Inherited forms of cataract are a heterogeneous group of eye disorders known in livestock species. Clinicopathological analysis of a single case of impaired vision in a newborn Original Braunvieh calf revealed nuclear cataract. Whole-genome sequencing of the parent-offspring trio revealed a de novo mutation of ADAMTSL4 in this case. The heterozygous p.Arg776His missense variant affects a conserved residue of the ADAMTSL4 gene that encodes a secreted glycoprotein expressed in the lens throughout embryonic development. In humans, ADAMTSL4 genetic variants cause recessively inherited forms of subluxation of the lens. Given that ADAMTSL4 is a functional candidate gene for inherited disorders of the lens, we suggest that heterozygosity for the identified missense variant may have caused the congenital cataract in the affected calf. Cattle populations should be monitored for unexplained cataract cases, with subsequent DNA sequencing a hypothesized pathogenic effect of heterozygous ADAMTSL4 variants could be confirmed.

**KEYWORDS**

Bos taurus, development, eye, lens, precision medicine, whole-genome sequencing
a mammalian species and added the affected gene to the list of candidate genes for inherited forms of nuclear cataract in humans (Murgiano et al., 2014). In human medicine, about 50% of cataracts are thought to be associated with genetic factors and causative variants for congenital or other early-onset forms of cataracts have been discovered in over 30 genes (Shiels & Hejtmancik, 2017). Congenital cataract has also been shown to be a heterogeneous group of diseases in cattle, as the reported CPAMD8 nonsense variant is obviously not sufficient to explain the majority of Holsteins suffering from Morgagnian congenital cataract (Braun et al., 2019).

In the present study, we investigated a 1.5-month-old male Original Braunvieh calf with clinical signs of cataract showing bilateral opaque eye lenses (Figure 1). According to the owner, the cataract had been present since birth. At first, the calf had problems finding the teats to suckle the milk and initially ran into walls and objects, but after a few days, it was able to orientate itself. Clinical examination revealed no further abnormalities and the calf was referred to an ophthalmologist. The calf presented to the ophthalmology service with open eyes. Dazzle and pupillary light reflexes were normal, but menace response was absent in both eyes. Intraocular pressure was within normal limits (right eye 20 mmHg, left eye 19 mmHg). The lens showed in both eyes a diffuse dense nuclear opacity and a focal axial anterior cortical opacity in addition with multifocal anterior capsular punctual opacities. Fundic examination was mostly blocked by lens opacities, the visible parts in the periphery were normal. Further ophthalmic examination showed no more abnormalities and the calf was diagnosed with an immature cataract in both eyes. The parents of the affected calf were clinically normal. At the time of slaughtering, the calf was 4.5 months old and appeared in a good general health condition.

Macroscopically, the regularly shaped ocular bulbs demonstrated normal sized lenses with moderately diffuse clouding with clear demarcation of the capsules and regularly developed zonula fibers (Figure 2a,b,d). Histologically, the anterior half of lental protein appears normal, whereas from the equator onwards posteriorly, a severe lental nuclear cataract composed of eosinophilic partly fibrillary, partly amorphous lental proteinaceous material with multifocal small foci of mineralisation (calcification) is present (Figure 2c.e,f). The anterior lens capsule is well developed, the anterior epithelium and equator show a regular formation whereas the posterior lens capsule reveals a moderate fibrillation (Figure 2g, right side). No evidence for infectious agents could be observed in hematoxylin and eosin as well as special stains. All other intraocular structures appeared histologically normal. The bovine embryonal lental development occurs during the first trimester of gestation with formation of the lens placode to development of the lens vesicle between 3.3- and 14-mm embryonal crown rump length (Schnorr & Kressin, 2006). Lenses show continuous lifelong growth with strongest growth rate during embryonal and morphologically non-altered development and the 1st year of life (Levin et al., 2011). The lenses presented here are fully developed with an intact lental capsule, zonula fibers, and regularly arranged and morphologically normal equatorial cells. Thus the opacification of the lental protein, i.e. the formation of a nuclear and posterior subcapsular cataract, must have developed in a relative late stage of the lental development, morphologically widely comparable to the findings demonstrated in the NID1-related nuclear cataract of Romagnolas (Murgiano et al., 2014) and Morgagnian cataracts in Red Holstein Friesian cattle (Braun et al., 2019).

