway was

evident. This inhibition could have been activated through the inhibition of β -catenin [39]. There has been a correlation established between Wnt/ β -catenin pathways and mGLUR1. The loss of mGLUR1 can cause Wnt to activate β -catenin. Although there has not been a direct correlation between *Grm4* and Wnt pathways, it has been shown that *Grm4* can mitigate medulloblastomas through suppression of granule cell neuroprogenitors. This is significant because canonical Wnt has also been linked to medulloblastomas. It is, therefore, suggested that continued exploration of *Grm4* and Wnt pathway may lead to an underlying connection between the two [40].

It should be noted that HHcy leads to neointimal hyperplasia and stroke [41, 42]. Further, moderate levels of HHcy are linked with an increased incidence of stenosis in carotid artery [43]. Many examples are there wherein HHcy has been shown to promote vascular smooth muscle proliferation and hyperplasia in vivo such as in minipigs with HHcy and in vitro findings from rat aortic smooth muscle cells [44, 45]. It has been documented that hypertrophy of cerebral vessels is a potential risk factor for stroke, and in this context, several morphological changes have been observed in the vessel walls of CBS-deficient mice [46]. Interestingly, cerebral arteriolar walls from CBS^{+/-} mice are ~25% thicker than the littermate control mice, and these vessels had lower ratios of nondistensible (collagen and basement membrane) to distensible (elastin and smooth muscle) components. Hence, CBS^{+/-} vessel walls hypertrophy was associated with a weaker myogenic response and was also accompanied by an increase in vessel distensibility during maximal dilatation with EDTA [47]. Increased extracellular matrix fiber deposition, elevated blood pressure, increased wall thickness, and fragmented elastic fibers have been demonstrated in the aortas of CBS^{+/-} mice on a methionine-enriched diet (simulating the red meat diet) compared with the control littermates [48]. These findings were attributed to matrix remodeling by increased expression and activities of matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9 [49].

Glutamate toxicity and the role of mechanistic target of rapamycin (mTOR) and regulation in development and DNA damage responses 1 (REDD1) molecules

Glutamate toxicity also affects the expression levels of both the mechanistic target of rapamycin (mTOR) and the regulation in development and DNA damage responses 1 (REDD1). REDD1 is an important regulator of Akt and mTOR, and plays a key role in neuronal function and survival. The protein mTOR is downregulated when amino acids are deficient, and its signaling pathway is important for the CNS) [50]. When there is an inhibition of mTOR, then

there appears to be a blockage within the NMDA receptor-dependent dendritic green fluorescent protein (GFP) synthesis meaning that there is a relationship between mTOR and glutamate receptor(s); however, the extent of this correlation has not been explored in detail especially in the eye [51]. REDD1 works as an inhibitor to the mTOR during hypoxic stress, and REDD1 degradation is crucially required for the restoration of mTOR signaling as cells recover from hypoxic stress [52]. During chronic stress, the expression of mTOR decreases despite the upregulation of REDD1 [53]. Also, in stressful situations, cell growth is depleted, and this leads to the inhibition of mTOR [54]. When glutamate toxicity affects mTOR, then it also simultaneously affects REDD1. There have been studies on an elevated expression of REDD1 in the retina during the stressful hyperglycemic environment, for example, in the diabetic mice, thus again supporting an important role for REDD1 in the diabetes-induced retinal neurodegeneration [55].

DNA methyltransferase 1 (DNMT1) and tight junction proteins (TJPs) and their implications in the retina during glutamate toxicity

Another issue which arises with the increase in glutamate levels is the changes in the expression of DNMT1, peripherin, cadherin, and occludin. All of these are tightly interlinked in the pathogenesis of retinal malfunctioning. First, DNMT1 gene responsibly creates DNMT1 that regulates DNA methylation. It is well known that DNA methylation is highly important in cellular functions including in determining whether a particular gene is remains to “on” or be suppressed (gene silencing), thereby intimately regulating reactions involving proteins and fats (lipids) and controlling the processing of chemicals that relay signals in the nervous system (neurotransmitters). DNMT1 is very active in the nervous system. Although not much is well understood in the eye itself, the enzyme helps regulate nerve cell (neuron) maturation and specialization (differentiation), the ability of neurons to move (migrate) and connect with each other, and most importantly the neuron survival. There are recent studies in schizophrenic patients showing an increase in DNMT1 binding to the glutamatergic promoters [56]. The latter could potentially mean that there could be an increase in the expression of DNMT1 in enriched glutamic environments. Next, the peripherin is a glycoprotein found within the photoreceptors, forming and stabilizing the rods and cones [57] that gene encodes a cytoskeletal protein found in neurons of the peripheral nervous system. Peripherin gene (PRPH) encodes a protein which is a type-III intermediate filament protein with homology to other cytoskeletal proteins, and any mutation(s) in it can lead to CNS disorders

such as amyotrophic lateral sclerosis affecting the nerves within the brain; however, not much is known about its functionality in the eye.

