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Detailed analysis of mortality rates in the female progeny of 1,001 Holstein bulls allows the discovery of new dominant genetic defects

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ABSTRACT

Reducing juvenile mortality in cattle is important for both economic and animal welfare reasons. Previous studies have revealed a large variability in mortality rates between breeds and sire progeny groups, with some extreme cases due to dominant mutations causing various syndromes among the descendants of mosaic bulls. The purpose of this study was to monitor sire-family calf mortality within the French and Walloon Holstein populations, and to use this information to detect genetic defects that might have been overlooked by lack of specific symptoms. In a population of heifers born from 1,001 bulls between 2017 and 2020, the average sire-family mortality rates were of 11.8% from birth to 1 year of age and of 4.2, 2.9, 3.1, and 3.2% for the perinatal, postnatal, preweaning, and postweaning sub-periods, respectively. After outlining the 5 worst bulls per category, we paid particular attention to the bulls Mo and Pa, because they were half-brothers. Using a battery of approaches, including necropsies, karyotyping, genetic mapping, and whole-genome sequencing, we described 2 new independent genetic defects in their progeny and their molecular etiology. Mo was found to carry a de novo reciprocal translocation between chromosomes BTA26 and BTA29, leading to increased embryonic and juvenile mortality because of aneuploidy. Clinical examination of 2 calves that were monosomic for a large proportion of BTA29, including an ortholo-

gous segment deleted in human Jacobsen syndrome, revealed symptoms shared between species. In contrast, Pa was found to be mosaic for a dominant de novo non-sense mutation of GATA 6 binding protein (*GATA6*), causing severe cardiac malformations. In conclusion, our results highlight the power of monitoring juvenile mortality to identify dominant genetic defects due to de novo mutation events.

Key words: bovine genetic defects, juvenile mortality, heart defects, *GATA6*, chromosomal rearrangements

INTRODUCTION

Juvenile mortality has a severe impact on the cattle industry, because calves are the major output in beef production and are necessary for replacement in dairy production. Beyond economics, juvenile mortality affects the environmental impact of cattle breeding and addresses serious animal welfare concern (Østerås et al., 2007; Uetake 2013; Knapp et al., 2014). For all these reasons, juvenile mortality is an increasingly important field of research.

Several cross-sectional studies have been carried out worldwide, generally focusing on different periods to accurately monitor juvenile mortality (Reiten et al., 2018; Hyde et al., 2020; Dachrodt et al., 2021). Causes of death change during the course of the first year of life, the main ones being calving conditions or problems of fetal maturity, insufficient colostrum intake, digestive troubles, and respiratory diseases for the perinatal, postnatal, preweaning and postweaning periods, respectively.

Studies conducted in Holstein cattle have found mortality rates of 6.8 to 7.3% in France and 8.8% in the

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US (Johanson et al., 2011; Raboisson et al., 2013) from birth to the second day of life, and of 12.9% from d 3 to 365 in the Chinese population (Zhang et al., 2019). In Norway, aggregating data from various dairy breeds, the mortality rate was only 7.8% over the whole first year of life in 2005 (Gulliksen et al., 2009), suggesting an influence of genetic components and farming systems on calves' survival. This assumption has been further supported by a comprehensive analysis of juvenile mortality in 19 French cattle breeds, which highlighted the breed purpose (beef or dairy), breed, sex, and sire progeny groups as the main factors influencing juvenile mortality across periods (e.g., Leclerc et al., 2016).

Heritability estimates for juvenile mortality are lower than 10% (Fuerst-Waltl and Sørensen 2010; van Pelt et al., 2012), reflecting the multiplicity of factors involved and the predominant effect of environment. However, as frequently observed for low-heritability traits, the genetic variability is large, with a genetic standard deviation around 5%, corresponding to a huge genetic coefficient of variation of 50%. The corresponding genetic variability is difficult to characterize biologically because the largest part of the mortalities results from common infections with undetermined, and probably underestimated, genetic components. Nevertheless, some genetic factors have been identified in the past, such as bovine leukocyte adhesion deficiency (Shuster et al., 1992), but most of them are recessive and explain a very small proportion of the mortalities. In some rare situations, their determinism is dominant and can explain large mortality rates in the progeny of carrier sires. These dominant conditions can be transmitted by carrier animals either because of incomplete penetrance or of mosaicism for de novo mutations (Bourneuf et al., 2017). One possible way to seek for dominant deleterious mutations is to analyze mortality rates in the progeny of artificial insemination sires used in multiple farms, assuming that extreme values hide congenital anomalies.

In this context, the purpose of this study was two-fold: to finely monitor calf mortality at the level of sire families within the French and Walloon Holstein populations, and to use this information to detect genetic defects that might have been overlooked by lack of specific externally visible symptoms.

MATERIALS AND METHODS

Mortality Rates in Sire Families at Different Ages

Data on the pedigree, sex, date of birth, date of death, and cause of death (either natural or slaughtering) of Holstein animals were recovered from the bovine French and Walloon databases. The data set included

calves born from 2017 to 2020. To focus on the most reliable data, only female calves that remained on their farm of birth until death or during their whole first year of life were selected. Sire families with fewer than 100 female progeny were disregarded. Accordingly, the final data set comprised 2.25 million daughters from 1,001 sires (with a mean of 2,246 and a maximum of 35,375 females per sire family). Natural mortality rates were computed during the first year of life and for 4 sub-periods known to correspond to distinct predominant causes of death: perinatal (d 0–2), postnatal (d 3–14), preweaning (d 15–55), and postweaning (d 56–365) mortality (Santman-Berends et al., 2019; Dachrodt et al., 2021). Mortality rates were calculated as the number of calves that died of natural causes during a window of time divided by the number of calves alive at the start date.

Then, we paid particular attention to the 5 bulls showing the highest mortality rates for each period, to identify sires potentially transmitting unreported dominant genetic defects to their progeny. Among them, 2 sires (Mo and Pa) were selected for subsequent analyses because they were half-brothers and potentially transmitted a common genetic defect.

Clinical Examination

Two affected calves of Mo (2 females) and 8 of Pa (4 females, 4 males) were necropsied by trained veterinarians in France, Belgium, and the UK. By “affected calves” we mean animals that have been reported by breeders as suffering from unexplained weakness, diminished growth rates, and often spontaneous death despite intensive care. Gross phenotypic description was also available for 5 additional clinically affected calves of Mo (see Supplemental Notes S1 and S2 for information on the age and symptoms of all calves examined; https://figshare.com/projects/Besnard_JDS_Supplementary_material/140747; Besnard, 2022). At the time of the study, biological material was still available for 2 affected calves of Mo and 8 of Pa.