We prepared a PCR-free DNA library of the affected calf and its dam and collected short read pairs (2 × 150 bp) to obtain roughly 25× coverage on an Illumina NovaSeq 6000 instrument. The whole genome sequencing data of the sire was publicly available. Variants in the genome of the affected calf were called with respect to the reference genome assembly ARS-UCD1.2 as described previously (Häfliger et al., 2020). A hard filtering approach was applied by comparing the variants to a cohort of 5115 cattle genomes containing both parents. Given that the mode of inheritance was unknown and assuming that the causative variant is rare, we filtered for variants that were only present in the affected calf, either in heterozygous or homozygous state. All 5116 genomes are included in the variant catalogue of run 9 of the 1000 Bull Genomes Project including animals of more than 130 genetically diverse breeds (Hayes & Daetwyler, 2019). Assuming recessive
inheritance, filtering for coding variants present only homozygous in the calf and heterozygous in the parental genomes identified no coding variant. Assuming a dominant mutation, filtering revealed a single private protein changing variant, a missense variant in the ADAMTSL4 gene (NM_001101061.1:c.2327G>A, p.Arg776His), with a heterozygous genotype in the affected calf that was not detected in any of the control genomes.

**FIGURE 2** Features of nuclear cataract in an Original Braunvieh calf. Normal eye, sagittal section (a). Altered right eye with severe lental nuclear cataract (rectangle), sagittal section (b). Histological overview (c) of the cataractous lens depicted in (b). Higher magnification (d) of the lens depicted in (b). Facies posterior of the lens with cloudy appearance of lental protein and multiple foci of mineralization (e). Higher magnification of the posterior lens capsule, with perilental collagenous membrane (asterisk) and fibrillar lental protein with mineralization (arrows) (f). Comparison of the anterior and posterior lens capsule, left side: normal bovine lens, right: altered fibrillary posterior capsule (g)
including both parents. This C-to-T transition at position 20146737 on chromosome 3 located in exon 12 of ADAMTSL4 is predicted to alter the encoded amino acid of ADAMTSL4 residue 776 (NP_001094531.1:p.Arg776His) to histidine, a less polar and more hydrophobic amino acid than arginine. The affected residue represents a probably functionally important and conserved residue located in the third of seven thrombospondin type 1 repeat domains (Figure 3). Although the arginine to histidine substitution was predicted to be neutral using PROVEAN software (Choi & Chan, 2015), it remains unclear whether this amino acid substitution affects protein folding or function. Sanger sequencing confirmed the mutant allele to be present in a heterozygous state in the affected calf and absent from its parents, given that the variant was not found in the paternal germline DNA analyzed (Figure 3). This means that the c.2327G>A variant probably arose de novo spontaneously during very early development of the calf.

The formal possibility exists, however, that the detected protein-changing de novo mutation is simply a functionally neutral change, but we regard this possibility as unlikely because of the following reasons. Various ADAMTS (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs) proteins are necessary for normal ocular development and eye function (Mead & Apte, 2018). ADAMTS-like proteins lack a metalloprotease domain, reside in the extracellular matrix and have regulatory roles and ADAMTSL4 has been implicated in fibrillin microfibril biogenesis (Gabriel et al., 2012). ADAMTSL4 is widely expressed in non-ocular tissues as well as in various eye components, particularly in the lens equatorial epithelium.

![Figure 3](image-url)

**Figure 3** Details of the detected ADAMTSL4 variant. (a) Integrative Genomics Viewer screenshot presenting the heterozygous single nucleotide variant (red arrow) present only in the affected calf. (b) Sanger sequencing results confirmed that the variant occurred de novo as sequencing of PCR products from DNA of both parents (for the sire semen) showed that the c.2327G>A variant was absent. (c) Schematic diagram of the bovine ADAMTSL4 protein that has a repetitive domain structure with seven thrombospondin type 1 repeat domains (blue). The p.Arg776His variant (red arrow) affects the third repeat. (d) Multiple species amino acid alignments encompassing the region of the variant demonstrates a high evolutionary conservation across species. The observed variant is indicated by an arrow and the respective position highlighted in gray.
when the zonule attaches (Chandra et al., 2013; Gabriel et al., 2012). Besides focal retinal pigment epithelium defects, homozygous disruption of murine Adamts4 resulted in a defect in the anchoring of zonule fibers to the lens surface, causing ectopia lentis, confirming its role in zonule formation (Collin et al., 2015). In humans, recessive isolated ectopia lentis (subluxation or dislocation of the human crystalline lens) and ectopia lentis et pupillae are caused by ADAMTSL4 loss-of-functions variants (OMIM 610113). Heterozygous carriers of the known nonsense or splice site variants were reported to be apparently normal probably due to nonsense-mediated decay of the aberrant transcripts. Finally, visual inspection for large structural variants in the genome, performed after plotting the average read depth across the entire genome for the sequenced trio, revealed no obvious evidence of this type of mutation either at the chromosomal level or in the region of the ADAMTSL4 gene (Figure S1).

