

Esha Afreen, Deepti Ajjampore, Max Czechowski, Adam El-Meanawy, Anthony Fung, Kamy Gopal, Jason Hubler, Tahmid Iqbal, Tarun Jella, Ram Karanam, Eugene Kim, Raga Komandur, Harshi Mogallapalli, Erik Nesler, Rishi Sachdev, Hafsa Shereen, Nikita Sood, Alice Zheng

Advisor: Mrs. Louise Thompson

Mentor: Joe Carroll, Ph.D., Eye Institute at the Medical College of Wisconsin

Abstract

One in every 60,000 children is born with Ocular Albinism Type 1. Ocular Albinism is a genetic disease in which pigmentation is lost in the eye, in the retinal pigment epithelium (RPE) located just below the photoreceptors in the retina. This reduced pigmentation affects the development of the fovea (an area of the retina responsible for 99% of vision) and leads to poor visual acuity (the capacity to see fine detail). The mutation causing Ocular Albinism occurs in the gene *Oa1*, which encodes a G-protein coupled receptor (GPCR) called Ocular Albinism Type 1 (OA1). OA1 is found in RPE cells, which normally absorb scattered light with melanin, allowing the eye to generate a high-contrast image. While its exact function is unknown, OA1 is central to melanin biosynthesis and foveal development in the retina. Under normal conditions, the highly-selective ligand L-DOPA binds to OA1, which triggers tyrosinase to increase melanin synthesis. Simultaneously, tyrosinase also triggers L-DOPA to further bind with OA1, which activates a positive feedback loop. However, in many cases of albinism, this pathway is disrupted and tyrosinase is not fully efficient. To counter this, scientists are researching the possibility of bypassing the enzyme and flooding the cells with L-DOPA. Further understanding of OA1 and its function could lead to more effective treatments for albinism¹. The Brookfield Central High School SMART (Students Modeling A Research Topic) Team created a physical model of OA1 using 3-D modeling printing technology to better understand its structure-function relationship.

Ocular Albinism—The Disorder

OA1 is a sex-linked disease that leads to a lack of melanin production in the eyes and affects the retina, cones, RPE cells, and the fovea (Figure 1). The retina is the light-sensitive tissue at the back of the eye that contains the cones. Cones are the color-sensing photoreceptors. It is the RPE cells, which protect the retina by absorbing stray photons of light, that lack melanin. The foveal pit is a depression in the retina where light is directed once in the eye (Figure 2). Patients with ocular albinism develop abnormal foveal pits. A mutation in the *Oa1* gene that codes for OA1 (the protein) results in Ocular Albinism. Also, a decreased depth in the foveal pit is found in these patients. Due to incomplete foveal development, vision correction with glasses is impractical. Although the exact cause of this disease is not known, common characteristics can be found among patients with ocular albinism¹.

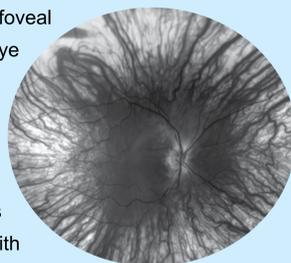


Figure 1: Human Fovea

Foveal Development and Ocular Albinism

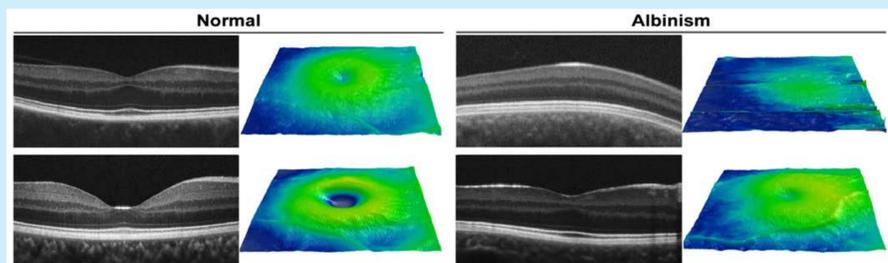


Figure 2: Foveal Pits

Individuals with normal vision (Left) have variable foveal pits. The top image is from an individual with relatively low melanin pigmentation, while the bottom image is from an individual with increased melanin pigmentation. Images from patients with Ocular Albinism (Right) also show variation. While both images are from patients with Ocular Albinism, note that the top image shows no foveal pit development while the bottom image shows a distinct foveal pit. This suggests that incomplete development of the foveal pit may not be the only cause of visual problems in patients with Ocular Albinism.