Karyotyping

Giemsa-stained karyotype of sire Mo and of 2 affected daughters of Pa were obtained from blood lymphocytes as described in Ducos et al. (1998). Of note, Pa was dead at time of the study and thus not available for sampling.

Analysis of Semen Quality and Fertility

Because chromosomal rearrangements can negatively affect spermatogenesis, 5 different traits were analyzed

for a cohort of 50 bulls, including Mo, that had their semen collected on a routine basis in the same artificial insemination center: the mean volume of the ejaculate (in mL measured by weighting), its concentration (in million spermatozooids per milliliter, measured by spectrophotometry), fresh mass motility and individual motility (in score and percentage, respectively, based on microscope observation), as well as post-freezing mean motility and progressive motility, measured with computer-assisted sperm analysis and IVOS II (O'Meara et al., 2022). The number of records per bull and trait ranged from 2 to 39. In addition, 2 fertility traits were calculated for the initial cohort of 1,001 sires mentioned previously. The nonreturn rate at 56 d corresponds to the percentage of cows inseminated with the semen of a given sire that were not reinseminated within the following 56 d, and the conception rate corresponds to the percentage of inseminations that led to the birth of a calf.

Analysis of Illumina SNP Array Genotypes

The bull Mo, 15 of his progeny and 1 of their dams, as well as Pa, 203 of his progeny and 89 of their dams, and finally Mogul (sire of both bulls) were genotyped with various Illumina arrays over time (Bovine SNP50, EuroG10K, and EuroGMD). Genotypes were phased and imputed to the Bovine SNP50 using FImpute3 (Sargolzaei et al., 2014) in the framework of the French genomic evaluation, as described in Mesbah-Uddin et al. (2019).

Following the detection by karyotyping of a chromosomal rearrangement in Mo, we analyzed along chromosomes BTA26 and BTA29 which of the paternal or maternal phases of this bull were transmitted to offspring, to detect recombination events. In parallel, we also mined the raw genotypes of affected daughters for increased rates of Mendelian transmission errors (a sign of monosomy) or increased rates of markers with null genotypes (“-/-”; a sign of trisomy).

For Pa, no chromosomal rearrangement was identified, and other investigations were carried out. Assuming a dominant inheritance with somatic mosaicism, we performed transmission disequilibrium tests for 16,487 informative markers, for 14 progeny that died during the preweaning period (including 5 already necropsied at that time), and 189 half-sib controls still alive at 2 years of age. The proportion of each of the paternal alleles transmitted to the case and control groups were compared using a Fisher test with Bonferroni correction. Finally, ggplot2 and Rcolorbrewer were used for data visualization with the R software (R version 4.1.2).

After the discovery of the causative mutation in the *GATA6* gene (see the Results section), we used allele transmission proportions for 2 flanking informative markers within the control population to estimate the proportion of mosaicism in Pa's germ cells. Given the deleterious consequences of the *GATA6* mutation on heart development, we expect that control calves carrying the at-risk haplotype inherited the ancestral version of this haplotype (i.e., predating the mutation event). The proportion of affected gametes was calculated as $(nHb - nHa)/(2 \times nHb)$, with nHa the number of carriers of the at-risk haplotype among half-sib controls and nHb the number of carriers of the alternative paternal haplotype within the same population. Finally, we used a chi-squared goodness-of-fit test to compare the observed proportion of affected gametes with those expected assuming mosaicism rates of 1/2, 1/4, 1/8, and 1/16 in Pa's germ cells.

Gene Content and Comparative Genomics

The gene content of specific regions was extracted from the bovine ARS-UCD1.2 and human GRCh38.p13 genome assemblies using the BioMart tool (Ensembl release 106; <https://www.ensembl.org/biomart/martview/>). In parallel, we used the synteny tool from Ensembl to identify conserved blocks between bovine and human chromosomes (https://www.ensembl.org/Bos_taurus/Location/Synteny/). Then we compiled the list of genes in common between the BTA29 segment deleted in Mo's affected calf and the core HSA11 deletion responsible for Jacobsen syndrome in human.

Analysis of Whole-Genome Sequences

The genome of 1 affected calf of Pa was sequenced at a coverage of 19.4× on an Illumina HiSeq3000 HWI-J00173 platform with 150 bp paired-end reads, after library preparation with an average insert size of 440 bp using the NEXTflex PCR-Free DNA Sequencing Kit (Bioo Scientific). The whole genome sequence data are available under the study accession no. ERR9669242 at the European Nucleotide Archive (www.ebi.ac.uk/ena). Reads were aligned on the ARS-UCD1.2 bovine genome assembly and processed in accordance with the guidelines of the 1000 Bull Genomes Project (Hayes and Daetwyler 2019) for the detection of SNPs and small InDels. Assuming that the causative mutation is dominant and occurred de novo, we retained only heterozygous variants that were (1) absent from 5,116 control genomes from run 9 of the 1000 Bull Genomes Project and (2) located within the mapping interval (positions 19,505,558 to 37,877,867 bp on BTA24).

The remaining variants were annotated using Variant Effect Predictor (Ensembl release 106; www.ensembl.org/Tools/VEP). In addition, we detected structural variants within the mapping interval using Pindel (Ye et al., 2009), Delly (Rausch et al., 2012), and Lumpy software (Layer et al., 2014), and applied the same filters after comparison with analogous data of 62 control genomes (Boussaha et al., 2015).

Genotyping of the GATA6 Candidate Variant

DNA samples from Pa (extracted from semen), 3 affected calves, and 3 controls carrying the same paternal haplotype but in the nonmutated version, as well as their 6 dams, were genotyped for variant g.34,187,181T > A on BTA24 using PCR and Sanger sequencing. A segment of 321 bp was PCR amplified in a Mastercycler Pro thermocycler (Eppendorf) using primers CAGTGGGCGCTAAACTACC and AGACCTGCTGGAGGACCTG and the Go-Taq Flexi DNA Polymerase (Promega), according to the manufacturer's instructions. Amplicons were purified and bidirectionally sequenced by Eurofins MWG (Hilden, Germany) using conventional Sanger sequencing, before analysis with NovoSNP software for variant detection (Weckx et al., 2005).

