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SHORT COMMUNICATION

Congenital microcephaly-linked CDK5RAP2 affects eye development

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Abstract

Biallelic mutations in the cyclin-dependent kinase 5 regulatory subunit-associated protein 2 gene *CDK5RAP2* cause autosomal recessive primary microcephaly type 3 (MCPH3). MCPH is characterized by intellectual disability and microcephaly at birth, classically without further organ involvement. Only recently, congenital cataracts were reported in four patients of one pedigree with MCPH3. Given the lack of a further pedigree with this phenotype, it remained unclear whether this was a true causal relationship. Here we support the link between CDK5RAP2 and eye development by showing that most *Cdk5rap2* mutant mice (*an/an*) exhibit eye malformations ranging from reduced size of one or both eyes (microphthalmia) to total absence of both eyes (anophthalmia). We also detected increased apoptosis in the *an/an* retinal progenitor cells associated with more mitotic cells. This indicates an important role of Cdk5rap2 in physiologic eye development.

KEYWORDS

cataract, CDK5RAP2, eye development, microcephaly, microphthalmia

1 | INTRODUCTION

Biallelic mutations in the cyclin-dependent kinase 5 regulatory subunit-associated protein 2 gene *CDK5RAP2* cause autosomal-recessive primary microcephaly type 3 (MCPH3) (reviewed in Kraemer et al., 2011; Zaqout, Morris-Rosendahl, & Kaindl, 2017b). Patients with MCPH3 display nonprogressive microcephaly at birth and nonsyndromic intellectual disability, classically without further organ involvement (Kaindl et al., 2010). Only recently, congenital cataracts were reported in four patients of one pedigree with MCPH3 (Alfares et al., 2018; Pagnamenta et al., 2012). Given the presence of only one pedigree with this finding, it remained unclear whether this was a true causal relationship.

Cdk5rap2 mutant or *Hertwig's anemia* mice (*an/an*) show congenital microcephaly, peripheral blood cytopenia, spontaneous aneuploidy, predisposition to hematopoietic tumors, and lack of germ cells leading to infertility (Lizarraga

et al., 2010; Zaqout, Bessa, Kramer, Stoltenburg-Didinger, & Kaindl, 2017a; reviewed in Kraemer et al., 2015). Because Cdk5rap2 is a centrosomal protein ubiquitously expressed in various tissues (Issa et al., 2013; Lizarraga et al., 2010), we hypothesized that other organs are also likely affected. Cdk5rap2 is also affecting cell-cycle progression (Zaqout et al., 2017a), and therefore abnormalities during retinal progenitor cell (RPC) proliferation and differentiation are highly expected. Here we report that *an/an* mice exhibit eye malformations already at early developmental stages. These include cataract and a spectrum of anomalies ranging from unilateral or bilateral microphthalmia (reduced size of one or both eyes) to anophthalmia (total absence of both eyes). Moreover, we found a potential mitotic delay in the *an/an* RPCs, which also show more apoptosis as compared to the wild type (+/+).

2 | MATERIALS AND METHODS

Cdk5rap2 mutant mice (*Hertwig's anemia* mice; *an/an*) were generated by crossing heterozygous (+/*an*) mice (*C57BL/6* background; Jackson Laboratory, stock no. 002306). The breeding was performed at the animal facility of the Charité – Universitätsmedizin Berlin, Berlin, Germany, and all experiments were carried out in accordance with national ethics principles (registration no. T0309.09).

