

Transcriptome of Myogenic Stem Cells from a Porcine Duchenne Muscular Dystrophy Model

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Data Note

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Abstract

Objectives

Here, we report transcriptomic profiles from myogenic satellite cells (SCs) derived from wild-type (WT), dystrophin-deficient (DMD), and heterozygous carrier (HET) pigs, providing a unique resource for studying the progression of Duchenne muscular dystrophy in a large animal model. The dataset enables the identification of any molecular alterations associated with dystrophin deficiency and supports comparative analyses to explore disease mechanisms and inform translational or therapeutic research. Parts of the data were previously published in a larger study, and the complete dataset is now made fully accessible for independent usage.

Data description

We collected *intercostalis* (*INT*) and *pectoralis* (*PECT*) muscle biopsies from WT, DMD, and HET pigs at two developmental stages (fetal and 3 days postnatal) and subsequently isolated SC. We cultivated SC *in vitro* free from the influence of their dystrophic environment, thus allowing us to detect cell-intrinsic changes. RNA was extracted from SC at pre-contractile stages of myogenesis before the development of a contraction-related machinery. RNA sequencing was performed using the Illumina NextSeq550Dx platform, and the resulting dataset was deposited at the NCBI's Gene Expression Omnibus (GEO) repository with the GEO Series accession numbers GSE287756 and at ArrayExpress, maintained by the European Bioinformatics Institute (EMBL-EBI), with the accession number E-MTAB-16179.

Objectives

Myogenic satellite cells (SCs), the stem cells of skeletal muscle [1, 2], are essential for muscle growth and regeneration, and their dysfunction is increasingly recognized as a contributing factor to Duchenne muscular dystrophy (DMD) [3]. DMD is a severe, X-linked disease caused by mutations in the *DMD* gene, resulting in the loss of dystrophin expression and a rapidly progressing muscle degeneration, which ultimately results in premature death in affected patients [4, 5].

Despite their importance, cell intrinsic impairments of dystrophin-deficient SCs remain poorly characterized, and heterozygous female carriers remain particularly underexplored. In humans, heterozygous females often remain asymptomatic, but approximately 8% develop muscle weakness and cardiac complications due to skewed X-chromosome inactivation [6]. A deeper investigation of both heterozygous and dystrophin-deficient SCs would provide valuable insights into intermediate phenotypes and clarify how *DMD* mutations alter SC biology, thereby driving disease onset and progression.

Here, we report transcriptomic data from SCs isolated from wild-type (WT), dystrophin-deficient (DMD), and heterozygous female carrier (HET) pigs, representing a large animal model with high translational relevance for human DMD [7, 8]. We analyzed SCs at pre-contractile stages of muscle development, before any contraction-related machinery is established, and compared proliferating cells (PROL) with those induced to differentiate into multinucleated myotubes (DIFF) to capture different stages during

myogenesis. To monitor disease progression longitudinally, gene expression was sequenced in SCs derived from fetal and 3-day-old animals.

By capturing these transcriptional signatures, our dataset provides novel insights into the molecular alterations underlying impaired muscle regeneration and represents the first comprehensive resource that includes heterozygous carriers. Parts were recently published in a larger study [9], and the complete dataset is now available as a reference for future investigations on SCs in the context of DMD pathogenesis. Access to high-quality transcriptomic data further supports the development of translational and pre-clinical research strategies [10].

Data description

For sample collection, fully anesthetized pigs (ketamine (Ursotamin®, Serumwerk Bernburg, Bernburg, Germany), azaperone (Azaporc®, Serumwerk Bernburg), propofol (Propofol 2%, Fresenius Kabi, Bad Homburg, Germany)) were euthanized by intravenous injection of potassium chloride (KCl 7.45%, Braun, Melsungen, Germany) [7, 11]. All animals used in this study were bred and housed at the Center for Innovative Medical Models (CiMM) at the Department of Veterinary Science of the Ludwig-Maximilians University Munich, Germany. Approximately 1 cm³ tissue biopsies of *INT* and *PECT* muscles were collected freshly from fetal and 3-day-old WT, DMD, and HET animals [8] immediately after euthanasia. The tissue was minced with a scalpel blade, enzymatically digested with protease (*Streptomyces griseus*, Sigma Aldrich, Darmstadt, Germany, 3.5 U/mg) and collagenase (*Clostridium histolyticum*, Sigma Aldrich, 800 U/mg), and subjected to magnetically activated cell sorting (MACS) using an indirect labelling approach and two sequential separation steps. Unwanted endothelial and hematopoietic cells were depleted by adding rabbit anti-CD31 (1:200, ab28364, Abcam, Cambridge, UK) and rabbit anti-CD45 (1:200, ab10558, Abcam) antibodies together with magnetically conjugated anti-rabbit IgG(-) secondary antibodies (1:15, Miltenyi Biotec, Bergisch Gladbach, Germany) to the cell suspension. Labelled cells were retained within a magnetic column placed on the MACS separator, and the flow-through was used for the subsequent separation step. SCs were then targeted with mouse anti-Integrin-β1 (1:200, abcam30388, Abcam), mouse anti-NCAM1 (1:200, ab9018, Abcam), and mouse anti-M-cadherin (1:200, sc-374093, Santa Cruz, Heidelberg, Germany) [12] and magnetically conjugated secondary anti-mouse IgG(-) antibodies (1:15, Miltenyi Biotec); again labelled cells retained in the column allowing enrichment of the desired SC population. Purified SCs were cultivated under standard conditions in Ham's F10 Medium (GIBCO, Thermo Fisher, Schwerte, Germany) supplemented with 20% Fetal Bovine Serum (GIBCO), 1% Penicillin-Streptomycin (GIBCO), and 10 µg recombinant human basic fibroblast growth factor (PEPro Tech, Cranbury, NJ, USA). Myoblast differentiation was induced by nutrient starvation using medium with 5% horse serum (Biozol, Eching, Germany). A comprehensive protocol has been published previously [9].

