Received: 3 March 2023 | Accepted: 6 March 2023

DOI: 10.1111/age.13317

BRIEF REPORT

ANIMAL GENETICS WILEY

MYH3-associated non-syndromic palatoschisis (cleft palate, CP) in Limousine cattle

BACKGROUND

Palatoschisis (also called cleft palate, CP) is an opening or cleft in the upper lip, the roof of the palate, or both, and is a common defect in both humans and domestic animals (Mossey & Modell, 2012; Mulvihill et al., 1980). It can also occur in cattle as an isolated (non-syndromic) birth defect (OMIA 000197-9913), owing to de novo mutations (Jacinto et al., 2021; OMIA 002483-9913) or associated with familial recessive syndromes (Agerholm et al., 2016; OMIA 000070-9913).

ANALYSIS

A 2-years-old Italian-bred Limousine heifer was referred for a non-syndromic form of palatoschisis involving both soft and hard palate, rhinitis and retarded growth (Figure S1). We hypothesized a genetic etiology for this congenital anomaly and performed whole-genome sequencing. Further details of the methodology are described in Appendix S1. Two homozygous private protein-changing variants were located in a putative candidate gene for the observed phenotype, *myosin heavy chain 3* (*MYH3*) (Table S1). Inherited forms of *MYH3*-related human syndromes, including cleft palate, are known (OMIM 160720). The homozygous variants at chr19:29609623A>G and at chr19:29609604TGTCAGCTCAAG>Trepresentamissense variant (NM_001101835.1:c.2864T>C; NP_001095305.1:p.Ile955Thr) and frameshift deletion (NM_001101835.1:c.2872_2882delCTTGAGCTGAC; NP_001095305.1:p.Leu958_Leu962delinsThrGlyGlnGlyTer), respectively, in exon 24 of bovine *MYH3* (Figure S2). The isoleucine-to-threonine substitution affects an evolutionarily highly conserved amino acid in the myosin tail domain, and the frameshift deletion was predicted to cause a premature stop codon. Sanger sequencing confirmed the presence of both homozygous variants in *MYH3* in the case (Figure S2).

Among the additional 28 private homozygous coding variants, 23 were probably common variants, while the remaining five were classified as variants of uncertain significance (Appendix S1). A recent publication from France (Vaiman et al., 2022) finally proved the assumption of causality of *MYH3* variants.

To estimate the prevalence of *MYH3* carriers within the Swiss Limousine population, we genotyped a population sample of purebred animals, including the bulls currently used for insemination (Table S2). The frequency of the variant allele in 1167 genotyped cattle was 1.5%.

COMMENTS

We have successfully identified the most likely cause for a rare autosomal recessive disorder with only one case. Our clinicopathologic and genomic findings for the *MYH3*-related recessively inherited form of non-syndromic cleft palate are consistent with those recently reported by Vaiman et al. (2022) (OMIA002590-9913). Interestingly, the 2-year-old Limousine female in our study appears to be the oldest known animal with *MYH3*-associated non-syndromic palatoschisis. According to Vaiman et al. (2022), life expectancy is short, with most animals euthanized in the first month of life. In addition, the variant allele frequency was found to be lower in the current Swiss population compared with the French Limousine (2.4%).

Whether the *MYH3* missense or the loss-of-function variant, or both, is actually causal remains open. The affected gene, *MYH3*, encodes the myosin heavy chain 3 protein, which is known to be responsible for muscle contraction. In addition, *MYH3* is expressed in a variety of human and mouse fetal tissues (Chong et al., 2015; Zieba et al., 2017). In particular, in the mouse, *Myh3* has been shown to be moderately expressed in head tissues during embryonic development (MGI: 1339709). There are several factors that can lead to the development of palatoschisis, such as impaired function and position of the tongue and surrounding muscles, which can disrupt the embryonic development

Correction added on 26 April 2023, after initial online publication. A duplicate of this article was published under the DOI 10.1111/age.13316. This duplicate has now been deleted and its DOI redirected to this version of the article.

of the palate (Rot & Kablar, 2013). Therefore, considering the rarity, the *in silico* effect prediction, the role of skeletal muscle in embryonic palate formation, the known function of *MYH3* and the report of Vaiman et al. (2022), the identified variants can be considered as the cause. Future studies evaluating the functional consequences will be valuable in understanding the biological impact of the *MYH3* variants during craniofacial development.

