International Research Journal of Human Resource and Social Sciences
ISSN(O): (2349-4085) ISSN(P): (2394-4218)
Impact Factor 6.924 Volume 9, Issue 09, September 2022
Website- www.aarf.asia, **Email** : editoraarf@gmail.com

DEVELOPMENT OF CANINE RETINA AND ITS FUNCTIONAL ANNOTATION

Dr.Rashmi

Assistant Professor, Department of Zoology, College of Commerce, Arts and Science, Patna, Bihar

ABSTRACT

The optics of the eye create a focused two-dimensional image of the visual world on the retina, which then processes that image within the retina and sends nerve impulses along the optic nerve to the visual cortex to create visual perceptionIn the majority of cases, the mode of inheritance is autosomal recessive, although some autosomal dominant and X-linked RP existThe choriocapillaris, a network of capillary vessels located under the retinal pigmented epithelium, is the main source of oxygen for the outer retina, and retinal vessels are the main supply for the inner retina.

KEYWORDS: Retinal, Oxygen**,** autosomal, Inheritance, Optics, Capillary

INTRODUCTION

The retina (from Latin: rete "net") is the innermost, light-sensitive layer of tissue of the eye of most vertebrates and some molluscs. The optics of the eye create a focused two-dimensional image of the visual world on the retina, which then processes that image within the retina and sends nerve impulses along the optic nerve to the visual cortex to create visual perception. The retina serves a function which is in many ways analogous to that of the film or image sensor in a camera.The neural retina consists of several layers of neurons interconnected by synapses and is supported by an outer layer of pigmented epithelial cells. The primary light-sensing cells in the retina are the photoreceptor cells, which are of two types: rods and cones. Rods function mainly in dim light and provide monochromatic vision. Cones function in well-lit conditions and are responsible for the perception of colour through the use of a range of opsins, as well as high-acuity vision used for tasks such as reading. A third type of light-sensing cell, the photosensitive ganglion cell, is important for entrainment of circadian rhythms and reflexive responses such as the pupillary light reflex.

Retinitis pigmentosa (RP) is a heterogeneous group of inherited retinopathies with varying genetic background and highly variable clinical consequences. RP is the leading cause of irreversible blindness in man with a worldwide prevalence of one in 4,000 people [1]. The disease first manifests as impaired vision in dim light (nyctalopia) resulting from progressive loss of the rod photoreceptor cells. As the disease progresses, complete blindness is expected due

to cone photoreceptor degeneration accompanied by changes in the retinal pigment epithelium (RPE), the retinal vasculature, the glial cells and neurons of the inner retina. To date, 67 genes and loci have been implicated to nonsyndromic RP according to the Retinal Information Network RetNet (http://sph.uth.edu/retnet/) [2]. In the majority of cases, the mode of inheritance is autosomal recessive, although some autosomal dominant and X-linked RP exist [2]. Despite a large number of implicated genes and variants, 30–80% of the patients have RP of unknown genetic cause and thus many genes remain still to be discovered [3].

RP is incurable at the moment and much is expected from gene therapy to treat this disease. Different eye diseases have been favorite targets of gene therapy for several reasons, including relatively easy access to treat and monitor the target organ. A recent study described patient derived induced pluripotent stem cell (iPSC) treatment with clustered regularly interspersed short palindromic repeats (CRISPR/Cas9) to treat a RP affected patient with an X-linked point mutation in the retinitis pigmentosa GTPase regulator (RPGR) gene [4] in addition to other attempts to treat retinal dystrophies. As a known genetic cause of disease is obligatory to any gene therapy and still many RP related genes remain unknown, continuous attempts are needed to discover new causative variants and understand the underlying biology.

