Genetics 564
Specific Aim Draft #2
April 16, 2024
Dianna Xie

Optic atrophy type 1 is a genetic disease caused by a mutation within the OPA1 gene [1]. The OPA1 protein can be found mainly in the retinal ganglion cells within the eyes, and it is responsible for maintaining the structure of mitochondria [3]. The mutation of the gene affects protein production and further affects the mitochondria structure and the function of retinal ganglion cells [5]. With the dysfunction of mitochondria, optic nerve cells such as the retinal ganglion cells, will lead to apoptosis [4]. As the cell lysis, it becomes impossible for optic nerves to transmit visual signals, and eventually cause optic atrophy type 1 [6]. It is **known** that patients with optic atrophy type 1 will experience blindness, blocked vision, or color vision deficiency [2]. Also, the OPA1 gene undergoes autosomal dominant inheritance and display phenotypes in offspring [7]. *However, the role of OPA1 in the maintenance of mitochondria during retinal development remains **unknown** [3].*

My **primary goal** is to research when the development of mitochondria is affected by the mutation of the OPA1 gene. I will use **Danio rerio (zebrafish)** as the model organism to conduct the experiments since the zebrafish have rapid and transparent embryonic development, which is beneficial for observation [8]. I **hypothesize** that the developmental stage of mitochondria could occur from the embryonic stage to the early childhood stage. My **long-term goal** is to research more on the Optic Atrophy Type 1 disease and find potential chemical compounds that can rescue the disease-like phenotypes, and further go on to drug developments.

Aim 1: Identify the amino acids in OPA1 that are necessary during the early development of the retina.
Rationale: I can determine the domains that mutations occur in the OPA1 gene and the developmental stage of optic atrophy type 1 in zebrafish models by data analysis.
Approach: I will use **domain analysis** on the zebrafish OPA1 gene sequence to determine the site that caused the mutation. Different developmental stages are selected for observation: embryonic stage, day 3 to 5 after hatching, and every week until the sixth month. **Genome sequencing** will be performed to analyze the mutant and wildtype genome. These two techniques would identify the domains and time windows where the mutation will happen.
Hypothesis: I hypothesize that the developmental stage of optic atrophy type 1 ranges from the embryo stage to the adult stage.

Aim 2: Identify chemical compounds that can rescue the phenotypes of optic atrophy type 1.
Rationale: Chemical screens can help in visualizing the mitochondria structure of the zebrafish models. I can further determine which chemical compounds work the best in rescuing the disease-like phenotype and use in drug discovery.
Approach: I will use **chemical screens** on zebrafish models, both the mutants and the wild types, to identify if any compounds can rescue the disease-like phenotype. The zebrafish models will be observed from the embryonic to adult stage. The level of recovery of the disease-like phenotypes with each compound can be visualized. The same number of zebrafish will be selected for each compound, the same concentration of each compound will be injected, and the observation will be made in the same time window. The chemical compound library selected for this research is the Stanford High-Throughput Screening Knowledge Center Library (HTSKC) since it contains chemicals suitable for high-throughput chemical screening.
Hypothesis: I hypothesize that different compounds rescue different phenotypes, and there are a few compounds that can rescue the phenotype and go onto drug discovery for the treatment of optic atrophy type 1.

Aim 3: Quantify proteins in mutant embryonic zebrafish models that associate with mitochondria development to identify potential novel protein interactions.
Rationale: Quantifying protein could identify how protein interactions are affected by the mutation of OPA1 in mitochondria during the embryonic stage.
Approach: **iTRAQ** will be used for conducting mass spectrometry to determine the protein abundance level. Mitochondria from both wildtype and mutant zebrafish will be separated at 24h and 48h during the embryonic stage for experiments,
Hypothesis: Proteins that associate with OPA1 and are involved in the embryonic developmental stage will exhibit an increase in protein abundance and new protein interactions may be identified.

Works Cited

1. Arruti, N., Rodríguez-Solana, P., Nieves-Moreno, et al. (2023). OPA1 Dominant Optic Atrophy: Diagnostic Approach in the Pediatric Population. *Current issues in molecular biology*, *45*(1), 465-478.
2. Delettre-Cribaillet, C., Hamel, C. P., & Lenaers, G. (2007). Optic Atrophy Type 1. In M. P. Adam (Eds.) et. al., *GeneReviews*®. University of Washington, Seattle.
3. Ferré, M., Bonneau, D., Milea, D., et al. (2009). Molecular screening of 980 cases of suspected hereditary optic neuropathy with a report on 77 novel OPA1 mutations. *Human mutation*, *30*(7), E692–E705. https://doi.org/10.1002/humu.21025
4. Formichi, P., Radi, E., Giorgi, E., et al. (2015). Analysis of opa1 isoforms expression and apoptosis regulation in autosomal dominant optic atrophy (ADOA) patients with mutations in the opa1 gene. *Journal of the neurological sciences*, *351*(1-2), 99–108. https://doi.org/10.1016/j.jns.2015.02.047
5. Lenaers, G., Hamel, C., Delettre, C. *et al.* Dominant optic atrophy. *Orphanet J Rare Dis* **7**, 46 (2012). https://doi.org/10.1186/1750-1172-7-46
6. Roubertie, A., Leboucq, N., Picot, M. C., et al. (2015). Neuroradiological findings expand the phenotype of OPA1-related mitochondrial dysfunction. *Journal of the neurological sciences*, *349*(1-2), 154–160. https://doi.org/10.1016/j.jns.2015.01.008
7. Yu-Wai-Man, P., Griffiths, P. G., Burke, et al. (2010). The prevalence and natural history of dominant optic atrophy due to OPA1 mutations. *Ophthalmology*, *117*(8), 1538–1546.e1. https://doi.org/10.1016/j.ophtha.2009.12.038
8. Zanna, C., Ghelli, A., Porcelli, A. M., et al. (2008). OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. *Brain : a journal of neurology*, *131*(Pt 2), 352–367. https://doi.org/10.1093/brain/awm335