Whole embryos of embryonic days E12.5 and E14.5 and isolated eyes/retinas of postnatal day 0 (P0) and adult mice were collected. The samples were fixed in 4% paraformaldehyde for 10 minutes (isolated eyes/retinas) or overnight (whole embryos). Embryos and tissues were then dehydrated in an ethanol series, cleaned with xylene, and embedded in paraffin. Matched 10-mm-thick sections from both *an/an* and wild-type (+/+) mice were stained for hematoxylin and eosin (H&E). For immunostaining, the following primary antibodies were used: mouse anti-phospho-histone H3 (1:100; Cell Signaling Technology, Frankfurt am Main, Germany, Antibody #9706) and rabbit anti-activated cleaved caspase-3 (1:200, Cell Signaling Technology, Antibody #9661). Goat Alexa Fluor 488 conjugates anti-mouse (IgG; 1:400, Invitrogen, Darmstadt, Germany) and donkey Cy3-conjugated anti-rabbit (IgG; 1:400, Jackson ImmunoResearch, Newmarket, United Kingdom) were used as secondary antibodies. Nuclei were stained with DAPI (1:1000, Sigma-Aldrich, St. Louis, MO). Bright-field images were taken with an Olympus BX60 microscope with an AxioCam MRc Zeiss camera and AxioVision 4.8 software (Zeiss, Jena, Germany). Fluorescent images were taken with an Olympus BX51 microscope with an Intas camera and MagnaFire 2.1B software (Olympus, Hamburg, Germany). All images were processed using Adobe Photoshop CS6 version 13.0×64.

3 | RESULTS

We observed the eye phenotype in *an/an* from E12.5 to adult age. Most *an/an* show either uni- or bilateral eye deformity ranging from microphthalmia (reduced eye size) to anophthalmia (absence of an eye) (Figure 1a–c). In around 20% of adult mice, we detected white lenses as correlates of cataracts (Figure 1a, white arrow). H&E-stained E14.5 transverse head sections revealed signs of developmental eye defects with some animals showing rudimentary eyes openly connected to the diencephalon (Figure 1b, E14.5, III), while others lacked eye tissue (Figure 1b, E14.5, IV). We also found a reduction in the *an/an* P0 retina thickness (Figure 1d,e).

We then looked for potential defects in the proliferation and/or apoptosis in RPCs that might lead to the resultant eye phenotype in *an/an*. We found an increase in the number of $pH3^+$ mitotic RPCs (Figure 2a,b) and $Caspase3^+$ apoptotic RPCs (Figure 2a,c) in P0 *an/an* retinas.

4 | DISCUSSION

MCPH3 is widely known as a model disorder for nonsyndromic congenital microcephaly, an isolated brain phenotype. However, Cdk5rap2 is ubiquitously expressed (Issa et al., 2013), and previous studies on *an/an* as a mouse model for MCPH3 indicate that Cdk5rap2 functions not only as a regulator of neural progenitor proliferation but also affects the development of other organs in mice (Kraemer et al., 2011; Megraw, Sharkey, & Nowakowski, 2011; Zaqout et al., 2017a). In support of a recent report linking CDK5RAP2 to congenital cataracts in humans (Alfares et al., 2018), here we found cataracts and further eye malformations in *an/an* from reduced retinal thickness, via microphthalmia to anophthalmia.

The eye development in vertebrate starts with the formation of an optic vesicle, which subsequently contacts the ectoderm and promotes the proliferation of the lens placode. The lens placode separates as a lens vesicle and invaginates within the enfolded optic vesicle to form the mature eye (Wawersik & Maas, 2000). Our findings of a wide variation of eye malformation from rudimentary eyes openly connected to the diencephalon to a lack of eye tissue in *an/an* indicate that these critical steps are likely affected. One factor that is critical for RPCs to reach normal eye size that could contribute to this phenotype is an abnormal control of proliferation and apoptosis. We report in *an/an* mice that the number of $pH3^+$ mitotic cells and $Caspase3^+$ apoptotic cells is increased. The increased number of mitotic cells in *an/an* can be the result of an accumulation and delay in mitotic progression as we recently demonstrated for germ cells in *an/an* mice (Zaqout et al., 2017a). Similarities between eye and brain development

