

## LncRNAs in genetic basis of glaucoma

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**ABSTRACT**

Glaucoma is an umbrella term used to designate a heterogeneous group of ocular disorders characterised by progressive excavation of the optic disc, optic atrophy and gradual loss of the visual field caused by the slow death of retinal ganglion cells and their axons. Glaucoma can potentially lead to blindness if left untreated. It usually starts from the periphery and progresses gradually toward the centre of the visual field. Vision loss caused by glaucoma is irreversible and causes a heavy burden on affected families and society, therefore the importance of early diagnosis and prevention should be emphasised. Genetic factors appear to play a role in glaucoma pathogenesis; it has been shown that individuals with a positive family history are at a greater risk because they are more likely predisposed to be affected. Notable advances have been recorded in the past decade concerning the genetic and environmental factors likely to contribute or cause glaucoma with the discovery of multiple glaucoma-associated genes and genetic loci. Thorough investigations by a handful of studies on the function of long non-coding RNAs discovered that, although lacking protein-coding potential, lncRNAs can still participate in the regulation of gene expression at various levels, thus their possible implication in different disease aetiologies. In this review, we focus on the lncRNAs characteristics and its regulation, and summarise these results from separate, independent, glaucoma-related studies in addition to discussing possible pathways by which lncRNAs might contribute to glaucoma.

**INTRODUCTION**

Glaucoma is an umbrella term used to designate a heterogeneous group of ocular disorders characterised by optic nerve damage that leads to vision loss and ultimately blindness if untreated and; usually starts from the periphery and progresses gradually towards the centre of the visual field.<sup>1</sup>

The number of people with glaucoma (primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG) combined) worldwide was estimated to be around 60.5 million in 2010. This number will increase significantly in the future, and it has been estimated that by 2020, approximately 79.6 million people will be affected, including 3 million in the USA.<sup>2-3</sup> According to recent studies, the number of people affected by glaucoma worldwide will reach 111.8 million by 2040, with Africa and Asia being most heavily affected.<sup>4</sup> Chinese patients with PACG account for nearly half of PACG

cases worldwide.<sup>3</sup> According to the WHO, it is currently the leading cause of irreversible blindness worldwide, despite the existence of an indisputable variation in its subtypes and risks among races and countries.<sup>5</sup>

Vision loss caused by glaucoma is irreversible and causes a heavy burden on affected families and society; therefore, the importance of early diagnosis through relevant examinations of suspicious cases to facilitate early detection and prevention; and avoid high intraocular pressure (IOP)-associated damage to the optic nerve should be emphasised.

Genetic factors seem to play an undeniable role in glaucoma pathogenesis; it has been shown that individuals with a positive family history are at a greater risk because they have a predisposition towards the condition.<sup>6-7</sup> Since the discovery of multiple glaucoma-associated genes and genetic loci in the past decade, notable advances have been recorded concerning the genetic and environmental factors likely to contribute to or cause glaucoma.<sup>8-10</sup>

Recent recognition of the crucial functional importance of the non-coding region of the genome in normal development and physiology has focused increasing attention on its potential to contribute towards disease aetiology. lncRNAs are defined as non-protein-coding transcripts with a size ranging from 200 to 100 000 nt; although lacking protein-coding potential, lncRNAs can still participate as RNA in the regulation of gene expression at various levels and are reportedly implicated in different disease aetiologies.<sup>11</sup>

Despite the fact that lncRNAs have been said to be implicated in normal neural development<sup>12</sup> and progression of neurodegenerative diseases, including Alzheimer disease,<sup>13</sup> and previously linked to multiple disease aetiologies, the proper connections and pathways they may use to influence the susceptibility to developing glaucoma, which is also characterised by nerve damage, have not yet been completely elucidated.

In this article, we review underlining mechanisms and risk factors for developing glaucoma, focus on the characteristics and regulation of lncRNAs and aim to



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demonstrate their implication in glaucoma pathogenesis by summarising results from separate, independent, glaucoma-related studies.

