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Insilico Identification of LncRNA and CircRNAs with a Prospective Role in the Diabetic Retinopathy Diagnosis

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Diabetic retinopathy, Noncoding RNAs, lncRNAs, circular RNA, RNACentral

Abstract
One of the serious complications of Type 2 Diabetes mellitus is Diabetic Retinopathy (DR) with fluctuating ranges of vision damage. DR is a polymorphic disorder with no complete information and understanding. Earlier studies signified the relation of DR development with long noncoding RNAs (lncRNAs), and their expression levels may trigger DR indicating that lncRNAs are acting as the regulatory elements and further research studies are in progress. Considering this, long noncoding RNAs and circular RNAs related to Diabetic Retinopathy are retrieved from various databases which include NCBI, RNA Central, LNCSEA2.0, LncRNA disease V2.0 database, Mammalian ncRNA disease repository (MNDR V3.1). The data retrieved is pooled and analyzed. Accordingly, the accessed lncRNAs are assessed in PubMed and other bibliography databases. A total of forty-three lncRNAs and thirty-nine circular RNAs were retrieved. Insilico analyses are made to interpret the expression profiles of lncRNAs and circRNAs predicted in relation to DR. In addition, the regulatory levels of depicted lncRNAs and their targets are noted using respective databases. The detection of lncRNAs which play a major role in DR starting and progressing states, could be treated as probable biomarkers for early diagnosis of Diabetic Retinopathy leading to innovative therapeutic and medication strategies.

1. Introduction

Diabetic Retinopathy (DR) is a problem with loss of vision in eye developed in diabetic patients eventually leading to blindness. The blood vessels of the eye and the sensitive layer of the tissue in the backside are highly affected. In general, diabetic patients should make regular eye checks annually in a comprehensive way as the major population develop DR in due course of time. DR is not specific to a particular type of diabetes and hence could be developed in any cases of Type 1, Type 2, or gestational diabetes. The severity of DR depends on the duration of the person being suffered from Diabetes. About 50% of diabetic patients are recorded to develop Diabetic Retinopathy as the high levels of glucose present in the blood will damage the retina stopping it from detecting light and receiving signals from brain. This happens due to blockages of glucose molecules in the blood vessels of retina, making them to bleed. There are many diagnoses conducts and treatments for DR, but still there exists the gap as it is identified in later stages where most the vision loss has already happened. Henceforth, there is need to develop alternate new methods for early diagnosis of DR using the emerging technologies.

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For the past few years, improvements in the genome studies and respective association studies of mammalian transcriptomes have signified the new approachable units of DNA called non-coding RNAs, especially Long Non-coding RNAs (lncRNAs) that are being transcribed (Qiaoyun Gong & Guanfang Su, 2017). LncRNAs are a stretch of 200plus nucleotide sequences. The significant ORF regions localized in both cytoplasm and nucleus are absent in lncRNAs (van Heesch S et al., 2014). Like coding genes, lncRNAs are also regulated using cell-specific expression systems (Guttman M et al., 2011). The complex characteristics of a cell in diseased state may be determined by RNA regulatory networks and most part of it is unexplored in the genetic analyses. A few research studies also revealed that lncRNAs play a key role in human diseases (Kaori Kashi et al., 2016). Nevertheless, the precise understanding of lncRNAs role in Diabetic retinopathy has persisted mostly unknown.

A novel approach to diabetic retinopathy detection through non-coding RNAs is the currently focussed study. As per Yan et al., 2015, the lncRNA named Myocardial infarction-associated transcript – MIAT plays a key role in the regulation of diabetes mellitus. Synonyms of MIAT include Gomafu, RNCR2 and is the first locus with susceptibility being noted in the retinal precursor cells (Ishii N et al., 2006; Blackshaw S et al., 2004). So, to design therapies for DR, efficient tools and modules need to be generated for lncRNA functional analyses.

From Gene ontology studies, it was depicted that lncRNA co-expressed mRNAs are integrated with eye membrane and structural activities and are related to its development. KEGG analyses also revealed the role of lncRNAs in axon guidance, coagulation cascades, signaling pathways, pyruvate metabolism. In turn, these signaling pathways are associated with neurodegeneration, inflammation signifying the role of lncRNA interconnections in the pathogenesis of Diabetic Retinopathy.

Further, the other type of non-coding RNAs namely Circular RNAs (circRNAs) are also retrieved in association with Diabetic Retinopathy to study their role in the DR progression. CircRNAs are covalently closed circlet structures with no 5' or 3' poles and no poly 'A' tails. They are responsible for some features like specificity, conservatism and stability depending on which they can be analysed for disease diagnosis studies.

Henceforth, the long noncoding RNAs and circular RNAs that are associated with Diabetic retinopathy are retrieved from the corresponding databases and various bioinformatic analyses are performed to explore strategies for early prediction and diagnosis of diabetic Retinopathy.

2. Methodology

Search Approaches of Study

The electronic search platforms namely PubMed and google scholar resources are used for the literature study keeping the publication duration ranging from 2018 – 2022. The search keywords include: 'lncRNA' and 'Diabetic Retinopathy,' 'CircRNA' and 'Diabetic Retinopathy' for retrieval of lncRNAs and circRNAs respectively. A total of 154 and 3650 published articles for lncRNAs and 50 and 575 published articles for circRNA are recognized in PubMed and Google Scholar, separately. Further, the related articles are reviewed physically to list out the lncRNAs and circRNAs associated with Diabetic Retinopathy.

Insertion and Elimination criteria

A few insertion and elimination criteria are followed for the current study that includes 1. the inclusion of different lncRNAs and circRNAs at different levels in DR, 2. upregulation and downregulation of lncRNAs in DR, predictive studies on the structural and functional aspects of lncRNAs and circRNAs. The elimination criteria include case studies, editorial communications, reviews, meta-analyses, and conference reports.