RESULTS AND DISCUSSION

Analysis of Mortality Rates at Different Stages in the Progeny of Individual Sires

The natural mortality rate of heifers during their first year of life was 11.8% on average in the population of 1,001 bulls analyzed, with 4.2% for perinatal, 2.9% for postnatal, 3.1% for preweaning, and 3.2% for postweaning mortalities. These rates were lower than most of those reported in the literature (e.g., Johanson et al., 2011; Raboisson et al., 2013; Leclerc et al., 2016; Zhang et al., 2019), probably because we considered only females. Sex is known to have a significant effect on juvenile mortality (Raboisson et al., 2013; Hyde et al., 2020), notably because females receive more care than males, due their higher financial value. The addition of vitality at birth in the French Holstein total merit index in 2009 may also have contributed to a reduction of perinatal mortality through selection.

Interestingly, natural mortality rates per period and per half-sib family showed approximately normal distribution, suggesting quantitative inheritance (Figure 1). Yet we observed outlier families with possible mono- or oligogenic inheritance of excess mortality and focused on the 5 worst sires per category (Table 1). Among

them, the 2 bulls Mo and Pa, ranked number 1 and number 5 for mortality rate over the first year of life, were half-brothers sired by the popular bull Mogul (HOLUSAM003006972816). Although they displayed distinct profiles (with, for example, 16.7 and 5.3% perinatal mortality versus 2.4 and 6.3% preweaning mortality, respectively), their close relationship raised the question of a common underlying pathophysiology, and therefore they were selected for further analysis.

Identification and Characterization of a Reciprocal Translocation Between BTA26 and BTA29 in Mo

Genetic Analyses of Mo and His Progeny. To gain insights into the causes of increased mortality within the Mo and Pa sire families, we carried out a series of investigations, starting with cytogenetic analyses. Although the karyotypes of 2 affected daughters of Pa were apparently normal (not shown), we observed a reciprocal translocation between chromosomes BTA26 and BTA29 in Mo [t(26;29)(q11;q19); Figure 2A].

Subsequent analysis of Illumina SNP array genotypes from Mo, his own sire Mogul, and 15 of Mo's progeny enabled us to define the approximate borders of chromosomal break and fusion points, and to determine that the affected chromosomes originated from Mogul (Figure 2B, C; Supplemental Figure S1, https://figshare.com/projects/Besnard_JDS_Supplementary_material/140747; Besnard, 2022). Considering that Mogul was extensively used as a bull sire and did not display abnormal juvenile mortality rates, these results suggest that the rearrangement occurred in the germ cells of Mogul during the meiosis that gave the spermatozoon at Mo's conception.

In addition, we demonstrated that 2 affected daughters of Mo with DNA samples available were monosomic for approximately the first 70% of BTA29 (767 markers, 36.7 Mb; Supplemental Figure S1). Interestingly, comparative genomics revealed synteny between part of the hemizygous region and the monosomy of the telomeric region of chromosome 11q responsible for Jacobsen syndrome (Figure 2C; Mattina et al., 2009). Both segments share a common set of 69 orthologous protein coding genes out of the 318 affected by monosomy in Mo's progeny and the ~100 of the core Jacobsen deletion (Supplemental Table S1, https://figshare.com/projects/Besnard_JDS_Supplementary_material/140747, Besnard, 2022; Rodríguez-López et al., 2021).

Phenotypic Characterization of Mo's Calves and Mo's Semen Characteristics. In human, Jacobsen syndrome has been extensively studied, with 200 cases compiled in the Human Phenotype Ontology da-

Table 1. Selection of the 5 bulls showing the highest mortality rates for each period and over the whole first year (bold number in table) versus average value of population; the bulls are sorted in descending order on the entire first-year mortality rate¹

Bull	No. of daughters	Mortality rates per period, %				
		Perinatal	Postnatal	Preweaning	Postweaning	First year
Mo ²	108	16.7	6.7	2.4	7.3	30.6
Lp	110	3.6	4.9	6.2	8.8	20.9
Ce	126	1.6	9.0	6.3	5.8	20.6
Sg	130	5.4	8.6	3.8	4.9	20.0
Pa ²	2,297	5.3	6.1	6.3	4.8	19.9
Mn	122	9.0	2.0	3.1	8.4	19.7
Jo	103	1.9	5.1	3.2	8.8	17.5
Ja	1,196	5.2	6.1	4.2	3.9	17.5
Wt	124	2.4	5.9	6.3	3.9	16.9
Pg	315	2.9	4.4	2.5	8.7	16.8
Ip	203	5.9	1.7	2.8	8.1	16.8
Co	1,749	9.4	3.9	3	2.2	16.5
Io	815	8.3	2.5	2.9	3.9	15.8
Me	117	2.6	4.5	6.6	3	15.4
Ln	2,399	8.1	2.6	3	2.7	14.9
Average value for 1,001 bulls	2,250	4.2	2.9	3.1	3.2	11.8

¹Each rate is calculated based on the calves alive at the start of the period. For this reason, summing up the mortality rates for the 4 subperiods does not give exactly the same number as the mortality rate for the whole period.

²Bulls are paternal half-brothers.

tabase (<http://human-phenotype-ontology.github.io>). As with the progeny of Mo, most of human cases are due to translocations between HSA11 and other chro-

mosomes (Basinko et al., 2011). The clinical features of Jacobsen syndrome include various symptoms that are more or less expressed depending of the patient,