### GENETIC ASSOCIATIONS OF LNCRNA AND GLAUCOMA CDKN2B-AS1

There are several genes in the 9p21 genomic region: CDKN2B-AS1, also known as ANRIL (antisense non-coding RNA in the INK4 locus); cyclin-dependent kinase inhibitor 2B (CDKN2B) and cyclin-dependent kinase inhibitor 2A (CDKN2A).<sup>14</sup>

Recent genome-wide association studies (GWAS) have remarkably shown the association of single nucleotide polymorphisms (SNPs) in this region with various human diseases, including notably, coronary artery disease,<sup>15</sup> diabetes,<sup>16</sup> ischaemic stroke,<sup>17</sup> cranial and aortic aneurysm,<sup>18</sup> glioma,<sup>19</sup> malignant melanoma<sup>20</sup> as well as many cancers.<sup>19</sup>

CDKN2B-AS1 is a long non-coding RNA in the antisense direction compared with CDKN2A and CDKN2B.<sup>21</sup> Its exact biological function is largely unknown. The molecular mechanisms underlying the association between ANRIL and POAG are still not well understood.<sup>22</sup> The occurrence of polymorphisms at these loci may alter the expression of the target genes responsible for regulating the cell cycle or act through epigenetic mechanisms, subsequently inducing a tendency towards retinal ganglion cell (RGC) apoptosis and glaucoma.<sup>23–25</sup> Another study identified associations between 9p21 variants and glaucoma features, suggesting that the ANRIL region modifies the vulnerability of the optic nerve to cause glaucomatous change, further implying the role of ANRIL in modulating optic nerve degeneration.<sup>26</sup>

#### Glaucoma-associated variants

Numerous genetic variations have been associated with an increased risk of POAG in the past decade. The corresponding SNPs have been identified by different GWAS and are located in and around the CDKN2B-AS1.<sup>24 27 28</sup> Actually, each glaucoma-associated SNP does in fact possess two alleles: one associated with a decreased POAG risk (protective variants) and the other one associated with an increased POAG risk (risk variants). Variants such as rs7049105 and rs2151280 were not only associated with a reduced POAG risk, but have also been linked with a smaller cup-to-disc ratio (CDR) observed at disease presentation. Although primarily associated with a reduced risk in case-control studies, an association was also established between rs573687 and the age at diagnosis of patients with POAG, with people carrying this allele developing the disease at least 5 years later than non-carriers.<sup>26</sup>

The rs2157719 allele was described as not only having an association with an increased chance of peripheral visual field loss and smaller CDR, but is widely known to play a major role in the occurrence of both normal tension glaucoma (NTG) and exfoliation glaucoma. It has further been reported to be implicated in reduced

POAG risk in Asian, African and Caucasian populations in diverse studies.<sup>23 29</sup> rs32177992 was found to increase the risk of POAG, with carriers more prone to develop the disease and potentially harbouring a larger CDR at diagnosis. A larger optic disc has been shown to be more susceptible to IOP-related stress. Thus, this suggests an implication in the occurrence of glaucoma and the progressive increase in vertical cup-to-disc ratio (VCDR) found in patients with POAG.<sup>30</sup>

Compared with patients who lack glaucoma risk alleles, patients carrying the risk alleles<sup>31</sup> (rs7049105 and rs10120688) have a lower IOP and a larger VCDR<sup>32</sup> and are predisposed to the development of POAG at lower IOP levels, with carriers recording nearly peak IOPs of 3 mm Hg less than non-carriers. In other words, these patients exhibit stronger associations with NTG and advanced glaucoma phenotypes.<sup>31</sup> This finding demonstrates out that they might also be classed as severity markers for POAG. A free of adverse effects and lowering intraocular therapy is beneficial in patients at risk of disease progression.<sup>33</sup>

The possible explanations for how these risk alleles may predispose or increase the risk of glaucoma are by conferring a risk non-dependent of IOP and by increasing the susceptibility to IOP, which means increasing the sensitivity of RGCs to IOP. Importantly, these risk alleles may manage to specifically affect both the regulation of IOP and the physiology of the RGC. Cell cycle regulation is known to be possibly affected by altered gene expression, leading the RGCs to a tendency towards apoptosis. Cells in carriers may then become more sensitive to IOP, such that pressures still contribute to optic nerve damage even when statistically normal.<sup>23</sup>

Individuals exhibiting an elevated IOP without the presence of optic nerve damage may only carry genetic variants that can influence IOP regulation, while those with NTG may primarily carry genetic variants predisposed to RGC death, as the nerve degeneration in this case does not have the added stress of elevated IOP.<sup>33</sup>

#### Regulation of CDKN2A and CDKN2B

Two proteins are encoded by *CDKN2A*: p16INK4A and p14<sup>ARF</sup>, which regulate the cell cycle and act as tumour suppressors. CDKN2A products regulate the Rb (retinoblastoma protein) and p53 (phosphoprotein p53) pathways, which reportedly involved in RGC apoptosis and the induction of cell cycle arrest, apoptosis and senescence.<sup>34</sup> *CDKN2B* encodes p15INK4B, a protein believed to play an important role in cell growth regulation through inducing G1 phase cell cycle arrest and is likely induced by transforming growth factor beta (TGF-β).<sup>35</sup> TGF-β modulates developmental and repair processes in several tissues, as well as participates to a considerable degree in programmed cell death in the developing retina and optic nerve.<sup>36 37</sup>