Total RNA was extracted using the NEB Monarch RNA Miniprep Kit (New England Biolabs, Ipswich, MA, USA) following the manufacturer's instructions. Samples were collected at passage (p) 6 or 7 under proliferation conditions (PROL) in growth medium or after ten days in medium with reduced serum content

to recollect differentiation into myotubes (DIFF). RNA concentration and purity were assessed with a spectrophotometer (Nanodrop 2000c, Thermo Fisher Scientific).

Gene expression was quantified using an adapted 3' mRNA-seq protocol [13–15]. Each sample was processed as a technical replicate, and 25 ng of total RNA per sample was used for library preparation, including polyA + selection with a unique molecular identifier (UMI) and well barcodes, second-strand synthesis, and amplification. Libraries were prepared with the NexteraXT DNA library prep kit (Illumina, San Diego, CA, USA) and sequenced on a NextSeq550Dx (75-cycle high-output kit). Demultiplexing was performed with bcl2fastq (Illumina), quality control with FastQC [16] and MultiQC [17], and deduplication with UMI-tools [18]. Reads were aligned to the *Sus scrofa* reference genome (susScr3) using STAR aligner [19] (v2.7.10b), and gene counts were obtained with STAR (quantMode GeneCounts).

This dataset enables the identification of stage-specific gene expression signatures during myogenesis, comparing transcriptional changes across healthy, dystrophin-deficient, and heterozygous genotypes, and supports cross-species comparison in DMD research.

The RNA-seq dataset is available at the NCBI's Gene Expression Omnibus (GEO) repository [20], accession number GSE287756 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756>) and at ArrayExpress, a platform for freely available data resources maintained by the European Bioinformatics Institute (EMBL-EBI), with the accession number E-MTAB-16179 (<https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179>). Table 1 summarizes the data files, repositories, and identifiers described in this study.

Table 1
Sequence data of transcriptome profiles of fetal and 3-day-old WT, DMD, and HET animals

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 1	SC, 2fWT, proliferation, biol rep 1	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 2	SC, 3fWT, proliferation, biol rep 2	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 3	SC, 4fWT, proliferation, biol rep 3	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 4	SC, 5fWT, proliferation, biol rep 4	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 5	SC, 2fWT, differentiation, biol rep 1	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 6	SC, 3fWT, differentiation, biol rep 2	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 7	SC, 4fWT, differentiation, biol rep 3	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 8	SC, 5fWT, differentiation, biol rep 4	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 9	SC, 2fDMD, proliferation, biol rep 1	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 10	SC, 3fDMD, proliferation, biol rep 2	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set	SC, 3fDMD, proliferation,	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
11	biol rep 3		E-MTAB-16179
Data set 12	SC, 2fDMD, differentiation, biol rep 1	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 13	SC, 3fDMD, differentiation, biol rep 2	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 14	SC, 3fDMD, differentiation, biol rep 3	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 15	SC, 1fHET, proliferation, biol rep 1	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 16	SC, 2fHET, proliferation, biol rep 2	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 17	SC, 3fHET, proliferation, biol rep 3	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 18	SC, 3fHET, proliferation, biol rep 4	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 19	SC, 1fHET, differentiation, biol rep 1	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 20	SC, 2fHET, differentiation, biol rep 2	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 21	SC, 3fHET, differentiation, biol rep 3	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 22	SC, 3fHET, differentiation, biol rep 4	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 23	SC, 31WT, proliferation, biol rep 1	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750877
Data set 24	SC, 33WT, proliferation, biol rep 2	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750878
Data set 25	SC, 34WT, proliferation, biol rep 3	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750879
Data set 26	SC, 35WT, proliferation, biol rep 4	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750880
Data set 27	SC, 31WT, differentiation, biol rep 1	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750881
Data set 28	SC, 33WT, differentiation, biol rep 2	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750882
Data set 29	SC, 34WT, differentiation, biol rep 3	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750883
Data set 30	SC, 35WT, differentiation, biol rep 4	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750884
Data set 31	SC, 14DMD, proliferation, biol rep 1	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750885
Data set 32	SC, 15DMD, proliferation, biol rep 2	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750886