Another protein severely affected by the glutamate toxicity is cadherin that is usually found with the retinal-pigmented epithelium (RPE) layer of the retina. It keeps the integrity of the epithelial cells by acting as an adhesive junction between the epithelial cells. It depends on calcium to properly adhere the cells to one another [58]. Calcium fluctuations have led to glutamate toxicity through voltage-gated calcium ion channels [59]. Glutamate toxicity leads to an increase in calcium ions by overstimulating the glutamate receptors [60]. The increase in calcium has detrimental effects on cadherin due to its functionality as it relies on calcium concentrations.

Lastly, occludin, a 65-kDa tetraspan integral membrane protein, contributes to tight junction (TJ) stabilization and optimal barrier function as an intercellular tight junction molecule thus providing a reliable connection between the adjacent epithelial cells. TJ is a complex molecular architecture and involves a regulated co-interaction of cytoplasmic adaptor proteins such as zonula occludens and integral membrane linker proteins like the occludin and claudins. They offer a structural integrity to epithelial and endothelial cells, and help create the highly polarized barrier(s) that is essential to homeostatic maintenance within the vertebrate physiological systems, while their dysregulation during degenerative or inflammatory changes is an established pathophysiological hallmark of various systems' diseases. The junctional complex itself is a highly dynamic signaling structure/entity wherein the participant proteins constantly undergo a blend of regulatory modifications in response to diverse physiological/pathophysiological cues, ultimately diversifying the overall adhesive properties of the TJ. In instances of high glutamine levels, glutamate can potentially break down the blood–brain barrier (BBB), leading to the degradation of these TJPs such as occludin causing to a decrease in the amount of TJPs which are essential to the functionality of the retina as a whole [61, 62].

Microvascular implications of glutamate toxicity in eyes

Major issues arising due to glutamate toxicity can have effects on macro- and microblood vessels' functioning. In this context, direct associations have been documented between the increased risk of coronary heart disease (CHD) vis-a-vis glutamate toxicity and also the enhancement of atherosclerosis [63, 64]. Briefly, atherosclerosis occurs when there is an imbalance in lipid metabolism leading to the accumulation of macrophages within the affected blood vessels in the host [65]. Glutamate has been found

to affect macrophage and atherogenicity in the blood vessels by altering triglyceride metabolism in the affected cells. Glutamate is known to be a pro-atherogenic agent due to its ability to stimulate the accumulation of triglyceride in the cell types such as macrophages [66]. The blood vessels accumulate macrophage-derived foam cells which lead to blockage/occlusion within the retinal blood vessels [64]. If there is a blockage in the retinal blood vessels, this eventually could lead to an increase in the intraocular pressure (IOP). This can occur as a result of the build-up of blood within the retinal parenchyma. Glutamate levels have been found to be one of the main differences between those with coronary artery disease patients and the non-coronary artery disease patients. It has also been seen that the stimulation of glutamatergic pathways can induce both the contraction and restriction of the blood vessels through the increase in calcium ions. Microvascular dysfunction, especially in the eye, can be a result of glutamate toxicity due to the harmful effects of excess glutamate [67]. An increase in calcium levels can lead to a subsequent increase in IOP as well. Further, when there is an accumulation of blood, this can invariably be caused by water accumulation via the aquaporin-4 (AQP4) since AQP4 has the ability to control water contents within both the blood–brain and blood–spinal barriers. During an interaction between AQP4 and calmodulin (CaM), a shift in the structure of AQP4 takes place. Consequently, an increase in calcium ions can certainly initiate CaM which in turn causes a specific conformational change in the shape of AQP4 allowing for an accumulation of water. An increase in water influx could cause a breakdown of the blood–brain barrier as a result of disturbance in the osmotic environment [68].

Impact of glutamate toxicity on mitochondrial fusion and fission

The functionality of the mitochondria is essential to the eye due to its ability to provide the retina with high energy demand through the process of oxidative phosphorylation [69]. If there is damage to the mitochondria, then it can lead to the depletion of energy needed for the retinal cells. Mitochondrial fusion and fission are crucial parts of the structure and biogenesis of the mitochondria. Further, the rate between fusion and fission determines the shape of the mitochondria, which can be affected depending upon the metabolic conditions [70]. For example, fission assists in building new mitochondria, whereas fusion is known to mix damaged mitochondria to alleviate the stressful environment. Drp1 is collected during fission in order to create spirals for the inner and outer membranes of the mitochondria, whereas Mfn2 mediates the fusion of mitochondrial outer membranes [69]. When there is downregulation of