The retina is a complex and highly metabolic extracranial part of the central nervous system that requires a continuous and self-regulated blood supply (Harris et al., 1998; Yu and Cringle, 2001). In vascularised retinas, oxygenation is ensured by a well-organised retinal and choroidal vascular network. The choriocapillaris, a network of capillary vessels located under the retinal pigmented epithelium, is the main source of oxygen for the outer retina, and retinal vessels are the main supply for the inner retina (Michaelson, 1954). While choroidal vasculature has been maintained throughout evolution in vertebrates, the retinal vasculature pattern differs widely between species (Rochon-Duvigneaud, 1943). Based on the presence and distribution of this vasculature, four patterns have been described: euangiotic/holangiotic, merangiotic, paurangiotic and anangiotic (Leber, 1875; Schaepdrijver et al., 1989). The canine retina is classified as holangiotic as its blood vessels extend from the optic nerve head to the far periphery. In the temporally located area centralis, they converge toward the highly specialized fovea-like area

LITERATURE REVIEW

Rueben G. Daset.al (2017) Defects in the cilia gene *RPGRIP1* cause Lebar congenital amaurosis and cone-rod dystrophy in humans. A form of canine cone-rod dystrophy (cord1) was originally associated with a homozygous insertion in *RPGRIP1* (*RPGRIP1* $^{ins/ins}$) as the primary disease locus while a homozygous deletion in *MAP9* (*MAP9* $^{del/del}$) was later identified as a modifier associated with the early onset form. However, we find further variability in cone electroretinograms (ERGs) ranging from normal to absent in an extended *RPGRIP1* $^{ins/ins}$ canine colony, irrespective of the *MAP9* genotype. Ophthalmoscopically, cone ERG ^{absent} *RPGRIP1* $^{ins/ins}$ eyes show discoloration of the tapetal fundus with varying onset and disease progression, while ds-OCT reveals atrophic changes. Despite marked changes in cone ERG and retinal morphology, photopic vision-guided behavior is comparable between normal and cone ERG ^{absent} *RPGRIP1* $^{ins/ins}$ littermates. Cone morphology of the dogs lacking cone ERG are truncated with shortened outer and inner segments. Immunohistochemically, cone ERG ^{absent} *RPGRIP1* $^{ins/ins}$ retinas have extensive L/M-opsin misvocalization, lack CNGB3 labelling in the L/M-cones, and lack GC1 in all cones. Our results indicate that cord1 is a multigenic disease

in which mutations in neither *RPGRIP1* nor *MAP9* alone lead to visual deficits, and additional gene(s) contribute to cone-specific functional and morphologic defects.

Matthew J Annear et.al (2021) The Rpe65-deficient dog has been important for development of translational therapies of Lebar congenital amaurosis type 2 (LCA2). The purpose of this study was to provide a comprehensive report of the natural history of retinal changes in this dog model. Rpe65-deficient dogs from 2 months to 10 years of age were assessed by fundus imaging, electroretinography (ERG) and vision testing (VT). Changes in retinal layer thickness were assessed by optical coherence tomography and on plastic retinal sections. ERG showed marked loss of retinal sensitivity, with amplitudes declining with age. Retinal thinning initially developed in the *area centralis*, with a slower thinning of the outer retina in other areas starting with the inferior retina. VT showed that dogs of all ages performed well in bright light, while at lower light levels they were blind. Retinal pigment epithelial (RPE) inclusions developed and in younger dogs and increased in size with age. The loss of photoreceptors was mirrored by a decline in ERG amplitudes. The slow degeneration meant that sufficient photoreceptors, albeit very desensitized, remained to allow for residual bright light vision in older dogs. This study shows the natural history of the Rpe65-deficient dog model of LCA2.

Maria Kaukonenet.al (2019) Retinitis pigmentosa (RP) is the leading cause of blindness with nearly two million people affected worldwide. Many genes have been implicated in RP, yet in 30–80% of the RP patients the genetic cause remains unknown. A similar phenotype, progressive retinal atrophy (PRA), affects many dog breeds including the Miniature Schnauzer. We performed clinical, genetic and functional experiments to identify the genetic cause of PRA in the breed. The age of onset and pattern of disease progression suggested that at least two forms of PRA, types 1 and 2 respectively, affect the breed, which was confirmed by genome-wide association study that implicated two distinct genomic loci in chromosomes 15 and X, respectively. Whole-genome sequencing revealed a fully segregating recessive regulatory variant in type 1 PRA. The associated variant has a very recent origin based on haplotype analysis and lies within a regulatory site with the predicted binding site of HAND1::TCF3 transcription factor complex. Luciferase assays suggested that mutated regulatory sequence increases expression. Case-control retinal expression comparison of six best HAND1:TCF3 target genes were analyzed with quantitative reverse-transcriptase PCR assay and indicated overexpression of EDN2 and COL9A2 in the affected retina. Defects in both EDN2 and COL9A2 have been previously associated with retinal degeneration. In summary, our study describes two genetically different forms of PRA and identifies a fully penetrant variant in type 1 form with a possible regulatory effect. This would be among the first reports of a regulatory variant in retinal degeneration in any species, and establishes a new spontaneous dog model to improve our understanding of retinal biology and gene regulation while the affected breed will benefit from a reliable genetic testing.

