Infertility due to defective sperm flagella caused by an intronic deletion in DNAH17 that perturbs splicing

Adéla Nosková,1,† Maya Hiltpold,1,† Fredi Janett,2 Thomas Echtermann,3 Zih-Hua Fang,1,† Xaver Sidler,3 Christin Selige,4 Andreas Hofer,4 Stefan Neuenschwander,5 and Hubert Pausch1,†

1Animal Genomics, Institute of Agricultural Sciences, ETH Zürich, 8315 Lindau, Switzerland
2Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland
3Division of Swine Medicine, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland
4SUISAG, 6204 Sempach, Switzerland
5Animal Genetics, Institute of Agricultural Science, ETH Zürich, 8092 Zurich, Switzerland
†These authors contributed equally to this work.

Keywords: sperm flagella defect; impaired male fertility; polypyrimidine tract; exon skipping; genetic disorder; Genetics of Sex

Abstract
Artificial insemination in pig (Sus scrofa domesticus) breeding involves the evaluation of the semen quality of breeding boars. Ejaculates that fulfill predefined quality requirements are processed, diluted, and used for inseminations. Within short time, eight Swiss Large White boars producing immotile sperm that had multiple morphological abnormalities of the sperm flagella were noticed at a semen collection center. The eight boars were inbred on a common ancestor suggesting that the novel sperm flagella defect is a recessive trait. Transmission electron microscopy cross-sections revealed that the immotile sperm had disorganized flagellar axonemes. Haplotype-based association testing involving microarray-derived genotypes at 41,094 SNPs of six affected and 100 fertile boars yielded strong association (P = 4.22 × 10−15) at chromosome 12. Autozygosity mapping enabled us to pinpoint the causal mutation on a 1.11 Mb haplotype located between 3,473,632 and 4,587,759 bp. The haplotype carries an intronic 13-bp deletion (Chr12:3,556,401–3,556,414 bp) that is compatible with recessive inheritance. The 13-bp deletion excises the polypyrimidine tract upstream exon 56 of DNAH17 (XM_021066525.1: c.8510–17_8510–5del) encoding dynein axonemal heavy chain 17. Transcriptome analysis of the testis of two affected boars revealed that the loss of the polypyrimidine tract causes exon skipping which results in the in-frame loss of 89 amino acids from DNAH17. Disruption of DNAH17 impairs the assembly of the flagellar axoneme and manifests in multiple morphological abnormalities of the sperm flagella. Direct gene testing may now be implemented to monitor the defective allele in the Swiss Large White population and prevent the frequent manifestation of a sterilizing sperm tail disorder in breeding boars.

Introduction
Artificial insemination is the most frequent method of breeding in pigs. The semen of breeding boars is collected once or twice per week at semen collection centers. Traits that are routinely measured in all ejaculates include ejaculate volume, sperm concentration, motility, and morphology. Only ejaculates that meet predefined quality requirements are processed, diluted, and used for inseminations (Colenbrander et al. 1993; Holt et al. 1997; Broekhuysje et al. 2011). Semen quality and insemination success vary within and between boars due to environmental, permanent environmental, and genetic effects (Marques et al. 2017).

Access to longitudinal data on standardized semen traits for large cohorts of males is unique to livestock populations (Thibier and Wagner 2002; Knox 2016). Boars suitable for breeding are selected based on semen quality and genomic predictions derived from dense SNP microarray-derived genotypes. Dense genotypes and repeated semen trait measurements for thousands of males are prerequisites to unravel the genetic architecture of male fertility. The comprehensive genetic analysis of male fertility is challenging in most species other than livestock. For instance, the analysis of male fertility in humans typically relies on correlated proxy phenotypes such as family size and birth rate (Kosova et al. 2012), small cohorts of males (Sato et al. 2018; Sato et al. 2020), and observations from a single ejaculate per individual (Rahban et al. 2019).

Boar semen quality is positively correlated with insemination success and litter size (Holt et al. 1997). Semen traits that are measured at semen collection centers have medium heritability (Marques et al. 2017). Thus, they are well-suited response variables in genome-wide association studies for male reproductive performance (Diniz et al. 2014; Hiltpold et al. 2020). Genome-wide association studies on male fertility carried out in livestock discovered novel phenotype-genotype associations that improved our biological understanding of mammalian fertilization (Pausch et al. 2011).
Ejaculates from hundreds of boars are evaluated every year at semen collection centers as a service to the pig breeding industry. This rich resource of semen quality records facilitates to investigate sporadically occurring sperm defects using case-control association testing (Sironen et al. 2006; Sironen et al. 2011; Noskova et al. 2020). Once causative variants have been identified, direct gene tests and genome-based mating strategies may be implemented to avoid the birth of infertile males. Moreover, the discovery of genes that harbor pathogenic alleles that compromise male fertility is important to enhance the diagnostic yield of genetic testing also in species other than livestock (Xavier et al. 2020).

Here, we investigate an autosomal recessive sperm tail defect of Swiss Large White boars. Using genome-wide association testing, we map the disorder to porcine chromosome 12. The analysis of Swiss Large White boars. Using genome-wide association testing, we map the disorder to porcine chromosome 12. The analysis of genome-wide DNA and RNA sequencing data of affected boars is causal for the morphological abnormalities of the sperm flagella.

Material and methods
Ethics approval and consent to participate
Semeno samples of five breeding boars were collected by laboratory technicians at an approved semen collection center as part of their regular service to the Swiss pig breeding industry. Semen samples of three boars were flushed from the epididymis post mortem. Testes were collected after regular slaughter at an approved slaughterhouse. Our study was approved by the veterinary office of the Canton of Zurich (animal experimentation permit ZH 070/20).

Consent for publication
SUISAG, the Swiss pig breeding and competence center provided written consent to the analyses performed and agreed to publish results and data.

Animals
Eight Swiss Large White boars with a sperm tail defect were considered in our study (Table 1). Five of them were noticed at the semen collection center of SUISAG because their ejaculates contained immotile spermatozoa that had multiple morphological abnormalities of the flagella. Because the five boars were healthy and pedigree analysis indicated that they were inbred on a common ancestor, recessive inheritance of the sperm tail defect was suspected. A mating between two suspected carrier animals was performed in the field to assess phenotypic manifestations in their offspring. The pregnant sow was purchased and maintained at the research barn of the Division of Swine Medicine, Vetsuisse Faculty, University of Zurich. The sow gave birth to a litter with eleven piglets (eight females, three males). One of the male piglets (Boar_1246) died at the age of 200 days due to hemorrhagic bowel syndrome (Grahofer et al. 2017). The other two male boars (Boar_1249, Boar_1254) were slaughtered at the age of 17 months at a regular slaughterhouse. All three male piglets from the mating of suspected carrier animals expressed the sperm tail defect (see below). Pedigree records were analyzed using the PyPedal software package (Cole 2007).

