Utilization of electroretinography and advanced retinal imaging to monitor development of retinal abnormalities in nonhuman primates with homozygous or heterozygous mutations in PDE6C

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Introduction

- The PDE6C gene is involved in the phototransduction pathway in cone cells of the retina. This gene encodes for phosphodiesterase-6-alpha enzyme, which hydrolyzes cGMP to GMP.[4]
- When this gene is mutated, it results in a condition known has achromatopsia. Those with achromatopsia have poor visual acuity, poor color vision, photophobia, and nystagmus.[4]
- There is currently no cure for this disorder, but researchers are working on developing gene and cell therapies that might show some promise.[4]

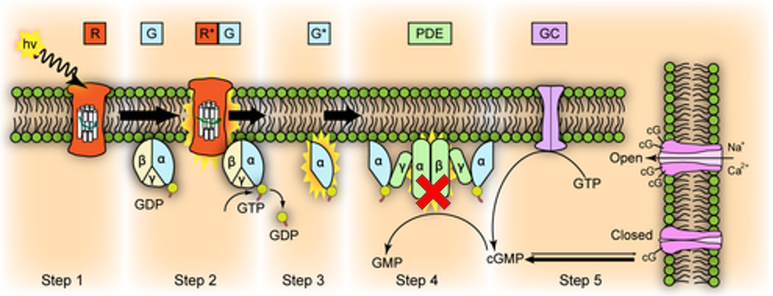


Figure 1. Steps of the phototransduction pathway. Image acquired from *https://en.wikipedia.org/wiki/Visual_phototransduction*

Aims

Specific aim 1: To determine when retinal abnormalities associated with a homozygous mutation in PDE6C appear in rhesus macaques and whether these abnormalities are stationary or progress over time.

Specific aim 2: To determine whether retinal abnormalities on electroretinography or multimodal retinal imaging are present in rhesus macaques with a heterozygous mutation in PDE6C .

Hypothesis

Rhesus macaques with the homozygous mutation will show reduced cone receptor function and macular abnormalities from birth and have slowly progressive macular disease over their lifetime. Also, rhesus macaques with the heterozygous mutations will show no discernible retinal abnormalities on electroretinography or retinal imaging.

Methods

Subjects

- 5 affected homozygous adult primates ranging from 4-13 years old with 5 age and sex-matched controls.
- 4 affected homozygous baby primates ranging from a few months to 1 years old with 4 age and sex-matched controls.
- 3 heterozygous primates ages 3 months, 4 months, and 12 years old.
- These primates were recruited from the California National Primate Research Center.

Electroretinography (ERG)

Measures electrical activity of the cone and rod photoreceptors of each eye when stimulated under different conditions.[2]

Spectral Domain Optical Coherence Tomography (SD-OCT)

Produces high resolution horizontal scans of the retina for measuring the thickness of various retinal layers.[2]

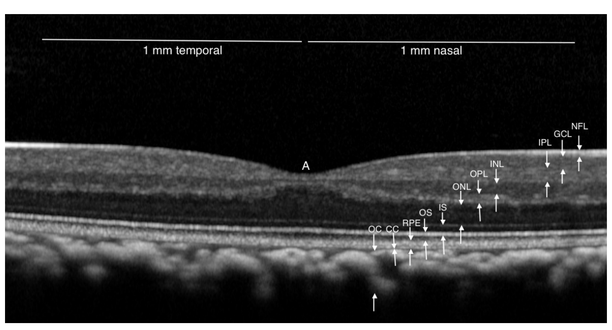


Figure 2. An OCT scan of the retina of a control primate. Layers of the retina are distinguished by the white arrows.

Fundus Photography

Produces color and red-free images of the fundus and macular structures.

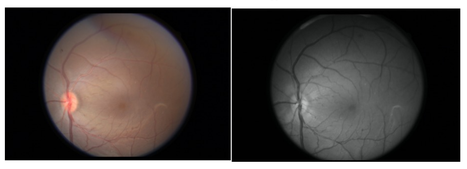


Figure 3. Color image (left) and red-free image (right) of the fundus of a control primate.