Retrieval of lncRNAs and circRNAs through Online databases

To retrieve the lncRNA and circRNA information related to DR, many bioinformatic tools and resources are accessed to study the expression profiles of lncRNAs, to predict their target regions such as Lncbook, lncipedia, RNACentral, lncRNAs-diseases, KEGG.

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National Centre for Biotechnology Information (NCBI) Gene tool was used to know the basic details of lncRNAs. RNACentral is used for the retrieval of lncRNAs related to DR. The lncRNAs and circRNAs associated diseases are recorded from LncRNA Disease v2.0 databse. The comprehensive annotation of predicted human genes are referred from UniProt and GeneCards databases. GWAS analysis is performed to understand the traits and genes involved in DR. KEGG pathway analysis is performed to know the related mechanisms involved and associated with Diabetic Retinopathy. The role of projected lncRNAs, circRNAs, and their corresponding targets are identified and assessed based on the retrieved data.

3. Results

LncRNAs & CircRNAs – Predicted and Experimentally validated

In this study, a total of 157 lncRNAs are available in RNACentral. Out of which 15 lncRNAs from RNACentral and 28 from PubMed are considered for further analysis. A total of 23 CircRNAs are retrieved using LnCRNA diseaseV2.0 database. 16 circRNAs from PubMed sources are also chosen with a total of 39 circRNAs for study. It is noted that lncRNAs that are not mentioned in NCBI Gene/UniProt are disregarded. The lncRNAs and circRNAs retrieved are PubMed and Google Scholar derived ones. Afterward, the lncRNAs are categorized into two groups. The lncRNAs or the circRNAs that are reported in a minimum one publication, are presented as experimentally validated lncRNAs and other ones lacking experimental proof are considered as predicted ones.

Thus, 43 lncRNAs and 39 circRNAs are established for the study. Figure 1 and figure 2 shows both 43 lncRNAs and 39 circRNAs respectively.

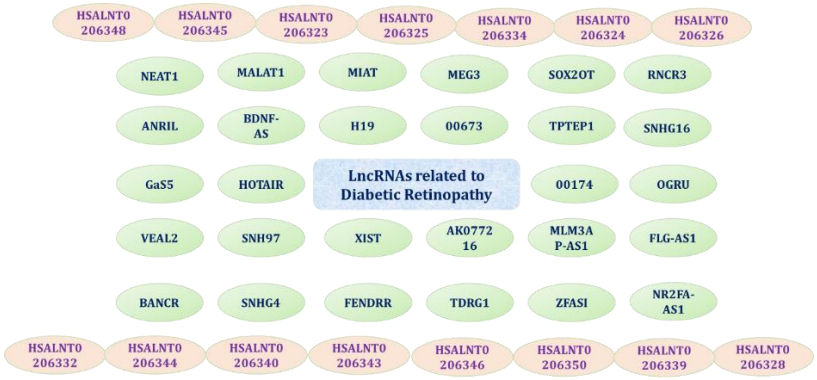

Figure:1. LncRNAs associated with Diabetic Retinopathy
(Green colour – Validated lncRNAs from PubMed; Orange colour – Predicted lncRNAs from RNACentral)

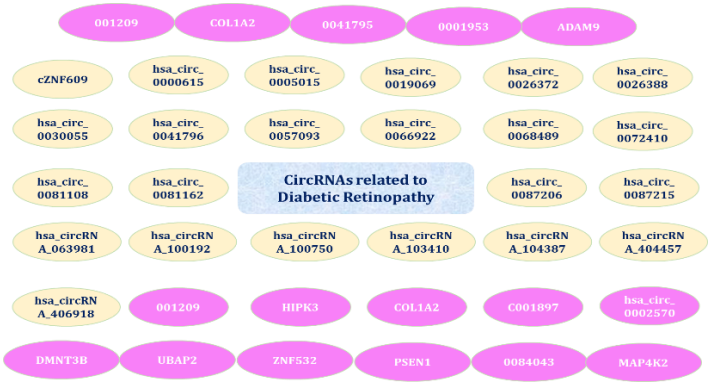

Figure:2. CircRNAs associated with Diabetic Retinopathy
(Lavender colour – Validated CircRNAs from PubMed; Yellow colour – Predicted CircRNAs from lncRNA disease)

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Dysregulation of LncRNAs

At the outset, validated lncRNAs are given a thorough reading. Using NCBI, the basic details of validated lncRNAs are obtained and their expression patterns were checked out in publications. The role of validated lncRNAs in Diabetic Retinopathy are explained in different articles and the expression profile which includes the dysregulation of lncRNAs is tabulated in table1.

Table 1: Experimentally validated lncRNAs regulation in Diabetic Retinopathy

S.No.	Name	Dysregulation
	00174	Upregulated
	00673	Downregulated
	AK077216	Downregulated
	ANRIL	Upregulated
	BANCR	Upregulated
	BDNF-AS	Upregulated
	FENDRR	Upregulated
	FLG-AS1-PR	Downregulated
	GAS5	Downregulated
	H19	Downregulated
	HOTAIR	Upregulated
	HOTTIP	Upregulated
	MALAT1	Upregulated
	MEG3	Downregulated
	MIAT	Upregulated
	MCM3AP-AS1	Downregulated
	NEAT1	Downregulated
	NR2F1-AS1	Upregulated
	OGRU	Upregulated
	RNCR3	Upregulated
	SNHG7	Upregulated
	SNHG16	Upregulated
	SNHG4	Downregulated
	SOX2OT	Downregulated
	TDRG1	Upregulated
	TPTEP1	Downregulated
	VEAL2	Upregulated
	XIST	Downregulated
	ZFAS1	Upregulated

Predicted lncRNAs and CircRNAs

The lncRNAs search in relation to Diabetic retinopathy is performed in RNACentral. A total of 157 Non-coding RNA molecules including Long Intergenic Non-protein coding (LINC) sequences. However, the long intergenic non-protein coding RNAs were not discussed in the current study. The predicted 15 lncRNAs are found on the same chromosome 13 and are tabulated in table 2.

