MITOCHONDRIAL GENE EXPRESSION IS ASSOCIATED WITH ORGAN AND TISSUE METABOLISM IN DAIRY CATTLE

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SUMMARY

Mitochondria and their genes (MGs) are central to cellular energy metabolism in eukaryotes. Mitochondrial DNA mutations are associated with mitochondrial diseases and affected productivity in animals. The variation in mitochondrial phenotypes are plausibly affected through differential expression of MGs, but currently, MG expression across tissues types are unknown. We profiled MG expression using RNAseq of 16 tissues of dairy cattle. Our results show MGs are differentially expressed among tissues. Specifically, upregulated in heart, leg muscles, tongue and downregulated in leukocytes, thymus, lymph node, and lung. Besides, all DE MGs were regulated in a single direction within a tissue.

INTRODUCTION

Mitochondria are autonomous organelles having their own maternally inherited haploid genome. The mitochondrial genome in cattle small (~16.6 kb) encoding 37 genes (13 proteins, 22 tRNAs and 2 rRNAs) and non-coding region (Anderson *et al.* 1982). Mitochondria are functionally responsible for cellular energy metabolism, and the proteins encoded by a mitochondrial genome are integral components of the complex electron transport chain (ETC). Energy (ATP) is generated by the transfer of electron through ETC in the process known as oxidative phosphorylation (OXIPHOS) and caters to varying energy demands across the tissue types in the body (Wang *et al*. 2012).

Mitochondrial mutations are associated to mitochondrial dysfunction and diseases, including diabetes, obesity, and aging (Taylor and Turnbull 2005) and are increasingly studied in humans to understand the underlying biology and to develop therapies for mitochondrial diseases. In livestock, mitochondrial mutations are indicated in productivity (Schutz *et al*. 1994), but little is known regarding the potential causal effects or underlying biology of mitochondrial mutations.These mitochondrial phenotypes are plausibly the result of differential expression of MGs. However, MG expression across the tissue in dairy cattle is less known.

Therefore, we characterized MG expression across 16 tissues in dairy cow using RNA sequencing technology to gain insights into the relationship between cellular energy metabolism and potential biological effects of mitochondrial mutations.

MATERIALS AND METHODS

We sampled 16 tissues from two adult lactating cows (2181 and 6819) aged 8 years from the Agriculture Victoria Research dairy herd at Ellinbank after euthanasia. Cows were born from different sire and dam. Cow 2181 was 208 days in milk and in the fifth lactation while cow 6819 was 173 days in milk and in the seventh lactation. Blood was collected by venipuncture of the coccygeal vein and processed according to the standard protocol in the RiboPure™ blood kit (Ambion by Life Technologies). Other tissue samples were dissected into 1cm squares, sealed in a 5ml tube and flash-frozen in liquid nitrogen and stored at -80 ºC. RNA was extracted from leukocytes using RiboPure Blood Kit (Ambion) following the manufacturer's instructions. RNA was extracted from ~30 mg of ground tissue using Trizol (Invitrogen) according to standard protocol. RNAseq libraries were prepared from

all samples with a RIN > 6 using the SureSelect Strand Specific RNA Library Prep Kit (Agilent) according to manufacturer's instructions. Each library was uniquely barcoded, randomly assigned to one of two pools and sequenced on a HiSeq™ 3000 (Illumina) in a 150-cycle paired-end run. One hundred and fifty bases paired-end reads were called with bcltofastq and output in fastq format.

Each library of paired-end reads was aligned to Ensembl bovine genome UMD3.1 using STAR version 2.5.3ab (Dobin *et al*. 2013), and quality checked for alignment. We used the SAM output file to generate gene expression counts in a tissue using R package featureCounts (Liao *et al*. 2014). The gene counts were filtered for lowly expressed reads. The gene counts were normalized (counts per million) and analyzed for differential expression using the R package edgeR (Robinson *et al*. 2010). A design matrix with the overall mean of gene expression across all tissues was used as the intercept and compared to gene expression in each sample. Specifically, we used glmQLTest method to identify DE genes (Log_2Fold Change (LFC) > |0.6|, $p<0.01$) and direction of regulation was determined by the sign of LFC values (i.e. +ve LFC as upregulated and -ve as downregulated).