Therefore, we propose the c.2327G>A variant as candidate causative variant for the observed congenital cataract phenotype based on the following arguments: (1) given that only one protein-changing de novo mutation event per generation is expected on average (Heinzen et al., 2015), this strongly supports the causality of the variant; (2) evolutionary conservation and expansion of ADAMTSL proteins in mammals indicates a crucial role in embryonic development (Mead & Apte, 2018); (3) in mice, it was shown that ADAMTSL4 is strongly expressed in the lens epithelium at the lens equator throughout embryonic development (Collin et al., 2015); and (4) patients with ADAMTSL4-related ectopia lentis commonly present with a marked loss in visual acuity in addition to a number of possibly accompanying ocular complications including early cataract development (Ahram et al., 2009; Christensen et al., 2010). Given that this is a single case investigation and that we have no functional confirmation, this result must be considered preliminary and should be interpreted with caution. However, it must also be emphasized that the analysis was not suitable for identifying larger structural variants. Further isolated cases of cataract in cattle could be investigated for ADAMTSL4 variants by DNA sequencing.

ACKNOWLEDGEMENTS
The authors would like to thank Cécile Meili (Braunvieh Schweiz) for contributing with information and contact to the farmer. The authors also wish to thank Swissgenetics for providing semen and Hubert Pausch, who made the sequencing data from the sire publicly available. Furthermore, we are thankful to Nathalie Besuchet-Schmutz and Simon Pot for expert assistance. We also acknowledge all bovine researchers contributing to the 1000 Bull Genomes Project and those who deposited cattle whole-genome sequencing data into public databases. This study was partially supported by the Swiss National Science Foundation, grant number 172911. Open access funding provided by Universität Bern. [Correction added on 23 May 2022, after first online publication: CSAL funding statement has been added.]

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT
The whole genome sequencing data of the animals generated in this study is publicly available at ENA project accession PRJEB18113 (https://www.ebi.ac.uk/ena/browser/view/PRJEB18113) with sample accessions SAMEA5159837 (case) and SAMEA6528886 (dam). The whole genome sequencing data of the sire was publicly available (ENA project accession PRJEB28191 (https://www.ebi.ac.uk/ena/browser/view/PRJEB28191), sample accession SAMEA5059753).

ORCID
Irene M. Häfliger https://orcid.org/0000-0002-5648-963X
Udo Hetzel https://orcid.org/0000-0001-9142-560X
Franz R. Seefried https://orcid.org/0000-0003-4396-2747
Cord Drögemüller https://orcid.org/0000-0001-9773-522X

REFERENCES
Ahram, D., Sato, T.S., Kohilan, A., Tayeh, M., Chen, S., Leal, S. et al. (2009) A homozygous mutation in ADAMTSL4 causes autosomal-recessive isolated ectopia lentis. American Journal of Human Genetics, 84, 274–278.
Bistner, S.I., Rubin, L.F. & Saunders, L.Z. (1970) The ocular lesions of bovine viral diarrhea-mucosal disease. Pathologia Veterinaria, 7, 275–286.
Braun, M., Struck, A.K., Reinartz, S., Heppelmann, M., Rehage, J., Eule, J.C. et al. (2019) Study of congenital Morgagnian cataracts in Holstein calves. PLoS One, 14, e0226823.
Chandra, A., Jones, M., Cottrill, P., Eastlake, K., Limb, G.A. & Charteris, D.G. (2013) Gene expression and protein distribution of ADAMTS-4 in human iris, choroid and retina. British Journal of Ophthalmology, 97, 1208–1212.
Choi, Y. & Chan, A.P. (2015) PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics, 31, 2745–2747.
Christensen, A.E., Fiskerstrand, T., Knappskog, P.M., Boman, H. & Redahl, E. (2010) A novel ADAMTSL4 mutation in autosomal recessive ectopia lentis et pupillae. Investigative Ophthalmology & Visual Science, 51, 6369–6373.
Collin, G.B., Hubmacher, D., Charette, J.R., Hicks, W.L., Stone, L., Yu, M. et al. (2015) Disruption of murine Adamts4 results in zonular fiber detachment from the lens and in retinal pigment epithelium dedifferentiation. Human Molecular Genetics, 24, 6958–6974.
Detlefson, J.A. & Yapp, W.W. (1920) The inheritance of congenital cataract in cattle. The American Naturalist, 54, 277–280.
Gabriel, L.A., Wang, L.W., Bader, H., Ho, J.C., Majors, A.K., Hollyfield, J.G. et al. (2012) ADAMTSL4, a secreted glycoprotein
widely distributed in the eye, binds fibrillin-1 microfibrils and accelerates microfibril biogenesis. *Investigative Ophthalmology & Visual Science*, 53, 461–469.