Patients with glaucoma tend to have a higher aqueous humour concentration of TGF-β2 (the predominant isoform of TGF-β in ocular tissue) than normal individuals,

suggesting a link between a high level of TGF- $\beta$ 2 and the pathogenesis of the disease.<sup>38 39</sup> CDKN2B-AS1 apparently participates in the transcription regulation of CDKN2A/CDKN2B, and it has been noted that ANRIL depletion (deletion of the 3'-end) in transgenic mice leads to excessive proliferation and diminished senescence in aortic smooth muscle cells, suggesting a key role in cell cycle regulation. Comparatively, the reduced cardiac expression of CDKN2A and CDKN2B in these mice was also pointed out and may be the mechanism through which CDKN2B-AS1 can alter cell cycle regulation.<sup>40 41</sup>

To further decipher the nature of their interaction and involvement in the pathogenesis of glaucoma, researchers performed a PCR analysis of their expressions in a rat model of glaucoma. One week after ocular hypertension induction, an upregulation in the expression in CDKN2A and CDKN2B was observed in the retina, which corresponds to a time point of ongoing RGC death, as indicated by the presence of axonal cytoskeleton damage in the optic nerve of the animals studied.<sup>24</sup> CDKN2A and CDKN2B seem to be involved in inducing apoptosis in response to stress in terminally differentiated neurons, which are of particular relevance to glaucoma, a disease characterised pathologically by the apoptosis of RGCs and loss of their axons.<sup>42</sup>

According to previous research on animal studies, elevated IOP is implicated in the overexpression of CDKN2B, which is responsible for abnormal cell proliferation because it disrupts the normal cell cycle.<sup>43</sup> CDKN2A, previously found to be down regulated in neurodegenerative disorders such as Alzheimer disease, can equally be regulated by CDKN2B-AS1, which suggest that regulation of CDKN2A by CDKN2B-AS1 could also contribute to degeneration of the optic nerve in glaucoma.<sup>25 44</sup>

## MALAT1

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also known as non-coding nuclear-enriched abundant transcript 2 (NEAT2), is a long non-coding RNA highly expressed in the nucleus and located on chromosome 11.<sup>45</sup> It acts as a critical regulator of neurodegeneration and has been successfully identified in diverse physiological processes, and a variety of evidence indicates its implication in various pathological processes, including diabetes-induced retinal microvascular dysfunction.<sup>46</sup>

MALAT1 participates in the regulation of the gene expression involved in nuclear and synapse function and synaptogenesis in neurons and has been demonstrated by previous studies to play a positive role in apoptosis, tumour cell proliferation, migration, invasion or the metastatic spread of tumour cells.<sup>47 48</sup> Recent studies conducted to determine and define its role in retinal neurodegeneration not only discovered a significant upregulation of MALAT1 in the cerebellum, hippocampus and brain stem of human alcoholics<sup>49</sup> and many solid tumours,<sup>50</sup> but also revealed its possible involvement in diabetes-induced retinal microvascular dysfunction.<sup>46</sup>

MALAT1 can promote cell proliferation by activating the PI3K/Akt pathway,<sup>51 52</sup> and MALAT1 downregulation can lead to decreased visual function and retinal cell apoptosis.<sup>53</sup> Further investigations on whether MALAT1 dysregulation can be equally observed in other neurological diseases such as glaucoma have been conducted by performing qRT-PCRs to compare MALAT1 levels in aqueous humour samples from glaucoma and cataracts patients without ocular neurodegenerative diseases. The results showed comparatively downregulated MALAT1 levels in the aqueous humour of patients with glaucoma.<sup>54</sup>

