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D9.40 – Long term Scheimpflug lens imaging

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Abstract

The lens of the eye is known to be more radiosensitive than previously thought but, despite a substantial reduction in occupational dose limits based on recent epidemiological information and reanalyses, the mechanisms of low dose radiation cataract induction are still unclear. This is an important current public health issue, for instance for medical radiation workers, many of whom will need to amend their working practices even though there is no clear understanding of the underlying process and ultimate effects of chronic, low dose, ionising radiation exposure.

The LDLensRad project aims to bring together experts from across Europe to answer a number of key research questions on this topic, including: how does low dose radiation cause cataracts; is there a dose rate effect, and how does genetic background influence cataract development after radiation exposure. CONCERT Deliverable 9.40, 1.0.7 of the project, describes progress to date on the long term Scheimpflug imaging of lenses from the various mouse models used at HMGU, ENEA and PHE.

The data collated to date indicate that while ionising radiation dose and dose rate influence cataract progression in some mouse models, genetic background and age dominate in most cases, demonstrating that genetic background significantly contributes to cataract risk overall.

The results are currently being collated and analysed for submission for publication.

Progress summary

1 INTRODUCTION

It is well known that ionizing radiation can cause cataracts of the ocular lens. However, the dose and dose rate effect, threshold and the mechanism of radiation-induced lens clouding is still not fully understood. The mouse is a good model organism to study eye disorders and especially cataract development. In the past, many mouse models for cataracts were characterized by slit lamp biomicroscopy. Even if the modern slit lamps are equipped with a digital camera for documentation, the quantification is subjective and different scoring systems are used, as discussed recently (Dalke et al. 2018). A major advantage of the Scheimpflug system is the software-based quantification of the images. The lens opacification is quantified by the densitometry tool of the provided Pentacam software. Such densitometric analysis allows a quantitative measurement of the light scatter in the single lens layers, and enables the early recognition of disturbances in transparency crucially important in cataract epidemiology (NCRP 2016).

2 METHODS

Wild-type and heterozygous mutant mice (*Ercc2*$^{+/S737P}$, *Ptch1*$^{+/-}$, 129Sv) of different strain background (B6C3F1, CD1, C57Bl/6) were whole body irradiated with 0, 0.5, 1 and 2 Gy of γ-rays (^{60}Co) at different dose rates (0.3 or 0.063 Gy/min) and examined monthly for lens density up to 18 months post irradiation. Scheimpflug imaging (Fig. 2) was used to investigate the lens density of the mice *in vivo*. Digital images of the lenses and corneas were taken with the OCULUS Pentacam® system (Oculus GmbH, Wetzlar, Germany) as previously described (Puk et al. 2013). In brief, after dilation of the pupils with one drop of atropine (0.5 %), the mice were held in front of a Scheimpflug camera with the LED light projected into the middle of the pupil. A well-focused image was adjusted with the help of the provided software and by optimizing the distance between the camera and the mouse eye. Evaluation of the images was performed using the Pentacam® software. The mean lens density across the central anterior-posterior lens axis was quantified with the provided ‘‘densitometry along a line’’ tool.

3 RESULTS

3.1 HMGU (wild-type B6C3F1 and *Ercc2*$^{+/S737P}$; 0, 0.5, 1, 2 Gy; 0.3 Gy/min; at 10 weeks of age)

Analysis of long-term cohorts over 19 months showed an increasing mean lens density of irradiated and control mice (Fig. 1). The significant increase of mean density as a function of time after irradiation have been fitted with a cubic polynomial starting around a value of 5.5 % and continuing to a range of 6.6-7 %. Representative Scheimpflug images for irradiation groups of 0 Gy (control), 0.5 Gy and 1 Gy at timepoint 1 and 18 months post irradiation (mth p.i.) are shown in Fig. 2. Data accrual for the mice irradiated with 2 Gy is not yet complete; Scheimpflug data from observations up to 15 mth p.i. have been analyzed.

ANOVA of the summed values of the last three months for every group revealed that no single factor, (e.g., dose, sex or genotype) was responsible for slight differences between the investigated groups. Only one interaction of two factors, the interaction of line and dose in the ANOVA of control mice and 0.5 Gy-irradiated mice was significantly relevant for differences in the dependent observable mean lens density.

