Received: 14 June 2022 | Accepted: 14 June 2022

DOI: 10.1111/age.13236

ANIMAL GENETICS WILEY

BRIEF REPORT

A germline de novo variant in *NUMB* associated with a double-outlet right ventricle in Chianina cattle

BACKGROUND

Congenital heart disease (CHD) comprises a wide spectrum of abnormalities in heart structure that occur in the fetus during pregnancy (Sun et al., 2015). Double-outlet right ventricle (DORV) is a rare form of ventriculoarterial connection reported in a few cattle breeds (Caivano et al., 2021; McManus et al., 2020; Newhard et al., 2017; Nourani et al., 2009; Prošek et al., 2005; Wilson et al., 1985). However, the etiology of DORV in cattle has not yet been clarified.

ANALYSIS

DNA was extracted from ear tissue of the 10-day-old male Chianina DORV-affected calf that was previously reported (Figure S1; Caivano et al., 2021), from EDTA blood from its dam and from semen from its sire. We hypothesized a genetic etiology for the present DORV. A trio-based whole-genome sequencing approach was performed as described before (Jacinto et al., 2022). Reads were mapped to the ARS-UCD1.2 assembly (Rosen et al., 2020), resulting in average read depths of 16.9× in the calf, 17.9× in the dam and 21.3× in the sire, and then processed as reported earlier (Jacinto et al., 2021). This identified two heterozygous private protein-changing variants present exclusively in the genome of the affected calf and absent in both parental genomes as well as in 5365 controls (Table S1). Only one of these variants was in a putative candidate gene for the observed phenotype. This heterozygous variant at chr10:84751870G>A (NM_001101951.1: c.416C>T) represents a missense variant in a splicing region located in exon 7 of the *NUMB endocytic adaptor protein* (*NUMB*) gene (Figure S1). The guanine to arginine substitution affects an evolutionary highly conserved amino acid (NP_001095421.1: p.Thr139Met) in the phosphotyrosine interaction domain (PTB/PID) and was predicted to be deleterious using four different tools (PROVEAN, −2.599; POLYPHEN, 2: 56%; SIFT, 79%; and MAPP, 57%). Sanger sequencing confirmed the presence of the heterozygous *NUMB* variant in the affected calf and its sire, which clearly carried the mutant allele at a low level in comparison with the wild type allele, representing a germinal mosaic (Figure S1). Moreover, we identified 30 homozygous protein-changing variants present exclusively in the genome of the affected calf, of which only one affects a putative candidate gene (*XRCC1*) and was predicted to be deleterious (Chr18:51749382G>A; c.1058C>T; p.Pro353Leu). As well as three heterozygous carriers in Chianina and Romagnola cattle (Table S1), subsequent genotyping of 217 normal Chianina bulls showed an allele frequency of 6.91% and three homozygous *XRCC1* mutants, which rules out a possible causal cause.

COMMENTS

We propose the heterozygous c.416C>T variant as a candidate causative variant for the observed congenital disorder and thereby *NUMB* as a novel candidate gene for DORV. The protein-altering nature of this de novo mutation inherited from a mosaic sire strongly suggests the causality of the variant. The affected gene encodes a membrane-bound protein that plays an important role in the determination of cell fates during development (O'Leary et al., 2016). In mice, homozygous *NUMB* mutant animals (MGI3783761) show a variety of abnormalities, including abnormal cardiac morphology, abnormal vascular regression, pericardial edema, abnormal central nervous system morphology, abnormal neuron differentiation, embryonic growth retardation and embryonic lethality during embryogenesis (Zilian et al., 2001). The calf reported herein was uniquely affected by a congenital malformation of the heart (Figure S1). We hypothesized that the observed phenotypical differences between the *NUMB* mutated mice and the calf in this study might be explained by the presence of two mutated alleles in mice and only one in the calf. Therefore, considering the rarity, *in silico* effect prediction and known function of *NUMB*, the identified missense variant

might be considered as a possible cause for the observed phenotype, although this gene has not been previously associated with DORV in mammals, including humans.

ACKNOWLEDGEMENTS

We thank the Interfaculty Bioinformatics Unit of the University of Bern for providing high-performance computational infrastructure. Open access funding provided by Universitat Bern.