Phenotypes
Semen samples of five breeding boars were macro- and microscopically evaluated by laboratory technicians as part of the routine service of SUISAG to the Swiss pig breeding industry. Spermatozoa from three boars (Boar_1246, Boar_1249, Boar_1254) were flushed from the epididymis post mortem. Semen samples from a fertile boar were provided by SUISAG.

Sperm concentration, total sperm count, and sperm motility were determined with an IVOS II CASA system (Hamilton Thorne Inc. Beverly, U.S.A.) using Leja 2-chamber slides (Leja, Nieuw-Vennep, the Netherlands). For morphological examination, semen was fixed in buffered formal saline solution (NaHPO4 4.93 g, KH2PO4 2.54 g, 38% formaldehyde 125 ml, NaCl 5.41 g, distilled water q.s. 1000 ml) and smears prepared (Hancock 1956). At least 200 spermatozoa were subsequently evaluated by phase-contrast microscopy using oil immersion (Olympus BX50, UplanFl 100×/1.30, Olympus, Wallisellen, Switzerland).

Assessment of sperm viability was performed using the eosin-nigrosin staining method (Blom 1950). In stained slides at least 200 spermatozoa were evaluated under oil immersion on a light microscope (Olympus BX50, UplanFl 100×/1.30, Olympus, Wallisellen).

To prepare sperm flushed from the epididymis for transmission electron microscopy (TEM), the samples were washed twice in phosphate-buffered saline (PBS) and subsequently centrifuged at 300 g. A semen sample from an unaffected boar was first centrifuged at 300 g to increase the sperm concentration and then washed in PBS. The pellet was fixed with equal volume of 6% glutaraldehyde, resuspended gently, and centrifuged at 6000 g. After

Table 1 Experimental design of the study

| Boar   | AI center | Research station | GWAS cohort | PCR-based genotyping | Whole-genome sequence data | RNA sequencing data | Macro- and microscopic sperm analyses | Eosin-nigrosin staining | TEM sections |
|--------|-----------|------------------|-------------|----------------------|----------------------------|---------------------|---------------------------------------|------------------------|--------------|
| Boar_78 | X         | X                | X           | SAMEA6813549         |                            |                     | X                                     |                        |              |
| Boar_79 | X         | X                | X           | SAMEA6813550         |                            |                     | X                                     |                        |              |
| Boar_80 | X         | X                | X           | SAMEA6813551         | SAMEA68132284             | X                   | X                                     |                        |              |
| Boar_81 | X         | X                | SAMEA6813552 | SAMEA68132285        | X                          |                     | X                                     |                        |              |
| Boar_82 | X         | X                | X           | SAMEA6813553         |                            |                     | X                                     |                        |              |
| Boar_1246a | X    | X                | X           | SAMEA6813550         |                            |                     | X                                     |                        |              |
| Boar_1249 | X    | X                | X           | SAMEA68132284        | X                          |                     | X                                     |                        |              |
| Boar_1254 | X    | X                | X           | SAMEA68132285        | X                          |                     | X                                     |                        |              |

a Died at the age of 200 days due to hemorrhagic bowel syndrome; sperm were flushed from the epididymis post mortem.

Details on the eight boars with a sperm tail defect. Genotypes and haplotypes for the GWAS cohort are available at Zenodo: https://doi.org/10.5281/zenodo.4081475. DNA and RNA sequencing data from the boars are available at the European Nucleotide Archive (ENA) of the EMBL at the BioProject PRJEB38156 under the accession numbers listed in the table.
removing the supernatant, the sperm were fixed for a second 
time with 3% glutaraldehyde, and finally pelleted at 6000 g. 
Pellets were washed three times in PBS, post-fixed in 1% osmium 
tetroxide, washed in ddH2O, stained in 1% uranyl acetate, dehy-
drated in graded series of ethanol (25, 50, 75, 90, and 100%), and 
embedded in Epoxy resin through increasing concentrations (25, 
50, 75, and 100%) using PEŁCO Biowave+ tissue processor, and 
then cured at 60°C for 3 days. Embedded blocks were sectioned 
using Leica FC6 microtome and a DIATOME diamond knife with 
45° angle into 60 nm sections and mounted on Quantifoil copper 
grids with formvar carbon films. Sections were post-stained with 
2% uranyl acetate followed by lead citrate. Grids were imaged us-
ing FEI Morgagni 268 electron microscope operated at 100 kV at 
20 k magnification.

Genotypes and haplotype inference
Genotypes of 9955 Large White boars (including six boars with 
the sperm tail defect) and sows were provided by SUISAG. All pigs 
were genotyped using Illumina PorcineSNP60 Bead chips that 
comprised between 62,163 and 68,528 SNPs. We considered only 
autosomal SNPs. Physical positions of the SNPs corresponded to 
the Sscrofa11.1-assembly of the porcine genome (Warr et al. 
2020). Quality control on the genotypes was carried using the 
PLINK (version 1.9) software (Chang et al. 2015). Animals and SNPs 
with more than 10% missing genotypes were excluded from sub-
sequent analyses. We removed SNPs with minor allele frequency 
(MAF) less than 0.005 and SNPs for which the observed genotype 
distribution deviated significantly ($P < 0.00001$) from Hardy-
Weinberg proportions. After quality control, our dataset con-
ferred 9848 pigs and genotypes at 43,254 autosomal SNPs. 
Weinberg proportions. After quality control, our dataset con-
ferred 9848 pigs and genotypes at 43,254 autosomal SNPs.

Genome-wide association testing
Six boars that produced sperm with multiple morphological ab-
normalities of the flagella were considered as case group for a 
genome-wide association study. The control group consisted of 
100 randomly selected boars that produced normal sperm and 
were fertile, i.e. each of them sired at least one litter in the Swiss 
Large White breeding unit.