RESULTS AND DISCUSSION

Differential expression of genes. We compared nuclear and MGs expression profiles across all tissue types. Sixteen MGs (13 protein-coding genes, 2 rRNAs and 1 tRNA) were DE. The highest number of upregulated MG was in heart followed by the tongue, leg muscle, spleen, and kidney cortex, while the highest number of downregulated genes were in leukocytes, thymus, lymph node and lung (Figure 1). The MGs were not DE for the rest of the tissues. Interestingly, all DE MGs were regulated in a single direction (i.e. regulated either all up or all down), while the overall nuclear gene expression was regulated in both directions.

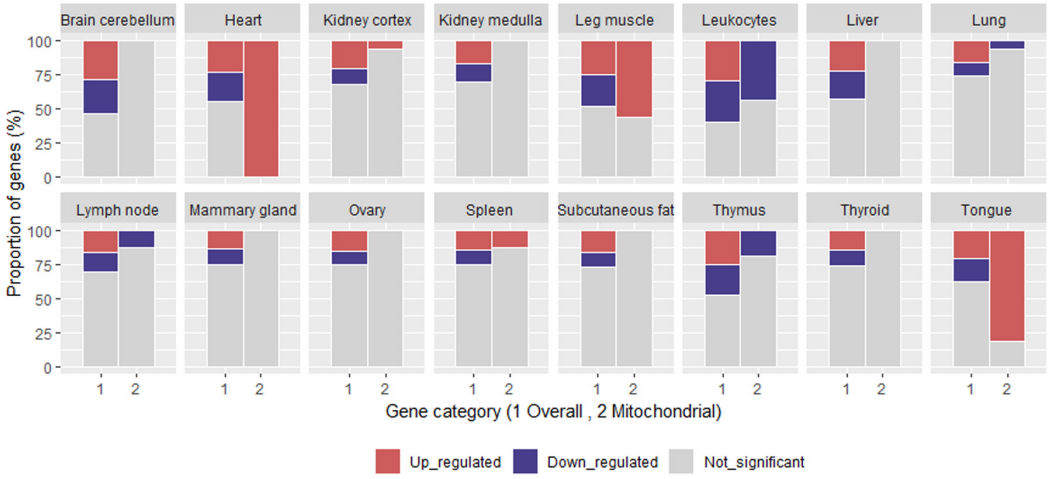

Figure 1. Proportion of differentially expressed overall gene and mitochondrial genes 16 tissue types of dairy cattle

This phenomenon of regulation of MGs in a single direction in tissue may be explained from the mechanism of transcription of mitochondrial DNA. The mitochondrial DNA is transcribed as a near-complete polycistronic unit (Shokolenko and Alexeyev 2017). The polycistronic transcription could result in an almost equal number of all MG transcripts, and thereby regulation of the genes in one direction compared to the mean.

The highest fold changes of MG expression across tissues were in heart, leg muscle, tongue, kidney and liver, and relatively lower fold changes in lung and adipose tissues (Figure 2). The consistency of MG expression in tissue is highlighted by clustering together of most tissues across two animals.