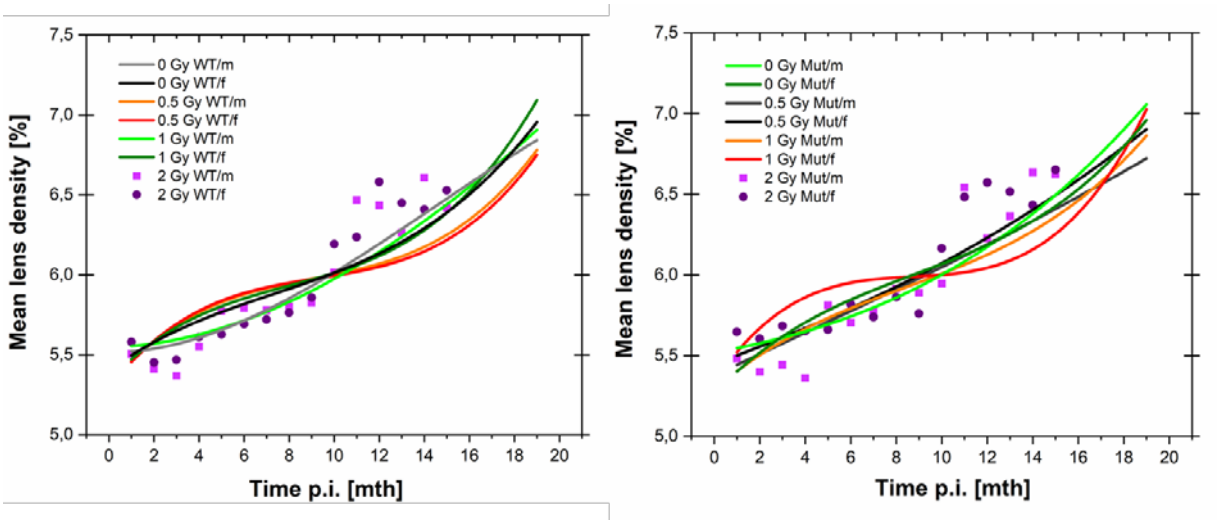

Fig. 1. Increase in mean lens density over 19 months of monthly Scheimpflug imaging.

