100 Moredun Centenary

Identification of serum proteins from Atlantic salmon with Cardiomyopathy Syndrome (CMS)

Janina Z. Costa *et al*

| Trination meeting 2021

The team

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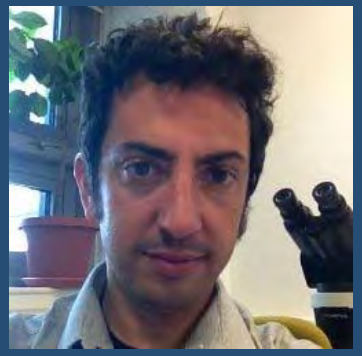

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Can we use innovative approaches to CMS diagnostic & prognostic?

Diagnosis of CMS is based on:

- Observation of cardiac lesions typical of CMS
- Detection of viral RNA by RT-qPCR
- Histopathology

Downside:

- Late detection and expensive methodology
- Requires sacrificing fish often near final stages of production cycle
- Limited number of individuals within large populations, providing fragmented information and little prognosis at a population level

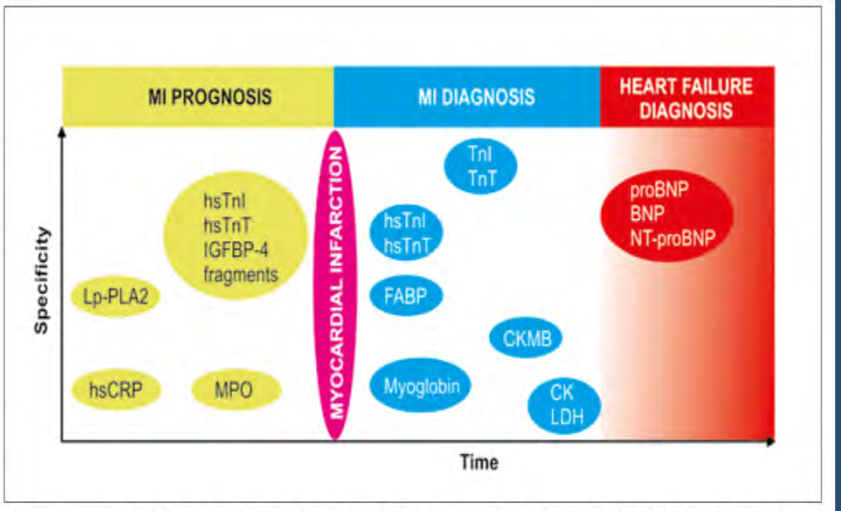
Achievable with biomarkers

Explore new possibilities… Seek a method that would

- Provide finer diagnostic and **prognostic** of CMS
- Avoid sacrificing fish
- Applicable to large numbers of individuals for better assessment
- Affordable and fast

The use of BIOMARKERS for diagnosis & prognosis of heart diseases in humans

According to Biomarkers Definitions Working Group (2001):
'*A biomarker is defined as a characteristic that is measured and evaluated as an indicator of normal biologic process, pathogenic process, or pharmacologic process to a therapeutic intervention*'.



Markers of myocardial infarction and heart failure. This schematic representation shows how they differ in timing and in specificity.

In human and veterinarian medicine are used to:

- identify disease state in individuals
- risks of developing the disease

- Troponins for cardiomyocyte injury

- C-reactive protein (CRP) for acute and chronic inflammation

The use of BIOMARKERS in salmon

POTENTIAL SALMON CARDIAC BIOMARKERS

- Creatine kinase (CK)
- Lactate dehydrogenase (LDH)
- Natriuretic peptides (Salmon cardiac peptide cSP)
- Troponins

CURRENT KNOWLEDGE IN SALMON BIOMARKERS

- CK levels are significantly increased in PD
- CK and LDH are increased in CMS and HSMI, although significant correlation with histopathological lesions was only seen for HSMI