We tested the association between the affection status of the 
boars and 41,094 autosomal SNPs for which the frequency of the 
minor allele was greater than 5%. Single marker-based case-con-
tral association testing was performed using Fisher’s exact tests 
of allelic association as implemented in the PLINK (version 1.9) 
software (Chang et al. 2015). To take population stratification into 
account, we performed also an SNP-based mixed linear model-

Analysis of positional candidate genes
The top association signal revealed a 1.11 Mb segment of ex-
tended homozygosity at chromosome 12. Genes located within 
the segment of homozygosity were obtained from the Refseq an-
notation of the porcine genome (version 10, available at ftp:// 
ftp.ncbi.nlm.nih.gov/genomes/refseq/vertebrate_mammalian/Su 
s_scrofa/annotation_releases/106/GCF_000003025.6_Sscrofa11.1/ 
GCF_000003025.6_Sscrofa11.1_genomic.gff.gz). Using publicly 
available RNA sequencing data (BioProject PRJNA506525, sample 
accession numbers SAMN10462191—SAMN10462197) from 
(Robic et al. 2019), we quantified mRNA abundance of the posi-
tional candidate genes in porcine testes. To this end, we down-
loaded between 47 and 58 million paired-end (2 x 100 and 2 x 
125 bp) sequencing reads that were generated from RNA 
extracted from testis tissue of seven pubertal boars from 
European Piétrain and Piétrain X Large White populations. The 
RNA sequencing reads were pseudo-aligned to an index of the 
porcine transcriptome (Refseq version 106) and transcript 
abundance was quantified using the KALLISEQ software (Bray et al. 2016). 
We used the R package TXIMPORT (Soneson et al. 2015) to aggregate 
these transcript abundances to the gene level.

Whole-genome sequencing and sequence variant genotyping
We sequenced five Swiss Large White boars that had multiple morphological abnormalities of the sperm flagella using 2 x 150 bp paired-end reads. Illumina TruSeq DNA 
PCR-free libraries with 400 bp insert sizes were prepared and se-
quenced at an Illumina NovaSeq6000 instrument. We used the 
FASTP software (Chen et al. 2018) to remove adapter sequences, 
poly-G tails and reads that had phred-scaled quality less than 15 
for more than 15% of the bases. Between 81,145,807 and 
114,616,836 filtered read pairs were aligned to the Sscrofa11.1 
as-
sembly of the porcine genome using the mem algorithm of the rwa 
software (Li 2013) with default parameter settings. We marked 
duplicates using the PICARD tools software suite (https://github. 
com/broadinstitute/picard) and sorted the alignments by coordi-
nates using SAMBA (Tarasov et al. 2015). The number of reads 
covering a position was extracted from the alignments using the 
MOIDPETH software (version 0.2.2) with default parameter settings 
(Pedersen and Quinlan 2018). The realized sequencing read depth 
of the five boars was 10.9-fold and it ranged from 8.9- 
to 12.5-fold.

Sequence variants (SNPs and Indels) of the five sequenced 
boars were genotyped together with 93 pigs from different breeds 
for which whole-genome sequence data were available from our 
in-house database using the multi-sample variant calling ap-
proach of the GENOME ANALYSIS TOOLKIT (GATK, version 4.1.0; Depristo et al. 2011). We filtered the sequence variants by hard-filtering 
according to best practice guidelines of the GATK. A detailed de-
scription of our reference-guided variant discovery and filtration 
approach is described in (Crysnanto et al. 2019). Functional
consequences of polymorphic sites were predicted according to the RefSeq annotation (version 106) of the porcine genome using the Variant Effect Predictor software from Ensembl (McLaren et al. 2016) along with the SpliceRegion.pm plugin (https://github.com/Ensembl/VEP_plugins/blob/release/101/SpliceRegion.pm). We considered 82 pigs with known pedigree as a control group for the present study.

Identification of candidate causal variants

We considered 14,806 SNPs and 3,856 Indels that were detected within the 1.11 Mb segment (between 3,473,632 and 4,587,759 bp) of extended homozygosity at chromosome 12 as positional candidate causal variants. To identify variants compatible with recessive inheritance of the sperm tail disorder, we applied a filtering strategy that takes into account flawed genotypes and the under-calling of heterozygous genotypes due to relatively low sequencing coverage. Specifically, we screened for alleles that had the following frequency:

- 0.8 in five affected boars (at least 8 out of 10 alleles),
- 0.05 in 82 control pigs from different breeds (less than 8 alleles).

This filtration resulted in only one compatible variant which was a 13-bp intronic deletion.

Whole transcriptome sequencing and read alignment

Tests were collected immediately after slaughter at an approved slaughterhouse from two boars (Boar_80, Boar_81) that were homozygous for the 13-bp deletion and produced sperm with multiple morphological abnormalities of the flagella. Tissue samples were frozen in liquid nitrogen and stored at -80°C until RNA extraction. Paired-end RNA libraries (2 x 150 bp) were prepared using total RNA purified from testis tissue using the Illumina TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA, USA). The libraries were sequenced at an illumina NovaSeq6000 instrument yielding 49,198,010 and 70,451,148 reads. Quality control on the raw RNA sequencing reads was performed using the fastq software using default parameter settings (see above). The filtered read pairs (48,889,296 and 69,988,376) were aligned to the Susc011.1 reference sequence and the RefSeq gene annotation (version 106) using the splice-aware read alignment tool STAR (version 2.7.3a) (Dobin et al. 2013). The number of RNA sequencing reads that covered a position was counted using the mosdepth software (see above).

Bioinformatic analysis

Putative branch point sequences within DNAH17 (XM_021066525.1) intron 55 were predicted using the BPP algorithm (Zhang et al. 2017). Putative 3’ splice acceptor sites were predicted using the NNSPLICE software tool (https://www.fruitfly.org/seq_tools/splice.html; Reese et al. 1997). Domains of porcine DNAH17 (protein-ID: A0A287AFU3) were retrieved from Uniprot.

Genotyping of the 13-bp deletion

DNA was isolated from either hair roots (living pigs) or spleen (slaughtered pigs) using DNeasy blood and tissue kit (Qiagen). A PCR test was established consisting of a FAM-labelled forward primer (Fw-DNAH17: 5’-TGAGCATCTTCTTGGCGAGG-3’) and a reverse primer (Re-DNAH17: 5’-GCTGTGTACGCACACGCCAGA-3’) which bind to the wild type and mutant alleles, as well as a wild type specific reverse primer (Re-wt-DNAH17: 5’-TCGAGCTAGAGCGGAGG-3’). PCR fragments were analyzed on a DNA analyzer 3130xl (Applied Bioscience).