CONFLICT OF INTEREST

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; calf SAMEA10833775, dam SAMEA10833776, sire SAMEA10833773).

Joana G. P. Jacinto[1,2]
Irene M. Häfliger[2]
Domenico Caivano[3]
Cord Drögemüller[2]

[1]*Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia (Bologna), Italy*
[2]*Vetsuisse Faculty, Institute of Genetics, University of Bern, Bern, Switzerland*
[3]*Department of Veterinary Medicine, University of Perugia, Perugia, Italy*

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

ORCID

Joana G. P. Jacinto https://orcid.org/0000-0002-6438-7975
Irene M. Häfliger https://orcid.org/0000-0002-5648-963X
Domenico Caivano https://orcid.org/0000-0001-8540-4853
Cord Drögemüller https://orcid.org/0000-0001-9773-522X

REFERENCES

Caivano, D., Marchesi, M.C., Boni, P., Venanzi, N., Angeli, G., Porciello, F. et al. (2021) Double-outlet right ventricle in a Chianina calf. *Animals*, 11, 318. Available from: https://doi.org/10.3390/ani11020318

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

Jacinto, J.G.P., Häfliger, I.M., Bernardini, M., Mandara, M.T., Bianchi, E., Bolcato, M. et al. (2022) A homozygous missense variant in laminin subunit beta 1 as candidate causal mutation of hemifacial microsomia in Romagnola cattle. *Journal of Veterinary Internal Medicine*, 36(1), 292–299. Available from: https://doi.org/10.1111/jvim.16316

McManus, A., Moloney, T., Kelly, P., Rowan, C., Skelly, C. & McAloon, C.I. (2020) An unusual presentation of developmental anomalies of the cardiovascular system including tetralogy of fallot, double outlet right ventricle, patent foramen ovale and persistent right aortic arch in a Friesian calf. *BMC Veterinary Research*, 16(1), 224. Available from: https://doi.org/10.1186/s12917-020-02439-8

Newhard, D.K., Jung, S.W., Winter, R.L., Kuca, T., Bayne, J. & Passler, T. (2017) Double-outlet right ventricle with an intact interventricular septum and concurrent hypoplastic left ventricle in a calf. *Journal of Veterinary Cardiology*, 19(2), 205–210. Available from: https://doi.org/10.1016/j.jvc.2016.11.002

Nourani, H., Parchami, A. & Bonyadian, M. (2009) Double outlet right ventricle in a calf. *Comparative Clinical Pathology*, 18(2), 187–189. Available from: https://doi.org/10.1007/s00580-008-0771-x

O'Leary, N.A., Wright, M.W., Brister, J.R., Ciufo, S., Haddad, D., McVeigh, R. et al. (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research*, 44(D1), D733–D745. Available from: https://doi.org/10.1093/nar/gkv1189

Prošek, R., Oyama, M.A., Church, W.M., Nagy, D.W. & Sisson, D.D. (2005) Double-outlet right ventricle in an Angus calf. *Journal of Veterinary Internal Medicine*, 19(2), 262–267. Available from: https://doi.org/10.1892/0891-6640(2005)19<262:drviaa>2.0.co;2

Rosen, B.D., Bickhart, D.M., Schnabel, R.D., Koren, S., Elsik, C.G., Tseng, E. et al. (2020) De novo assembly of the cattle reference genome with single-molecule sequencing. *GigaScience*, 9(3), 1–9. Available from: https://doi.org/10.1093/gigascience/giaa021

Sun, R., Liu, M., Lu, L., Zheng, Y. & Zhang, P. (2015) Congenital heart disease: causes, diagnosis, symptoms, and treatments. *Cell Biochemistry and Biophysics*, 72(3), 857–860. Available from: https://doi.org/10.1007/s12013-015-0551-6

Wilson, R.B., Cave, J.S., Horn, J.B. & Kasselberg, A.G. (1985) Double outlet right ventricle in a calf. *Canadian Journal of Comparative Medicine*, 49(1), 115–116.

Zilian, O., Saner, C., Hagedorn, L., Lee, H.Y., Säuberli, E., Suter, U. et al. (2001) Multiple roles of mouse Numb in tuning developmental cell fates. *Current Biology*, 11(7), 494–501. Available from: https://doi.org/10.1016/s0960-9822(01)00149-x

SUPPORTING INFORMATION

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