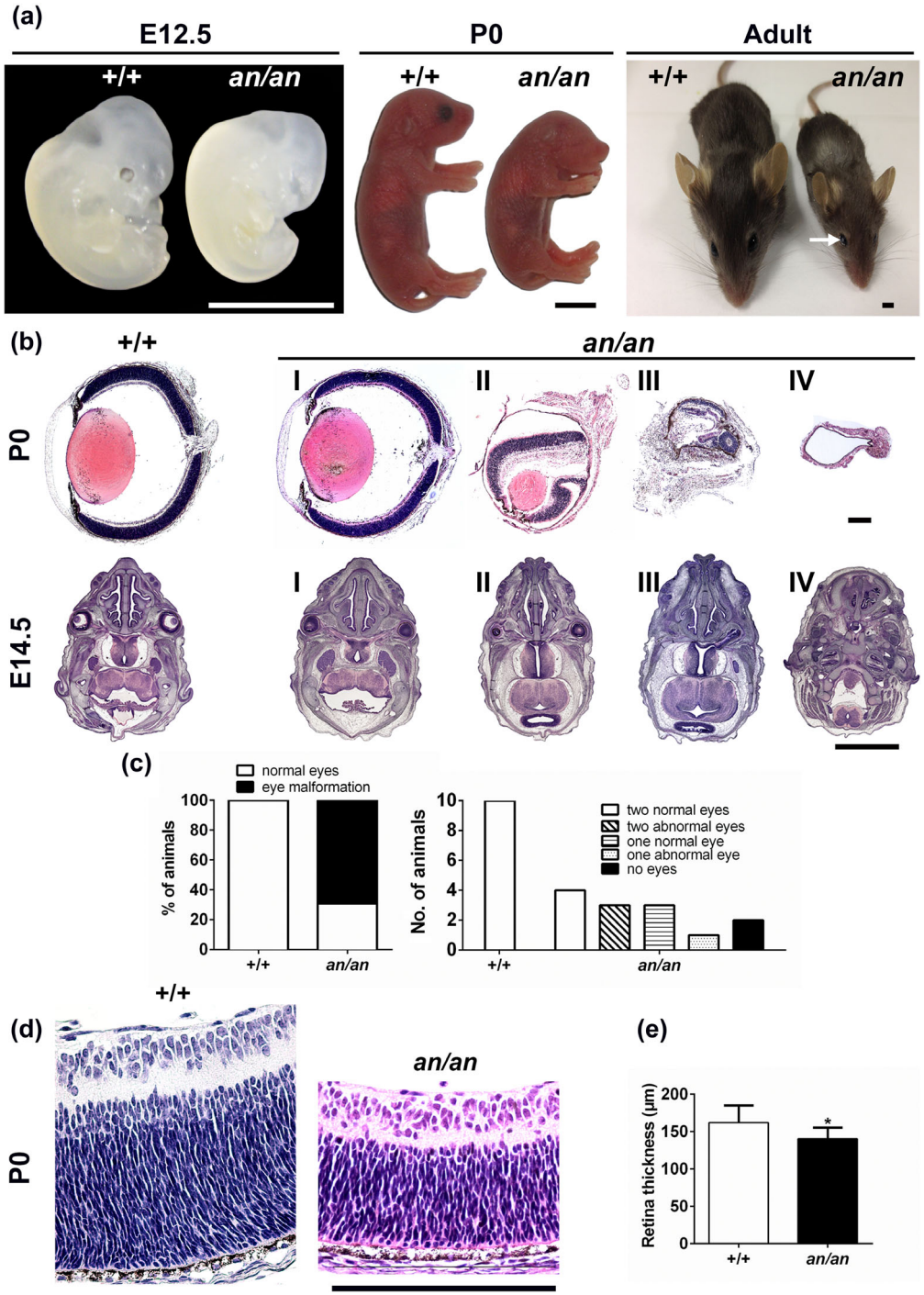

FIGURE 1 (a) Gross phenotype of E12.5, P0 and adult *an/an* mice. Note the anophthalmia in E12.5 and P0 *an/an* mice and the presence of cataract sign (white arrow) in the adult *an/an* mouse (scale bars, 5 mm). (b) hematoxylin and eosin (H&E) staining for P0 sagittal eye sections and E14.5 transverse head sections showing the variation of eye phenotype detected in *an/an* mice (scale bars, 200 µm, upper panel; 2 mm, lower panel). (c) Bar graphs show the percentage and type of detected *an/an* eye malformation. (d,e) H&E stained P0 retina sections displaying a reduction in *an/an* retina thickness (scale bar, 200 µm). DIC images: $n = 8$ animals/group, error bar indicates SD, Student's *t*-test, $*p < 0.05$ [Colour figure can be viewed at wileyonlinelibrary.com]