ISSN: 2309-5288 (Print)
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Journal of Coastal Life Medicine

Table:2. Predicted lncRNAs associated with Diabetic retinopathy

S.No	ID	Length (Nucleotides)	Annotation databases	Location in Genome
1	HSALNT0206323	1765	MalaCards; LncBook; GeneCards	Chr 13
2	HSALNT0206325	1526	MalaCards; LncBook; GeneCards; Noncode	Chr 13
3	HSALNT0206334	1446	MalaCards; LncBook; GeneCards	Chr 13
4	HSALNT0206324	1276	MalaCards; LncBook; GeneCards	Chr 13
5	HSALNT0206326	1236	MalaCards; LncBook; GeneCards	Chr 13
6	HSALNT0206328	1086	MalaCards; LncBook; GeneCards	Chr 13
7	HSALNT0206332	880	MalaCards; LncBook; GeneCards	Chr 13
8	HSALNT0206344	864	MalaCards; LncBook; GeneCards	Chr 13
9	HSALNT0206340	806	MalaCards; LncBook; GeneCards	Chr 13
10	HSALNT0206343	774	MalaCards; LncBook; GeneCards	Chr 13
11	HSALNT0206348	735	MalaCards; LncBook; GeneCards	Chr 13
12	HSALNT0206345	485	MalaCards; LncBook; GeneCards; Noncode	Chr 13
13	HSALNT0206346	485	MalaCards; LncBook; GeneCards	Chr 13
14	HSALNT0206350	402	MalaCards; LncBook; GeneCards	Chr 13
15	HSALNT0206339	329	MalaCards; LncBook; GeneCards	Chr 13

ISSN: 2309-5288 (Print)
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Journal of Coastal Life Medicine

The CircRNAs search in relation to Diabetic retinopathy is performed in LncRNA disease. A total of 23 circRNAs are predicted and is detailed in table 3.

Table: 3. Predicted circRNAs associated with Diabetic Retinopathy

Database ID	ncRNA Symbol	Species	Disease Name	Detection Method	Score
LDA0178802		Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178803	hsa_circ_0000615	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178804	hsa_circ_0005015	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178805	hsa_circ_0019069	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178806	hsa_circ_0026372	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178807	hsa_circ_0026388	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178808	hsa_circ_0030055	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178809	hsa_circ_0041796	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178810	hsa_circ_0057093	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178811	hsa_circ_0066922	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178812	hsa_circ_0068489	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178813	hsa_circ_0072410	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178814	hsa_circ_0081108	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178815	hsa_circ_0081162	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178816	hsa_circ_0087206	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178817	hsa_circ_0087215	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178818	hsa_circRNA_063981	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178819	hsa_circRNA_100192	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178820	hsa_circRNA_100750	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178821	hsa_circRNA_103410	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178822	hsa_circRNA_104387	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178823	hsa_circRNA_404457	Homo sapiens	diabetic retinopathy	qPCR	0.7311
LDA0178824	hsa_circRNA_406918	Homo sapiens	diabetic retinopathy	qPCR	0.7311

ISSN: 2309-5288 (Print)
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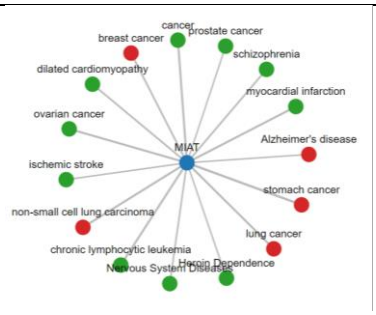
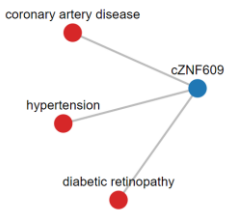
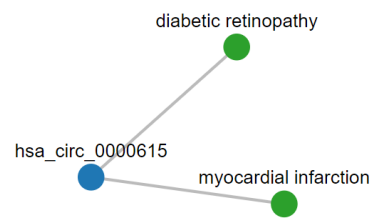
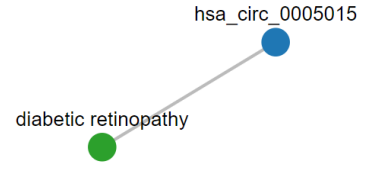
CODEN: JCLMC4
Journal of Coastal Life Medicine

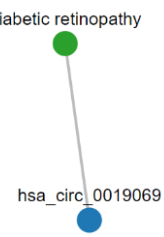
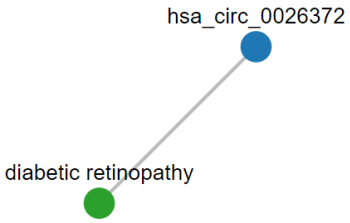
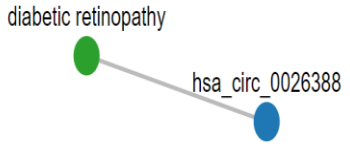
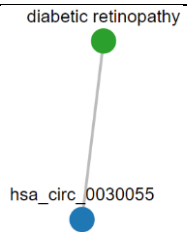
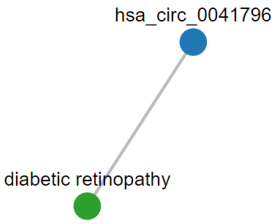
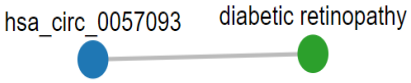
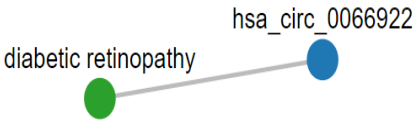
Predicted lncRNAs and CircRNAs - Regulatory relationships

