Progressive Focal Retinal Degeneration in *Ccl2/Cx3cr1* Double Deficient Mice with Pathological Features of Human Age-Related Macular Degeneration

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BACKGROUND & PURPOSE

Age-related macular degeneration (AMD) is the leading cause of irreversible central visual loss among the elderly in the world. AMD is a chronic and progressive degenerative disease primarily affecting the photoreceptors and underlying retinal pigment epithelium (RPE) in the macula, which constitutes the central region of the retina that is critical to fine and central vision. Clinical and pathological features of AMD include drusen formation within Bruch's membrane, hypo- and/or hyperpigmentation, and photoreceptor loss leading to geographic atrophy and/or choroidal neovascularization in the macula. Considerable efforts have been made to establish animal models that mimic human AMD. Even though mice have no macula, existing mouse AMD models with AMD-like lesions in the retina can develop cardinal pathological features of AMD.

RATIONALE

- **Loss of function variation in *CX3CR1* (T280M/V249I) is associated with AMD**
  Tuo et al: FASEB J. 2004 Aug;18(11):1297-9
- **Mouse AMD model of single KO of *Ccl2* or *Ccr2***
  Ambati J et al: Nat Med. 2003 Nov;9(11):1390-8
- **Hypothesis: KO of these two genes might have a synergistic effect**
  Early onset and easily reproducible

MATERIALS & METHODS

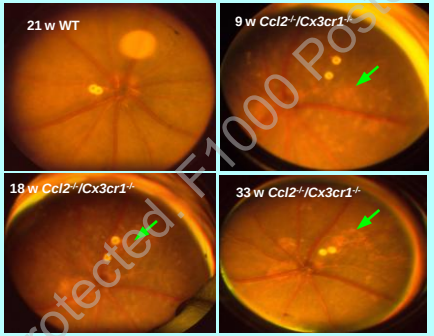
- *Cx3cr1*$^{-/-}$ mice on the C57BL/6 background were crossbred with Ccl2$^{-/-}$ C57BL/6 mice to generate *Ccl2*$^{+/-}$*/Cx3cr1*$^{+/-}$ mice.
- *Ccl2*$^{+/-}$*/Cx3cr1*$^{+/-}$ mice were intercrossed to generate various combinations of the 4 alleles including *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$.

RESULTS

- **Genotype and Phenotypes**
  - Lack *of Ccl2* and *Cx3cr1* transcripts was confirmed by RT-PCR
  - Among the 400 F2 pups analyzed, 12 animals were *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$, indicating an abnormal Mendelian segregation (1 in 16 expected).
  - *Ccl2*$^{+/-}$*/Cx3cr1*$^{+/-}$ mice were within normal size. *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice showed 20% weight decrease.
  - Double KO mice appeared to be less fertile, with an average of 6 pups per litter as compared to 9 in the controls.
  - 100% mice developed retinal AMD-like lesions.
- **Fundoscopy**

Ocular examination on a total of *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice appeared normal except within the retina and choroid. Sequential fundoscopy examinations were performed on *Ccl2* $^{-/-}$*/Cx3cr1*$^{-/-}$ mice and age-matched wild-type mice. Unlike WT mice, all 6-9 week old *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice spontaneously developed deep retina lesions characterized by heterogenous, round or domed-shaped, soft-bordered dots within the subretina. With aging, these lesions became large and confluent. Some of the lesions progressed to form chorioretinal scars and depigmented atrophic areas.

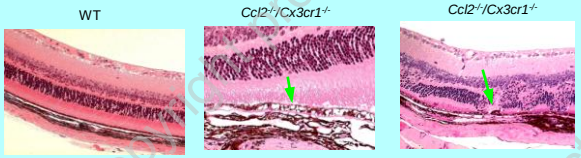
Representative Fundus pictures



- **Histopathology**

The eyes of the age-matched wild-type mice were entirely normal and lacked deep retina lesion, neovascularization, photoreceptor degeneration and RPE atrophy. All *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ eyes showed focal RPE hypopigmentation and vacuolation, and photoreceptor outer segment disorganization and/or photoreceptor atrophy. Intraretinal neovascularization was seen in some aged mice. Choroidal neovascularization was found in 15% of *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mouse eyes with the earliest onset at 12 weeks of age.

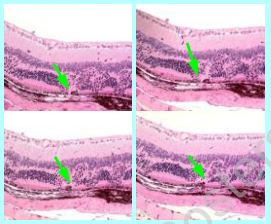
Representative Histological pictures



- **Choroidal neovasculization**

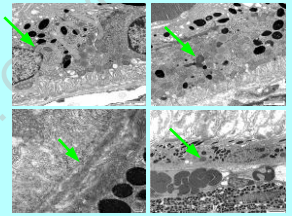
Series sections

Ccl2$^{-/-}$*/Cx3cr1*$^{-/-}$



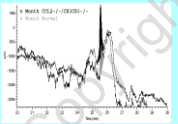
- **Ultrastructure**
  - RPE: decrease of melanosomes; increase of lipofuscin,
  - Bruch's membrane: Thick
  - Photoreceptor degeneration

Ccl2$^{-/-}$*/Cx3cr1*$^{-/-}$



- **Increase of A2E, major component of lipofuscin, in RPE in DKO**

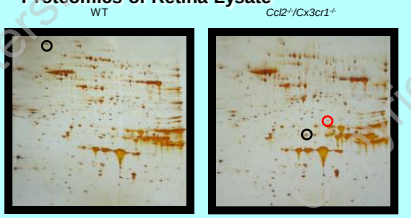
HPLC determination



Dark line: *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$, Grey line: WT

Auto fluorescence imaging on I yr old mice

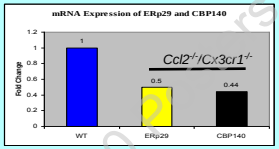
- **Proteomics of Retina Lysate**



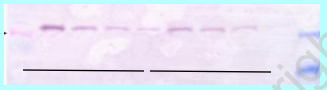
1: Calcium binding 140k protein,

2: ERp29 precursor,

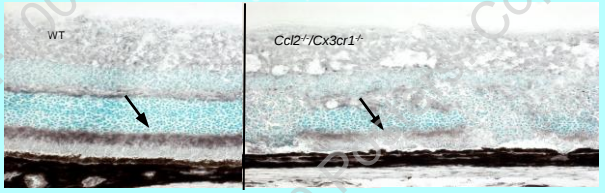
3.: RIKEN cDNA 2210010C04.



Western Blotting of ERp29



Immunostaining of ERp29 on mice retina



SUMMARY

- *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice show a broad spectrum of pathological features of human AMD.
- The phenotype is highly reproducible.
- The disease onset is earlier than most genetically engineered AMD mice reported in the literature.
- These observations implicate the important roles of certain chemokines in AMD pathogenesis.
- Reduced ERp29 might be one of the downstream pathways for causing the retina lesion.

REFERENCES

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- Tuo J, Ross RJ, Herzlich AA, et al. A high omega-3 fatty acid diet reduces retinal lesions in a murine model of macular degeneration. Am J Pathol 2009; 175:799-

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