Additional clinical and animal experiments discovered that MALAT1 knockdown may potentially affect the regenerative ability of Müller cells and contribute to the reduced expression of neurotrophic factor, thus decreasing the protection of the retina.<sup>54</sup> The knockout of MALAT1 results in delayed vessel extension, reduced density at the vascular front and decreased proliferating cells. Microarray and quantitative PCR analyses further suggest that the promigratory and antiproliferative effects of silencing MALAT1 may be due to its downregulation of the S-phase cell cycle cyclins and upregulation of cell cycle inhibitory genes. These results implicate that during normal development, the high expression of lncRNA-MALAT1 in endothelial cells may help maintain proliferative state of these cells.<sup>55</sup> Müller cells are the major glial component of the retina and participate in retinal homeostasis and intervene in the regulation of retinal blood flow.<sup>56</sup> They are said to be reactivated (gliosis) in the retinas of patients with POAG, and their activation actually consists of releasing neurotrophic factors favouring the protection of the retina from a wide variety of pathological stimuli, including ischaemia, trauma and degeneration.<sup>57-59</sup> Nonetheless, The disturbance of retinal metabolism or a primary Müller cell insufficiency, such as in certain forms of glaucoma, may participate in the production and release of cytotoxic factors, increase the susceptibility of neurons to stressful stimuli, and cause and/or aggravate neuronal cell death after undergoing a variety of changes in morphological, physiology and biochemistry features.<sup>60 61</sup>

Nakazawa *et al* created an ischaemic model of optic nerve injury with optic nerve crush and found that PI3K/Akt pathway activation is actually responsible for the production of a neuroprotective effect against retinal and optic nerve injury.<sup>53</sup>

Recent investigations on this protective effect and mechanism of MALAT1 in glaucomatous RGCs<sup>62</sup> did provide strong evidence that MALAT1 could also be implicated in the inhibition process of RGC apoptosis through the activation of the PI3K/Akt signalling pathway. Since this inhibition process protects RGCs, inhibits the occurrence of apoptosis and promotes axonal regeneration in both glaucoma and optic neuropathy,<sup>63 64</sup> it provides a new approach for the clinical treatment of these conditions.<sup>62</sup>

## LOXL1-AS1

Exfoliation syndrome (XFS) or pseudoexfoliation syndrome (PXF) is an age-related blinding disorder characterised by the production and accumulation of abnormal extracellular material in both ocular and non-ocular tissues.<sup>65</sup> XFS is the most common identifiable cause of open-angle glaucoma and affects 60–70 million people worldwide.<sup>66</sup> Patients with XFS are allegedly at a greater risk of developing a specific form of glaucoma called exfoliation glaucoma (XFG), with roughly half of the eyes with XFS developing the disease within 15 years of diagnosis.<sup>67</sup> XFG which accounts for approximately 25% of all glaucomas possesses comparatively advanced visual field defects, a higher IOP as well as a reduced response to medical treatment as main characteristics compared with POAG.<sup>68</sup>

During pupillary movement, exfoliation material is scraped from the lens surface by the iris, causing a rupture of iris pigment epithelial cells and leading to pigment dispersion into the anterior chamber and its deposition on anterior chamber structures.

The white deposit appearance of the material in the eye can be perceived on the anterior lens surface and/or pupillary border.<sup>69</sup> Behavioural factors, such as caffeine intake, latitude of residence and vitamin deficiency, have been demonstrated to influence the risk of developing XFS and XFG.<sup>70–72</sup>

The LOXL1 gene, a member of the lysyl oxidase family, participates in the formation and maintenance of elastic tissue and, by inducing cross-linking in collagen and elastin molecules, plays a significant role in maintaining the equilibrium of the extracellular matrix (ECM).<sup>73</sup> It is expressed in ocular tissues, such as the cornea, ciliary muscle, lens epithelium, lamina cribrosa and trabecular meshwork, all of which are supposedly involved in the formation of the ECM. An alteration in its activation, processing and/or substrate specificity can affect the function and synthesis of extracellular tissues.<sup>74 75</sup>

LOXL1 polymorphisms have previously been suggested by a large number of studies to be associated with XFS in different populations, including Caucasians (Europe, Australia and North America), Asians (China and Japan) and South Africans.

Unfortunately, these studies primarily produced inconclusive results because alleles that reportedly increase the risk of developing XFS and XFG among Caucasians (rs1048661 and rs3825942) were also shown to have a protective effect in Japanese populations.<sup>76–78</sup> Further investigations into LOXL1 and its involvement in XFS pathogenesis discovered that the strongest evidence for an association with an increased XFS risk was located within the first intron of LOXL1, which contains variants that possibly alter the promoter for LOXL1-AS1.<sup>79</sup>

LOXL1-AS1 (LOXL1 antisense RNA 1) is a long non-coding RNA that is encoded by the opposite strand of LOXL1. It is broadly expressed in tissues known to be affected in XFS, and its expression can be altered by XFS-relevant cell stressors, such as oxidative stress

in lens epithelial cells and cyclic mechanical stress in Schlemm's canal, suggesting a critical role in the cell stress response.<sup>79 80</sup> The expression of many genes involved in various processes and XFG pathophysiology, such as collagen fibril formation (COL6A3, LOXL4), ECM degradation (TIMP3), cytoskeleton integrity (ACTA2), calcium ion binding (EFHD2), and response to oxidative stress (HMOX1), are actually modulated by the LOXL1-AS1.