The main functional aspects of lncRNAs and circRNAs are accredited miRNA-lncRNA interactions. Thus, it is significant to identify and know the target regions of predicted lncRNAs and the cellular mechanisms in which they are involved. The targets of predicted lncRNAs in DR (Table 4) are considered in GeneCards and UniProt databases. These verdicts might enable the researcher to study the biological function of target genes. The regulatory relationship of predicted lncRNA (01) and circRNAs (23) are accessed from LncRNA Disease v2.0 database. The targets of the predicted lncRNAs in Diabetic Retinopathy and their interactions with miRNAs are extracted. The various mRNA and miRNA targets of the ncRNAs and their association with other diseases along with diabetic retinopathy were also listed (table 4). It is also considered that there are no target molecules for a few predicted circRNAs, considering their sense strand as target, according to NCBI.

Table:4. Regulatory relationships of predicted lncRNA and circRNAs with miRNA and mRNA in DR

Non-coding RNA	mRNA Target	miRNA target	Association with diseases
LncRNA			
MIAT	CRYBA4, TPST2, CRYBB1	MIR548J	
CircRNA			
cZNF609	OAZ2, RBPMS2, PIF1, PLEKHO2, AC069368.1	MIR1272	
hsa_circ_0000615	OAZ2, RBPMS2, PIF1, PLEKHO2, AC069368.1	MIR1272	
hsa_circ_0005015	-	-	

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

hsa_circ_0019069	PCGF5	-	
hsa_circ_0026372	KRT72, KRT73, KRT2, KRT1, KRT77, KRT76	-	
hsa_circ_0026388	KRT76, KRT3, KRT4, KRT79, KRT78, KRT8	-	
hsa_circ_0030055	VWA8	-	
hsa_circ_0041796	CLEC10A, ASGR2, ASGR1, DLG4, ACADVL, DVL2, PHF23, GABARAP, AC120057.2, CTDNEP1, AC003688, ELP5	MIR324	
hsa_circ_0057093	CDCA7	-	
hsa_circ_0066922	GPR156, LRRC58, FSTL1	MIR198	

ISSN: 2309-5288 (Print)
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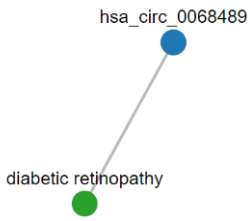
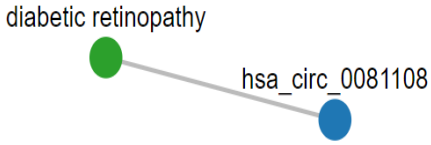
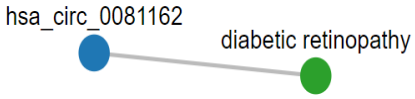
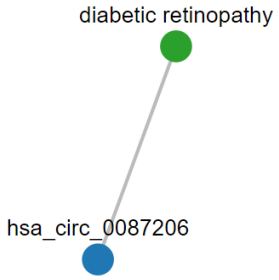
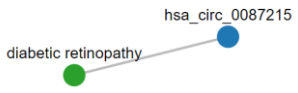
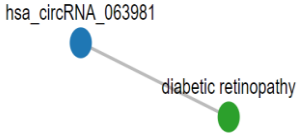
CODEN: JCLMC4
Journal of Coastal Life Medicine

hsa_circ_0068489	ADIPOQ, ST6GAL1	-	
hsa_circ_0072410	PAIP1, NNT	-	
hsa_circ_0081108	BET1	-	
hsa_circ_0081162	BET1	-	
hsa_circ_0087206	-	-	
hsa_circ_0087215	-	-	
hsa_circRNA_063981	-	-	

ISSN: 2309-5288 (Print)
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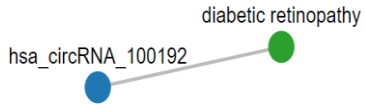
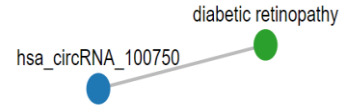
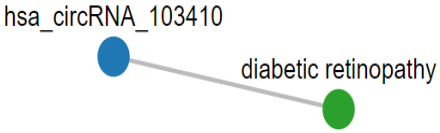
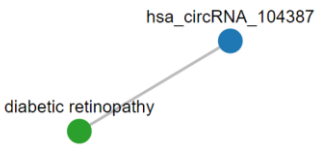
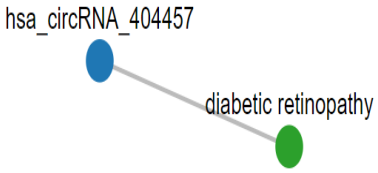
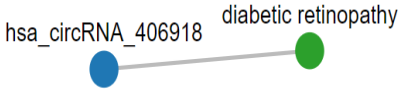
Journal of Coastal Life Medicine

hsa_circRNA_100192	-	-	
hsa_circRNA_100750	-	-	
hsa_circRNA_103410	-	-	
hsa_circRNA_104387	-	-	
hsa_circRNA_404457	-	-	
hsa_circRNA_406918	-	-	

The predicted circRNAs that are associated with DR are displayed in the network form (Fig:3).