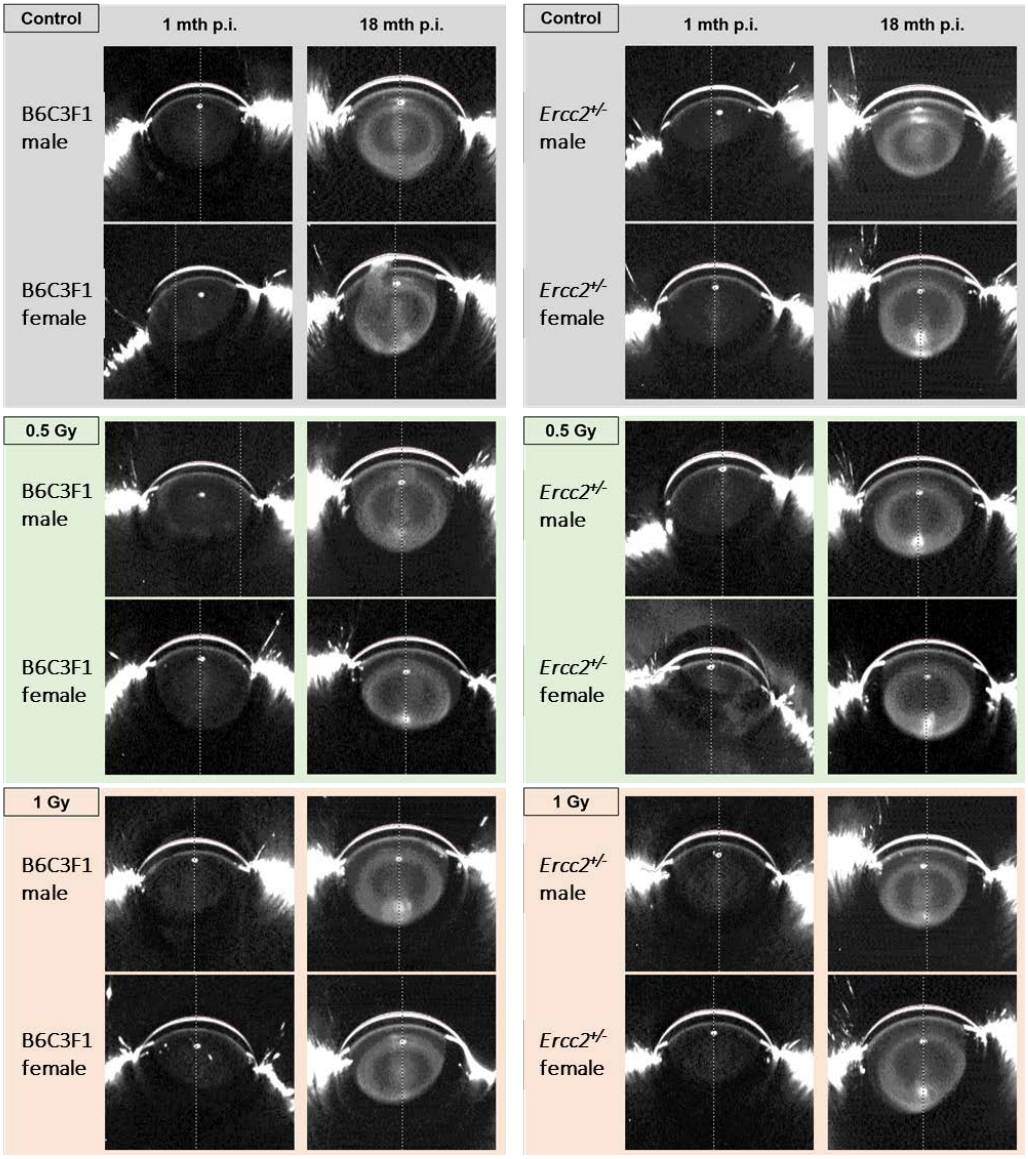

Fig. 2. Representative Scheimpflug images 1 and 18 months post irradiation (mth p.i.).

3.2 ENEA (Mice: CD1-Ptch1+/-, C57Bl/6-Ptch1+/-, wild-type (WT) CD1 and C57Bl/6; Radiation doses: 0, 0.5, 1, 2 Gy; dose rate: 0.3 Gy/min and 0.063 Gy/min; age at irradiation: postnatal day 2 (P2) and 10 weeks (10wks)

The whole experimental plan has been concluded and Scheimpflug images analyzed for mean and maximum lens opacity. Data were elaborated as temporal dynamics and statistically analyzed by linear regression curve fit (95% CI; best fit value using 2 parameters: y-intercept and slope). Results obtained from adult irradiated mice (irradiated at 10 wks) highlight that all tested doses of ionizing radiation, regardless of genotype and dose rate, are able to induce a significant increase in the mean and maximum lens opacity, compared with controls, when mice are bred on CD1 background (Fig. 3A and C). Notably, no increase in lens opacity was found in both C57Bl/6-*Ptch1*$^{+/-}$ and WT C57Bl/6 mice, at all doses examined, showing a significant effect of genetic background (Fig. 1A and C). In addition, irradiation of mice at P2 showed a clear effect of age at the time of irradiation in accelerating dramatically cataractogenesis in CD1-*Ptch1*$^{+/-}$ and WT-CD1 mice, but not in C57Bl/6 regardless of genotype, strengthening the sensitivity of the CD1 background to induction of radiogenic lens opacity (Fig. 3B and D).

3.3 PHE (Mice: Wildtype C57Bl/6; Radiation doses: 0, 0.5, 1, 2 Gy; dose rate: 0.3 Gy/min and 0.063 Gy/min; age at irradiation: 10 weeks (10 wks)

Irradiation of C57 mice has been completed according to the experimental plan; due to unexpected problems with the irradiation facilities, irradiation of 129Sv mice has been delayed but will be completed before the combined intrastrain analysis is carried out. Data are still being processed, however, but analysis has been carried out as described above. Fig. 4 illustrates the 0.3 Gy/min data for mean and max lens opacity (+/- SD) collected for the C57Bl/6 mice over the course of 17 months, for doses of 0 – 1 Gy and up to 4 months for 2 Gy (the data processed so far). For mean opacity, ANOVA reveals no effect of dose over this time period ($p=0.378$) but for max opacity, a significant effect is detected ($p=0.006$), albeit with a lack of fit in this simple ANOVA model ($p>0.999$; further analysis is needed here). No effect of dose rate has been (p all>0.05) detected in the data analysed to date. Time is significant (p all<0.001) and posthoc testing (Tukey's test) reveals significant increases between 0 and 10 - 17 months ($p<0.05$) overall.

