Investigating Impacts of the E736D COL22A1 Mutation Upon Vascular Stability and Hemorrhagic Incidence in an Embryonic Zebrafish Model

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Intracranial aneurysms (IAs) are a vascular disorder characterized by the bulging of weakened cerebral blood vessel walls. Affecting 166 million people globally, IAs pose a serious risk due to their potential to rupture and develop into subarachnoid hemorrhages (45% mortality rate). The E736D mutation in the COL22A1 protein has been identified as a promising genetic driver of IAs, with recent research establishing a correlation between E736D COL22A1 protein overexpression and vascular instability in zebrafish embryos. However, the previously utilized model exhibited limited protein overexpression, lacked experimental modularity, and was not investigated thoroughly. This project addresses these issues by generating a novel mutant transgenic zebrafish model that leverages heat-shock-inducible promoters to elicit enhanced protein overexpression via the binary Gal4/UAS system. Novel mutants were tested alongside flk1:GFP primary controls and wild-type COL22A1 secondary controls. Successful inheritance of the Gal4/UAS system was confirmed by expression of DsRED. Following heme staining at 72 hours post-fertilization (hpf) for model validation, a significant increase ($p < 0.05$) in the hemorrhagic incidence rate of heat-stressed E736D COL22A1 embryos was observed compared to controls. The recorded phenotype is stronger than in any past research, attributable to implementation of the Gal4/UAS system. Analysis of intracranial pericyte coverage - a potential pathological factor of IAs - in the three groups of embryos after fluorescent in situ hybridization revealed insignificant variations ($p > 0.05$), necessitating further exploration. Quantification of blood vessel metrics from confocal images offered unique insight into the destabilizing effect of the E736D COL22A1 mutation.

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