Chao Wang et.al (2021) We present GSD_1.0, a high-quality domestic dog reference genome with chromosome length scaffolds and contiguity increased 55-fold over CanFam3.1. Annotation with generated and existing long and short read RNA-seq, miRNA-seq and ATAC-seq, revealed that 32.1% of lifted over CanFam3.1 gaps harboured previously hidden functional elements, including promoters, genes and miRNAs in GSD_1.0. A catalogue of canine “dark” regions was made to facilitate mapping rescue. Alignment in these regions is difficult, but we demonstrate that they harbour trait-associated variation. Key genomic regions were completed, including the

Dog Leucocyte Antigen (DLA), T Cell Receptor (TCR) and 366 COSMIC cancer genes. 10x linked-read sequencing of 27 dogs (19 breeds) uncovered 22.1 million SNPs, indels and larger structural variants. Subsequent intersection with protein coding genes showed that 1.4% of these could directly influence gene products, and so provide a source of normal or aberrant phenotypic modifications.

Maria Kaukonen et.al (2021) Retinitis pigmentosa (RP) is a blinding eye disease affecting nearly two million people worldwide. Dogs are affected with a similar illness termed progressive retinal atrophy (PRA). Lapponian Herders (LHs) are affected with several types of inherited retinal dystrophies, and variants in PRCD and BEST1 genes have been associated with generalized PRA and canine multifocal retinopathy 3 (cmr3), respectively. However, all retinal dystrophy cases in LHs are not explained by these variants, indicating additional genetic causes of disease in the breed. We collected DNA samples from 10 PRA-affected LHs, with known PRCD and BEST1 variants excluded, and 34 unaffected LHs. A genome-wide association study identified a locus on CFA20 (praw=2.4x10 -7,pBonf=0.035), and subsequent whole-genome sequencing of an affected LH revealed a missense variant, c.3176G>A, in the intraflagellar transport 122 (IFT122) gene. The variant was also found in Finnish Lapphunds, in which its clinical relevancy needs to be studied further. The variant interrupts a highly conserved residue, p.(R1059H), in IFT122 and likely impairs its function. Variants in IFT122 have not been associated with retinal degeneration in mammals, but the loss of ift122 in zebrafish larvae impaired opsin transport and resulted in progressive photoreceptor degeneration. Our study establishes a new spontaneous dog model to study the role of IFT122 in RP biology, while the affected breed will benefit from a genetic test for a recessive condition.

METHODOLOGY

A female dog of unknown breed and age (roughly four to five years) was involved in an automobile accident and subsequently died while being treated at the Department of Veterinary Surgery & Radiology at Anand Agricultural University (AAU) in Anand, Gujarat, India. The retinal tissues from both eyes were collected for study. After being rinsed with phosphate buffer saline solution, tissues were placed into the "RNA later" and frozen in liquid nitrogen for further processing. Both sets of produced reads were combined and screened for quality using PRINSEQ. Any readings that were less than 60 bases, had a mean read quality of less than 20, or were duplicates were discarded. We used the online programme ORF Predictor to find open reading frames in the assembled contigs at an e-value threshold of 10^{-5}.