Availability of data

Whole-genome sequence data of 87 pigs including five boars with a sperm tail defect have been deposited at the European Nucleotide Archive (ENA) of the EMBL at BioProject PRJEB38156, PRJEB37956, PRJEB39374, and PRJNA622908, under-sample accession numbers listed in Supplementary File S1. The genotypes of five affected and 82 unaffected boars at 14,806 SNPs and 3,856 Indels that were detected within the segment of autozygosity are available from Zenodo (https://doi.org/10.5281/zenodo.4081475). RNA sequencing data of testicular tissue samples of two boars homozygous for the 13-bp deletion have been deposited at the European Nucleotide Archive (ENA) of the EMBL at the BioProject PRJEB38156 under-sample accession numbers SAMEA6832284 and SAMEA6832285. RNA sequencing data of testicular tissue samples of seven pubertal boars are accessible via the European Nucleotide Archive (ENA) of the EMBL at the BioProject PRJNA506525 under-sample accession numbers SAMN10462191—SAMN10462197. The microarray-derived genotypes and haplotypes of cases and controls, the eigenvectors considered to account for stratification, the R script used to carry out haplotype-based association testing, and the results from the haplotype-based association study are available from Zenodo (https://doi.org/10.5281/zenodo.4081475). Supplementary material is available at figshare: https://doi.org/10.6084/m9.figshare.13353605

Results

Phenotypic manifestation of a sperm tail disorder in Large White boars

Five Swiss Large White boars maintained at a semen collection center produced ejaculates that contained spermatozoa with defective tails. Ejaculate volume and sperm concentration were normal. Microscopic semen analysis revealed multiple morphological abnormalities of the sperm flagella including rudimentary, short, coiled, and irregularly shaped tails (Figure 1A). Proximal cytoplasmic droplets were frequently observed at the junction of sperm head and tail. Progressively motile sperm were not detected in the ejaculates. Eosin-nigrosin staining of semen flushed from the epididymis of two affected boars indicated that 48 and 60% of the sperm were viable (Supplementary File S2). Apart from producing sperm with defective tails, the boars were healthy and had no testicular abnormalities. Due to the absence of motile sperm and the high degree of abnormalities of the flagella, the boars were not suitable for breeding and were slaughtered. The sperm tail defect of the boars is commonly referred to as asthenoteratozoospermia.

Transmission electron microscopy cross-sections of sperm flagella from a fertile boar revealed the typical axonemal arrangement of nine outer microtubule doublets surrounding the central pair (9x2 + 2) (Figure 1D). In contrast, cross-sections of sperm that were flushed from the epididymis of two affected boars revealed multiple ultrastructural abnormalities including completely disorganized axonemes (Figure 1C). Central and outer microtubule doublets were absent in some flagella. Some sperm had superfluous but disorganized axonemal structures. The cytoplasmic bags that were detected at the junction between sperm head and tail using light microscopy contained unassembled axonemal components or multiple flagellar-like structures within the cell membrane of one sperm (Supplementary File S3).
The boars that produced defective sperm were closely related (Figure 1E). Two affected boars were fullsibs (Boar_80, Boar_81). The average relationship coefficient between the boars was 0.28 and it ranged from 0.14 to 0.63. Their average coefficient of inbreeding was 0.087, which is slightly higher than in the fertile boars ($F = 0.06$). All affected boars were inbred on a boar born in 2005. This common ancestor was present in both the paternal and maternal ancestry of significantly less ($N = 10$, $P_{\text{Fisher's exact}} = 4.69 \times 10^{-5}$) fertile boars. Three sires of affected boars were used in artificial insemination. None of their ejaculates ($n = 168$) contained an anomalous amount of sperm with defective flagella. These observations are compatible with an autosomal recessive inheritance of the sperm tail defect.

To verify the presumed recessive inheritance and monitor phenotypic manifestations in homozygous boars, a mating between two suspected carrier animals was performed in the field (Figure 1E). The sow gave birth to a litter with eight female and three male piglets (Boar_1246, Boar_1249, Boar_1254). The three male piglets were maintained at a research barn. Boar_1246 died at the age of 200 days due to hemorrhagic bowel syndrome. Testes and epididymis were collected post mortem. Testicular abnormalities were not detected. Microscopic analysis of sperm...
flushed from the epididymis revealed that the sperm flagella had multiple morphological abnormalities. Boar_1249 and Boar_1254 were healthy and developed normal at the research barn. At 17 months, both boars were slaughtered at a regular slaughterhouse and testes and epididymis were collected. Microscopic analysis of sperm flushed from the epididymis revealed that both boars produced sperm with multiple morphological abnormalities of the flagella.

Haplotype-based association testing maps the sperm tail defect to a 1.11 Mb interval on porcine chromosome 12

Six affected boars (Table 1) were genotyped with the Illumina PorcineSNP60 microarray. As a control group, we considered 100 randomly selected fertile boars of the Swiss Large White breeding population that also had Illumina PorcineSNP60-derived genotypes. Following quality control, genotypes at 41,094 autosomal SNPs were used for association testing. Single-marker-based case/control-association testing using Fisher’s exact test of allelic associations revealed eleven SNPs located on porcine chromosomes 11, 12 and 13 that exceeded the Bonferroni-corrected significance threshold (Figure 2A). The strongest association signal (P = 1.87 × 10⁻⁷) resulted from SNP ASGA0052524 that is located at 2,785,333 bp on porcine chromosome 12. An inflation factor of 1.55 indicated that the SNP-based association study was enriched for false-positive association signals. Inspection of the top principal components of the genomic relationship matrix revealed clustering of the boars with the sperm tail defect (Supplementary File S4), suggesting that population stratification confounded the association analysis, thereby producing spurious association signals.

To take population stratification into account, we repeated the association analysis using either an SNP-based mixed linear model or a haplotype-based linear model that included 30 principal components of a genomic relationship matrix as covariates. An inflation factor of 1.03 indicated that the SNP-based mixed linear model successfully controlled population stratification. Two SNPs (ASGA0052524 at 2,785,333 bp and ALGA0118426 at 8,504,544 bp) at SSC12 were significantly associated with the sperm tail defect (Figure 2B).

An inflation factor of 1.15 indicated that the principal components-based control of population stratification was mostly successful in the haplotype-based association study. Twenty-one haplotypes located between 94,345 and 9,256,260 bp on porcine chromosome 12 were located between 2,166,472 and 3,755,956 bp. The top haplotype was significantly associated with the sperm tail defect (Figure 2B).

In silico analysis of sperm flushed from the epididymis revealed that the 13-bp deletion excises an intronic region from the DNAH17 gene (XM_021066525.1: c.8510-17_8510-5del) located in an intron of DNAH17. The 13-bp location resides within the top window from the haplotype-based association study. Sequence variant genotyping using GATK indicated that the five sequenced boars with the sperm tail defect were homozygous for the deletion. Only the three boars from the control group, that carried the top haplotype in the heterozygous state, also carried the 13-bp deletion in the heterozygous state. The deletion did not occur in the homozygous state in animals from the control group. According to the variant classification algorithm of the vrn software, the intronic 13-bp deletion was annotated as “splice_region_variant & intron_variant.” Its impact on protein function was predicted to be low.

