The significance of serum proteome analysis in diagnosis of canine idiopathic epilepsy

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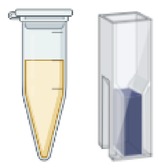
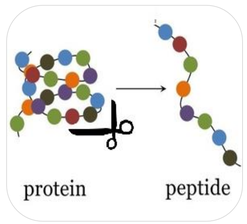
INTRODUCTION

- Epilepsy is a brain disorder affecting both humans and animals and its clinical manifestations include epileptic seizures.
- Epileptic seizure etiology includes metabolic disturbances (metabolic epilepsy), structural brain abnormalities (structural epilepsy), genetic or strongly suspected genetic factors (idiopathic epilepsy), and unknown etiology (cryptogenic epilepsy).
- Idiopathic epilepsy (IE) is the most common cause of epileptic seizures in young dogs. Seizure control is achieved with antiepileptic medication (AEM).
- Diagnosis of different types of epilepsy involves history-taking, physical and neurological examination, blood chemistry tests, brain diagnostic imaging and cerebrospinal fluid (CSF) analysis.
- Proteomic analysis is an advanced tool which detects different proteins abundances in a sample, contributing and interpreting the pathophysiological mechanisms of a disease at the level of protein translation.
- The purpose of this research was to identify and compare different protein abundances in serum samples of two groups of dogs with IE with healthy controls using TMT based-shotgun methods.

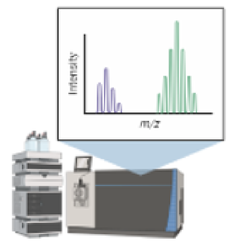
MATERIALS- METHODS



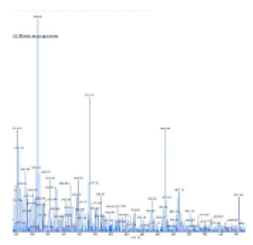
Serum sample collection and measurement of total protein concentration (BCA assay)



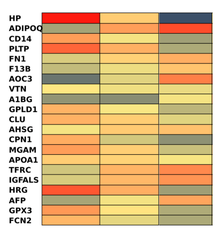
Sample preparation
- **Reduction**
- **Alkylation**
- **Digestion**
- **Label**



Peptide separation *(PepMap™ RSLC C18)* & LC-MS/MS analysis *(Q-exactive Plus, Thermo Scientific)*



Data processing
- **Proteome Discoverer with SEQUEST algorithm implemented**



Data Visualization
- **Volcano Plots**
- **Heatmap**
- **Microsoft Excel**

RESULTS

A. STUDY POPULATION DATA

Descriptive statistics of epileptic dogs (history data)

Group A: *Healthy Controls*

Group B*: Dogs with IE under antiepileptic medication (AEM)*

Group C: *Dogs with IE without receiving AEM*

	Group A	Group B	Group C
Number of dogs	9	9	8
Median age on admission (months)	24	48	45
Median weight (kg)	26.5	10	28.55
Type of epileptic seizures			
Generalized, Tonic-clonic		5	7
Generalized, Tonic		1	
Focal		2	
Focal evolved to generalized		2	
Complex partial		2	1
Frequency of epileptic seizures			
Single seizures		4/month	3/3 months
Cluster seizures		1/month	1/3 months*
Status epilepticus		1/month	
Post-ictal clinical manifestation			
Aggressiveness			1
Ataxia			4
Cognitive dysfunction	2		
Depression			2
Disorientation	4		1
Head pressing			
Increased appetite			1
Pacing	2		
Temporary blindness	2		1
Vomiting	1		
Walking disorders	1		
No detectable signs	3	3	