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

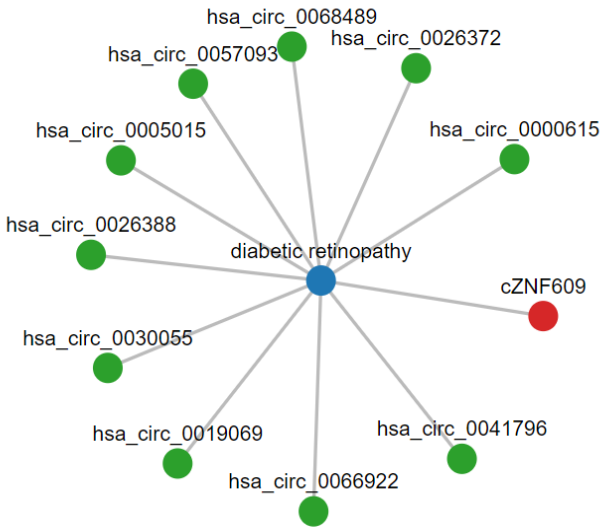


Figure: 3. DR Disease Association Statistics – predicted circRNAs (LncRNAdisease database)

LncRNAs - Survival analysis in DR

As per the expression levels of validated lncRNAs, box plot diagram plotted for lncRNAs expression. A total of 8 out of 29 experimentally validated lncRNAs (MALAT1, MIAT, MEG3, ANRIL, BDNF-AS1, RNCR3, HOTAIR, BANCR) have been significantly associated with Diabetic Retinopathy (Fig: 4). MALAT1 is upregulated in DR retinas and has a role in the regulation of retinal endothelial cell function. MIAT expression is high in DR retina tissues and its downregulation alleviates retinal vessel impairment. MEG3 is significantly downloaded in DR conditions and its knockdown contributes to retinal endothelial cell proliferation and migration. ANRIL overexpresses in DR conditions and upregulates VEGF factor. RNCR3 inhibition makes retinal glial reactivity suppression and stops retinal neurodegeneration. Low expression level of BDNF is observed in DR patients, while BDNF-AS1 is upregulated. The knock-down of BDNF-AS shields retinal cells under diabetic conditions providing a novel therapeutic way for the medication of diabetes-associated retinal degeneration.

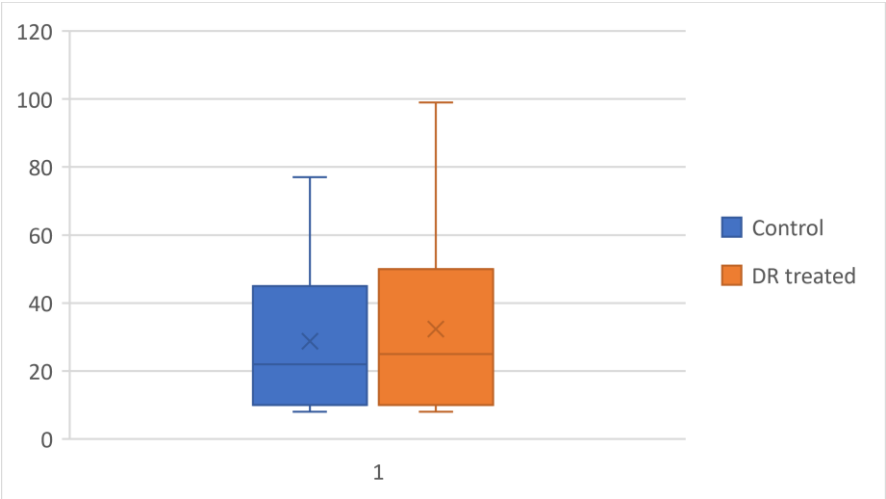


Figure: 4. Box plot graph of lncRNAs expression

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

GWAS Analysis

Genome-wide association studies with variant and risk alleles for proliferative diabetic retinopathy have been retrieved and analyzed (table 5; Fig.5). DR, the prevalent vascular complication of diabetes is divided into two stages namely non-proliferative diabetes (NPD) and proliferative diabetes (PD). NPD, the 1st stage is asymptomatic and basically cannot be identified upon eye examination. Still, microaneurysms, narrowing of blood vessels can be detected. PD, the 2nd stage is symptomatic, severe and causes neovascularization leading to fragility of blood vessels and further leakage or bleeding causing blindness ultimately.

Table: 5. Variants & Risk alleles – Diabetic retinopathy (PDR & DR)

Variant & risk allele	P-value	RAF	OR	CI	Mapped gene	Reported trait	Study accession
rs9362054	1 x 10-6	0.291	1.4	'-	LINC01611	DR in type 2 diabetes	GCST002684
rs1000708	7 x 10-7	NR	'-	'-	SLC16A7	PDR (vs no DR)	GCST007291
rs114921230	5 x 10-7	NR	1.9	[NR]	STUM	PDR (vs no DR)	GCST007291
rs116396065	1 x 10-6	NR	'-	'-	'-	PDR (vs no DR)	GCST007291
rs200197449	2 x 10-6	NR	1.44	[NR]	LINC00523, DLK1	PDR (vs no DR)	GCST007291
rs200295620	7 x 10-8	NR	1.39	[NR]	EGFEM1P, GOLIM4	PDR (vs no DR)	GCST007291
rs201584991	1 x 10-6	NR	1.63	[NR]	SQLE, ZNF572	PDR (vs no DR)	GCST007291
rs71354195	8 x 10-7	NR	2.42	[NR]	ZFP82	PDR (vs no DR)	GCST007291
rs74705672	1 x 10-6	NR	1.449275	[NR]	OC90	PDR (vs no DR)	GCST007291
rs11575234	2 x 10-6	NR	1.754386	[NR]	STAT2	PDR (vs no DR)	GCST007291
rs12447665	2 x 10-6	NR	1.83	[NR]	RBFOX1	PDR (vs no DR)	GCST007291
rs1566115	2 x 10-6	NR	1.87	[NR]	FAXC, BDH2P1	PDR (vs no DR)	GCST007291
rs184340784	4 x 10-8	NR	'-	'-	LINC01646	PDR (vs no DR)	GCST007291
rs2064196	1 x 10-6	NR	1.818182	[NR]	UTRN, TPT1P4	PDR (vs no DR)	GCST007291
rs4129798	2 x 10-6	NR	1.818182	[NR]	TBC1D5	PDR (vs no DR)	GCST007291
rs4726066	5 x 10-8	NR	'-	'-	PRKAG2	PDR (vs no DR)	GCST007291
rs61811867	1 x 10-6	NR	'-	'-	KCNN3	PDR (vs no DR)	GCST007291
rs74161190	7 x 10-7	NR	3.125	[NR]	'-	PDR (vs no DR)	GCST007291

