AAP China Scholarship Council - CSC 2023

PROJET DE RECHERCHE DOCTORALE (PRD)

Titre du PRD : MADLESS: Reducing oxidative damage in age-related macular degeneration

DIRECTION de THESE

Porteuse ou porteur du projet (***doit être titulaire de l'HDR***) :

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CO-TUTELLE INTERNATIONALE envisagée : ☒ OUI ☐ NON

DESCRIPTIF du PRD :

*Ce texte sera affiché en ligne à destination des candidates et candidats chinois : il ne doit pas excéder **2 pages** doit être rédigé en **ANGLAIS***

1. Scientific backgroud and reliminary results: We have identified with the IAMDG consortium 34 loci carrying risk alleles for age-related macular degeneratio (AMD), including risk alleles at the SLC16A8 locus encoding for the lactate transporter MCT3. The first risk allele identified (rs8135665-T) is located within intron 4 of the gene (intronic) 1. A rare splice variant in SLC16A8 is predicted to interrupt splicing of intron 2 2. GT is the sequence at 99% of human 5' splice sites and consequently rs77968014-G is a putative splice-site mutation (splicing). We hypothesize that the presence of risk alleles reveals a novel molecular mechanism for development of AMD pathology through a deficit in the clearance of lactate produced by photoreceptors through aerobic glycolysis 3.

We have study the possible implication of a metabolic imbalance associated with risk alleles within the SLC16A8 gene that encodes for a retinal pigment epithelium (RPE)-specific lactate transporter MCT3 and its consequences for vision. As a first approach, we report the deficit in transepithelial lactate transport of the rare splicing allele. We produced induced pluripotent stem cells (iPSCs) from the unique patient in our cohort that carries two copies of this allele. After in vitro differentiation of the iPSCs into RPE cells (iRPE) and their characterization, we demonstrated that the splicing allele results in the retention of intron 2 of the SLC16A8 gene leading to the absence of MCT3 protein and a consequently a deficit of transepithelial lactate transport 4.

We hypothesize that the coding allele induces a deficit in lactate transport resulting in elevation of lactate concentration in the extracellular space surrounding cone photoreceptors in the fovea that antagonizes the stimulation of aerobic glycolysis in cones by rod-derived cone viability factor (RdCVF), encoded by the nucleoredoxin-like (NXNL1) gene 3,5 and triggers oxidative stress in the RPE 6. The flux of glucose metabolites in the cones diverts to produce the hydrophilic heads of phospholipids incorporated into the cone outer segments during their daily renewal. Reduction of aerobic glycolysis flux leads to the shortening of the outer segments leading progressively to central vision loss, as observed for AMD. Excessive metabolism of lactate by the RPE mitochondria would theoretically produces reactive oxygen species (ROS) 7.

We have created using CRISPR-Cas9 technology a mouse carrying the presumed risk allele rs77968014-G (splicing), the metabolic model of AMD (MAD) mouse as a model of MCT3-mediated lactate transport deficit. We confirmed that MCT3 is not expressed by RPE cells of the Scl16a8MAD/MAD mouse using western blotting and immunohistochemistry. As expected, we found that the RPE of the Scl16a8MAD/MAD mouse have an overload of lactate that triggers a similar lactate increase in the outer retina, presumably by the reduced activity of the facilited transporter MCT1, resulting from change in lactate concentration across the RPE plasma membrane 8. This lead to a deficit in the function of rods measured by electroretinography (ERG) on 2-month Scl16a8MAD/MAD mice and a deficit in the function of cones measured by ERG and optometry. Interestingly, an intermediate visual phenotype was observed for the heterozygous Scl16a8MAD/+ mouse. By 3 months, the rods dye as shown by optical coherence tomography (OCT) as the cones by measuring their density 9. At 8 months, the retina of the Scl16a8MAD/MAD mouse is inflammatory as shown by the presence of microglial cells expressing ionized calcium binding adaptor molecule 1 (IBA1). Fluorescence-activated cell sorting (FACS) of the retina and the RPE of these mice show that the inflammation is local rather than triggers by the infiltration of immune cells. The MAD mouse is a model of the MCT3 deficit in lactate transport in AMD.

We studied the regulation of the SLC16A8 promoter. Indeed, a stimulation of the SLC16A8 promoter activity in these patients could have a therapeutic effect 3. To identify promoter elements tested a 5'

region of the gene that drives specific expression by RPE and made a bioinformatic analysis of that sequence. In a 2.1 kilo base (kb) construct, we showed that one element binding SOX9 is essential by the restrict expression of GFP by the RPE. In addition, we found two active elements binding OTX2, reminiscent of our study on the regulation of SLC16A8 expression by OTX2 10.

2. General objective of the proposal: The objective of MADLESS is to quantify oxidative damage and inflammation of the RPE of the MAD mouse, a model of AMD and to provide the proof of concept of benefit for vision of the gene delivery of the thioredoxin RdCVFL, the second product of the NXNL1 gene in that model.

3. Details of the proposal: The student will first quantify oxidative damage in sections of the Scl16a8MAD/MAD versus Slc16a8+/+ eyes by immunohistochemistry using anti-acrolein, and by measuring the concentration of malondialdehyde (MDA) in cell extracts from the RPE and the retina at different ages (2, 3, and 6 months). 11,12.

In parallel, the student will quantify microglial activation (inflammation) on the contralateral eyes of these mice using IBA1 immunohistochemistry and FACS.

The thioredoxin RdCVFL will be delivered to groups of 8 Scl16a8MAD/MAD mice at post-natal day 15 using adeno-association virus (AAV) serotype 8 by subretinal injection 13. The student will evaluate the benefit of delivering RdCVFL under the control of ubiquitous promoter, the cytomegalovirus-chicken β-actin (AAV2/8-CMV/CBA-RdCVFL) or driven by the SLC16A8 promoter AAV2/8-SLC16A82.1-RdCVFL on mouse vision (ERG and optometry) as compared to the respective negative control (AAV2/8-CMV/CBA-GFP and AAV2/8-SLC16A82.1-RdCVFL) at 2, 3, and 6 months.

At 6 months, after euthanasia, the student will quantify oxidative damage and microglial activation of the treated Scl16a8MAD/MAD mice with RdCVFL as compared to its negative control (GFP).

4. References

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