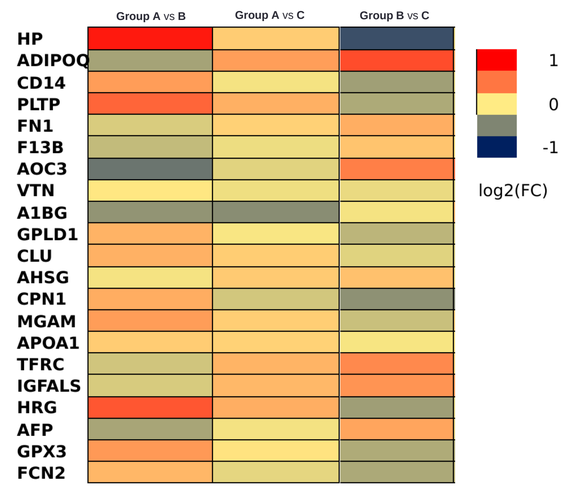
B. PROTEOMIC ANALYSIS

Significantly different abundance of Master proteins among the three groups

Gene name	Protein name (Protein accession)	Log 2 fold change (P/ FDR)		
		B vs A	*C vs A*	*C vs B*
APIDOQ	Adiponectin D (A0A0B4J198)	-0.35 (0.03/0.02)	0.32 (0.04/0.03)	0.67 (0/0)
607076	Monocyte differentiation antigen CD14 (A0A1S7J0A8)	0.33 (0/0)		-0.37 (0/0)
PLTP	Phospholipid transfer protein (A0A5F4BQ47)	0.57 (0/0)		-0.31 (0.02/0.01)
FN1	Fibronectin (A0A5F4BW32)			0.26 (0/0)
	Uncharacterized protein (A0A5F4BYJ5)	-0.41 (0/0)	-0.32 (0.01/0.01)	
F13B	Coagulation factor XIII B chain (A0A5F4C0A8)	-0.23 (0/0)		0.16 (0.01/0.01)
AOC3	Amine oxidase (A0A5F4C6Q3)	-0.57 (0/0)		0.46 (0/0)
VTN	Vitronectin (A0A5F4C7K2)			
	Ig-like domain-containing protein (A0A5F4CSP7)		-0.42 (0.02/0.02)	
A1BG	Alpha-1-B glycoprotein (A0A5F4CTD0)	-0.42 (0/0.01)	-0.46 (0/0.01)	
	Ig-like domain-containing protein (A0A5F4CWK7)	-0.77 (0/0)	-0.62 (0/0)	
LOC479668	Peptidase S1 domain-containing protein (A0A5F4DB38)	0.89 ((0/0)		-0.76 (0.01/0.01)
GPLD1	Glycosyl-phosphatidylinositol-specific phospholipase D (A0A5F4DEN6)	0.23 (0/0)		-0.26 (0/0)
	Ig-like domain-containing protein (A0A5F4DGD5)	-0.49 (0/0)		0.20 (0.07/0.04)
CLU	Clusterin (A0A5S6DEE6)	0.24 (0/0)	0.13 (0.03/0.02)	-0.11 (0.04/0.03)
AHSG	Alpha-2-HS-glycoprotein (E2QUV3)			
CPN1	Carboxypeptidase N subunit 1 (E2RNG3)			-0.44 (0.01/0.01)
MGAM	Maltase-glucoamylase (F1PAQ3)	0.33 (0/0.01)		
APOA1	Apolipoprotein A-I (F1PDJ5)	0.13 (0/0.01)		
TFRC	Transferrin receptor protein 1 (F1PEN6)	-0.18 (0.03/0.02)	0.23 (0.03/0.02)	0.41 (0/0)
IGFALS	Insulin like growth factor binding protein acid labile subunit (F1PYM4)		0.21 (0.05/0.03)	0.37 (0/0)
HRG	Histidine rich glycoprotein (F1PZC6)	0.63 (0/0)	0.26 (0.04/0.03)	-0.37 (0.04/0.03)
AFP	Alpha fetoprotein (F2Z4Q6)	-0.34 (0.04/0.02)		0.29 (0.06/0.04)
GPX3	Glutathione peroxidase (J9P028)	0.34 (1.05E-05/0)		-0.31 (0/0)
FCN2	Fibrinogen C-terminal domain-containing protein (J9P7F7)	0.22 (0.04/0.03)		-0.32 (0/0)

Volcano Plots

Heatmap showing abundance differences



- Eighty-one proteins had significant different abundances between the three groups and 25 of them were master proteins. Although HP was not a master protein, it was included in the heatmap.
- CLU and APOA1 had higher abundance in epileptic groups (B and C) compared to controls (group A).
- Amine oxidase (AOC3) had higher abundance in group B compared to group C and lower in group B than in group A.
- Haptoglobin (HP) had higher abundances in group B compared to group A and lower abundance than in group C.
- ADIPOQ had higher adundance in group B, followed by group C and the lowest in group A.
- FN1 abundance was higher in group B compared to group C.

CONCLUSIONS

- Haptoglobin (HP) abundance changes in serum may be indicative of high seizure frequency and poor seizure control despite proper AEM administration.
- Clusterin (CLU) abundance changes in serum may be indicative of severe nerve tissue cell apoptosis in canine idiopathic epilepsy, clinically manifested with *status epilepticus*.
- Apolipoprotein A1 (APOA1) and CLU abundance changes may be indicative of nerve tissue regeneration.
- Amine oxidase (AOC3) may contribute to the seizure-induced damage to the brain in epileptic dogs with severe and frequent epileptic seizures through its effect in brain vasculature activation.
- AEM administration could alter matrix protein fibronectin (FN1) abundance through its inhibitory effect on GABA receptors' function.
- Adiponectin (ADIPOQ) abundance changes in serum may be affected by illness duration (time from first epileptic seizure occurence until sampling) in dogs with IE.

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