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

rs3081219	1 x 10-9	0.13	1.29	[1.19-1.41]	WDR72	PDR	GCST008668
rs1065386	5 x 10-8	0.43	1.17	[1.11-1.24]	HLA-B	PDR	GCST008668
rs10560003	3 x 10-6	0.02	1.52	[1.28-1.81]	GAP43	PDR	GCST008668
rs72740408	2 x 10-8	0.03	1.52	[1.31-1.76]	HNRNPA1P46	PDR	GCST008668
rs11201335	8 x 10-7	NR	1.3	[NR]	'-	PDR (vs NPDR)	GCST007289
rs11488711	1 x 10-6	NR	1.428572	[NR]	LINC02814	PDR (vs NPDR)	GCST007289
rs138683663	1 x 10-6	NR	4.9	[NR]	GRP, SEC11C	PDR (vs NPDR)	GCST007289
rs1414474	1 x 10-7	NR	1.6	[NR]	C1orf94	PDR (vs NPDR)	GCST007289
rs71354195	5 x 10-7	NR	1.5	[NR]	ZFP82	PDR (vs NPDR)	GCST007289
rs7139429	2 x 10-6	NR	1.5	[NR]	RN7SL865P,UBA52P7	PDR (vs NPDR)	GCST007289
rs73050171	5 x 10-7	NR	1.5	[NR]	NAALADL2	PDR (vs NPDR)	GCST007289
rs75348186	1 x 10-6	NR	1.9	[NR]	'-	PDR (vs NPDR)	GCST007289
rs7604016	1 x 10-6	NR	2.5	[NR]	COMMD1	PDR (vs NPDR)	GCST007289
rs1144964	4 x 10-7	NR	1.492537	[NR]	CPM	PDR (vs NPDR)	GCST007289
rs115523882	9 x 10-9	NR	3.1	[NR]	EGFEM1P, GOLIM4	PDR (vs NPDR)	GCST007289
rs137949823	6 x 10-8	NR	2.325581	[NR]	NNT	PDR (vs NPDR)	GCST007289
rs149201869	9 x 10-7	NR	5.57	[NR]	FN1	PDR (vs NPDR)	GCST007289
rs184340784	1 x 10-6	NR	'-	'-	LINC01646	PDR (vs NPDR)	GCST007289
rs2037601	2 x 10-6	NR	1.44	[NR]	RNU6-169P	PDR (vs NPDR)	GCST007289
rs73228199	1 x 10-6	NR	2.777778	[NR]	CD96	PDR (vs NPDR)	GCST007289
rs73347124	2 x 10-6	NR	5	[NR]	Y_RNA, LINC02672	PDR (vs NPDR)	GCST007289
rs7533141	3 x 10-7	NR	1.47	[NR]	GPATCH2	PDR (vs NPDR)	GCST007289
rs78340493	3 x 10-7	NR	1.66	[NR]	RN7SL691P	PDR (vs NPDR)	GCST007289
rs78464534	3 x 10-7	NR	'-	'-	DPP10	PDR (vs no DR)	GCST007291

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

rs9446832	2 x 10-6	NR	'-	'-	KCNQ5	PDR (vs no DR)	GCST007291
rs918519	4 x 10-6	0.769	2.86	[1.85-4.55]	IL12B, LINC01845	PDR in type 2 diabetes	GCST006283
rs1158314	4 x 10-6	0.4	2.16	[1.56-3.00]	NRXN3	PDR in type 2 diabetes	GCST006283

Note: PDR – Proliferative Diabetic Retinopathy; NPDR – Non-proliferative Diabetic Retinopathy

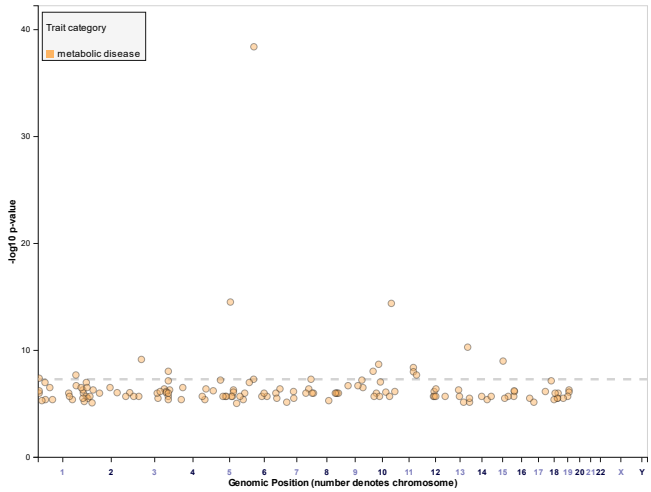


Figure: 5 Graph indicating the traits and the genomic position

KEGG pathway analysis

To understand the underlying and associated mechanisms of Diabetic retinopathy, Kyoto Encyclopedia of Genes Genomes (KEGG) database search is performed. The KEGG search is made by providing the disease name as 'Diabetic Retinopathy'. This resulted in one hit with Id: H01457. It is recorded that vascular endothelial growth factor A (VEGF-A), angiotensin-converting enzyme (ACE), paraoxonase, erythropoietin are associated with DR development and progression. Accordingly, three pathways are connected with DR and they are showed in the below figures (6, 7, & 8).

