Dogs and Humans Share Unique Gene Expression Signatures in the Macular and Peripheral Retina

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

Michele M. Salzman[1]*, Tetsuya Takimoto[2], Melanie L. Foster[3], Freya M. Mowat[1,3,4]

[1]Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison;
[2]Medical Genetics, School of Medicine and Public Health, University of Wisconsin-Madison;
[3]College of Veterinary Medicine, North Carolina State University, Raleigh, NC,
[4]Ophthalmology and Visual Sciences, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI

M o w a t L a b

msalzman2@wisc.edu

Background

The macula is a photoreceptor-enriched region of the central retina in humans, critical for high quality vision, and susceptible to age-related disease, including age-related macular degeneration (AMD) and glaucoma, as well as environmental toxicants such as heavy metals.[1]

The tissues that support the retina include the retinal pigmented epithelium (RPE), choroid and sclera.

Previous work using normal human donor eyes determined the macula has unique gene expression signatures compared to the periphery, with 2,051 differentially expressed (DE) genes in the macular retina and 926 differentially expressed genes in the macular RPE/choroid.[2] These differences between the macula and periphery may help to explain why some diseases affect the macular region while others affect the periphery.[2]

We have shown that the dog also has a central photoreceptor-enriched macula-like region similar to the human macula, whereas laboratory rodents lack a similar region.[1,3,4]

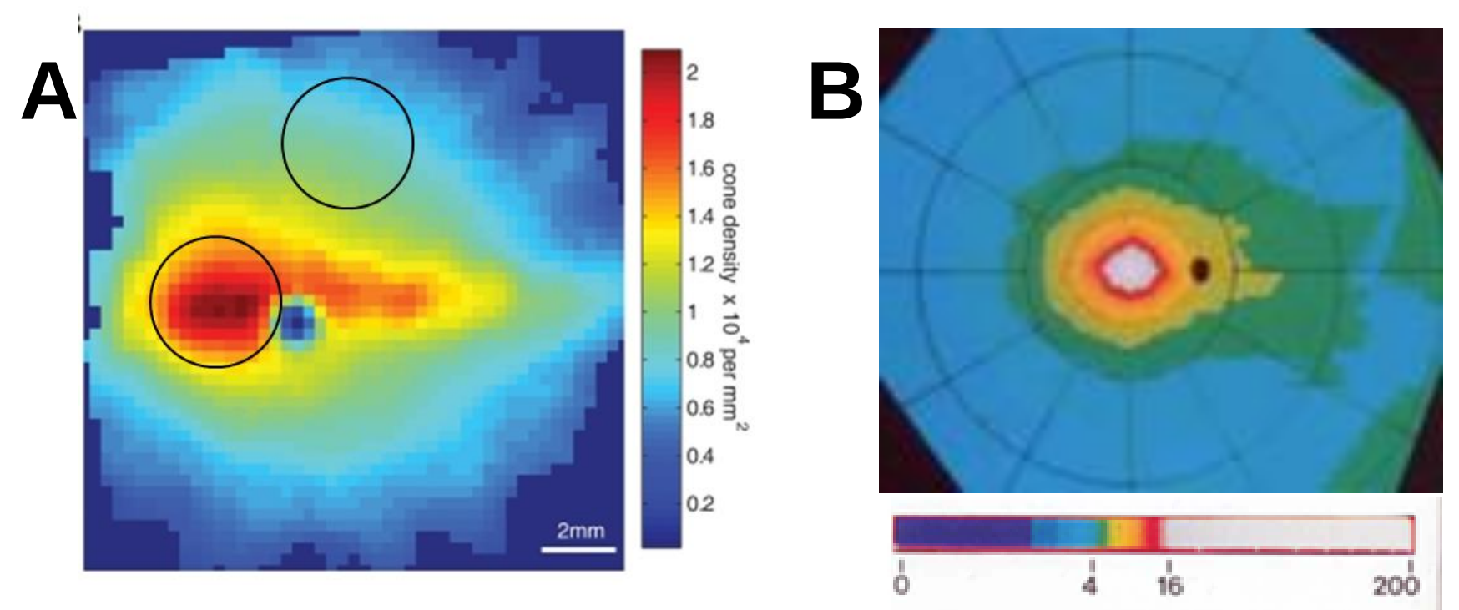


Figure1. A) Cone density map with correlation to predicted macula in dogs (n = 9 eyes from 5 adult dogs).[2] Circles represent where macula and periphery samples were taken. B) Cone density map showing macula and periphery in humans (n = 8 eyes from 7 adult humans).[1]