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 33	SC, 16DMD, proliferation, biol rep 3	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750887
Data set 34	SC, 17DMD, proliferation, biol rep 4	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750888
Data set 35	SC, 14DMD, differentiation, biol rep 1	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750889
Data set 36	SC, 15DMD, differentiation, biol rep 2	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750890
Data set 37	SC, 16DMD, differentiation, bio rep 3	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750891
Data set 38	SC, 17DMD, differentiation, biol rep 4	FASTQ (.fastq.gz)	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756 GSM8750892
Data set 39	SC, 6HET, proliferation, biol rep 1	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 40	SC, 7HET, proliferation, biol rep 2	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 41	SC, 8HET, proliferation, biol rep 3	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 42	SC, 9HET, proliferation, biol rep 4	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 43	SC, 6HET, differentiation, biol rep 1	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 44	SC, 7HET, differentiation, biol rep 2	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 45	SC, 8HET, differentiation, biol rep 3	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data set 46	SC, 9HET, differentiation, biol rep 4	FASTQ (.fastq.gz)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data file 47	Raw_counts_3d-WT-DMD-HET	TSV (.tsv)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data file 48	Raw_counts_fetal-WT-DMD-HET	TSV (.tsv)	https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179 E-MTAB-16179
Data file 49	All_samples	Excel Sheet (.xlsx)	https://figshare.com/s/363f8c3f78fd884308ab
Data file 50	Methodology	Word Document (.docx)	https://figshare.com/s/363f8c3f78fd884308ab

Limitations

1. **Sample size:** Although technical replicates were included for each sample, the number of biological replicates remains limited. This may affect statistical power and generalizability, but the inclusion of technical replicates ensures consistent and reliable data.
2. **Model:** The porcine DMD model offers high translational relevance, but species-specific differences in SC biology and dystrophin function may restrict direct extrapolation to human DMD patients.
3. **Tissue:** The data reflect transcriptome profiles of SCs derived from *INT* and *PECT* muscles, which are important for respiration and locomotion and are highly affected in DMD, but their characteristics may differ from SC of distinct muscle groups.
4. **Multi-omics approach:** The dataset is limited to RNA expression; a complementary approach measuring proteomics and epigenomics, together with functional assays, would provide additional layers of insights into the effects of dystrophin-deficiency on SC behavior.

Abbreviations

DIFF Differentiation

DMD Duchenne muscular dystrophy

EMBL-EBI European Bioinformatics Institute

HET Heterozygous female carrier

GEO Gene Expression Omnibus

INT Intercostalis muscle

MACS Magnetically activated cell sorting

p Passage

PECT Pectoralis muscle

PROL Proliferation

SCs Satellite cells

UMI Unique molecular identifier

WT Wild type

Declarations

Ethics approval and consent to participate

Ethical review was performed as required by German law, and the animal study protocol was approved by the competent governmental authority, the Government of Upper Bavaria (Regierung von Oberbayern), which serves as the responsible animal welfare authority under the German Animal Welfare Act (TierSchutzgesetz), permission number ROB.55.2-2532.Vet_02-19-195, granted on 20 July 2020. All experiments were conducted in accordance with the German Animal Welfare Act and Directive 2010/63/EU on the protection of animals used for scientific purposes.

Consent for publication

Not applicable.

Availability of data and materials

The data described in this Data note can be freely and openly accessed on NCBI's GEO repository under the accession number GSE287756 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287756>) and on the EMBL-EBI ArrayExpress under the accession number E-MTAB-16179

(<https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-16179>). Please see Table 1 and references [21], [21], and [22] for details and links to the data.

Competing interests

The authors declare no competing interests.

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Authors' contributions

S.F. conceived and designed the project. S.F., S.C., and E.M. contributed to data collection and analysis. N.P., E.W., and K.M. provided resources and funding. S.F. prepared and wrote the original draft with intellectual input from J.S., E.W. and K.S. K.M. supervised the whole study. All authors read and agreed to the final version of the manuscript.

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