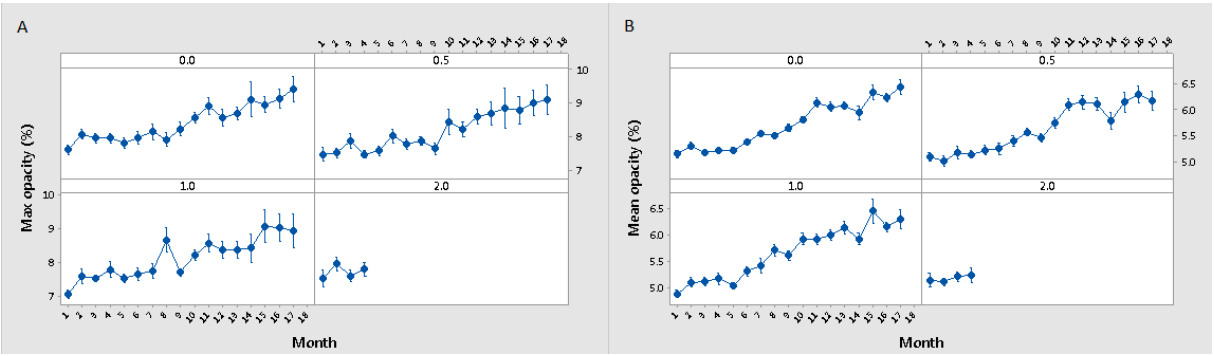

Fig. 4. C57Bl/6 maximum (A) and mean (B) opacity over 17 months for mice irradiated with 0 – 1 Gy and 4 months for mice irradiated with 2 Gy at 0.3 Gy/min. Panel variables are dose in Gy; Error bars are SE.