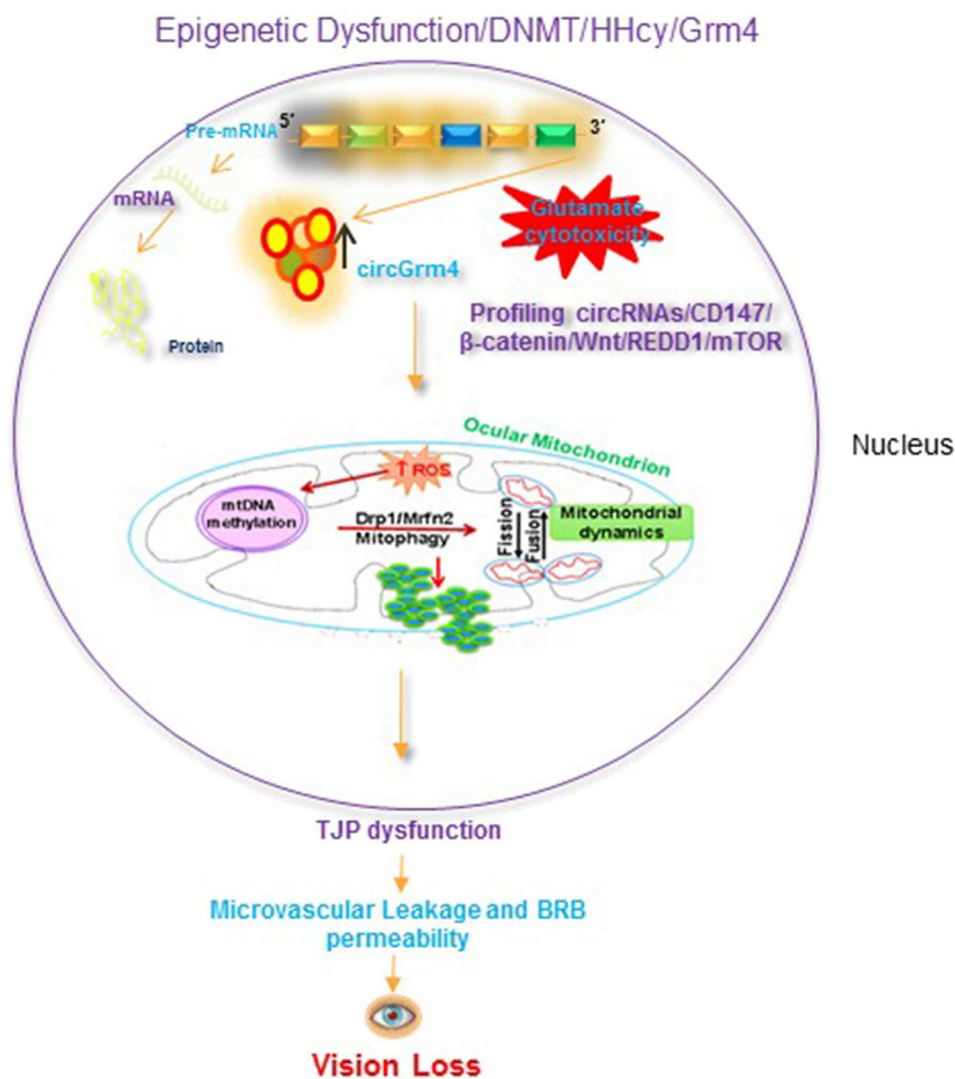
Drp1, then the mitochondrial dysfunction induced by glutamate also decreases and this occurs presumably through the preservation of the mitochondria by ensuring the volume of calcium ions that does not exceed the inherent limit of the mitochondria [71]. Interestingly, glutamate toxicity is known to fluctuate the calcium ion concentrations of the cells, for instance, in high concentrations of the calcium ions, there is a collapse of the mitochondrial membrane potential which leads to the degradation of the mitochondria itself [72]. In simple words, glutamate toxicity can in fact determine the fate of the mitochondria in the retina [24].

Conclusion and perspective

As an indispensable intermediary molecule, the mRNA plays a central role in the flow of genetic information; however, structural and functional versatilities of numerous other RNA species (e.g., catalytic RNAs, small microRNAs, long non-coding RNAs, circular RNAs—circRNAs, etc.) offer crucial support for the cell to function properly. For example, the abundance of relatively less characterized circRNAs has important regulatory functions including development, differentiation, and growth. After initiation of the transcription, the very first step in gene regulation, many downstream events are critically important in establishing the functionality of mature mRNAs and proteins. In fact, it has become increasingly obvious now since the past decade that post-initiation regulatory mechanisms are much more common than previously realized by researchers. These post-initiation regulatory strategies exert their influence over several processes, including transcription elongation, translation efficiency, mRNA stability, and even the ultimate function of proteins. The circRNAs, especially their diverse and multifaceted roles in gene regulation, make them novel candidates for developing them as potentially novel diagnostics and therapeutic targets in human diseases.