LOXL1-AS1 knockdown has been demonstrated to alter the expression of up to 109 genes, including those related to ECM constituents. The dysregulated expression of LOXL1-AS1 may contribute to XFS and XFG pathogenesis because genetic risk variants and environmental stressors altering the level of LOXL1-AS1 disrupt the equilibrium of the ECM by affecting the gene expression of other XFS-relevant genes, potentially contributing to the pathogenesis of these complex inherited diseases.<sup>81</sup>

Additionally, it has been proven that the altered expression of LOXL1-AS1 levels has functional relevance in other organs and tissues affected in XFS including stem cells. This implies the possible implication of LOXL1-AS1 in increasing the risk of cerebrovascular diseases in patients with XFS. Similarly, increased oxidative stress is observed in neurodegenerative disorders, which are also associated with XFS, suggesting that LOXL1-AS1 dysregulation and abnormal response to oxidative stress play a role in these disease states.<sup>82</sup>

## ASMM10P055228 and ASMM10P040128

Optineurin, also called optic neuropathy-inducing protein, is a protein that is encoded by the *OPTN* gene and the second identified glaucoma causative gene through investigations of large NTG pedigrees.<sup>83</sup> The *OPTN* gene is located on chromosome 10p15-14, ubiquitously expressed in both non-ocular and ocular tissues<sup>84</sup> that include the trabecular meshwork, non-pigmented ciliary epithelium, retina, brain, heart, skeletal muscle, placenta and kidney; and mutations in this gene have been linked to POAG and NTG.<sup>85</sup>

*OPTN* mutations in transgenic mice models, including E50, have been identified as a key element in RGC apoptosis in previous studies; however, specific mechanisms remain largely unknown.<sup>86</sup> Recent studies have aimed to investigate the expression of differentially expressed lncRNAs in *OPTN* (E50K) transgenic and wild-type mice. For this purpose, six retinas from 8-month-old transgenic and wild-type mice were collected, divided into different groups and analysed independently for levels of lncRNA expression, which were later compared between mutant and wild groups by performing a two-tailed t-test.

The results revealed various differentially expressed lncRNAs between the *OPTN* (E50K) transgenic and wild-type mice; in addition, the pathway analysis contributed to the annotation of **ASMM10P055228** and **ASMM10P040128**, two lncRNAs suspected to be responsible for the negative regulation of oxidative stress-induced cell death and regulation of the execution

phase of apoptosis, which may be the underlying mechanism for POAG.

Although observations about the discovery of differentially expressed lncRNAs in the retinas between OPTN (E50K) transgenic and wild-type mice remain preliminary; and require further investigations to help clarify pathogenetic pathways, this finding is still attracting increasing interest as it creates a paradigm regarding the function of lncRNAs for detecting POAG pathogenesis.<sup>87</sup>

## CONCLUSION

Glaucoma is a complex disease because genetic and environmental factors both play important roles in its pathogenesis, therefore and despite the tremendous progress achieved in the last decade, the genetic basis of glaucoma is still not completely understood. Although lncRNAs have previously been linked with diverse diseases, however the proper connections and pathways they may use to influence the susceptibility to developing glaucoma have not yet been completely elucidated.

By summarising results from separate independent glaucoma-related studies, we reviewed lncRNAs characteristics and regulation, and tried to discuss potential pathways by which CDKN2B-AS1, MALAT1, LOXL1-AS1, ASMM10P055228 and ASMM10P040128 may contribute to glaucoma.

However, further researches and investigations are needed to discover vast number of glaucoma-associated lncRNAs, their characteristics and expression patterns, and decipher pathways of their involvement and role in the pathogenesis of this blinding disease. Since most glaucomas are symptomless at early stages, we stress that this article creates a paradigm for future studies of lncRNAs in the prevention, early determination and monitoring of the evolution of glaucoma and may prove to be useful to define high-risk or low-risk alleles for early determination of whether a patient with a suspected glaucoma allele should receive prioritised treatment to help slow the progression of the disease, reduce further damage to the optic nerve and subsequently avoid blindness, and retain quality of life.

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