Fundus Autofluorescence

Detects the retina's natural fluorophores located in the retinal pigment epithelium. This allows us to discern the quality of retinal metabolism and health.[1]

Fluorescein Angiography

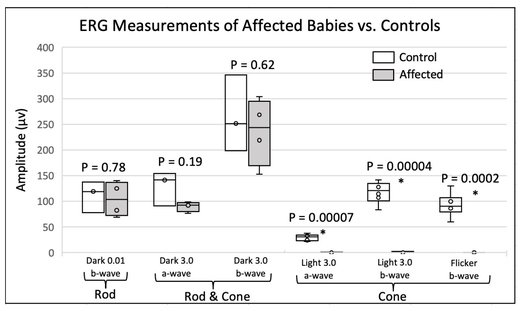
Highlights retinal blood vessels, allowing us to detect abnormalities in vascular perfusion and integrity

Statistical Analyses

Two-sample t-test

$P < 0.05$ was statistically significant

Results



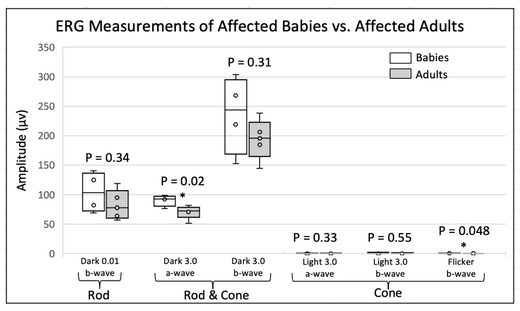
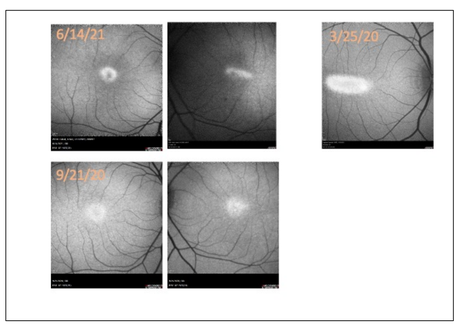


Figure 4. ERG boxplots comparing affected babies vs. controls and affected babies vs. affected adults.



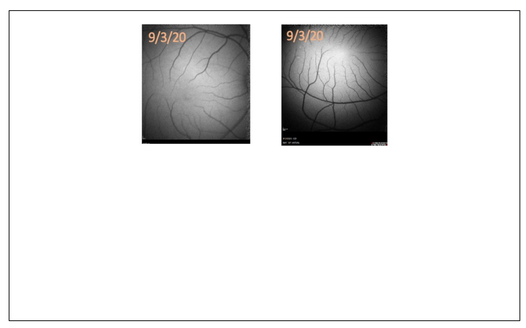


Figure 5. Fundus autofluorescence of affected babies (left) and control babies (right).

Mean Thickness of Retinal Layers from OCT of Affected Baby Primates vs. Control Baby Primates (Nasal)

Retinal Layer	Control (µm)	Affected (µm)	P-values
Total Retinal Thickness	338.47	279.45	0.002
NFL	21.48	20.52	0.51
GCL	40.10	32.42	0.04
IPL	42.83	37.86	0.25
INL	38.64	38.87	0.95
OPL	26.99	20.89	0.04
ONL	61.33	44.18	0.045
IS	41.94	38.19	0.11
OS	29.37	26.44	0.08
RPE	29.64	18.25	0.008
CC	10.86	8.76	0.11
OC	186.91	141.59	0.42

NFL = nerve fiber layer; GCL = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = inner nuclear layer; IS = inner segments of photoreceptors; OS = outer segment of photoreceptors; RPE = retinal pigment epithelium; CC = choriocapillaris; OC = outer choroid