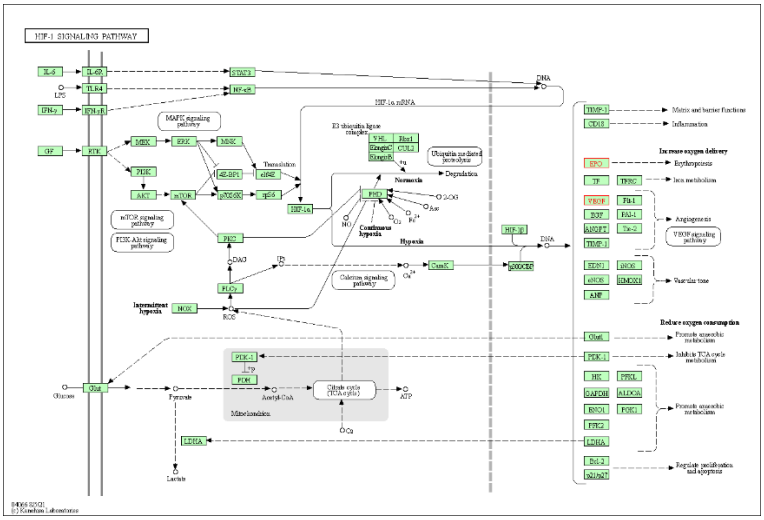


Figure: 6. HIF-1 Signalling Pathway (Red color VEGF, EPO genes – associated with DR)

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

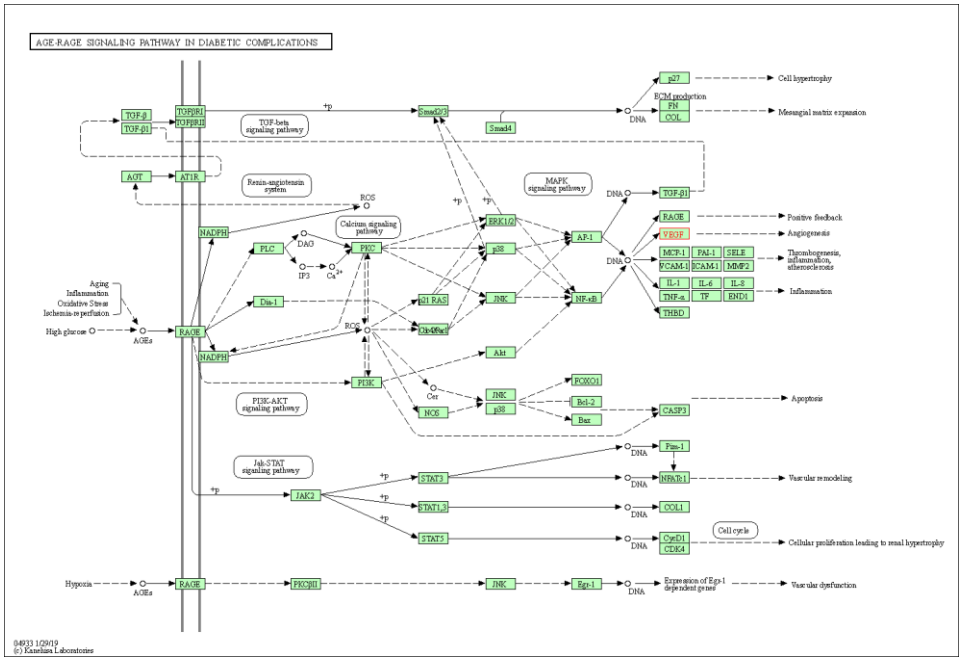


Figure: 7. AGE - RAGE Signalling Pathway (Red color VEGF, gene – associated with DR)