Ocular dysfunctionality remains a serious concern due to glutamic toxicity. Therefore, a well-balanced regulation of both the circGrm4 transcripts and the mGLUR4-specific miRNAs is vital due to its effect on abundant aspects of eye functions. Some of the deleterious effects are clearly manifested as glutamate neurotoxicity, disruption of the mitochondrial fusion and fission dynamics, and dysfunction in the microvascular network especially in the retina. Glutamate, being an excitatory neurotransmitter, overexcites the neurons when its concentration is in excess leading to dispatch of messages even when there is no signal present/exists. This happens when there is an absolute excess amount of extracellular glutamate that binds to its receptor(s) [69]. Over-excitation ultimately causes glutamate toxicity, and it is known to induce significant neural damage. Glutamate

Fig. 4 A schematic depicting the implications of circGrm4 over-expression during hyperhomocysteinemia. The circGrm4 induces glutamate neurotoxicity during hyperhomocysteinemia (HHcy) resulting in CD147/Wnt signaling that in turn leads to abnormal mitophagy, blood-retinal barrier (BRB) breakdown, vascular leakage/microvascular dysfunction, ocular hypertension, vision impairment, and finally blindness



toxicity in the eye affects mitochondrial fusion and fission, microvasculature, and even the integrity of TJPs. It can in fact potentially deteriorate the integrity of the BBB in the eye and thus derailment of the microvascular functions [73, 74].

The tight regulation of transcription initiation by transcription factors has long been presumed to be the most important mode of gene-function regulation in the cells so far. However, recent discovery of the circRNAs that are embodied with a significant diversity in their structures, are coupled with highly crucial roles they play including but not limited to the transcriptional regulation that make them truly novel types of diagnostic, and therapeutic targets for a host of metabolic diseases that have no or limited options for cure such as hyperhomocysteinemia (HHcy). Therefore, epigenetics via circRNAs involvement can change the expression of specific genes in the eye, and the epigenetic memory can also be altered through methylation or demethylation

processes. Since circRNAs make them important due to their abilities to regulate gene expression via miRNAs, in part, understanding of how circRNAs in the eye could influence genes/genetic sequences that are responsible for disease causation during chronic metabolic syndromes. For example, if the expression of circRNA or its target is controlled, then expression of the genes can also be controlled successfully in these syndromes. In this way, eyes affected by glutamate toxicity can also be treated if the circGRM4 or its target could be regulated appropriately.

Based on the foregoing discussion, it seems that an increase in circGRM4 levels leads to glutamate toxicity, and this increase is the result of hyperhomocysteinemia (HHcy) since HHcy is responsible for the changes in the epigenetic profile of the affected cells in the eye. The toxic amount of glutamate leads to a decrease in the expression of TJPs such as peripherin, cadherin, and occludin. It also weakens the integrity of the mitochondria based on the irregular rate

of fusion and fission. Not only does the glutamate toxicity affect the cells' tight junctions and mitochondria, but it can also lead to the degradation of the extracellular matrix of the ocular microvasculature. In severe cases, the toxicity can lead to blindness as depicted in Fig. 4. The prevalence of visual impairment increases rapidly with age. According to the National Institutes of Health (NIH), USA, the number of people who will be blind will double in the next 3 decades. By age 60, around 1 in 9 people will be either blind or have moderate-to-severe visual impairment (MSVI). By age 80, the ratio increases considerably: around 1 in 3 people will be either blind or have MSVI. Furthermore, 1.1 billion people are estimated to have functional presbyopia [75]. In 2015, there were 216 million people from 98 countries with moderate-to-severe vision impairment (Flaxman SR, Bourne RRA). Due to blindness growing at exponential rates, we must find newer ways of tackling this epidemic. We believe that recent advances in epigenetics will provide newer insights into the fields of RNA biology in general and diseases of the eye in particular. Keeping pace with the rapid development in the field of RNA "omics"-centered epigenetics and to bring uniformity and consistency among the research community, we recently introduced a new term "cromics" (circular ribonucleic acids-based omics [3, 4].

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Author contributions MS conceived, edited, and finalized the manuscript while WE and AKG worked on the experiments and drafted the manuscript. The other co-authors helped in providing reagents and the feedback in moving the project forward. All authors approved the final version of the manuscript before its submission.