RNA sequencing reveals that the 13-bp deletion causes skipping of DNAH17 exon 56

Closer inspection of the 13-bp deletion revealed that it exsices an intronic pyrimidine-rich sequence containing a continuous stretch of eight pyrimidine bases between 5 and 17 bases upstream of a canonical 3’ splice site of DNAH17 exon 56 (Figure 3A). The vrn plugin SpliceRegion.pm indicated that the deletion coincides with a putative poly(pyrrimidine tract. A branch point consensus sequence (yUnAy) (Gao et al. 2008) was predicted (z-score: 4.79) 2-6 nucleotides upstream the 13-bp deletion. The pyrimidine content between the predicted branch point adenosine and the splice acceptor site at the 3’ end of intron 55 is 68.4%. Considering that the poly(pyrrimidine tract is an important cis-acting element for spliceosome assembly in canonical "GT-AG"-type acceptor splice sites (Coolidge et al. 1997), we suspected that the 13-bp deletion perturbs pre-mRNA splicing. In silico splicing
site prediction using NNSPLICE indicated (prediction score 0.46) that the 13-bp deletion likely prevents recognition of the 3’ splice acceptor site at the intron-exon boundary of exon 56, thus possibly causing exon skipping.

Exon 56 of porcine DNAH17 is regularly expressed in testis tissue of pubertal boars (Supplementary File S5). To examine if the loss of the polypyrimidine tract upstream exon 56 perturbs splicing, we sequenced RNA purified from testis tissue of two boars that produced defective sperm and were homozygous for the 13-bp deletion. We aligned 49 and 70 million paired-end (2x150 nt) RNA sequencing reads to the porcine transcriptome to quantify transcript and exon abundance. An average of 49 DNAH17 transcripts per million (TPM) were detected in the two boars homozygous for the 13-bp deletion. However, no reads...
aligned to the sequence of exon 56 corroborating that the 13-bp deletion prevents recognition of the 3’ splice site, thereby causing exon skipping (Figure 3B).

The skipping of exon 56 causes an in-frame loss of 89 amino acids (residues 2836–2924) representing 2% of porcine DNAH17 (XP_020922184.1). DNAH17 contains six evolutionarily conserved “ATPase associated with a variety of cellular activities (AAA)” domains that are linked together as an asymmetric hexameric ring required to power the beating movement of sperm flagella (Snider et al. 2008; Gleave et al. 2014). The 89 amino acids are truncated from AAA4 of DNAH17 (Figure 3C).

Discussion

Eight Swiss Large White boars produced sperm with multiple morphological abnormalities of the flagella. Considering that all sperm were immotile, the boars were judged to be infertile and not used for breeding. Because a majority of the sperm were viable, fertilization might be possible using intracytoplasmic sperm injection (ICSI) (Nijia et al. 1996; Ortega et al. 2011). Although ICSI is not routinely applied in pig breeding programs, it could enable the reproduction of boars with sperm tail defects that prevent fertilization otherwise. However, success rates after ICSI may vary considerably in individuals with asthenoteratozoospermia (Wambargue et al. 2016). Interestingly, ICSI is successful in some but not all individuals with pathogenic DNAH17 alleles (Whitfield et al. 2019). In any case, the application of ICSI in livestock populations seems unwarranted if the underlying male-factor infertility follows recessive inheritance, because a defective allele would be transmitted to the offspring and might increase allele frequency in the population.

Apart from producing sperm with defective tails, the boars were healthy. Diseases and environmental stressors were less likely to cause the defective sperm flagella (Jurewicz et al. 2009), because the boars were maintained at a semen collection center with many other boars that produced normal semen. Idiopathic infertility and poor semen quality in healthy males may result from pathogenic alleles in genes that are specifically expressed in the male reproductive tract (Pausch et al. 2014; Pausch et al. 2016; Iso-Touru et al. 2019; Noskova et al. 2020). The eight affected boars were inbred on a common ancestor supporting the hypothesis of a shared genetic etiology. Although the presence of common ancestors in the pedigrees of all affected boars is compatible with recessive inheritance, it does not rule out other modes of inheritance (Bourneuf et al. 2017). Considering that all affected but only few fertile boars were inbred on the common ancestor, a recessive mode of inheritance was likely. A mating between two heterozygous carriers resulted in the birth of eight female and three male piglets (5 wt/wt, 2 wt/mt, 4 mt/mt). The three male piglets were homozygous carriers of the 13-bp deletion and manifested the sperm tail defect, thus corroborating recessive inheritance.

The morphological and ultrastructural flagella defects of the eight Swiss Large White boars are similar to those observed in Finnish Yorkshire boars that are homozygous for a recessive loss-of-function allele in KPL2 (a.k.a. SPEF2) (Andersson et al. 2000; Sironen et al. 2006). Although cross-breeding between Finnish Yorkshire and Swiss Large White pigs had not been documented recently, the defective allele could be located on an ancient shared haplotype (Pausch et al. 2016a; Schwarzenbacher et al. 2016). However, the genomic region on chromosome 16 encompassing SPEF2 is not associated with the sperm defect of the Swiss Large White boars, indicating genetic heterogeneity.

SNPs on chromosomes 11, 12, and 13 were significantly associated with the sperm disorder using Fisher’s exact test of allelic association. This result was puzzling because an oligogenic inheritance of the sperm flagella defect was unlikely. Phenotypic misclassification and genetic heterogeneity could lead to an inconclusive association study (Manchia et al. 2013). However, the morphological abnormalities of the sperm flagella were strikingly
similar in the ejaculates of eight boars. Moreover, pedigree analysis suggested monogenic recessive inheritance. Principal components analysis and a genomic inflation factor of 1.55 indicated that population stratification confounded our SNP-based association study (Price et al. 2010). Although Fisher’s exact tests have been widely applied to map binary traits (Balding 2006), these tests are prone to type I errors when cases and controls differ in their ancestries. A linear mixed model-based association study successfully controlled for population stratification and revealed two associated SNPs at SSC12. However, the SNPs were 6 million bp away from each other and the association signal was not very strong (P = 1.2 × 10^{-9}). Following the approach from (Hiltold et al. 2020), we fitted the top principal components of a genomic relationship matrix as covariates in a haplotype-based association model to take population stratification into account. A compelling association signal (P = 4.22 × 10^{-15}) at chromosome 12 remained, whereas all other association signals disappeared. In agreement with previous studies (Price et al. 2006; Pausch et al. 2011; Hiltold et al. 2020) and evidenced by a low inflation factor, the principal components-based correction successfully eliminated spurious signals that arose due to population stratification. Considering that the most significantly associated haplotype also encompasses the causal variant for the sperm flagella defect, our findings corroborate that genome-wide haplotype-based association testing offers a powerful approach to identify trait-associated regions in stratified mapping cohorts (Kadri et al. 2014; Pausch et al. 2016a; Hiltold et al. 2020).