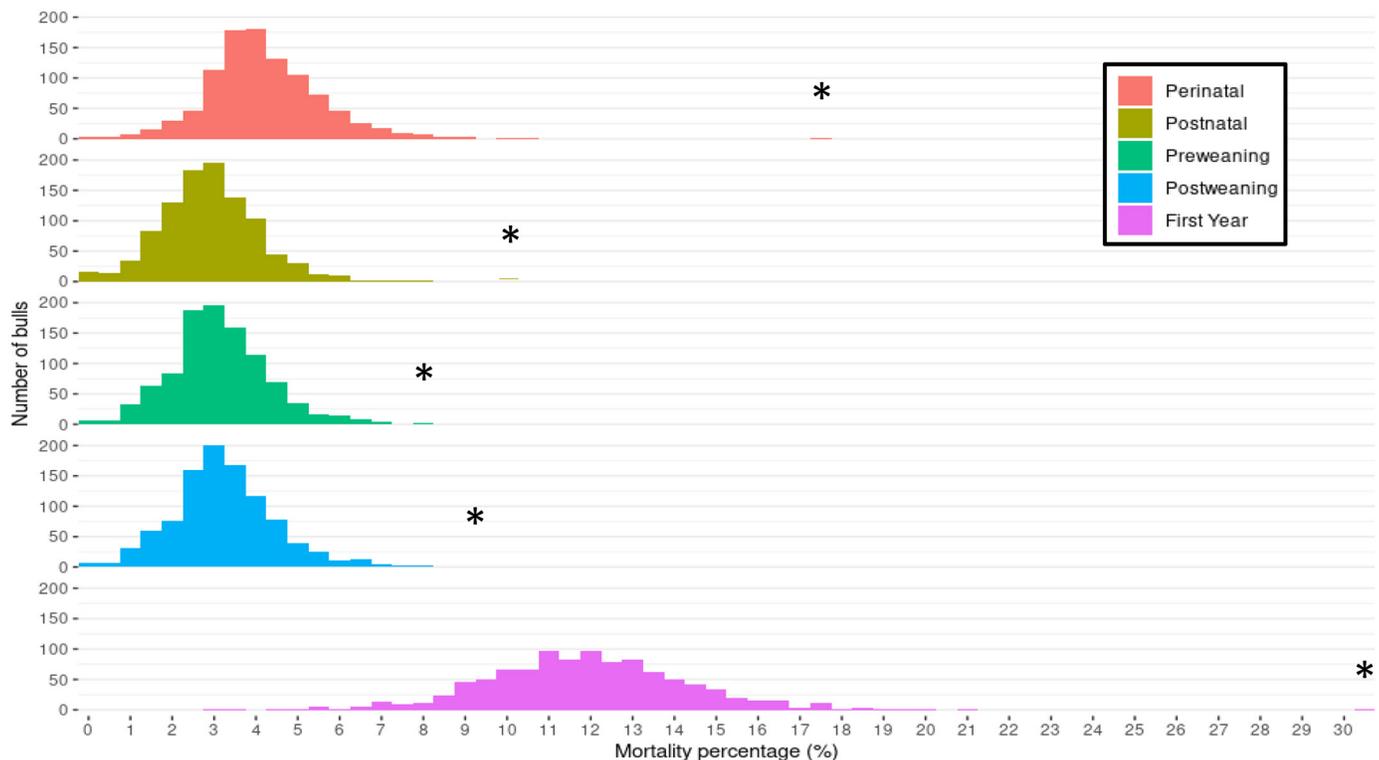


Figure 1. Distribution of daughters' mortality rates of 1,001 bulls for 5 periods of death. Asterisks highlight barely visible outliers.

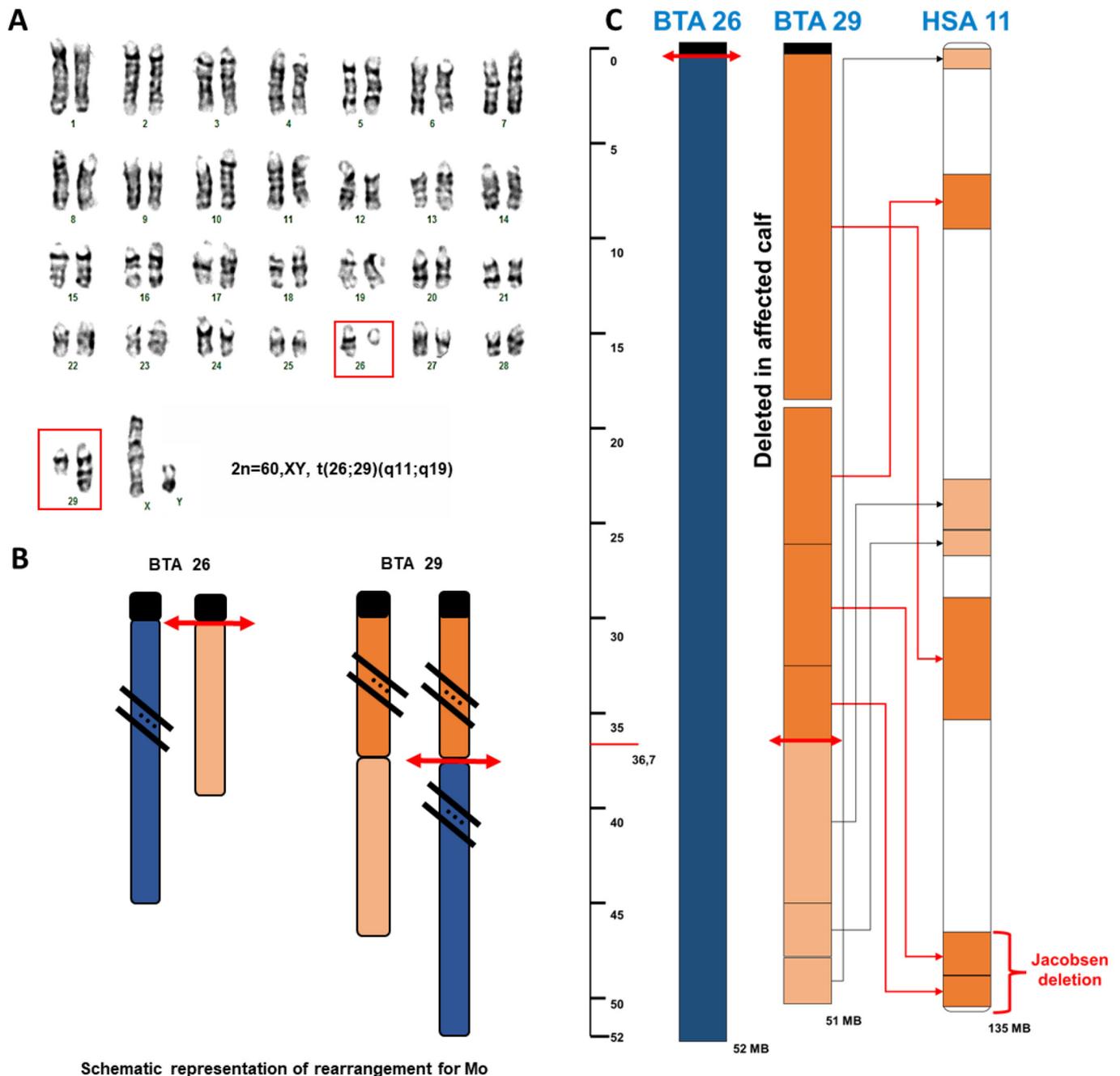


Figure 2. Karyotype of sire Mo and details on the chromosomal segments involved. (A) Giemsa-stained karyotype of Mo showing a reciprocal translocation between BTA26 and BTA29. (B) Schematic representation of the chromosomal rearrangement with mutant chromosomes on the right of each pair. (C) Approximate localization of the breakpoints (based on the analysis of SNP array genotypes from 15 progeny of Mo; Supplemental Figure S1, https://figshare.com/projects/Besnard_JDS_Supplementary_material/140747; Besnard, 2022) and synteny between BTA29 and human HSA11 chromosomes.

such as Paris-Trousseau thrombocytopenia, growth rate reduction, and psychomotor impairment, as well as cardiac, craniofacial, gastrointestinal, renal, genitourinary, ophthalmic, and orthopedic anomalies (<https://www.omim.org/entry/147791>). In agreement with the

observations made in humans, clinical examination of the 2 calves partially monosomic for BTA29 and of 5 additional cases for which no DNA was available revealed very similar symptoms (Figure 3; Supplemental Note S1).