References


1. Yorston D (2003) Retinal diseases and VISION 2020. *Community Eye Health* 16:19–20
2. Malek G, Busik J, Grant MB, Choudhary M (2018) Models of retinal diseases and their applicability in drug discovery. *Expert Opin Drug Discov* 13:359–377. <https://doi.org/10.1080/17460441.2018.1430136>
3. George AK, Master K, Majumder A, Homme RP, Laha A, Sandhu HS, Tyagi SC, Singh M (2019) Circular RNAs constitute an inherent gene regulatory axis in the mammalian eye and brain (1). *Can J Physiol Pharmacol* 97:463–472. <https://doi.org/10.1139/cjpp-2018-0505>
4. Singh M, George AK, Homme RP, Majumder A, Laha A, Sandhu HS, Tyagi SC (2018) Circular RNAs profiling in the cystathionine-beta-synthase mutant mouse reveals novel gene targets for hyperhomocysteinemia induced ocular disorders. *Exp Eye Res* 174:80–92. <https://doi.org/10.1016/j.exer.2018.05.026>
5. Singh M, George AK, Homme RP, Majumder A, Laha A, Sandhu HS, Tyagi SC (2019) Expression analysis of the circular RNA molecules in the human retinal cells treated with homocysteine. *Curr Eye Res* 44:287–293. <https://doi.org/10.1080/02713683.2018.1542005>
6. D'Urso A, Brickner JH (2014) Mechanisms of epigenetic memory. *Trends Genet* 30:230–236. <https://doi.org/10.1016/j.tig.2014.04.004>
7. Chaturvedi P, Tyagi SC (2014) Epigenetic mechanisms underlying cardiac degeneration and regeneration. *Int J Cardiol* 173:1–11. <https://doi.org/10.1016/j.ijcard.2014.02.008>
8. Kalani A, Kamat PK, Tyagi SC, Tyagi N (2013) Synergy of homocysteine, microRNA, and epigenetics: a novel therapeutic approach for stroke. *Mol Neurobiol* 48:157–168. <https://doi.org/10.1007/s12035-013-8421-y>
9. Tyagi SC, Joshua IG (2014) Exercise and nutrition in myocardial matrix metabolism, remodeling, regeneration, epigenetics, microcirculation, and muscle. *Can J Physiol Pharmacol* 92:521–523. <https://doi.org/10.1139/cjpp-2014-0197>
10. Veeranki S, Winchester LJ, Tyagi SC (2015) Hyperhomocysteinemia associated skeletal muscle weakness involves mitochondrial dysfunction and epigenetic modifications. *Biochim Biophys Acta* 1852:732–741. <https://doi.org/10.1016/j.bbdis.2015.01.008>
11. Weber GJ, Pushpakumar S, Tyagi SC, Sen U (2016) Homocysteine and hydrogen sulfide in epigenetic, metabolic and microbiota related renovascular hypertension. *Pharmacol Res* 113:300–312. <https://doi.org/10.1016/j.phrs.2016.09.002>
12. Perla-Kajan J, Jakubowski H (2019) Dysregulation of epigenetic mechanisms of gene expression in the pathologies of hyperhomocysteinemia. *Int J Mol Sci*. <https://doi.org/10.3390/ijms20133140>
13. Moretti R, Caruso P (2019) The controversial role of homocysteine in neurology: from labs to clinical practice. *Int J Mol Sci*. <https://doi.org/10.3390/ijms20010231>
14. Machado FR, Ferreira AG, da Cunha AA, Tagliari B, Mussulini BH, Wofchuk S, Wyse AT (2011) Homocysteine alters glutamate uptake and Na⁺, K⁺-ATPase activity and oxidative status in rats hippocampus: protection by vitamin C. *Metab Brain Dis* 26:61–67. <https://doi.org/10.1007/s11011-011-9232-3>
15. O'Brien J, Hayder H, Zayed Y, Peng C (2018) Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. *Front Endocrinol (Lausanne)* 9:402. <https://doi.org/10.3389/fendo.2018.00402>
16. Yang S, Sun Z, Zhou Q, Wang W, Wang G, Song J, Li Z, Zhang Z, Chang Y, Xia K, Liu J, Yuan W (2018) MicroRNAs, long non-coding RNAs, and circular RNAs: potential tumor biomarkers and targets for colorectal cancer. *Cancer Manag Res* 10:2249–2257. <https://doi.org/10.2147/cmar.S166308>
17. Rong D, Sun H, Li Z, Liu S, Dong C, Fu K, Tang W, Cao H (2017) An emerging function of circRNA-miRNAs-mRNA axis in human diseases. *Oncotarget* 8:73271–73281. <https://doi.org/10.18632/oncotarget.19154>
18. Ishikawa M (2013) Abnormalities in glutamate metabolism and excitotoxicity in the retinal diseases. *Scientifica (Cairo)* 2013:528940. <https://doi.org/10.1155/2013/528940>
19. Majtan T, Park I, Bublil EM, Kraus JP (2018) Enzyme replacement therapy prevents loss of bone and fat mass in murine homocystinuria. *Hum Mutat* 39:210–218. <https://doi.org/10.1002/humu.23360>
20. Scott JW, Hawley SA, Green KA, Anis M, Stewart G, Scullion GA, Norman DG, Hardie DG (2004) CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. *J Clin Invest* 113:274–284. <https://doi.org/10.1172/jci19874>
21. Gupta S, Kruger WD (2011) Cystathionine beta-synthase deficiency causes fat loss in mice. *PLoS ONE* 6:e27598. <https://doi.org/10.1371/journal.pone.0027598>
22. Kruger WD, Gupta S (2016) The effect of dietary modulation of sulfur amino acids on cystathionine beta synthase-deficient