Hypothesis

There will be similarities in the differential gene expression and biological pathways implicated comparing dog and human macular/peripheral retina and RPE/choroid/sclera.

Methods

Dogs were humanely euthanized for purposes unrelated to this study.

8mm punches of retina and RCS taken < 3 hrs after death from macula and superior periphery (see Fig 1, circles indicate locations samples taken); MR = macular retina, MRCS = macular RPE/choroid/sclera, PR = peripheral retina, PRCS = peripheral RPE/choroid/sclera.

Total RNA isolation using RNeasy mini kit (Qiagen) with DNase treatment.

RNA sequencing on 4 eyes (mixed breed dogs, 2M, 2F); Illumina directional libraries (Illumina HiSeq 2500, 125bp SE); RIN 9.35 ±0.18, depth 33.6 ±1.0 million reads; QC analysis with FaFastQC v.0.11.8 after trimming Tru-seq adapter with Trimmomatic v.0.39; passed sequences mapped on CanFam3 by Hisat2 v.2.1.0; raw counts extracted from mapped data by FeatureCounts v.2.0.0; ~80% of sequences mapped on CanFam3.

Human data: tissue punches taken from 8 eyes from normal human donors (74 yrs old ±13 yrs; 5M, 3F) < 6 hrs after death; RNA-Seq data aligned to the hg19 reference genome using GSNAP.[4]

Using our dog data and the retrospective human data, detection of differentially expressed genes (false discovery rate: FDR < 0.1 > 2FC) was performed with edgeR v.3.26.8, followed by enrichment analysis using DAVID bioinformatics database v.6.8.

Quantitative RT-PCR of key pathway genes that were differentially expressed (n = 7 eyes, Beagle and mixed breed dogs, 4M, 3F); cDNA synthesized using the iScript cDNA Synthesis kit (Bio-Rad); primers designed to CanFam4 using NCBI reference sequence and PrimerBlast; SYBR green chemistry; HPRT1 as the endogenous control.

Results

Group	Dog (n = 4 eyes)	Human (n = 8 eyes)
MR	15721	18318
MRCS	15856	17334
PR	16213	18850
PRCS	17564	19022

Table 1. Numbers of expressed genes.
The mean number of expressed genes were calculated based on the number of samples for each eye part.

Comparison of groups	Number of DE genes	Number of DE genes with higher expression in macula	Number of DE genes with higher expression in periphery
	Dog (n = 4)		
MR vs PR	554	120	434
MRCS vs PRCS	671	286	385
	Human (n = 8)		
MR vs PR	2,051	1,239	812
MRCS vs PRCS	926	653	273

Table 2. Numbers of DE genes identified from each comparison.
A gene was considered differentially expressed if the FDR-adjusted p-value was < 0.1 and > 2FC.

Figure 2. Volcano plots of DE genes.
FDR < 0.1 > 2FC; FC is fold change; vertical lines represent boundaries for > or < 2FC.