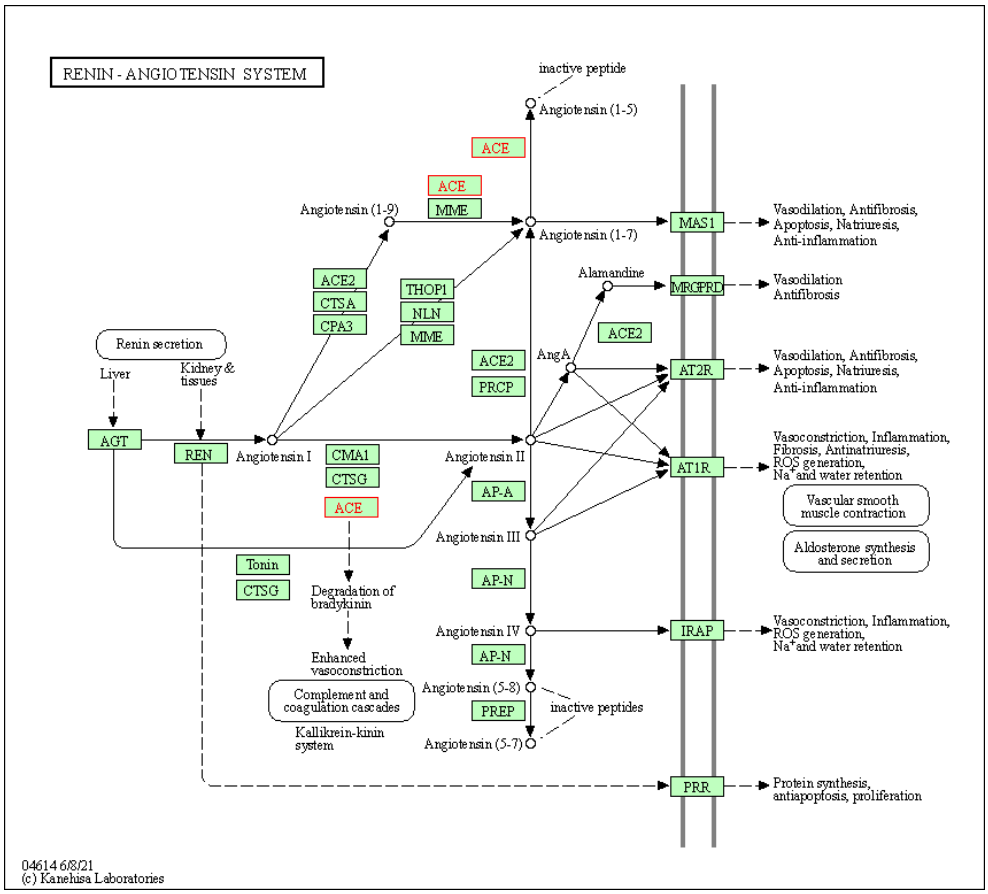


Figure: 8. Renin – Angiotensin System (Red color ACE gene – associated with DR)

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Further, OMIM database analysis is performed. OMIM is a free, comprehensive, authoritative compilation having human genes and genetic phenotypes. All mendelian disorders and more than 16000 genes are collected in OMIM database with complete text and published overviews. The main motto for using OMIM is to study the relation between genotype and phenotype. In this study, microvascular complications interlinking with other disorders is depicted (Fig.9) which displayed Erythrocytosis, Gastric cancer, Alzheimer's disease, hemochromatosis, varied porphyria.

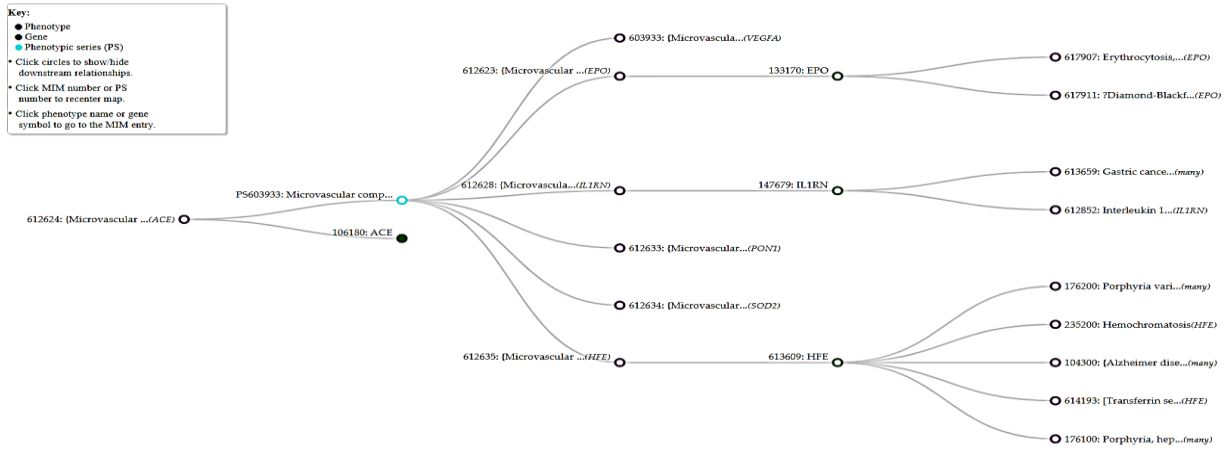


Figure: 9. Schematic Showing the Microvascular (DR) interconnections with other diseases

4. Discussion

For the past two decades, several attempts and studies have been done to realize and understand the molecular changes in Diabetic Retinopathy. By Bioinformatics analysis, a total of 43 lncRNAs and 39 circRNAs involved in DR are identified. The study on the analysis of predicted and experimentally validated lncRNAs and circRNAs involved in DR is the first of its kind. The various targets of lncRNAs and their expression levels are discussed. Genome-wide association studies and pathway analysis have been interpreted. A few predicted lncRNAs in DR are also known to be involved in other types of diseases and are tabulated (Table 4), especially Cancer and Hypertension. Consequently, varied regulations of the lncRNAs can be noticed with varying disease types. These findings expose the role of lncRNAs in DR in various cellular mechanisms and functions such as retinal endothelial cell proliferation, and other related mechanisms by aiming at associated proteins.

5. Conclusion

Diabetic Retinopathy is one of the major complications of Diabetes mellitus, interrelated with various pathogenic processes. An advanced understanding of the disease will lead to new therapy identification and to make efficient compounds for the diagnosis of DR. In our study, 43 lncRNAs and 39 circRNAs are retrieved from specific databases. These lncRNAs can be furthermore studied for therapeutic applications with experimental validations. The studies on miRNAs and lncRNAs have gained more significance and attention for the past two decades, especially in medical fields like cancer, genetic disorders and other complications. A large number of lncRNAs included in DR are identified and mentioned in various publications, however, lncRNAs are needed to be explored more to understand the biological mechanism and the pathogenesis in DR. RNA Sequencing and Microarray analysis offer wide-ranging methods for lncRNA detection which are differentially expressed in DR>

To conclude, a summary and bioinformatic analysis, including the regulatory roles and the expression

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

profiles of lncRNAs and circRNAs in DR are provided. In addition, the target mRNAs and the crosstalk with miRNAs are also stated. To develop novel and efficient targets against Diabetic Retinopathy, the study and understanding of lncRNAs and CircRNAs are very significant. Efficient lncRNA interpretation and a better knowledge on cellular mechanisms involved would pave a way for early detection of DR and for its improved therapeutics.

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Journal of Coastal Life Medicine

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