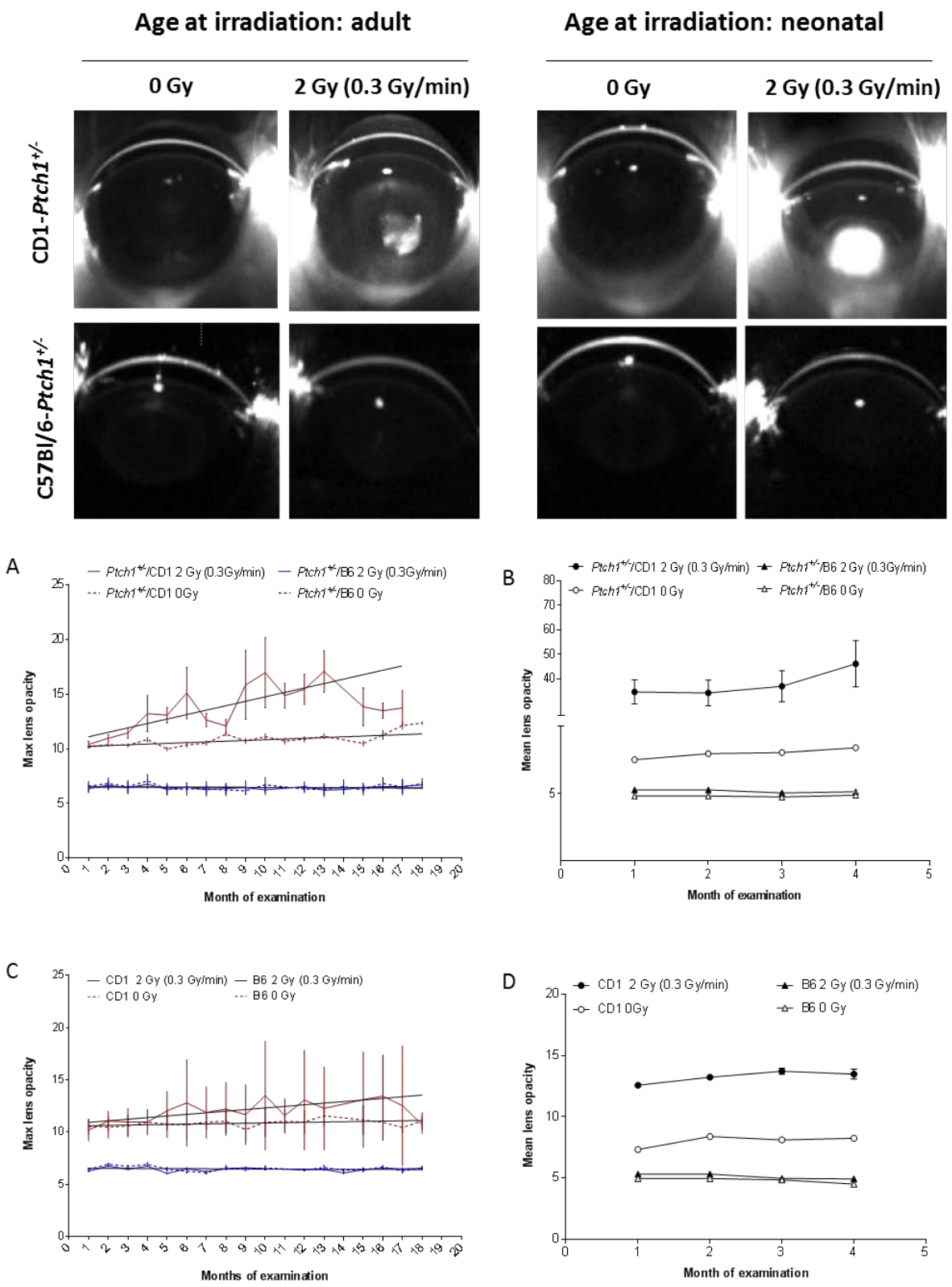

Fig. 3. Representative graphs of data obtained at ENEA showing the role of CD1 and C57Bl/6 genetic backgrounds in the control of radiogenic lens opacity induction in *Ptch1*$^{+/-}$ mice.

4 INITIAL DISCUSSION

Although significant effects of ionizing radiation dose and dose rate have been detected in some models, for all models apart from the P2 *Ptch1*$^{+/-}$, all measured lens densities at 19 months post exposure, are below the LOCS III criterion of 14.1% for a type 2 cataract in humans, which was defined as the beginning for vision impairment according to Pei et al. (2008). This data observed in the LDLensRad experiments thus far indicates that, in most mouse models, the effect of aging outweighs the effect of radiation dose or dose rate up to 2 Gy.

For the Ptch1$^{+/-}$ mice, irradiation of mice at P2 showed a clear effect of age at irradiation in accelerating dramatically cataractogenesis in *Ptch1*$^{+/-}$ and significantly in WT mice on CD1 background, but not on C57Bl/6 background. The effect of age at irradiation is thus strongly influenced by genetic background. Altogether these results show that the genetic background significantly contribute to alter the risk for cataract and, importantly, suggest that interactions between genetic and modifying factors are able to completely abrogate the induction of lens opacification also in the presence of a penetrant germline gene mutation representing a paradigm for radiation hypersensitivity, such as *Ptch1.*

When the data from each strain are all fully complete, further analysis will be carried out across all strains and the results will be collated into at least one publication.

REFERENCES

Dalke C, Neff F, Bains SK, Bright S, Lord D, Reitmeir P, Rößler U, Samaga D, Unger K, Braselmann H, Wagner F, Greiter M, Gomolka M, Hornhardt S, Kunze S, Kempf SJ, Garrett L, Hölter SM, Wurst W, Rosemann M, Azimzadeh O, Tapio S, Aubele M, Theis F, Hoeschen C, Slijepcevic P, Kadhim M, Atkinson M, Zitzelsberger H, Kulka U, Graw J. 2018. Lifetime study in mice after acute low-dose ionizing radiation: a multifactorial study with special focus on cataract risk. Radiat Environ Biophys 57(2):99-113. doi: 10.1007/s00411-017-0728-z.

NCRP 2016. Guidance on radiation dose limits for the lens of the eye. NCRP Commentary No. 26. National Council on Radiation Protection and Measurements. Bethesda, Maryland.

Puk O, de Angelis MH, Graw J. 2013. Lens density tracking in mice by Scheimpflug imaging. Mamm Genome. 24(7-8):295-302. doi: 10.1007/s00335-013-9470-2.

Pei X, Bao Y, Chen Y, Li X. 2008. Correlation of lens density measured using the Pentacam Scheimpflug system with the Lens Opacities Classification System III grading score and visual acuity in age-related nuclear cataract. Br J Ophthalmol. 92(11):1471-5. doi: 10.1136/bjo.2007.136978.