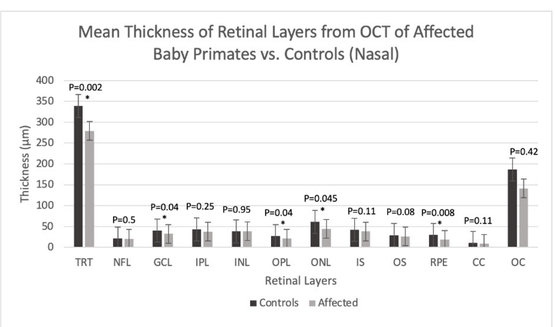


Figure 6. Measurements of the thickness of the various retinal layers of affected babies vs. controls.

Mean Thickness of Retinal Layers from OCT of Affected Babies vs. Affected Adults (Nasal)

Retinal Layer	Babies (µm)	Adults (µm)	P-values
Total Retinal Thickness	279.45	272.00	0.50
NFL	20.52	27.06	0.01
GCL	32.42	34.88	0.10
IPL	37.86	39.33	0.73
INL	38.88	42.12	0.39
OPL	20.89	17.52	0.07
ONL	44.18	32.56	0.00002
IS	38.19	33.81	0.02
OS	26.44	24.42	0.13
RPE	18.25	28.01	0.01
CC	8.76	10.08	0.27
OC	141.59	188.42	0.03

NFL = nerve fiber layer; GCL = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = inner nuclear layer; IS = inner segments of photoreceptors; OS = outer segments of photoreceptors; RPE = retinal pigment epithelium; CC = choriocapillaris; OC = outer choroid

Mean Thickness of Retinal Layers from OCT of Affected Babies vs. Affected Adults (Temporal)

Retinal Layer	Babies (µm)	Adults (µm)	P-values
Total Retinal Thickness	260.01	244.72	0.11
NFL	18.12	19.39	0.37
GCL	22.89	31.27	0.02
IPL	37.06	35.05	0.37
INL	34.09	37.49	0.15
OPL	23.38	18.63	0.10
ONL	40.96	27.04	0.0002
IS	38.38	34.49	0.18
OS	28.57	25.56	0.04
RPE	18.61	28.87	0.004
CC	8.55	11.54	0.02
OC	158.73	214.38	0.02

Figure 7. Measurements of the thickness of the various retinal layers of affected babies vs. affected adults. Measurements were done 1 mm nasally (left) and 1 mm temporally (right)

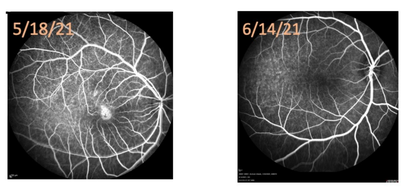


Figure 8. Average foveal avascular zone (FAZ) for control babies (left): **3.22 mm²**
Average foveal avascular zone (FAZ) for affected babies (right) : **1.95 mm²**
Sample size of the measurements for FAZ of control babies (n=2) were too few for meaningful statistical analyses

Discussion

1. Rhesus macaques with achromatopsia have reduced cone photoreceptor function and macular abnormalities from birth, implying that this disease is congenital. This is evident in the ERG data showing significantly decreased cone receptor activity in affected babies compared to control babies. The affected babies also seem to have unhealthy retinal pigment epithelium (RPE) cells compared to the controls as seen in the hypofluorescent and hyperfluorescent areas of the fundus autofluorescence. Certain layers of their retina are also significantly thin compared to the controls in the OCT data.
2. There appears to be progression of the disease over time, although subtle. A decrease in cone function as babies become adults can be seen with the flicker test in the ERG. Significant thinning of some layers the retina can be appreciated in babies compared to adults.
3. We need a larger sample size of rhesus macaques with the heterozygous mutation to make any meaningful analyses or definitive conclusions on whether there is an intermediate phenotype of the disease. Some data were also missing in this cohort, resulting in their exclusion from the study and a sample size of only 2.

References

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