The haplotype associated with multiple morphological abnormalities of the sperm flagella carries a 13-bp deletion in an intron of DNAH17 encoding dynein axonemal heavy chain 17. Our results show that DNAH17 is abundant in porcine testis tissue. Dynein axonemal heavy chains are required for the axonemal assembly and the beating movement of the flagella (Inaba 2003; Ben Khelifa et al. 2014; Tu et al. 2019; Whitfield et al. 2019; Liu et al. 2020; Sha et al. 2020; Sironen et al. 2020; Zhang et al. 2020).

Pathogenic alleles in human and murine DNAH17 manifest in sperm with an abnormal mitochondrial sheath and cyttoplasmic droplets, as well as axonemal disorganization including absence of the central pair and missing peripheral microtubule doublets (Whitfield et al. 2019; Sha et al. 2020; Zhang et al. 2020). Boars that are homozygous for a 13-bp deletion in intron 55 of DNAH17 also produce sperm with multiple morphological and ultrastructural abnormalities of the flagella. Although annotated as a low impact variant, the intronic 13-bp deletion perturbs pre-mRNA splicing because it disrupts the polypyrimidine tract in intron 55 of DNAH17. The mutant DNAH17 lacks part of the AAX domain which likely compromises axonemal assembly and the beating movement of the sperm flagellum in the homoygous state. The assembly of the flagellar axoneme is less disorganized when other domains of DNAH17 are affected by pathogenic variants (Whitfield et al. 2019; Sha et al. 2020; Zhang et al. 2020), likely because an intact AAX domain is essential to physiological DNAH17 function (Snider et al. 2008). Thus, our findings suggest that the intronic 13-bp deletion is a loss-of-function allele.

We previously identified mutations at splice donor sites and nearby splice acceptor sites that manifest in male reproductive disorders due to aberrant splicing (Iso-Touru et al. 2019; Hiltold et al. 2020). Our present study reveals an intronic deletion from 5 to 17 nucleotides upstream a canonical 3’ splice site that perturbs splicing as the most likely causal variant for a novel porcine sperm flagella defect. The 13-bp deletion compromises splicing because it excises a polypyrimidine tract upstream DNAH17 exon 56. Phenotypic consequences arising from polypyrimidine tract mutations had rarely been described so far (LeFevre et al. 2002; Sartelet et al. 2015; Abramowicz and Gos 2018). To the best of our knowledge, our study reports the first phenotype-genotype association for a mutation affecting an intronic polypyrimidine tract in pigs. We provide evidence that the loss of the polypyrimidine tract in porcine DNAH17 intron 55 prevents recognition of the 3’ splice site which leads to the skipping of exon 56, thus causing defective sperm flagella. Sequence motifs that govern the assembly of the spliceosome may be more distant to 3’ splice sites than the polypyrimidine tract in porcine DNAH17 intron 55 (Zhang et al. 2017). However, standard sequence variant annotation tools are largely blind to putative consequences arising from mutations within intronic motifs. Thus, the systematic characterization of branch points and polypyrimidine tracts seems warranted to refine the functional classification of intronic variants.

Our findings enable the monitoring of the 13-bp deletion and unambiguous identification of carrier and homozygous animals using direct gene testing. Although homozygosity for the 13-bp deletion is easily recognized in artificial insemination boars using microscopic semen analysis, it may remain undetected in sows and natural service boars. Affected natural service boars will be noticed after few matings due to low fertility. In agreement with previous findings on loss-of-function alleles in human and murine DNAH17 (Whitfield et al. 2019; Sha et al. 2020; Zhang et al. 2020), homozygous mutation carriers were healthy. Considering that the frequency of the 13-bp deletion is low and the majority of sows is inseminated artificially, economic losses due to unsuccessful breeding with homozygous boars are negligible in the Swiss Large White population.

The number of recessive conditions detected in livestock populations increases steadily (Nicholas and Hobbs 2014). Low effective population size, founder effects and inbreeding favor the manifestation of recessive conditions. Recent advances in genome-wide genotyping and sequencing offer powerful tools for rapid detection of causal variants underpinning inherited conditions (Bourneuf et al. 2017). The phenotypic and economic consequences of the 13-bp deletion in DNAH17 are less detrimental than mutations that manifest in malformed and non-viable piglets (Derk et al. 2018; Fang et al. 2020). However, alleles that compromise semen quality and male fertility in otherwise healthy individuals may attain high frequency in the absence of deleterious manifestations in females (Pausch et al. 2014; Hiltold et al. 2020). The 13-bp deletion in DNAH17 adds to a catalogue of sequence variants that cause male-factor infertility in pigs (Sironen et al. 2006, 2011; Noskova et al. 2020). It remains an open question how an increasing number of undesired recessive alleles may be considered appropriately in livestock populations (Cole 2015; Upperman et al. 2019). Although the newly identified 13-bp deletion does neither compromise the welfare of homozygous animals nor result in huge economic losses, it’s frequency should be kept at a low level to prevent the birth of homozygous boars that manifest a sterilizing sperm tail disorder.

Acknowledgments

We thank Dr. Cecilia Bebeacua (ScopeM) for support in transmission electron microscopy. We are thankful for the excellent technical support provided by the ETH Zurich technology platforms FGZZ (https://fgcz.ch/), ScopeM (https://scope.ethz.ch/), and Genetic Diversity Centre (https://gdc.ethz.ch/), respectively, for sequencing, microscopy and DNA fragment analysis.
Funding

We acknowledge financial support from SUISAG, Micarna SA and the ETH Zürich Foundation.

Conflicts of interest

Christin Selige and Andreas Hofer are employees of SUISAG (the Swiss pig breeding and competence center).

Literature cited

Abramowicz A, Gos M. 2018. Splicing mutations in human genetic disorders: examples, detection, and confirmation. J Appl Genet. 59:253–268.

Andersson M, Peltoniemi O, Makinen A, Sukura A, Rodriguez-Martinez H. 2000. The hereditary ‘short tail’ sperm defect—a new reproductive problem in Yorkshire boars. Reprod Domest Anim. 35:59–63.

Aulchenko YS, Ripke S, Isaacs A, van Duijn CM. 2007. GenABEL: an R library for genome-wide association analysis. Bioinformatics. 23:1294–1296.

Balding DJ. 2006. A tutorial on statistical methods for population association studies. Nat Rev Genet. 7:781–791.

Ben Khelifa M, Coutton C, Zouari R, Karaouze `ne T, Rendu J, et al. 2014. Mutations in DNAH1, which encodes an inner arm heavy chain dynein, lead to male infertility from multiple morphological abnormalities of the sperm flagella. Am J Human Genet. 94:95–104.

Blom E. 1950. A one-minute live-dead sperm stain by means of eosin-nigrosin. Fertil Steril. 1:176–177.