- mice. *Ann N Y Acad Sci* 1363:80–90. <https://doi.org/10.1111/nyas.12967>
23. Koutmos M, Kabil O, Smith JL, Banerjee R (2010) Structural basis for substrate activation and regulation by cystathionine beta-synthase (CBS) domains in cystathionine [beta]-synthase. *Proc Natl Acad Sci USA* 107:20958–20963. <https://doi.org/10.1073/pnas.1011448107>
 24. Albertini E, Koziel R, Durr A, Neuhaus M, Jansen-Durr P (2012) Cystathionine beta synthase modulates senescence of human endothelial cells. *Aging (Albany NY)* 4:664–673. <https://doi.org/10.18632/aging.100491>
 25. Bordone MP, Salman MM, Titus HE, Amini E, Andersen JV, Chakraborti B, Diuba AV, Dubouskaya TG, Ehrke E, Espindola de Freitas A et al (2019) The energetic brain. *J Neurochem* 151(2):139–165. <https://doi.org/10.1111/jnc.14829>
 26. Sun J, Li B, Shu C, Ma Q, Wang J (2020) Functions and clinical significance of circular RNAs in glioma. *Mol Cancer* 19:34. <https://doi.org/10.1186/s12943-019-1121-0>
 27. Sekar S, Liang WS (2019) Circular RNA expression and function in the brain. *Noncoding RNA Res* 4:23–29. <https://doi.org/10.1016/j.ncrna.2019.01.001>
 28. Lasda E, Parker R (2014) Circular RNAs: diversity of form and function. *RNA* 20:1829–1842. <https://doi.org/10.1261/rna.047126.114>
 29. Piwecka A, Glazar P, Hernandez-Miranda LR, Memczak S, Wolf SA, Rybak-Wolf A, Filipchuk A, Klironomos F, Cerda Jara CA, Fenske P, Trimbuch T, Zywitzka V, Plass M, Schreyer L, Ayoub S, Kocks C, Kuhn R, Rosenmund C, Birchmeier C, Rajewsky N (2017) Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function. *Science*. <https://doi.org/10.1126/science.aam8526>
 30. Cartmell J, Schoepp DD (2000) Regulation of neurotransmitter release by metabotropic glutamate receptors. *J Neurochem* 75:889–907. <https://doi.org/10.1046/j.1471-4159.2000.0750889.x>
 31. Crupi R, Impellizzeri D, Cuzzocrea S (2019) Role of metabotropic glutamate receptors in neurological disorders. *Front Mol Neurosci* 12:20. <https://doi.org/10.3389/fnmol.2019.00020>
 32. Wery M, Kwapisz M, Morillon A (2011) Noncoding RNAs in gene regulation. *Wiley Interdiscip Rev Syst Biol Med* 3:728–738. <https://doi.org/10.1002/wsbm.148>
 33. George AK, Homme RP, Majumder A, Laha A, Metreveli N, Sandhu HS, Tyagi SC, Singh M (2019) Hydrogen sulfide intervention in cystathionine-beta-synthase mutant mouse helps restore ocular homeostasis. *Int J Ophthalmol* 12:754–764. <https://doi.org/10.18240/ijo.2019.05.09>
 34. Muramatsu T, Miyauchi T (2003) Basigin (CD147): a multifunctional transmembrane protein involved in reproduction, neural function, inflammation and tumor invasion. *Histol Histopathol* 18:981–987. <https://doi.org/10.14670/hh-18.981>
 35. Wei M, Li H, Shang Y, Zhou Z, Zhang J (2014) Increased CD147 (EMMPRIN) expression in the rat brain following traumatic brain injury. *Brain Res* 1585:150–158. <https://doi.org/10.1016/j.brainres.2014.06.018>
 36. Grass GD, Toole BP (2015) How, with whom and when: an overview of CD147-mediated regulatory networks influencing matrix metalloproteinase activity. *Biosci Rep* 3:e00283. <https://doi.org/10.1042/bsr20150256>
 37. Fujimura N (2016) WNT/beta-catenin signaling in vertebrate eye development. *Front Cell Dev Biol* 4:138. <https://doi.org/10.3389/fcell.2016.00138>
 38. Lutgen V, Narasipura SD, Sharma A, Min S, Al-Harathi L (2016) beta-Catenin signaling positively regulates glutamate uptake and metabolism in astrocytes. *J Neuroinflammation* 13:242. <https://doi.org/10.1186/s12974-016-0691-7>