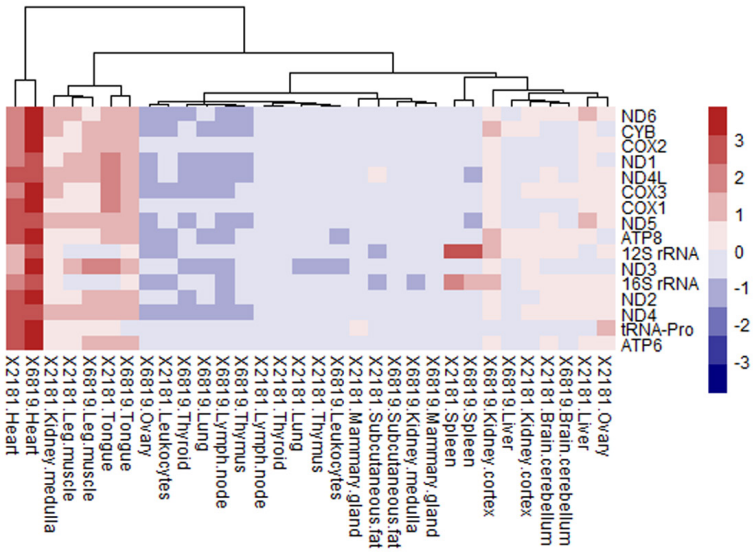

Figure 2. Heatmap showing mitochondrial gene expression across the tissues of two cows (X6819 and X2181) highlighting higher expression in heart, skeletal muscles and tongue

Specific organs and tissues. Heart tissue had the highest number of DE MG as well as the highest fold change compared to the mean expression of all 16 tissues. This coincides with the specific metabolic rate of the organ, which is the highest among tissues in mammals (Table 1). The overall DE genes in the heart were significantly enriched for OXIPHOS pathway (adj $p < 1.2e\text{-}52$). This is logical because a heart requires a continuous and reliable energy supply for the contraction of cardiac muscle to pump blood through the body. Almost 95% of the energy demand of the heart is met from OXIPHOS in mitochondria (Stanley and Chandler 2002).

Tongue (muscular organ) and leg muscle followed heart in the number of DE MGs as well as fold changes with significant enrichment for OXIPHOS pathways. The skeletal muscle has a low basal metabolic rate but has the capacity to increase depending on the activity (Glaister 2005) by as high as 1000-fold during exercise (Spriet 1992). The muscular strength and movement of the tongue are vital for harvesting forage, chewing and regurgitation: typically, in use for up to 20 hours per day.

Kidney, liver and brain despite relatively higher specific metabolic rates compared to the residual tissues neither show higher fold MG expression nor DE MGs (except kidney cortex). This indicates that the OXIPHOS may not be a primary energy generation process in these tissues.

Table 1. Specific metabolic rates of organs and tissues across species (kcal/kg/day)

Species	Heart	Kidney	Brain	Liver	Skeletal	Adipose	Residuals	Source
Human	440	440	240	200	13	4.5	12.0	(Wang *et al.* 2010)
Cattle	429	412	185	130	-	-	10.7	(Wang *et al.* 2012)
Sheep	588	496	255	200	-	-	15.5	(Wang *et al.* 2012)

It follows that leukocytes were the tissue with the most downregulated MG because the role of mitochondria in leukocytes are primarily for non-energy production roles (Kramer *et al*. 2014). The leukocytes mostly derive their energy from glycolysis.

Differential expression of mitochondrial genes and mitochondrial copy number across tissues. The number of mitochondria per cell and the amount of Mitochondrial DNA per cell are closely regulated within a given cell type but differ widely between cell types (Robin and Wong 1988). Currently, the effect of mitochondrial content on the variation of MG expression across the tissue remains poorly known. Tissues with high MG expression in this study are concurrently reported to have high mitochondrial content and vice versa. For example, heart, skeletal muscle, omental fat and breast cells contained 6970, 3650, 400-600 and 25 copies respectively (Miller *et al*. 2003; Lindinger *et al*. 2010; Yu *et al*. 2007).

Differential expression of MGs across tissues and association with energy metabolism indicate gene expression of mitochondrial protein genes (from both nuclear and mitochondrial genomes) can be extended to identify candidate genes involved in energy use efficiency and related traits (e.g. feed efficiency). The genetic markers identified for a trait can be used in genomic selections.

CONCLUSIONS

This study shows that mitochondrial genes are differentially expressed across the tissues in dairy cattle. Mitochondrial gene expression is upregulated in tissues with high energy demand and vice versa. Within a tissue, the mitochondrial genes are regulated in a single direction. Because mitochondrial gene expression was associated with tissues metabolic rates, we are now using RNA sequencing to determine if there are associations between mitochondrial protein gene expression and specific cattle phenotypes.

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