DATA ANALYSIS

Totaling 569,066 quality reads with a mean read length of 145.79 bp, RNA sequencing performed on an Ion Torrent, and a total of 231,088 quality reads with a mean read length of 373.19 bp, were obtained on a 454 GS-FLX. We found that 222,296 reads matched uniquely to the reference genome out of a total of 226,684 fragments counted (Table 1). Out of the 28,455 reference genes in the canine transcriptome, 10,360 were shown to be functional. The CanFam3.1 reference genome assembly with annotations was obtained from the NCBI Genome browser. In total, 34.05% of the reads mapped inside exons and 14.63% % mapped on exonexon boundaries to protein-coding genes. About 48.00% are in the introns, whereas 2.75 %

are in the exon-intron junctions. When sequencing library preparation involves random priming of the mRNA, it is not unusual for a relatively high percentage of reads to be allocated to introns.

Table 1: Distribution of mapped reads to different transcript types and gene regions

	Uniquely mapped	Nonspecifically mapped	Mapped reads	
	Number of reads	Number of reads	Number of reads	%
Total exon reads	75,883	1,305	77,188	34.05
Exon-exon reads	32,385	768	33,153	14.63
Exon-intron reads	6,210	35	6,245	2.75
Total intron reads	107,818	2,280	110,098	48.57
Total gene reads	222,296	4,388	226,684	100.00

Totaling 569,066 quality reads with a mean read length of 145.79 bp, RNA sequencing performed on an Ion Torrent, and a total of 231,088 quality reads with a mean read length of 373.19 bp, were obtained on a 454 GS-FLX. We found that 222,296 reads matched uniquely to the reference genome out of a Total of 226,684 fragments counted (Table 1). Out of the 28,455 reference genes in the canine transcriptome, 10,360 were shown to be functional. The CanFam3.1 reference genome assembly with annotations was obtained from the NCBI Genome browser. In total, 34.05% of the reads mapped inside exons and 14.63&percent;% mapped on exonexon boundaries to protein-coding genes. About 48.00% are in the introns, whereas 2.75 % are in the exon-intron junctions (Table 2). When sequencing library preparation involves random priming of the mRNA, it is not unusual for a relatively high percentage of reads to be allocated to introns.

TABLE 2. Predictive Value of the Vision Test

	Frequency of Correct Choice		Prediction of Disease Status		
Dog	Full Light	Dim Light	By Choice	By Time	Actual Disease Status
1	5/7	4/7	U $P = 0.573$	U $P = 0.996$	U
2	6/7	5/7	U $P = 0.508$	U $P = 0.417$	U
3	4/7	5/7	U $P = 0.573$	U $P = 0.359$	U
4	6/7	5/7	U $P = 0.508$	U $p = 0.359$	U
5	6/7	7/7	U $P = 0.280$	U $P = 0.471$	U
6	9/10	6/10	U $P = 0.099$	U $P = 0.732$	U
7	5/8	6/8	U $P = 0.586$	U $P = 0.383$	U
8	6/7	4/7	U $P = 0.212$	U $P = 0.932$	U
9	6/7	5/7	U $P = 0.508$	U $P = 0.850$	U
10	6/7	7/7	U $P = 0.280$	A $P = 0.017$	U
11	N/A	N/A	N/A	N/A	U
12	6/7	1/7	A $P = <0.0005$	A $P = <0.001$	A (RPE65)
13	12/13	6/11	A $P = 0.024$	A $P = <0.0005$	A (RPE65)
14	7/7	0/7	A $P = <0.0005$	A $P = <0.001$	A (RPE65)
15	6/7	1/7	A $P = <0.0005$	A $P = <0.0005$	A (PDE6A)
16	7/7	4/7	A $P = 0.022$	A $P = 0.001$	A (PDE6A)
17	6/7	3/9	A $P = 0.011$	A $P = <0.0005$	A (PDE6A)

CONCLUSION

In this study, we shortened the departure time data for low light levels by stopping the experiments early if the dog hadn't left the room within 60 seconds. This article presents an instance in which canines routinely and rapidly emerged from the device when it was still bright outside. It took the dogs more than a minute to leave the cage because of the low lighting. The average escape time in low-light settings would have been greater if we hadn't shortened the data, but the truncation had no effect on the average exit time in high-light conditions. That's why we were able to cut out the excess information and provide an upper bound on the likelihood. Eliminating data points based on departure time will exaggerate visual performance in a common study technique when researchers need to more precisely estimate visual performance. We find that this uncertainty is removed from the first-choice tunnel result, making it more amenable to examination. The F-test establishes statistical significance for the strength of the correlation between dependent and independent variables, and the technique's output, rmcorr, indicates this strength. Measurements of eccentricity in increments of 1 mm were taken using SD-OCT and analysed using a mixed effect model implemented in the Python environment.