Autocrine Loop between OA1 and Tyrosinase

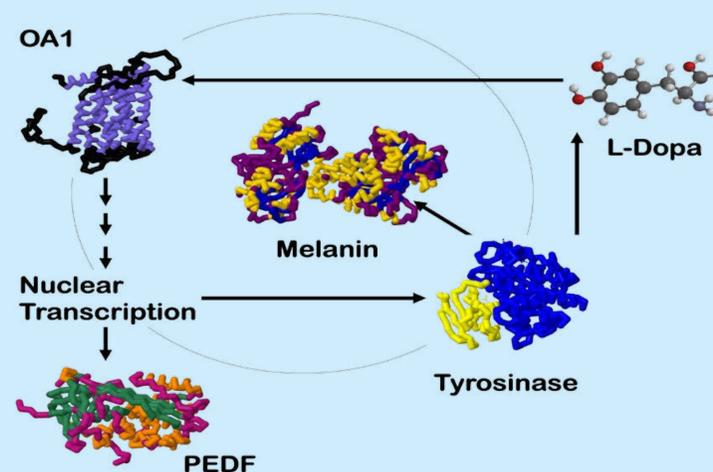


Figure 3: Feedback Loop

An autocrine loop between OA1 and tyrosinase and linked through L-DOPA includes the secretion of at least one very potent retinal neurotrophic factor, PEDF. Changes in OA1 signaling may underlie the disruptions in retinal development that cause albinism-related vision problems².

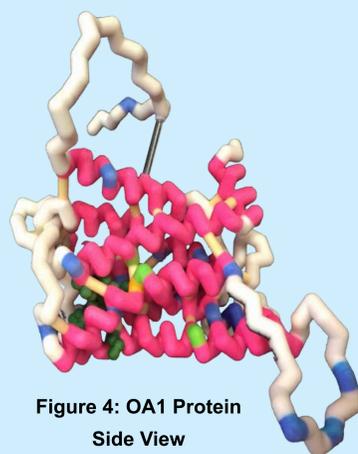


Figure 4: OA1 Protein Side View

OA1 Protein

Left: OA1 is a GPCR located on an intracellular melanosomal membrane. Residues colored light green are the conserved residues common to proteins of the GPCR superfamily (Asp140, Ala141, Tyr142, Trp162, Cys184, Pro210, Cys256, Trp257). Those colored light blue represent points of mutation leading to the disease of ocular albinism (Arg5, Gly35, Leu39, Asp78, Glu233, Arg245, Gly84, Cys116, Gly118, Gln124, Trp133, Ala138, Ser152, Ala173, Glu229, Thr232, Glu235, Ile244, Ile261, Glu271, Thr290, Trp292)³.

OA1 Protein Binding Site

Right: This image highlights the binding site of L-Dopa to OA1. Residues colored dark green represent amino acids that bind to L-Dopa (Asn268, Glu264, Tyr127, Met198, Phe293)³.