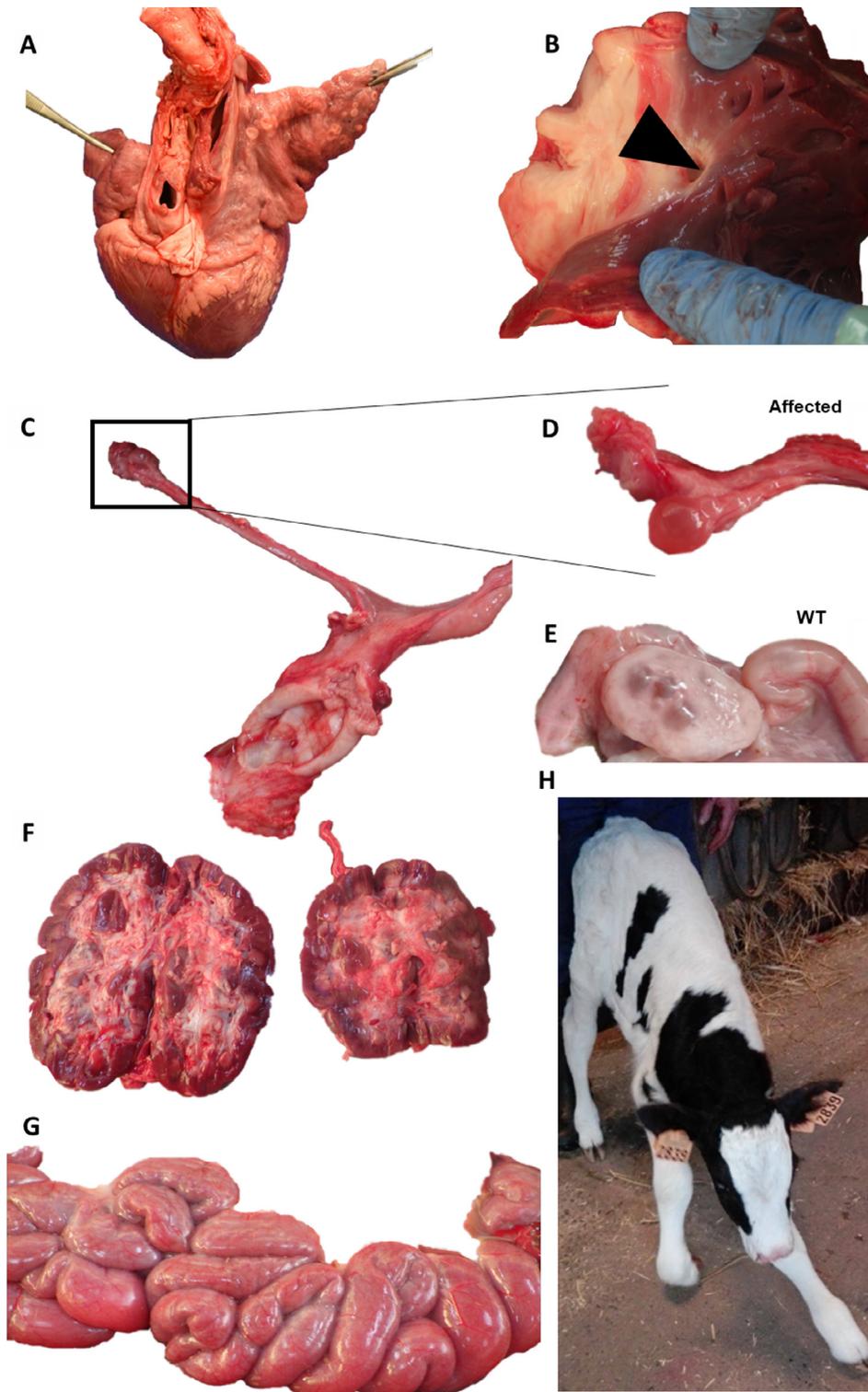


Figure 3. Clinical features observed in the progeny of Mo. (A–G) Symptoms displayed by 2 calves partially monosomic for BTA29 who died at birth (male case 1, A) or was euthanatized at 3.5 mo (female case 2, B–G). (A) Heart with tetralogy of Fallot. (B) Open heart, with black arrow pointing to an interventricular septal defect of 1-cm diameter located high under the sigmoid valves. (C) Blind and hypoplastic uterine horn ending with an atrophied and cystic ovary. (D) Detail of the affected ovary in comparison with a matched control (E). (F) Moderate hypogenesis of the left kidney, whose volume is 2/3 of the right kidney. (G) Abnormally hydrated content in the colon and rectum. (H) Picture of female case 3, showing articular defects and difficulties standing. For further information, see Supplemental Note S1 (https://figshare.com/projects/Besnard_JDS_Supplementary_material/140747; Besnard, 2022).