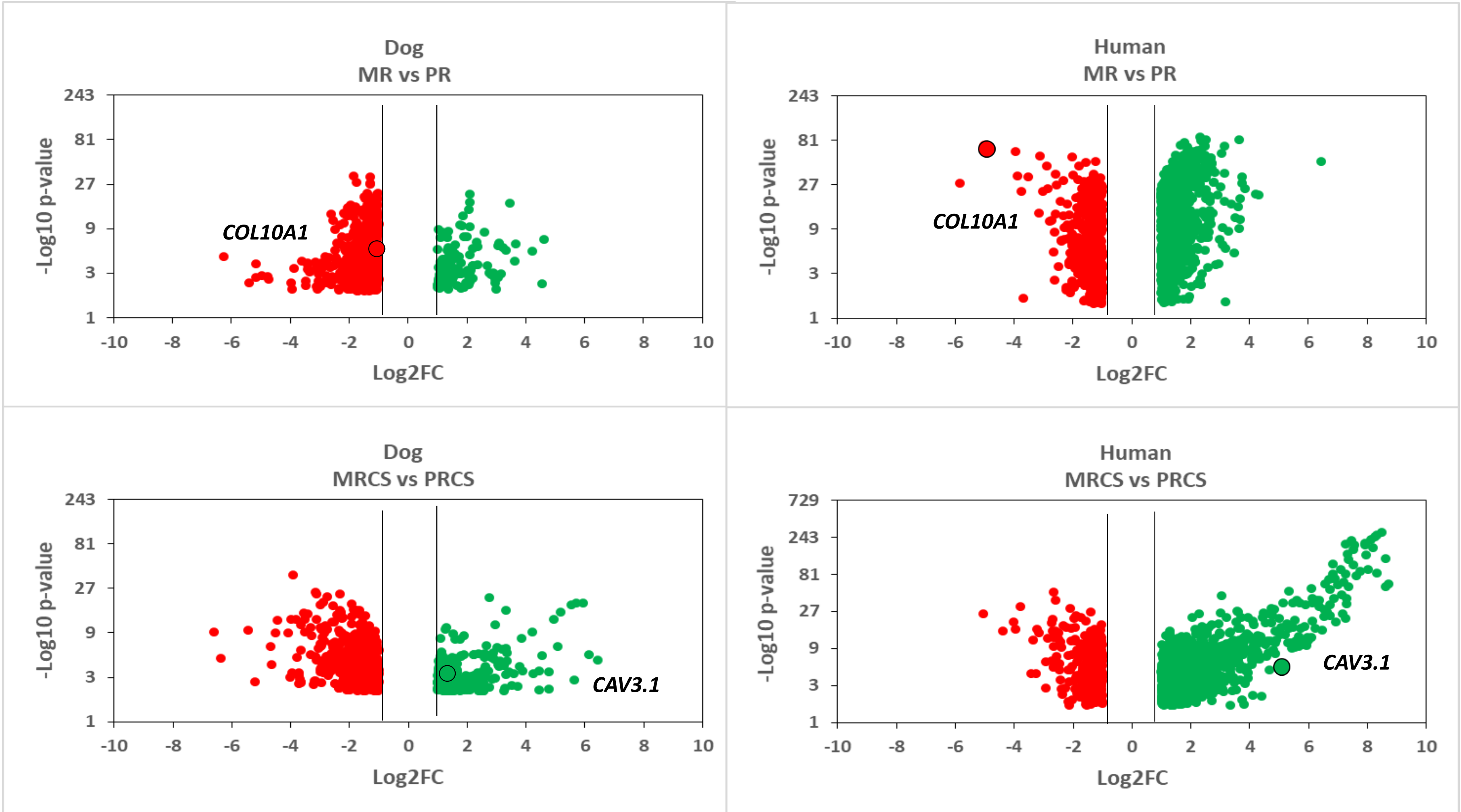


Table 3. DAVID enrichment analysis for DE genes between the macular and periphery.
Displayed are the top four enriched terms for each category. Enriched terms shared between dogs and humans are highlighted in color; green = up-regulated in the macula, red = down-regulated in the macula.