Bourneuf E, Otz P, Pausch H, Jagannathan V, Michot P, et al. 2017. Rapid discovery of de novo deleterious mutations in cattle enhances the value of livestock as model species. Sci Rep. 7:11466.

Bray NL, Pimentel H, Melsted P, Pachter L. 2016. Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol. 34:525–527.

Broekhuijse MLWJ, Feitsma H, Gadella BM. 2011. Field data analysis of boar semen quality. Reprod Domest Anim. 46:59–63.

Brown CL, Zhou Y, Browning SR. 2018. A one-penny imputed genome from next-generation reference panels. Am J Human Genet. 103:338–348.

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, et al. 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 4:7.

Chen S, Zhou Y, Chen Y, Gu J. 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 34:i884–i890.

Clark EL, Bush SJ, McCulloch ME, Farquhar II, Young R, et al. 2017. A high resolution atlas of gene expression in the domestic sheep (Ovis aries). PLoS Genet. 13:e1006997.

Cole JB. 2007. PyPedal: a computer program for pedigree analysis. Comput Electron Agricul. 57:107–113.

Cole JB. 2015. A simple strategy for managing many recessive disorders in a dairy cattle breeding program. Genet Sel Evol. 47:94.

Colenbrander B, Feitsma H, Grooten HJ. 1993. Optimizing semen production for artificial insemination in swine. J Reprod Fertil Suppl. 48:207–215.

Coolidge CJ, Seely RJ, Patton JG. 1997. Functional analysis of the poly-pyrimidine tract in pre-mRNA splicing. Nucleic Acids Res. 25:888–896.

Crysnanto D, Wurmser C, Pausch H. 2019. Accurate sequence variant genotyping in cattle using variation-aware genome graphs. Genet Sel Evol. 51:21.

Depristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, et al. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 43:491–501.

Derks MFL, Lopes MS, Bosse M, Madsen O, Dibbits B, et al. 2018. Balancing selection on a recessive lethal deletion with pleiotropic effects on two neighboring genes in the porcine genome. PLoS Genet. 14:e1007661.

Diniz DB, Lopes MS, Broekhuijse MLWJ, Lopes PS, Harlizius B, et al. 2014. A genome-wide association study reveals a novel candidate gene for sperm motility in pigs. Anim Reprod Sci. 151:201–207.

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, et al. 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 29:15–21.

Fang L, Cai W, Liu S, Canela-Xandri O, Gao Y, et al. 2020. Comprehensive analyses of 723 transcriptomes enhance genetic and biological interpretations for complex traits in cattle. Genome Res. 790–801. 30.

Gao K, Masuda A, Matsuura T, Ohno K. 2008. Human branch point consensus sequence is yUnAy. Nucleic Acids Res. 36:2257–2267.

Gleave ES, Schmidt H, Carter AP. 2014. A structural analysis of the AAA+ domains in Saccharomyces cerevisiae cytoplasmic dynein. J Struct Biol. 186:367–375.

Grahofer A, Gurtner C, Nathues H. 2017. Haemorrhagic bowel syndrome in fattening pigs. Porcine Health Manag. 3:1–6.

Hancock JL. 1956. The morphology of boar spermatozoa. J R Microsc Soc. 76:84–97.

Hiltmold P, Niu G, Kadri NK, Crysnanto D, Fang Z-H, et al. 2020. Activation of cryptic splicing in bovine WDR19 is associated with reduced semen quality and male fertility. PLoS Genet. 16:e1008804.

Holt C, Holt WV, Moore HD, Reed HC, et al. 1997. Objectively measured boar sperm motility parameters correlate with the outcomes of on-farm inseminations: results of two fertility trials. J Androl. 18:312–323.

Inaba K. 2003. Molecular architecture of the sperm flagella: molecules for motility and signaling. Zool Sci. 20:1043–1056.

Iso-Touru T, Wurmser C, Venhoranta H, Hiltmold M, Savolainen T, et al. 2019. A splice donor variant in CDDC189 is associated with asthenospermia in Nordic Red dairy cattle. BMC Genomics. 20:286.

Jurewicz J, Hanke W, Radwan M, Bonde JP. 2009. Environmental factors and semen quality. Int J Occup Med Environ Health. 22:305–329.

Kadri NK, Sahana G, Charlier C, Iso-Touru T, Wurmser C, Venhoranta H, Hiltmold M, Savolainen T, et al. 2019. A splice donor variant in CDDC189 is associated with asthenospermia in Nordic Red dairy cattle. BMC Genomics. 20:286.

Knox RV. 2016. Artificial insemination in pigs today. Theriogenology. 85:83–93.

Kosova G, Scott NM, Niederberger C, Prins GS, Ober C. 2012. Genome-wide association study identifies candidate genes for male fertility traits in humans. Am J Hum Genet. 90:950–961.

Lamas-Toranzo I, Hamze JG, Bianchi E, Fernández-Fuertes B, Pérez-Cerezales S, et al. 2020. TMEM95 is a sperm membrane protein essential for mammalian fertilization. eLife. 9:e53913.

Lefèvre SH, Chauvein C, Stoppa-Lyonnet D, Michon J, Lumbroso L, et al. 2002. A T to C mutation in the poly-pyrimidine tract of the exon...
9 splicing site of the RB1 gene responsible for low penetration hereditary retinoblastoma. J Med Genet. 39:E21.

Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:13033997.

Liu C, Miyata H, Gao Y, Sha Y, Tang S, et al. 2020. Bi-allelic DNAH8 Variants Lead to Multiple Morphological Abnormalities of the Sperm Flagella and Primary Male Infertility. Am J Hum Genet. 107:330–341.

Manchia M, Cullis J, Turecki G, Rouleau GA, Uher R, et al. 2013. The impact of phenotypic and genetic heterogeneity on results of genome wide association studies of complex diseases. PLoS One. 8: e76295.

Marques DBD, Lopes MS, Broekhuijse MLWJ, Guimarães SEF, Knol EF, et al. 2017. Genetic parameters for semen quality and quantity traits in five pig lines. J Anim Sci. 95:4251–4259.

McLaren W, Gil I, Hunt SE, Riat HS, Ritchie GRS, et al. 2016. The ensembl variant effect predictor. Genome Biol. 17:122.

Ni X, Wang J, Lv M, Liu C, Zhong Y, McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GRS, et al. 2016. Mutation discovery for Mendelian inheritance of spermatogenic arrest in mice. Proc Natl Acad Sci. 113:4588–4593.

Nicholas FW, Hobbs M. 2014. Mutation discovery for Mendelian traits in non-laboratory animals: a review of achievements up to 2012. Anim Genet. 45:157–170.

Nijs M, Vanderzwalmen P, Vandamme B, Segal-Bertin G, Lejeune B, et al. 2014. Ancestral asthenozoospermia and ICSI: what are the options? Hum Reprod Update. 17:684–692.