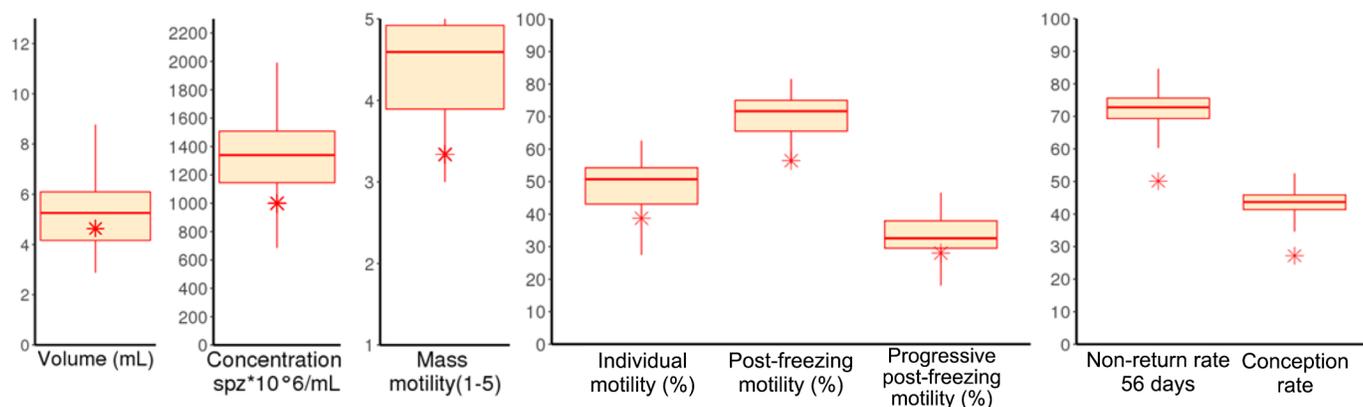


Figure 4. Analysis of the quality and fertility of Mo's semen compared with control bulls. Boxplots of the distribution of 50 bulls for 6 traits measured in the same artificial insemination center and of 1,001 bulls for the nonreturn rate at 56 d and the conception rate. Mo is indicated with a red asterisk. Traits are as follows, from left to right: average volume of ejaculate (mean = 5.21 mL, Mo = 4.64), average spermatozoid concentration in ejaculate [mean = 1,329 million spermatozooids (spz)/mL, Mo = 998], average mass motility score is manually assessed by artificial insemination operators from 1 to 5 (mean = 4.36, Mo = 3.34), average individual motility (mean = 70%, Mo = 56), average post-freezing motility (mean = 49%, Mo = 39), 56-d nonreturn rate (mean = 71%, Mo = 50), and conception rate (mean = 43%, Mo = 27). Boxplots should be read as follows: the horizontal line contained within each box marks the median value (second quartile) of the data. Lower and upper lines correspond to the first and third quartiles. Whiskers correspond to the first and third quartiles ± 1.5 interquartile range, respectively. The units of y-axes are indicated below each figure.

Instances of reciprocal translocations are rare in cattle, with only 20 reports counted in a recent review of literature by Iannuzzi and coauthors, none of which affected chromosome 29 (Iannuzzi et al., 2021).

Regarding aneuploidies affecting BTA29, only 1 complete trisomy has been reported before this study, in a stillborn Braunvieh calf showing preterm delivery, dwarfism, and severe craniofacial malformations (Häfliger et al., 2020). The absence of other reports on trisomy for BTA29 despite the segregation of a BTA1–29 Robertsonian fusion in various cattle breeds (Gustavsson, 1979), as well as the lack of human patients trisomic for the Jacobsen segment on HSA11 orthologous to part of BTA29 (e. g. Pylyp et al., 2018), suggest that this condition would lead to embryonic death in both species.

Because chromosomal abnormalities affect not only the viability of conceptuses but also meiosis and gametogenesis (Raudsepp and Chowdhary, 2016), we investigated several traits related to semen volume, quality, and fertility in Mo and 2 groups of Holstein bulls (Figure 4). Among 50 bulls reared and sampled in the same artificial insemination center, Mo showed normal average volume of ejaculate but low semen quality, with average concentration of semen and fresh and post-freezing motility trait records in the lowest quartile. The influence of the chromosomal rearrangement was even more severe with regard to fertility, Mo ranking as the worst sire for nonreturn rate at 56 d and the fifth worst for conception rate among our cohort of 1,001 Holstein sires. This major degradation of fertility,

observed on both early and late indicators of insemination success, is most probably the result of the premature death of a substantial proportion of aneuploid conceptuses throughout the gestation.

Thus, we report the first large animal model for Jacobsen syndrome in humans, and the first instance of partial monosomy for BTA29 in cattle, to our knowledge.

Identification of a Mosaic GATA6 Nonsense Mutation in Pa

Despite their close relationship, a different etiology was suspected for the excess of mortality observed among the daughters of Pa, because the peak of mortality occurred later in life than for Mo's offspring. This assumption was rapidly confirmed by clinical examination and karyotyping of Pa's descendants.

Clinical Examination of Pa's Progeny. A survey of French and British veterinarians allowed us to collect phenotypic information on Pa's descendants, among which 8 showed symptoms compatible with severe heart defects either leading to premature death or justifying euthanasia on humane grounds (Supplemental Note S2). Autopsies gave results strikingly similar to the systematic observation of a persistent *truncus arteriosus* (TA; i.e., a malformation of the large vessels at the base of the heart, characterized by the development of a single arterial trunk straddling the 2 ventricles, above a large interventricular communication, which gives rise to the aorta and the 2 branches of the pulmonary