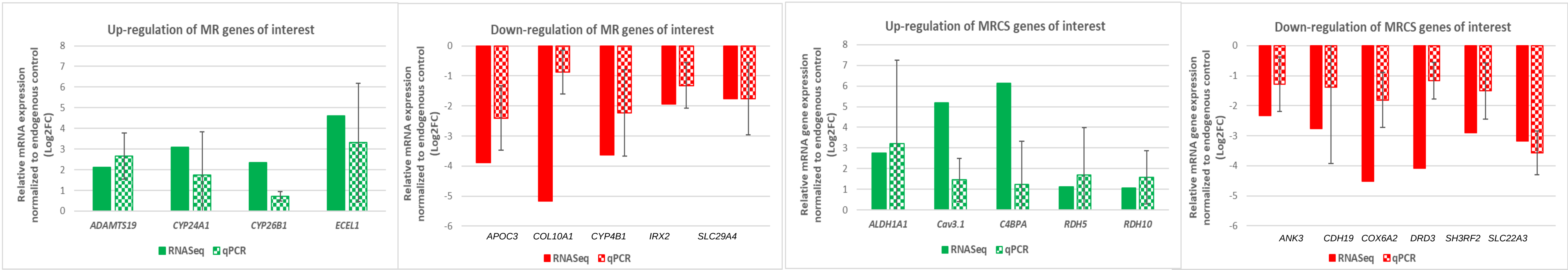
Dogs

Category	MR > PR term	Gene count	p-value	MR < PR term	Gene count	p-value
Gene Ontology	Extracellular space	11	1.6E-2	Integral component of membrane	102	8.3E-5
	Proteinaceous extracellular matrix	6	2.3E-3	Integral component of plasma membrane	35	4.0E-6
	Scavenger receptor activity	4	2.0E-3	Calcium ion binding	23	1.8E-3
	Heparin binding	4	1.7E-2	Protein homooligomerization	12	3.3E-5
KEGG Pathway	Phagosome	7	1.7E-4	Neuroactive ligand-receptor interaction	14	2.0E-4
	Tuberculosis	6	3.1E-3	Pathways in cancer	11	8.1E-2
	HTLV-1 infection	6	1.7E-2	Rap1 signaling	8	4.2E-2
	Neuroactive ligand-receptor interaction	6	2.3E-2	Ras signaling	8	5.3E-2
Category	**MRCS > PRCs term**	**Gene count**	**p-value**	**MRCS < PRCS term**	**Gene count**	**p-value**
Gene Ontology	Integral component of membrane	79	9.4E-5	Integral component of membrane	87	2.4E-2
	Calcium ion binding	15	3.4E-2	Plasma membrane	48	8.8E-3
	Extracellular region	12	4.9E-3	Calcium ion binding	32	6.7E-8
	Negative regulation of apoptotic process	7	6.9E-2	Small GTPase mediated signal transduction	11	4.3E-3
KEGG Pathway	Adrenergic signaling	7	1.0E-2	Neuroactive ligand-receptor interaction	22	9.3E-9
	Retinol metabolism	6	4.5E-4	PI3K-Akt signaling	13	2.1E-2
	Oxytocin signaling	6	5.0E-2	Calcium signaling	12	4.2E-4
	Calcium signaling	6	9.7E-2	cGMP-PKG signaling	10	2.3E-3

Humans

Category	MR > PR term	Gene count	p-value	MR < PR term	Gene count	p-value
Gene Ontology	Integral component of membrane	237	7.1E-6	Integral component of membrane	103	1.4E-3
	Plasma membrane	203	4.1E-7	Plasma membrane	87	7.7E-4
	Calcium ion binding	60	1.0E-9	Positive regulation of transcription, DNA-templated	22	8.9E-5
	Positive regulation of GTPase activity	33	7.6E-3	Oxidation-reduction process	21	1.4E-3
KEGG Pathway	Neuroactive ligand-receptor interaction	49	9.5E-2	Hippo signaling	9	6.9E-3
	Pathways in cancer	24	3.5E-5	Protein digestion and absorption	8	1.2E-3
	Adrenergic signaling	20	5.8E-2	Signaling pathways regulating pluripotency of stem cells	7	4.6E-2
	cAMP signaling	16	4.E-5	Mineral absorption	6	1.3E-3
Category	**MRCS > PRCs term**	**Gene count**	**p-value**	**MRCS < PRCS term**	**Gene count**	**p-value**
Gene Ontology	Integral component of membrane	296	6.7E-3	Integral component of membrane	82	3.4E-4
	Plasma membrane	277	7.6E-8	Plasma membrane	69	3.3E-4
	Signal transduction	76	2.3E-2	Cell adhesion	20	1.3E-6
	Calcium ion binding	74	4.3E-9	Proteolysis	19	1.7E-5
KEGG Pathway	Neuroactive ligand-receptor interaction	53	1.1E-17	Neuroactive ligand-receptor interaction	11	1.7E-3
	Calcium signaling	37	2.3E-13	Cell adhesion molecules (CAMs)	6	2.8E-2
	Adrenergic signaling	31	3.1E-12	ECM-receptor interaction	5	2.1E-2
	Oxytocin signaling	27	1.6E-8	TGF-beta signaling	4	8.1E-2