Gregory, P.W., Mead, S.W. & Regan, W.M. (1943) A congenital hereditary eye defect of cattle. *Journal of Heredity*, 34, 125–128.

Häfliger, I.M., Wiedemar, N., Švara, T., Starič, J., Cociancich, V., Šest, K. et al. (2020) Identification of small and large genomic candidate variants in bovine pulmonary hypoplasia and anasarca syndrome. *Animal Genetics*, 51, 382–390.

Hayes, B.J. & Daetwyler, H.D. (2019) 1000 Bull Genomes Project to map simple and complex genetic traits in cattle: applications and outcomes. *Annual Review of Animal Biosciences*, 7, 89–102.

Heinzen, E.L., Neale, B.M., Traynelis, S.F., Allen, A.S. & Goldstein, D.B. (2015) The genetics of neuropsychiatric diseases: looking in and beyond the exome. *Annual Review of Neuroscience*, 38, 47–68.

Hollmann, A.K., Dammann, I., Wemheuer, W.M., Wemheuer, W.E., Chilla, A., Tipold, A. et al. (2017) Morgagnian cataract resulting from a naturally occurring nonsense mutation elucidates a role of CPAMD8 in mammalian lens development. *PLoS One*, 12, e0180665.

Krump, L., O’Grady, L., Lorenz, I. & Grimes, T. (2014) Congenital cataracts in an Ayrshire herd: a herd case report. *Irish Veterinary Journal*, 67, 2.

Levin, L.A., Nilsson, S.F.E., Ver Hoeve, J., Wu, S., Kaufman, P.L. & Alm, A. (2011) *Adler’s physiology of the eye e-book*, 11th edition. Amsterdam, The Netherlands: Elsevier Health Sciences. ISBN 978-0-323-08116-0.

Mead, T.J. & Apte, S.S. (2018) ADAMTS proteins in human disorders. *Matrix Biology : Journal of the International Society for Matrix Biology*, 71-72, 225–239. https://doi.org/10.1016/j.matbio.2018.06.002.

Murgiano, L., Jagannathan, V., Calderoni, V., Joechler, M., Gentile, A. & Drögemüller, C. (2014) Looking the cow in the eye: deletion in the NID1 gene is associated with recessive inherited cataract in Romagnola cattle. *PLoS One*, 9, e10628.

Osinchuk, S., Petric, L., Leis, M., Schumann, F., Bauer, B., Sandmeyer, L. et al. (2017) Congenital nuclear cataracts in a Holstein dairy herd. *Canadian Veterinary Journal*, 58, 488–492.

Schnorr, B. & Kressin, M. (2006) *Embryologie der Haustiere: Ein Kurzlehrbuch*. Leipzig: Georg Thieme Verlag.

Shiels, A. & Hejtmancik, J.F. (2017) Mutations and mechanisms in congenital and age-related cataracts. *Experimental Eye Research*, 156, 95–102.

Siepker, C.L., Zimmer, J.L., Bedard, K.M., Hart, K.A., Czerwinski, S.L. & Carmichael, K.P. (2021) Congenital cataracts and microphakia with retinal dysplasia and optic nerve hypoplasia in a calf. *Case Reports in Veterinary Medicine*, 2021, 2064103.

Williams, D.L. (2010) Congenital abnormalities in production animals. *The Veterinary Clinics of North America. Food Animal Practice*, 26, 477–486.

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

**How to cite this article:** Häfliger, I.M., Wolf-Hoßfetter, S., Casola, C., Hetzel, U., Seefried, F.R. & Drögemüller, C. (2022) A de novo variant in the bovine *ADAMTSL4* gene in an Original Braunvieh calf with congenital cataract. *Animal Genetics*, 53, 416–421. Available from: https://doi.org/10.1111/age.13178