Our aim

To examine the differential expression of proteins in the serum of field samples (fish with CMS and clinically healthy fish), in an attempt to identify putative biomarker candidates that may be further developed to allow early diagnosis of CMS

Materials and Methods: Biological samples

2 marine production sites in Scotland

On-going CMS outbreak
(CMS+ site)

CMS-free farm
(CMS- site)

Biological samples: blood and heart tissue

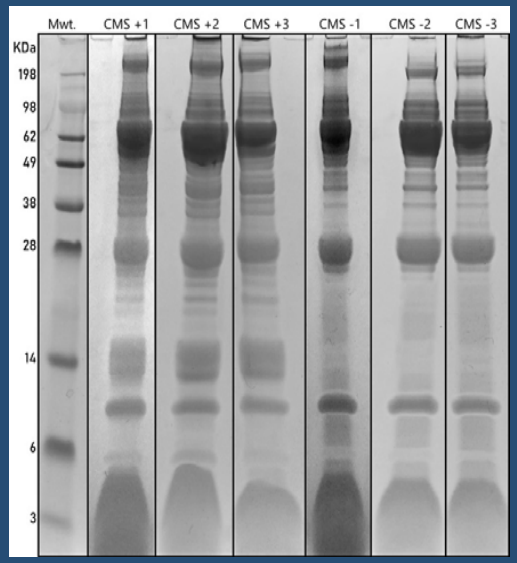
Disease status of fish: gross pathology, cardiac histopathology, and PMCV specific RT-qPCR

CMS+ if histology and RT-qPCR were consistent with CMS and PCMV infection
CMS- if this was not the case

Materials and Methods: Proteomics

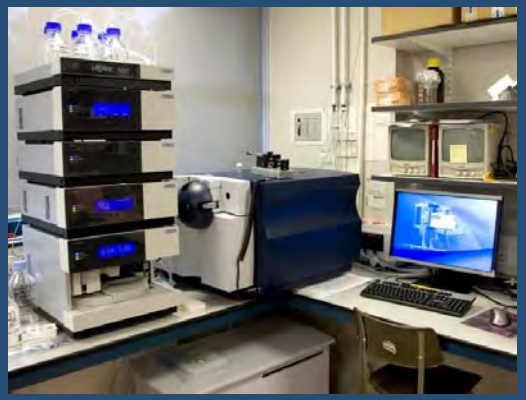
Biological samples: 3 pools of CMS+ and 3 pools of CMS- serum (n=4 fish/pool)

SDS gel



LC-ESI-MS/MS

Liquid chromatography-electrospray ionization-tandem mass spectrometry



Data mining

ProteinScape™ V3.1 (Bruker) for downstream mining with annotated Atlantic salmon genome sequence

Materials and Methods: Proteomics

Data Analysis

Common proteins between:

3 CMS+ pool samples
3 CMS- pool samples

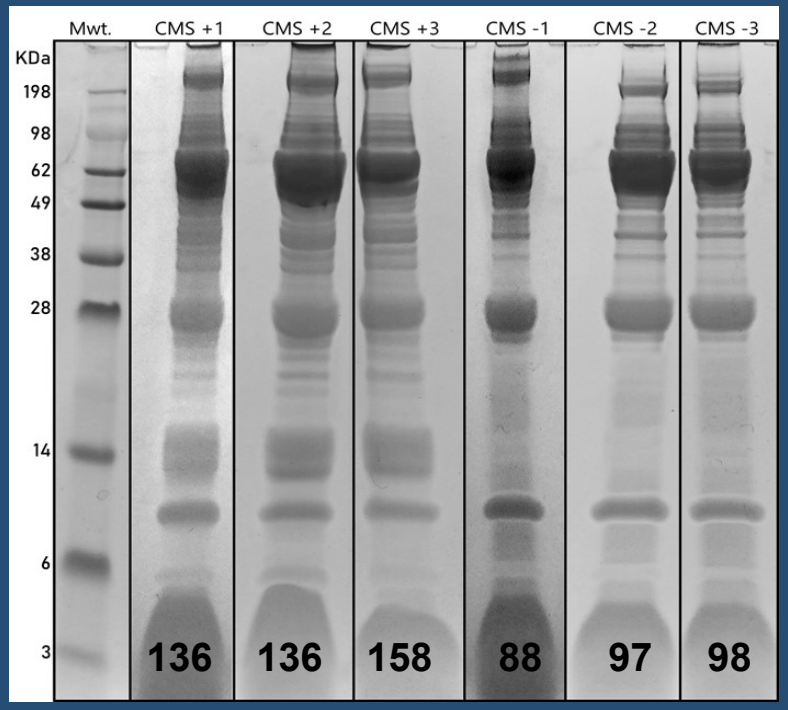
↓

Compared and produce a list of unique proteins for CMS+ serum

Blast2Go: Functional annotation and mapping of unique protein sequences

- Sequences were blasted with NCBI Blast Service (QBlast)
- InterProScan used to classify them by family and to identify main domains;
- Functional annotation was performed by mapping homologue sequences and annotating them with Gene Ontology databases

Results



No. proteins identified

Common CMS+ proteins: 85

Common CMS- proteins: 57

27 unique proteins to CMS+

7 unique proteins to CMS-

Results

Unique proteins of CMS + serum

Description	Biological process	Cellular component	Molecular function
Alpha-2-macroglobulin like	-	extracellular region	endopeptidase inhibitor activity
Carbonic anhydrase-like	-	-	carbonate dehydratase activity; zinc ion binding
Ceruloplasmin	iron ion transport; cellular iron ion homeostasis	extracellular space	copper ion binding; ferroxidase activity
Complement factor B-like	complement activation	extracellular region	serine-type endopeptidase activity
Cofilin-2-like	actin filament depolymerisation	actin cytoskeleton	actin binding
Creatine kinase M-type	phosphocreatine biosynthetic process, phosphorylation	-	creatine kinase activity; catalytic activity; kinase activity; transferase activity; transferring phosphorus-containing groups, ATP binding
Enolase	glycolytic process	phosphopyruvate hydratase activity	magnesium ion binding; phosphopyruvate hydratase activity
Fibronectin-like	-	-	protein binding
Fibrinogen alpha & gamma chain like	protein polymerization, platelet activation	fibrinogen complex, blood coagulation	signalling receptor binding
Fibrinogen beta chain-like	protein polymerization; platelet activation; blood coagulation; fibrin clot formation	fibrinogen complex	signalling receptor binding
Fructose-bisphosphate aldolase A	glycolytic process	-	catalytic activity fructose-bisphosphate aldolase activity
Glycogen phosphorylase, muscle form	carbohydrate metabolic process	-	glycogen phosphorylase activity; pyridoxal phosphate binding; 1,4-alpha-oligoglucan phosphorylase activity
Haptoglobin – like	proteolysis	-	serine-type endopeptidase activity
Histone H4	nucleosome assembly; DNA-template transcription, initiation	nuclear chromosome	DNA binding; histone binding; protein heterodimerization activity
Keratin type II cytoskeletal cochleal-like	-	keratin filament	-
Kininogen-1-like	-	-	cysteine-type endopeptidase inhibitor activity
L-lactate dehydrogenase B chain	oxidation-reduction process	cytoplasm	L-lactate dehydrogenase activity
Lipocalin-like	-	-	small molecule binding
Lumican-like	collagen fibril organization, visual perception	collagen-containing extracellular matrix	protein binding
Mannose-binding protein C-like	-	-	-
Parvalbumin beta 1	-	-	calcium ion binding
Pyruvate kinase PKM-like	glycolytic process	-	potassium ion binding; pyruvate kinase activity; magnesium ion binding; catalytic activity; kinase activity
Retinol-binding protein 4-B	retinol transport	-	retinoid binding, retinol transmembrane transporter activity
Serine protease-like protein	Notch signalling pathway, complement activation alternative pathway	extracellular space	serine-type endopeptidase activity
Sex hormone-binding globulin	-	-	-
Triosephosphate isomerase B	gluconeogenesis	-	triose-phosphate isomerase activity
2-peptidylprolyl isomerase	protein folding, protein peptidyl-prolyl isomerization	-	peptidyl-prolyl cis-trans isomerase activity