Figure 3. Quantitative RT-PCR confirmation of gene expression for key pathway genes of interest.



A portion of the differentially expressed genes were replicated by qRT-PCR to show our RNA-Seq data analysis was reproducible.

Conclusions

Differentially expressed genes in the retina and supporting tissues in dogs and humans share several pathways. The macular retina shares the neuroactive ligand-receptor interaction pathway, and the macular RCS shares the adrenergic, calcium, and oxytocin signaling pathways.

Key DE genes up-regulated in the macular retina of dogs include *CYP24A1* (cytochrome P450, family 24, subfamily A, polypeptide 1) involved in a number of metabolic pathways and biological oxidation, and *CYP26B1* (cytochrome P450, family 26, subfamily B, polypeptide 1) involved in retinol metabolism.

Key DE genes in the macular retina shared between dogs and humans include the down-regulated *COL10A1* (collagen type x alpha 1 chain) implicated in collagen formation and extracellular matrix organization.

Key DE genes up-regulated in the macular RCS include *CAV3.1* (caveolin 3) involved in the regulation of calcium and sodium channel activity and focal adhesion, as well as *ALDH1A1* (aldehyde dehydrogenase 1 family member 1) and *RDH10* (retinol dehydrogenase 10) involved in retinol metabolism and visual phototransduction.

Key DE genes in the macular RCS shared between dogs and humans include the up-regulated *CACNA1S* (calcium voltage-gated channel subunit alpha 1S) which encodes a subunit of the channel, and the down-regulated *S1PR5* (sphingosine-1 phosphate receptor 5) which regulates cell proliferation, apoptosis, motility, and neurite retraction.

The work adds to the growing body of evidence drawing similarities between canine and human neurologic tissues.

Future Directions

Investigation of novel differentially expressed genes for involvement in macular disorders.

Study of structural and functional decline in the canine visual system with aging and age-related retinal diseases, as well as in association with environmental neurodegenerative risk factor exposure such as heavy metals, since cadmium has been found in higher concentrations in the retinal tissues of donor eyes from patients with AMD.[5]

Acknowledgements

This work was supported by NIH K08EY028628 (FM) and a North Carolina State University Center for Human Health and the Environment pilot grant.

Author disclosures: None

References

1. C.A. Curcio, et al. Human photoreceptor topography. *J Comp Neurol* 1990; 292(4): 497-523.
2. M. Li, et al. Comprehensive analysis of gene expression in human retina and supporting tissues. *Hum Mol Genet* 2014; 23(15): 4001-4014.
3. F.M. Mowat, et al. Topographical characterization of cone photoreceptors and the area centralis of the canine retina. *Mol Vis* 2008; 14: 2518-2527.
4. W.A. Beltran, et al. Canine retina has a primate fovea-like bouquet of cone photoreceptors which is affected by inherited macular degenerations. *PLoS One* 2014; 9(3): e90390.
5. S. Aberami, et al. Elemental concentrations in choroid-RPE and retina of human eyes with age-related macular degeneration. *Exp Eye Res* 2019; 186: 107718.