ACKNOWLEDGEMENTS

We thank the Interfaculty Bioinformatics Unit of the University of Bern for providing high-performance computational infrastructure. We acknowledge Isabella Aebi-Huber for expert technical assistance. We are grateful to IFN Schönow GmbH, Germany for the genotyping of the Swiss Limousine population control cohort. Open access funding provided by Universitat Bern.

FUNDING INFORMATION

This study was financially supported by the Arbeitsgemeinschaft Schweizerischer Rinderzüchter, Zollikofen, Switzerland and the Federal Office for Agriculture, Bern, Switzerland.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The whole genome sequencing data are available under the study accession no. PRJEB18113 at the European Nucleotide Archive (www.ebi.ac.uk/ena; SAMEA111531537).

Joana G. P. Jacinto[1,2]
Eliana Schiavon[3]
Irene M. Häfliger[2]
Patrizio Coin[3,4]
Franz R. Seefried[5]
Cord Drögemüller[2]

[1]*Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy*
[2]*Vetsuisse Faculty, Institute of Genetics, University of Bern, Bern, Switzerland*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*
[4]*Dipartimento Veterinario e Sicurezza Alimenti di Origine Animale Agenzie di Tutela della Salute, Brescia, Italy*
[5]*Qualitas AG, Zug, Switzerland*

Correspondence
Cord Drögemüller, Vetsuisse Faculty, Institute of Genetics, University of Bern, 3001 Bern, Switzerland.
Email: cord.droegemueller@unibe.ch

ORCID

Joana G. P. Jacinto https://orcid.org/0000-0002-6438-7975
Eliana Schiavon https://orcid.org/0000-0002-1471-6144
Irene M. Häfliger https://orcid.org/0000-0002-5648-963X
Franz R. Seefried https://orcid.org/0000-0003-4396-2747
Cord Drögemüller https://orcid.org/0000-0001-9773-522X

REFERENCES

Agerholm, J.S., McEvoy, F.J., Menzi, F., Jagannathan, V. & Drögemüller, C. (2016) A *CHRNB1* frameshift mutation is associated with familial arthrogryposis multiplex congenita in red dairy cattle. *BMC Genomics*, 17, 479. Available from: https://doi.org/10.1186/s12864-016-2832-x

Chong, J.X., Burrage, L.C., Beck, A.E., Marvin, C.T., McMillin, M.J., Shively, K.M. et al. (2015) Autosomal-dominant multiple pterygium syndrome is caused by mutations in *MYH3*. *American Journal of Human Genetics*, 96, 841–849. Available from: https://doi.org/10.1016/j.ajhg.2015.04.004

Jacinto, J.G.P., Häfliger, I.M., Akyürek, E.E., Sacchetto, R., Benazzi, C., Gentile, A. et al. (2021) *KCNG1* -related syndromic form of congenital neuromuscular channelopathy in a crossbred calf. *Genes*, 12, 1792. Available from: https://doi.org/10.3390/genes12111792

Mossey, P.A. & Modell, B. (2012) Epidemiology of oral clefts 2012: an international perspective. *Frontiers of Oral Biology*, 16, 1–18. Available from: https://doi.org/10.1159/000337464

Mulvihill, J.J., Mulvihill, C.G. & Priester, W.A. (1980) Cleft palate in domestic animals: epidemiologic features. *Teratology*, 21, 109–112. Available from: https://doi.org/10.1002/tera.1420210115

Rot, I. & Kablar, B. (2013) Role of skeletal muscle in palate development. *Histology and Histopathology*, 28, 1–13. Available from: https://doi.org/10.14670/HH-28.1

Vaiman, A., Fritz, S., Beauvallet, C., Boussaha, M., Grohs, C., Daniel-Carlier, N. et al. (2022) Mutation of the *MYH3* gene causes recessive cleft palate in Limousine cattle. *Genetics, Selection, Evolution*, 54, 71. Available from: https://doi.org/10.1186/s12711-022-00762-2

Zieba, J., Zhang, W., Chong, J.X., Forlenza, K.N., Martin, J.H., Heard, K. et al. (2017) A postnatal role for embryonic myosin revealed by *MYH3* mutations that alter TGFβ signaling and cause autosomal dominant spondylocarpotarsal synostosis. *Scientific Reports*, 7, 41803. Available from: https://doi.org/10.1038/srep41803

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.