**SUPPLEMENTARY INFORMATION**

**Shelton, et al.** ***Functional and Metabolic Consequences of a Mitochondrial Transporter SLC25A12 Gene Mutation in a family of Dutch Shepherd Dogs with Inflammatory Myopathy***

**Supplementary Results**

*1-Carbon and Folate Metabolism.* The second most increased metabolite was 5-methyltetrahydrofolic acid (5-mTHF; Fig. 5B, Fig. 7P). Chronic perturbations to cellular redox produce sustained reductions in flux of metabolites through the transmethylation reactions needed to recycle folate and S-adenosylmethionine [1]. The AGC1 mutation produced primary redox disturbances that led to accumulation of 5-methyltetrahydrofolic acid (Table 1, Table S1).

*Phospholipids.* A prominent secondary effect of the p.L349P substitution in AGC1 was the significant increases in phospholipids and their biosynthetic precursors as a group in affected muscle samples (Table 1, Figure 7). Among these were the precursors ethanolamine, CDP-ethanolamine (Figure 7A), phosphoethanolamine (Figure 7B), and glycerophosphocholine (Figure 7C). Lysophosphatidylcholine (18:0), a product of phosphatidylcholine lipid turnover, was also increased (Figure 7F). LysoPC lipids are the natural ligand for a damage associated molecular pattern (DAMP), G-protein coupled receptor that is also responsive to acid conditions associated with persistent inflammation or wound healing called GPR132 [2]. Interestingly, the only class of phospholipids that was decreased was a phosphocholine plasmalogen PC(20:5/P-16:0) (Fig. 7G). Plasmalogens are enriched in tissues with heavy oxidative burdens like muscle, nerve, and brain. The loss of PC plasmalogens combined with an increase in precursors for phosphoethanolamine plasmalogens, increased oxidized glutathione (**Fig**. 6G) and cystine (Fig.6E) suggests that compensatory up-regulation of oxidative defenses has reached its limits in the affected animals, and oxidative damage and inflammation have become prominent.

*Glycosphingolipids.* Other secondary effects of the p.L349P substitution in AGC1 included a decrease in dihexosylceramide (DHC) species (Fig. 7E,H), and an increase in trihexosylceramide (THC) species (Fig. 7I). The non-glycosylated precursor ceramide(d18:1/24:1, Fig. 7J) was unchanged, but its product sphingomyelins were increased (Figure 7K, Table 1, Table S1). A common dihexosylceramide, DHC(d18:1/24:1, Fig. 7E) made from the corresponding ceramide contains the sugars glucose and galactose and is called lactosylceramide. Lactosylceramide creates an antigenic epitope on cells known as the CD17w antigen. DHC d18:1/24:1 is the two-sugar glycosphingolipid derived from the corresponding trihexosylceramide (THC) d18:1/24:1 via the lysosomal enzyme alpha-galactosidase-A. This THC is also known as a globotriaosylceramide (Gb3) and the glycolipid antigen CD77. Gb3/CD77 is a B-cell differentiation antigen that can lead to apoptosis [3]. N-acetyl glucosamine-1-phosphate (N-AG-1P) is a precursor of Gb4 (globotetraosylceramide) synthesis and is known to play an important role on the post-synaptic membrane of the neuromuscular junction [4], and TNF-alpha stimulated inflammation [5]. N-AG-1P was also increased (Table S1). Gb4 was not directly measured in this analysis. The increase in THC glycosphingolipids associated with a decrease in the DHCs derived from the larger glycolipids is consistent with an effect of the AGC1 mutation to impair lysosomal processing and recycling of glycosphingolipids.

**Supplementary References**

[1] Naviaux RK. Metabolic features of the cell danger response. Mitochondrion 2014; 16; 7-17.

[2] Weiss KT, Fante M, Kohl G, Schreml J, Haubner F, Kreutz M et al.Proton-sensing G protein-coupled receptors as regulators of cell proliferationand migration during tumor growth and wound healing. Exp Dermatol 2017; 26: 127-132.

[3] Tetaud C, Falguieres T, Carlier K, Lecluse Y, Garibal J, Couland E et al. Two distinct Gb3/CD77 signaling pathways leading to apoptosis are triggered by anti-Gb3/CD77 mAB and verotoxin-1. J Biol Chem 2003; 278:45200-45208.

[4] Parkhomovskiy N, Kammesheidt A, Martin PT. N-acetyllactosamine and the CT carbohydrate antigen mediate agrin-dependent activation of MuSK and acetylcholine receptor clustering in skeletal muscle. Mol Cell Neurosci 2000; 15:380-397.

[5] Park SY, Kwak CY, Shayman JA, Kim JH. Globoside promotes activation of ERK by interaction with the epidermal growth factor receptor. Biochim Biophys Acta 2012; 1820:1141-1148.