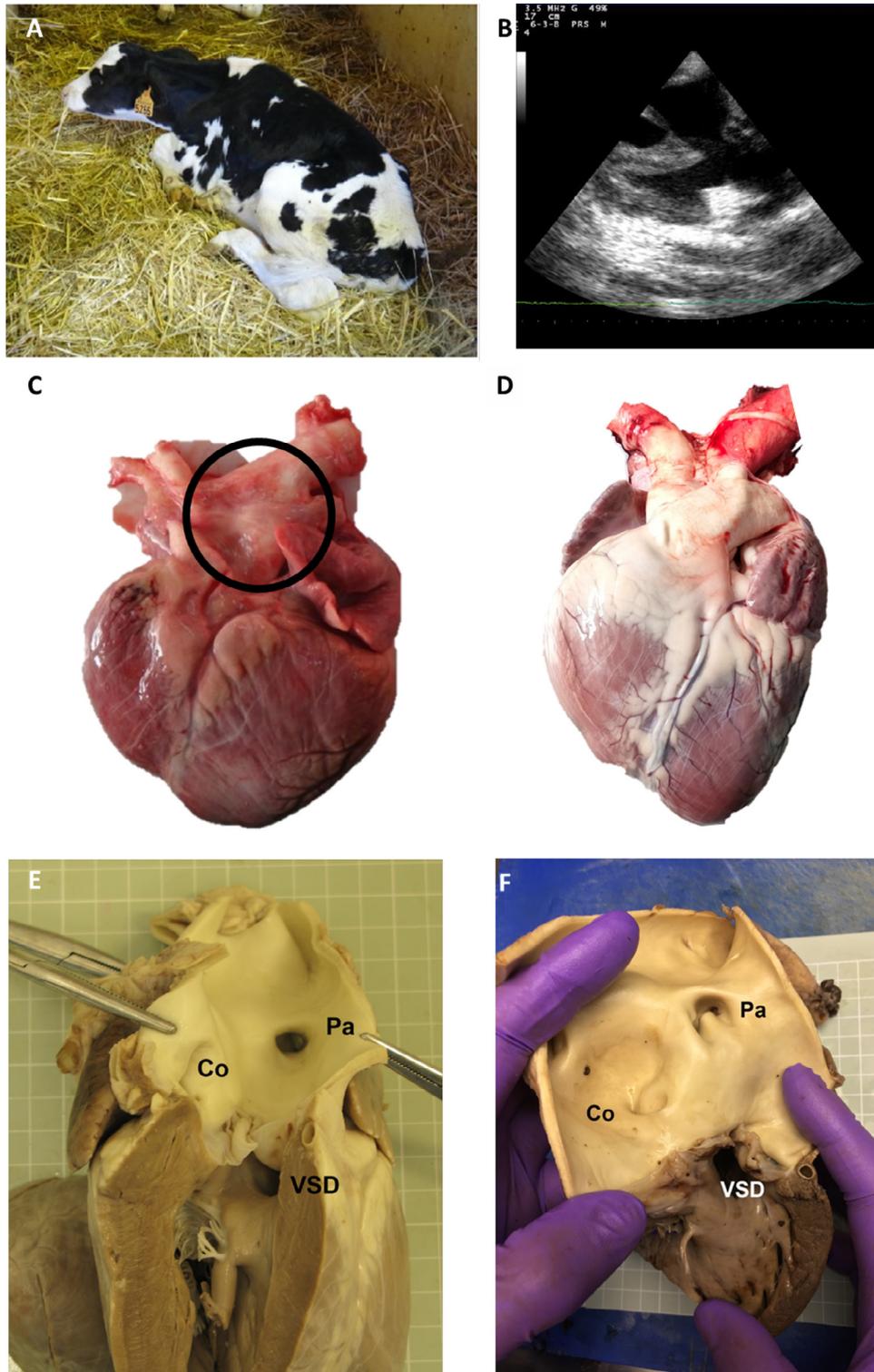


Figure 5. Clinical findings in Pa calves. (A–C) Pictures of case 1, euthanatized at 42 d of age. (A) Live calf on farm. (B) Ultrasonography showing communications between the auricles and the common arterial trunk. (C, D) Hearts of case 1 and of a matched control, respectively. Note the persistent *truncus arteriosus* (circle) and the modification of the general shape of the heart in C vs. D. (E) Right lateral view of the dissected right ventricular outflow tract and common arterial trunk of case 6. Note the thickened right ventricular wall and ventricular septal defect (VSD); the *truncus arteriosus* is situated over the ventricular septum and has a single common arterial valve with 3 leaflets; the coronary arteries arise from an ostium on the left side of this vessel (Co); and the pulmonary arteries arise from a common ostium on the right side (Pa). (F) Right dorsolateral view of the open *truncus arteriosus* in case 7 showing the VSD and ostia of the coronary arteries (Co) and pulmonary arteries (Pa).