REFERENCES

1. Rueben g. Daset.al (2017) variabilities in retinal function and structure in a canine model of cone-rod dystrophy associated with rpgrip1 support multigenic etiology

2. Matthew j annear et.al (2021) a comprehensive study of the retinal phenotype of rpe65-deficient dogs

3. Maria kaukonenet.al (2019) a putative silencer variant in a spontaneous canine model of retinitis pigmentosa

4. Chao wang et.al (2021) a novel canine reference genome resolves genomic architecture and uncovers transcript complexity

5. Maria kaukonen et.al (2021) a missense variant in ift122 associated with a canine model of retinitis pigmentosa

6. Forman, o. P. Et al. Canine genome assembly correction facilitates identification of a map9 deletion as a potential age of onset modifier for rpgrip1-associated canine retinal degeneration. Mammalian genome: official journal of the international mammalian genome society 27, 237–245, https://doi.org/10.1007/s00335-016-9627-x (2016).

7. Downs, l. M. *Et al*. Overlap of abnormal photoreceptor development and progressive degeneration in leber congenital amaurosis caused by nphp5 mutation. *Human molecular genetics*, doi:https://doi.org/10.1093/hmg/ddw254 (2016).

8. Kondo, m. Et al. A naturally occurring canine model of autosomal recessive congenital stationary night blindness. Plos one 10, e0137072, https://doi.org/10.1371/journal.pone.0137072 (2015).

9. Miyadera, k., brierley, i., aguirre-hernandez, j., mellersh, c. S. &sargan, d. R. Multiple mechanisms contribute to leakiness of a frameshift mutation in canine cone-rod dystrophy. Plos one 7, e51598, https://doi.org/10.1371/journal.pone.0051598 (2012).

10. Bainbridge, j.w.; mehat, m.s.; sundaram, v.; robbie, s.j.; barker, s.e.; ripamonti, c.; georgiadis, a.; mowat, f.m.; beattie, s.g.; gardner, p.j.; et al. Long-term effect of gene therapy on leber's congenital amaurosis. *N. Engl. J. Med.* 2015, *372*, 1887–1897.

11. Gardiner, k.l.; cideciyan, a.v.; swider, m.; dufour, v.l.; sumaroka, a.; komaromy, a.m.; hauswirth, w.w.; iwabe, s.; jacobson, s.g.; beltran, w.a.; et al. Long-term structural outcomes of late-stage rpe65 gene therapy. *Mol. Ther.* 2020, *28*, 266–278.

12. Annear, m.j.; gornik, k.r.; venturi, f.l.; hauptman, j.g.; bartoe, j.t.; petersen-jones, s.m. Reproducibility of an objective four-choice canine vision testing technique that assesses vision at differing light intensities. *Vet. Ophthalmol.* 2013, *16*, 324–328.

13. Dai, x.; zhang, h.; he, y.; qi, y.; chang, b.; pang, j.j. The frequency-response electroretinogram distinguishes cone and abnormal rod function in rd12 mice. *Plos one* 2015, *10*, e0117570.

14. Palczewski, k.; kiser, p.d. Shedding new light on the generation of the visual chromophore. *Proc. Natl. Acad. Sci. Usa* 2020, *117*, 19629–19638

15. Le meur, g.; lebranchu, p.; billaud, f.; adjali, o.; schmitt, s.; bézieau, s.; péréon, y.; valabregue, r.; ivan, c.; darmon, c.; et al. Safety and long-term efficacy of aav4 gene therapy in patients with rpe65 leber congenital amaurosis. *Mol. Ther.* 2018, *26*, 256–268.