 39. Salman MM, Kitchen P, Woodroffe MN, Bill RM, Conner AC, Heath PR, Conner MT (2017) Transcriptome analysis of gene expression provides new insights into the effect of mild therapeutic hypothermia on primary human cortical astrocytes cultured under hypoxia. *Front Cell Neurosci* 2017(11):386. <https://doi.org/10.3389/fncel.2017.00386>
 40. Teh J, Chen S (2012) Metabotropic glutamate receptors and cancerous growth. *WIREs Membr Transp Signal* 1:211–220. <https://doi.org/10.1002/wmts.21>
 41. Eikelboom JW, Hankey GJ, Anand SS, Lofthouse E, Staples N, Baker RI (2000) Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. *Stroke* 31:1069–1075. <https://doi.org/10.1161/01.str.31.5.1069>
 42. Liu F, Zhang J, Yu S, Wang R, Wang B, Lai L, Yin H, Liu G (2008) Effect of folate on neointima formation and matrix metalloproteinase-9 expression after balloon injury in hyperhomocysteinemic rabbits. *Int J Cardiol* 131:59–65. <https://doi.org/10.1016/j.ijcard.2007.08.138>
 43. Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PW, Belanger AJ, O'Leary DH, Wolf PA, Schaefer EJ, Rosenberg IH (1995) Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med* 332:286–291. <https://doi.org/10.1056/nejm199502023320502>
 44. Rolland PH, Friggi A, Barlatier A, Piquet P, Latrille V, Faye MM, Guillou J, Charpiot P, Bodard H, Ghiringhelli O et al (1995) Hyperhomocysteinemia-induced vascular damage in the minipig. Captopril-hydrochlorothiazide combination prevents elastic alterations. *Circulation* 91:1161–1174. <https://doi.org/10.1161/cir.91.4.1161>
 45. Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, Lee ME (1994) Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci USA* 91:6369–6373. <https://doi.org/10.1073/pnas.91.14.6369>
 46. Iadecola C, Davisson RL (2008) Hypertension and cerebrovascular dysfunction. *Cell Metab* 7:476–484. <https://doi.org/10.1016/j.cmet.2008.03.010>
 47. Baumbach GL, Sigmund CD, Bottiglieri T, Lentz SR (2002) Structure of cerebral arterioles in cystathionine beta-synthase-deficient mice. *Circ Res* 91:931–937. <https://doi.org/10.1161/01.res.0000041408.64867.1d>
 48. Ovechkin AV, Tyagi N, Sen U, Lominadze D, Steed MM, Moshal KS, Tyagi SC (2006) 3-Deazaadenosine mitigates arterial remodeling and hypertension in hyperhomocysteinemic mice. *Am J Physiol Lung Cell Mol Physiol* 291:L905–L911. <https://doi.org/10.1152/ajplung.00543.2005>
 49. Beard RS Jr, Bearden SE (2011) Vascular complications of cystathionine beta-synthase deficiency: future directions for homocysteine-to-hydrogen sulfide research. *Am J Physiol Heart Circ Physiol* 300:H13–26. <https://doi.org/10.1152/ajpheart.00598.2010>
 50. Chong ZZ, Shang YC, Zhang L, Wang S, Maiese K (2010) Mammalian target of rapamycin: hitting the bull's-eye for neurological disorders. *Oxid Med Cell Longev* 3:374–391. <https://doi.org/10.4161/oxim.3.6.14787>
 51. Gong R, Park CS, Abbassi NR, Tang SJ (2006) Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. *J Biol Chem* 281:18802–18815. <https://doi.org/10.1074/jbc.M512524200>
 52. Katiyar S, Liu E, Knutzen CA, Lang ES, Lombardo CR, Sankar S, Toth JJ, Petroski MD, Ronai Z, Chiang GG (2009) REDD1, an inhibitor of mTOR signalling, is regulated by the CUL4A-DDB1 ubiquitin ligase. *EMBO Rep* 10:866–872. <https://doi.org/10.1038/embor.2009.93>
 53. Duman RS, Sanacora G, Krystal JH (2019) Altered connectivity in depression: GABA and glutamate neurotransmitter deficits