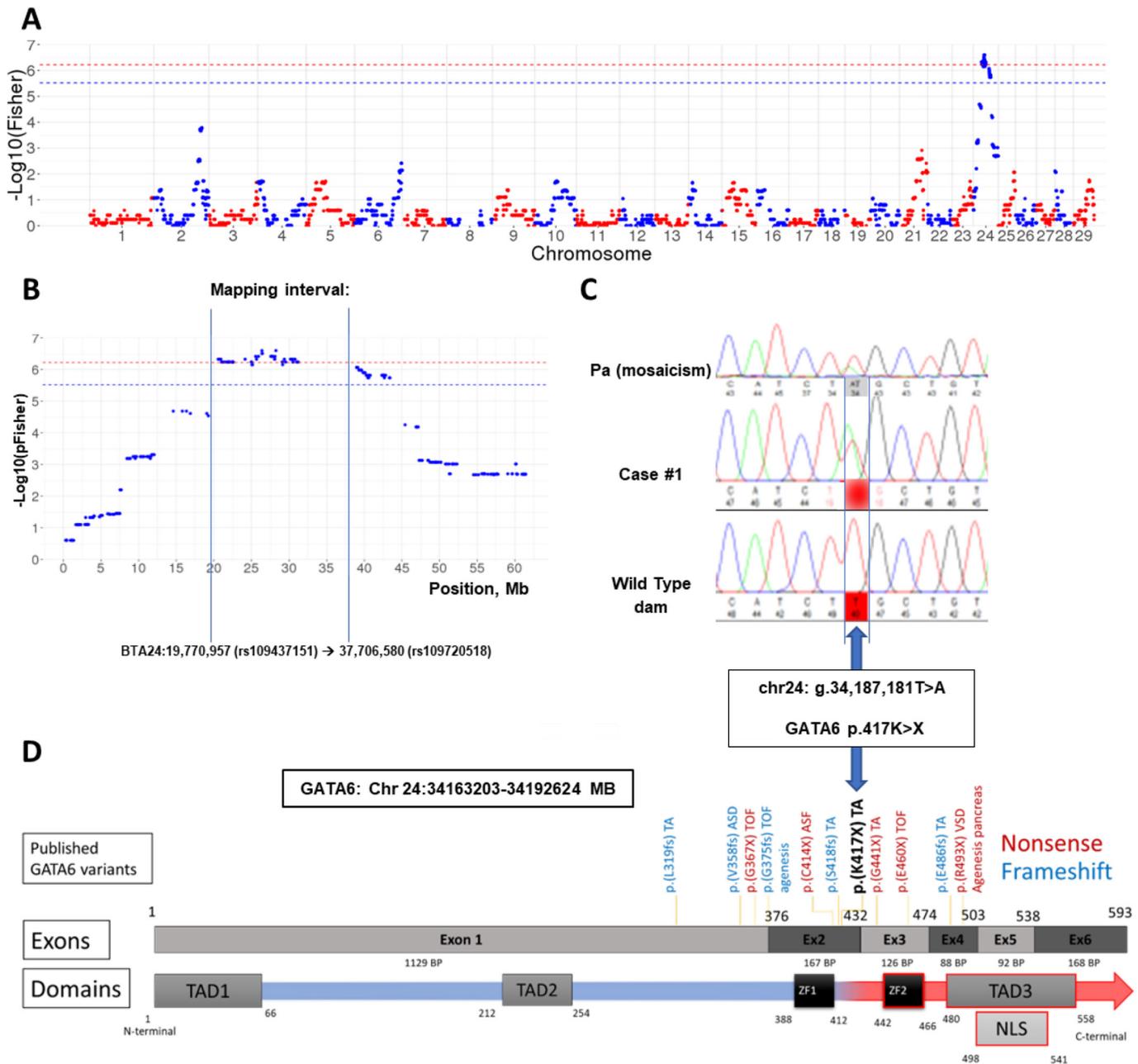


Figure 6. Mapping and identification of a de novo nonsense mutation of GATA6. (A, B) Manhattan plot of the results of mapping of the *truncus arteriosus* locus using transmission disequilibrium test, with a zoom on BTA24 (B). The blue and red lines represent the significance threshold for $P < 0.05$ and $P < 0.01$ after Bonferonni correction for multiple testing. (C) Electropherogram of the sire Pa, one affected calf, and its nonaffected dam, for a segment of BTA24 encompassing variant g.34,187,181T > A. Note the small proportion of allele A versus T in Pa as compared with case 1, supporting mosaicism. (D) Domain and region information for GATA6, with phenotype information for the bovine mutation p.417K > X and truncating variants reported in the orthologous protein in human. TAD: transcription activation domain; ZF: zinc finger domain; NLS: nuclear localization signal domain; TOF: tetralogy of Fallot; ASD: atrial septal defect; VSD: ventricular septal defect. Information obtained from the from the UniProt database (<http://www.uniprot.org/>; accession numbers A0A4W2FXQ7 and Q92908).

artery), sometimes associated with additional heart septation defects (Figure 5).

Mapping and Identification of the Causative Mutation. Given the fact that Pa was apparently un-

affected and that TA has never been reported outside of his progeny among thousands of genetic defects reported to the French National Observatory for Bovine Abnormalities (Grohs et al., 2016) over the last 20

years, we assumed a dominant inheritance associated with germline or somatic mosaicism, or both, in the sire. Therefore, we analyzed SNP array genotypes of 14 progeny that died during the preweaning period (including 5 necropsied) and 189 half-sib controls still alive at 2 years of age, via transmission disequilibrium test. We mapped the TA locus on BTA24 between positions 19,505,558 (rs453420861) and 37,877,878 (rs723126921) bp on the ARS-UCD1 assembly. Then we sequenced the genome of one TA-affected animal with Illumina technology and used up to 5,116 genomes from run 9 of the 1000 Bull Genomes Project (Hayes and Daetwyler 2019) as controls.

Filtering for heterozygous SNP, InDels, and structural variations that were absent from controls yielded only 29 positional candidates within the interval (Supplemental Table S2, https://figshare.com/projects/Besnard_JDS_Supplementary_material/140747; Besnard, 2022). Only one of them appeared as a bona fide functional candidate variant: a thymine-to-adenine substitution in exon 2 of *GATA6* predicted to introduce a premature stop codon (chr24: g.34,187,181T > A; *GATA6* p.K417X). If translated, the mutant protein would be shortened by approximately 30% and would lack 3 domains essential for the proper function of this transcription factor, controlling heart development in vertebrates (Brewer and Pizzey 2006; Lentjes et al., 2016; Figure 6D). Experiments in mice have demonstrated that the conditional inactivation of *GATA6* in heart progenitor cells causes embryonic lethality due to interrupted aortic arch and persistent TA (Lentjes et al., 2016). In humans about 80 dominant mutations of *GATA6* have been described to date, which cause various heart or pancreatic development anomalies depending on their nature and location (for a review see Škorić-Milosavljević et al., 2019). Remarkably, the 2 orthologous human truncating mutations located closest to the present bovine nonsense variant (pS418fs and pG441X) have been reported to cause exactly the same phenotype, that is, persistent TA, supporting the causality of the latter mutation (Figure 6D).

Validation of the Causality of the *GATA6* Mutation. For verification, we genotyped this *GATA6* nonsense variant by PCR and Sanger sequencing in Pa, 3 affected calves, and 3 controls carrying the same paternal haplotype but supposedly in the nonmutated version, as well as their 6 dams. As expected, the mutant allele was found in the heterozygous state only in the 3 cases and in Pa's semen, thus confirming the de novo nature and therefore the causality of the mutation (Figure 6C).

Then we analyzed the segregation distortion for 2 markers adjacent to the mutation among the 189 control calves of Pa that were still alive at 2 years of

age. We found 57 controls carrying the same paternal haplotype as the affected animals but presumably in its ancestral version (i.e., without the de novo mutation) and 132 with the second paternal haplotype. From this 57:132 ratio, we estimated a proportion of 28.4% of mutant spermatozooids $[(132 - 57)/(2 \times 132)]$ and thus 56.8% of mutant germ cells. Comparing the proportion observed in controls with those expected for various degrees of mosaicism using a chi-squared goodness-of-fit test, we demonstrated that this distortion was compatible with a degree of mosaicism of 1/2 ($P = 0.28$) and rejected lower levels of mosaicism (proportions of 1/4, 1/8, and 1/16; $P = 0.00012$ and lower). These results suggest that the mutation occurred either early in the germline progenitor cells of Pa, or possibly at the first division of the egg cell. Unfortunately, Pa was dead at time of the study, and we did not have access to tissues other than semen to answer this question.

CONCLUSIONS

With a few exceptions, we observed a nearly normal distribution of juvenile mortality rates among the daughters of 1,001 Holstein sires. By focusing on the progeny of 2 outlier bulls, we identified 2 de novo mutations consisting of a balanced translocation between chromosomes 26 and 29, and a mosaic nonsense mutation of *GATA6* (see Online Mendelian Inheritance in Animals entries OMIA 002558-9913, <https://www.omia.org/OMIA002558/9913/>, and 002559-9913, <https://www.omia.org/OMIA002559/9913/>). Furthermore, we described the first large animal models for human Jacobsen syndrome and persistent *truncus arteriosus* due to *GATA6* haploinsufficiency, to our knowledge. These results demonstrate the suitability of our approach to reveal genetic defects that are hardly detectable with traditional heredo-surveillance in the absence of specific externally visible symptoms. Beyond this proof of concept, the calculation of mortality rates at different ages for the whole population of bulls paves the way for future detection of QTL influencing juvenile mortality.

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