Discussion

27 unique proteins to CMS+ serum

24 are associated with cardiac disease

2 with cell structure

1 with sexual endocrine function

Discussion

Leakage enzymes

- Creatine kinase
- Lactate dehydrogenase
- Glycogen phosphorylase
- Carbonic anhydrase

include **myocardial injury biomarkers** used in other species

CK

In CMS there is an increase in CK levels but are not correlated with lesions (Yousaf et al. 2012)
In PD potential used for diagnosed (Rodger et al.,1991) and there is a correlation between CK and PD lesions (Braceland et al., 2013)

LDH

Increased levels are observed but are not correlated with CMS lesions (Yousaf et al. 2012)

Discussion

complement related proteins and **acute phase** proteins

e.g. haptoglobin, fibrinogen, α2-macroglobulin and ceruloplasmin are used as **myocardial infarction, stroke or heart failure biomarkers**

Host reaction

- Complement factor B^{+}
- Serine protease-like
- Haptoglobin **rapidly binds to haemoglobin after haemolysis and tissue damage event**
- Ceruloplasmin
- Fibrinogen
- Kininogen
- Kininogen
- α2-macroglobulin

platelet activation, coagulation, fibrin clot formation, clotting cascade, inhibitor of thrombin and plasmin

in Atlantic salmon has prevented the pro-coagulant effect of serine protease from *A. salmonicida* (Salte et al., 1993)

Discussion

cell adhesion, differentiation, migration, growth, collagenous matrix, cardiomyocyte proliferation

Regeneration/ Remodelling	
•Lumican	in high levels in Humans with HF
• Retinol-binding	zebrafish - injured cardiomyocytes induce the expression of a retinoic acid-synthesizing enzyme promoting cardiomyocyte proliferation and heart regeneration
• Lipocalin	
• Fibronectin	zebrafish - epicardium regeneration after a cardiac injury mediates the attachment and entry of IHNV into cells

Conclusion

Candidate BIOMARKERS from a list of PROTEINS unique to CMS+ sera

Leakage enzymes	Host reaction	Regeneration/ Remodelling
• Creatine kinase	• Complement factor B^+	• Lumican
• Lactate dehydrogenase	• Serine protease-like	• Retinol-binding
• Glycogen phosphorylase	• Haptoglobin	• Lipocalin
• Carbonic anhydrase	• Ceruloplasmin	• Fibronectin
	• Fibrinogen	
	• Kininogen	
	• Kininogen	
	• α2-macroglobulin	

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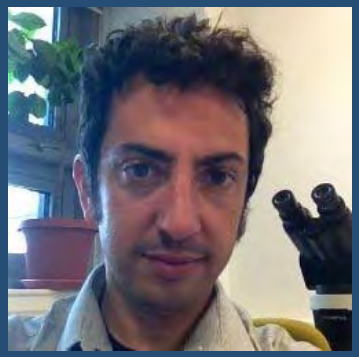

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Moredun Research Institute

Funding

Cooke Aquaculture Scotland biomarkers pump priming project

Leading to **SAIC** projects:

Assessing the use of cardiac biomarkers for early diagnostic of cardiomyopathy syndrome (CMS) (July 2019 - December 2020)

*Use of cardiac **b**iomarkers **Troponin** will be presented on the next talk by Dr. Jorge del Pozo*

Use of serum biomarkers for early differential diagnostics of cardiomyopathies of Atlantic salmon: field and challenge assessment (July 2021- September 2022)

Questions?

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https://brettfish.wordpress.com

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