- and reversal by novel treatments. *Neuron* 102:75–90. <https://doi.org/10.1016/j.neuron.2019.03.013>
54. Tan CY, Hagen T (2013) mTORC1 dependent regulation of REDD1 protein stability. *PLoS ONE* 8:e63970. <https://doi.org/10.1371/journal.pone.0063970>
 55. Miller WP, Yang C, Mihailescu ML, Moore JA, Dai W, Barber AJ, Dennis MD (2018) Deletion of the Akt/mTORC1 repressor REDD1 prevents visual dysfunction in a rodent model of Type 1 diabetes. *Diabetes* 67:110–119. <https://doi.org/10.2337/db17-0728>
 56. Dong E, Ruzicka WB, Grayson DR, Guidotti A (2015) DNA-methyltransferase1 (DNMT1) binding to CpG rich GABAergic and BDNF promoters is increased in the brain of schizophrenia and bipolar disorder patients. *Schizophr Res* 167:35–41. <https://doi.org/10.1016/j.schres.2014.10.030>
 57. Kalyanasundaram TS, Black GC, O'Sullivan J, Bishop PN (2009) A novel peripherin/RDS mutation resulting in a retinal dystrophy with phenotypic variation. *Eye (Lond)* 23:237–239. <https://doi.org/10.1038/eye.2008.33>
 58. Yang X, Chung JY, Rai U, Esumi N (2018) Cadherins in the retinal pigment epithelium (RPE) revisited: P-cadherin is the highly dominant cadherin expressed in human and mouse RPE in vivo. *PLoS ONE* 13:e0191279. <https://doi.org/10.1371/journal.pone.0191279>
 59. Sucher NJ, Lipton SA, Dreyer EB (1997) Molecular basis of glutamate toxicity in retinal ganglion cells. *Vision Res* 37:3483–3493. [https://doi.org/10.1016/s0042-6989\(97\)00047-3](https://doi.org/10.1016/s0042-6989(97)00047-3)
 60. Mizuno F, Barabas P, Krizaj D, Akopian A (2010) Glutamate-induced internalization of Ca(v)1.3 L-type Ca(2+) channels protects retinal neurons against excitotoxicity. *J Physiol* 588:953–966. <https://doi.org/10.1113/jphysiol.2009.181305>
 61. Cummins PM (2012) Occludin: one protein, many forms. *Mol Cell Biol* 32:242–250. <https://doi.org/10.1128/mcb.06029-11>
 62. Andras IE, Deli MA, Veszelka S, Hayashi K, Hennig B, Toborek M (2007) The NMDA and AMPA/KA receptors are involved in glutamate-induced alterations of occludin expression and phosphorylation in brain endothelial cells. *J Cereb Blood Flow Metab* 27:1431–1443. <https://doi.org/10.1038/sj.jcbfm.9600445>
 63. Paynter NP, Balasubramanian R, Giulianini F, Wang DD, Tinker LF, Gopal S, Deik AA, Bullock K, Pierce KA, Scott J, Martinez-Gonzalez MA, Estruch R, Manson JE, Cook NR, Albert CM, Clish CB, Rexrode KM (2018) Metabolic predictors of incident coronary heart disease in women. *Circulation* 137:841–853. <https://doi.org/10.1161/circulationaha.117.029468>
 64. Grajeda-Iglesias C, Aviram M (2018) Specific amino acids affect cardiovascular diseases and atherogenesis via protection against macrophage foam cell formation: review article. *Rambam Maimonides Med J*. <https://doi.org/10.5041/rmmj.10337>
 65. Moore KJ, Sheedy FJ, Fisher EA (2013) Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol* 13:709–721. <https://doi.org/10.1038/nri3520>
 66. Rom O, Grajeda-Iglesias C, Najjar M, Abu-Saleh N, Volkova N, Dar DE, Hayek T, Aviram M (2017) Atherogenicity of amino acids in the lipid-laden macrophage model system in vitro and in atherosclerotic mice: a key role for triglyceride metabolism. *J Nutr Biochem* 45:24–38. <https://doi.org/10.1016/j.jnutbio.2017.02.023>
 67. Rancillac A, Rossier J, Guille M, Tong XK, Geoffroy H, Amatore C, Arbault S, Hamel E, Cauli B (2006) Glutamatergic control of microvascular tone by distinct GABA neurons in the cerebellum. *J Neurosci* 26:6997–7006. <https://doi.org/10.1523/jneurosci.5515-05.2006>
 68. Kitchen P, Salman MM, Halsey AM, Clarke-Bland C, MacDonald JA, Ishida H, Vogel HJ, Almutiri S, Logan A, Kreida S, Al-Jubair T, Missel JW, Gourdon P et al (2020) Targeting aquaporin-4 sub-cellular localization to treat central nervous system edema. *Cell* 181(4):784–799. <https://doi.org/10.1016/j.cell.2020.03.037>
 69. Youle RJ, van der Blik AM (2012) Mitochondrial fission, fusion, and stress. *Science* 337:1062–1065. <https://doi.org/10.1126/science.1219855>
 70. Van der Blik AM, Shen Q, Kawajiri S (2013) Mechanisms of mitochondrial fission and fusion. *Cold Spring Harb Perspect Biol*. <https://doi.org/10.1101/cshperspect.a011072>
 71. Zhang C, Yuan XR, Li HY, Zhao ZJ, Liao YW, Wang XY, Su J, Sang SS, Liu Q (2014) Downregulation of dynamin-related protein 1 attenuates glutamate-induced excitotoxicity via regulating mitochondrial function in a calcium dependent manner in HT22 cells. *Biochem Biophys Res Commun* 443:138–143. <https://doi.org/10.1016/j.bbrc.2013.11.072>
 72. Vaarmann A, Kovac S, Holmstrom KM, Gandhi S, Abramov AY (2013) Dopamine protects neurons against glutamate-induced excitotoxicity. *Cell Death Dis* 4:e455. <https://doi.org/10.1038/cddis.2012.194>
 73. Lewerenz J, Maher P (2015) Chronic glutamate toxicity in neurodegenerative diseases-what is the evidence? *Front Neurosci* 9:469. <https://doi.org/10.3389/fnins.2015.00469>
 74. Lee KE, Kang YS (2018) L-Citrulline restores nitric oxide level and cellular uptake at the brain capillary endothelial cell line (TR-BBB cells) with glutamate cytotoxicity. *Microvasc Res* 120:29–35. <https://doi.org/10.1016/j.mvr.2018.05.010>
 75. Ackland P, Resnikoff S, Bourne R (2017) World blindness and visual impairment: despite many successes, the problem is growing. *Community Eye Health* 30:71–73

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