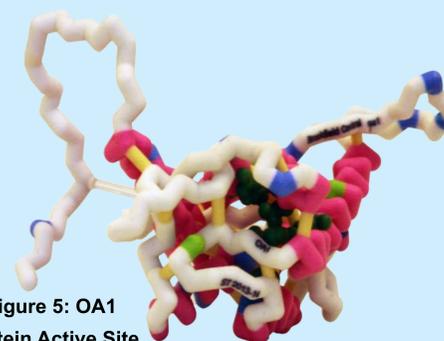


Figure 5: OA1 Protein Active Site

Evidence That L-Dopa Binds to OA1

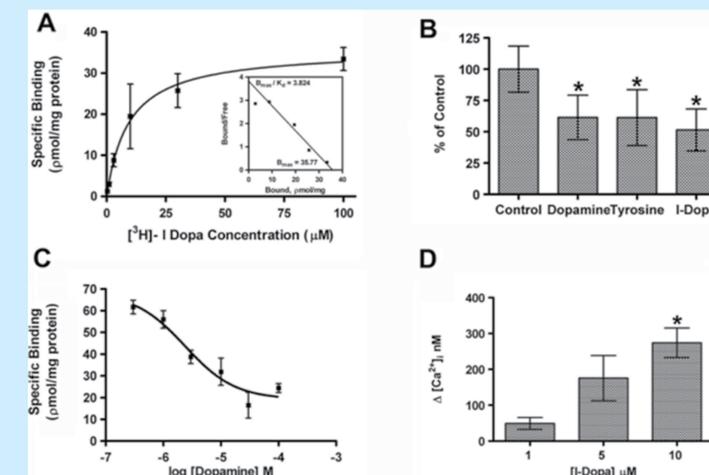


Figure 6: Evidence That L-Dopa Binds to OA1

- A) OA1 receptors were exposed to various concentrations of L-DOPA. The assays were labeled with radioactive isotopes that glow, making observation and data collection easier. Since the data points were close to a curve fit, a one to one binding relationship between L-DOPA and OA1 was determined.
- B) When OA1 was exposed to Tyrosine or Dopamine as well as L-DOPA, each of them was able to bind to OA1.
- C) To bolster the observation found in B), specific binding of 5µM L-DOPA was analyzed in the presence of various concentrations of Dopamine. The results confirm Dopamine as an antagonist for L-DOPA that competes for the same binding site, OA1.
- D) Calcium ions are released in response to the binding of a ligand to a GPCR. The graph illustrates the positive correlation between L-DOPA concentration and Calcium ion concentration in the cytoplasm⁴.

Conclusion

An interruption in the L-DOPA-induced metabolic pathway responsible for melanin synthesis due to a mutant form of the OA1 receptor protein inhibits the synthesis of RPE pigmentation agents and reduces the presence of other products of the pathway, namely PEDF. Involved with the allocation of vascularization in tissue, PEDF is responsible for the formation of the foveal pit of the retina. It was previously accepted that the absence of PEDF prevented the formation of a foveal pit, which in turn reduced visual acuity. However, recent evidence suggests that the foveal pit will form even in the absence of PEDF. Such evidence prompts scientists to re-investigate the true histological condition which results in the reduced visual acuity observed in patients with the *Oa1* gene mutation.

Citations

- ¹Oetting, W. S. (2002). New insights into ocular albinism type 1 (*oa1*): Mutations and polymorphisms of the *oa1* gene. *Human Mutation*, 19(2), 85-92. Retrieved from <http://onlinelibrary.wiley.com/doi/10.1002/humu.10034/pdf>
- ²Gross, L. (2008) A Molecular Link between Albinism and Visual Deficits. *PLoS Biol* 6(9): e248. doi:10.1371/journal.pbio.0060248. Retrieved from <http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.0060248>
- ³Ghosh, A., Sonavane, U., Andhirka, S.K., Aradhya, G. K., Joshi, R. (2012). Structural insights into human GPCR protein OA1: a computational perspective. *J Mol Model* 18: 2117-2133
- ⁴Lopez VM, Decatur CL, Stamer WD, Lynch RM, McKay BS (2008) L-DOPA is an endogenous ligand for OA1. *PLoS Biol* 6(9): e236. doi:10.1371/journal.pbio.0060236