Pausch H, Ammermüller S, Wurmser C, Hamann H, Tetens J, et al. 2016a. A nonsense mutation in the COL7A1 gene causes epidermolysis bullosa in Vorderwald cattle. BMC Genet. 17:149.

Pausch H, Flisikowski K, Jung S, Emmerling R, Edel C, et al. 2011. Genome-wide association study identifies two major loci affecting calving ease and growth-related traits in cattle. Genetics. 187:289–297.

Pausch H, Kölle S, Wurmser C, Schwarzenbacher H, Emmerling R, et al. 2014. A nonsense mutation in TMEM95 encoding a nonsecreted transmembrane protein causes idiopathic male subfertility in cattle. PLoS Genet. 10:e1004044.

Pausch H, Venhoranta H, Wurmser C, Hakala K, Iso-Touru T, et al. 2016b. A frameshift mutation in ARMC3 is associated with a tail stump sperm defect in Swedish Red (Bos taurus) cattle. BMC Genet. 17:49.

Pedersen BS, Quinlan AR. 2018. Mosdepth: Quick coverage calculation for genomes and exomes. Bioinformatics. 34:867–868.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, et al. 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 38:904–909.

Price AL, Zaitlen NA, Reich D, Patterson N. 2010. New approaches to population stratification in genome-wide association studies. Nat Rev Genet. 11:459–463.

Rahban R, Priskorn L, Senn A, Stettler E, Galli F, the NICER Working Group, et al. 2019. Semen quality of young men in Switzerland: a nationwide cross-sectional population-based study. Andrology. 7:818–826.

Reese MG, Eckman FH, Kulp D, Haussler D. 1997. Improved splice site detection in Genie. J Comput Biol. 4:311–323.

Robic A, Faraut T, Djebali S, Weikard R, Feve K, et al. 2019. Analysis of pig transcriptomes suggests a global regulation mechanism enabling temporary bursts of circular RNAs. RNA Biol. 16:1190–1204.

Sartelet A, Li W, Pailhoux E, Richard C, Tamma N, et al. 2015. Genome-wide next-generation DNA and RNA sequencing reveals a mutation that perturbs splicing of the phosphatidylinositol glycan anchor biosynthesis class H gene (PIGH) and causes arthrogryposis in Belgian Blue cattle. BMC Genomics. 16:316.

Sato Y, Tajima A, Kiguchi M, Kogusuri S, Fuji i A, et al. 2020. Genome-wide association study of semen volume, sperm concentration, testis size, and plasma inhibin B levels. J Hum Genet. 65:683–691.

Sato Y, Tajima A, Sato T, Nozawa S, Yoshiiike M, et al. 2018. Genome-wide association study identifies ERBB4 on 2q34 as a novel locus associated with sperm motility in Japanese men. J Med Genet. 55:1–7.

Schwarzenbacher H, Burgstaller J, Seefried FR, Wurmser C, Hilbe M, et al. 2016. A missense mutation in TUBD1 is associated with high juvenile mortality in Brauneck and Fleckvieh cattle. BMC Genomics. 17:400.

Sha Y, Wei X, Ding L, Mei L, Huang X, et al. 2020. DNAH17 is associated with asthenozoospermia and multiple morphological abnormalities of sperm flagella. Ann Hum Genet. 84:271–279.

Sironen A, Shoemark A, Patel M, Loebingier MR, Mitchisson HM. 2020. Sperm defects in primary ciliary dyskinesia and related causes of male infertility. Cell Mol Life Sci. 77:2029–2048.

Sironen A, Tomthen B, Andersson M, Ahola V, Vilikki J. 2006. An intronic insertion in KPL2 results in aberrant splicing and causes the immotile short-tail sperm defect in the pig. Proc Natl Acad Sci USA. 103:5006–5011.

Sironen A, Uimar i P, Venhoranta H, Andersson M, Vilikki J. 2011. An exonic insertion within Tex14 gene causes spermatogenic arrest in pigs. BMC Genomics. 12:591.

Snider J, Thibault G, Houry WA. 2008. The AAA+ superfamily of functionally diverse proteins. Genome Biol. 9:216.

Soneson C, Love MI, Robinson MD. 2015. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Res. 4:1521.

Tarasov A, Vilella AJ, Cuppen E, Nijman IJ, Prins P. 2015. Sambamba: fast processing of NGS alignment formats. Bioinformatics. 31:2032–2034.

Thibier M, Wagner H-G. 2002. World statistics for artificial insemination in cattle. Livest Prod Sci. 74:203–212.

Tu C, Nie H, Meng L, Yuan S, He W, et al. 2019. Identification of DNAH6 mutations in infertile men with multiple morphological abnormalities of the sperm flagella. Sci Rep. 9:15864.

Upperman LR, Kinghorn BP, MacNeill MD, Van Eenennaam AL. 2019. Management of lethal recessive alleles in beef cattle through the use of mate selection software. Genet Sel Evol. 51:36.

Venhoranta H, Pausch H, Flisikowski K, Wurmser C, Taponen J, et al. 2014. In frame exon skipping in UBE3B is associated with developmental disorders and increased mortality in cattle. BMC Genomics. 15:890.

Wambergue C, Zouari R, Fourati Ben Mustapha S, Martinez G, Devillard F, et al. 2016. Patients with multiple morphological abnormalities of the sperm flagella due to DNAH1 mutations have a good prognosis following intracytoplasmic sperm injection. Hum Reprod. 31:1164–1172.
Warr A, Affara N, Aken B, Beiki H, Bickhart DM, et al. 2020. An improved pig reference genome sequence to enable pig genetics and genomics research. GigaScience. 9:giaa051.

Whitfield M, Thomas L, Bequignon E, Schmitt A, Stouvenel L, et al. 2019. Mutations in DNAH17, encoding a sperm-specific axonemal outer dynein arm heavy chain, cause isolated male infertility due to asthenozoospermia. Am J Hum Genet. 105:198–212.

Xavier MJ, Salas-Huetos A, Oud MS, Aston KI, Veltman JA. 2020. Disease gene discovery in male infertility: past, present and future. Hum Genet. doi:10.1007/s00439-020-02202-x

Yang J, Lee SH, Goddard ME, Visscher PM. 2011. GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet. 88:76–82.

Zhang B, Ma H, Khan T, Ma A, Li T, et al. 2020. A DNAH17 missense variant causes flagella destabilization and asthenozoospermia. J Exp Med. 217:e20182365.

Zhang Q, Fan X, Wang Y, Sun M, Shao J, et al. 2017. BPP: a sequence-based algorithm for branch point prediction. Bioinformatics. 33:3166–3172.

Communicating editor: D.-J. De Koning