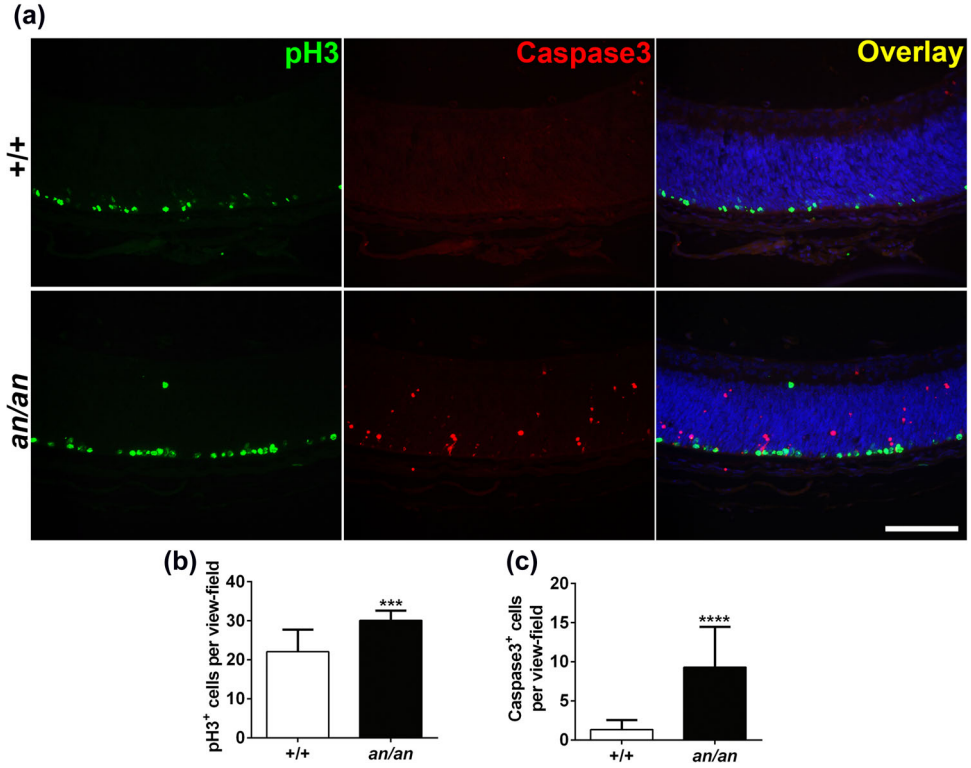

FIGURE 2 (a) P0 retina sections stained for anti-phospho-histone H3 (pH3; mitotic cells marker) and activated cleaved caspase-3 (Caspase3$^+$; apoptotic cells marker). Immunofluorescence images: scale bar, 100 μm. (b,c) Bar graphs indicate an increase in the number of mitotic and apoptotic retinal progenitor cells (RPCs) (pH3 and Caspase3-positive cells, respectively). $n = 11$ +/+ and 9 *an/an* animals, error bar indicates SD, Student's *t*-test, ***$p < 0.001$, ****$p < 0.0001$ [Colour figure can be viewed at wileyonlinelibrary.com]

will open the door to study the mechanism behind eye defects in *an/an* on the one hand and to further understand the role of Cdk5rap2 in central nervous system development on the other hand.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

A.M.K. and S.Z. are responsible for the project conception. A.M.K. and S.Z. wrote the manuscript. S.Z., E.R., and G.S.-D. performed and analyzed the experiments. All authors read, revised, and approved the final manuscript.

DATA AVAILABILITY

All original data are saved under the Charité – Universitätsmedizin Berlin computer server and available for any special requests.

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