Muscle Wasting in Patients with Early-Stage Lung Cancer: Identification of an Atrophying Programme

Forum de la Recherche en Cancérologie
Lyon, 4-5 Avril 2019

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Muscle loss: from an adaptive to a deleterious mechanism

Muscle main functions:

- **Force generation (locomotion, etc.)**
- **Amino acids reservoir**

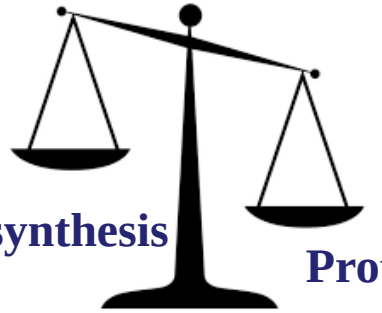
Normal situation (adult)

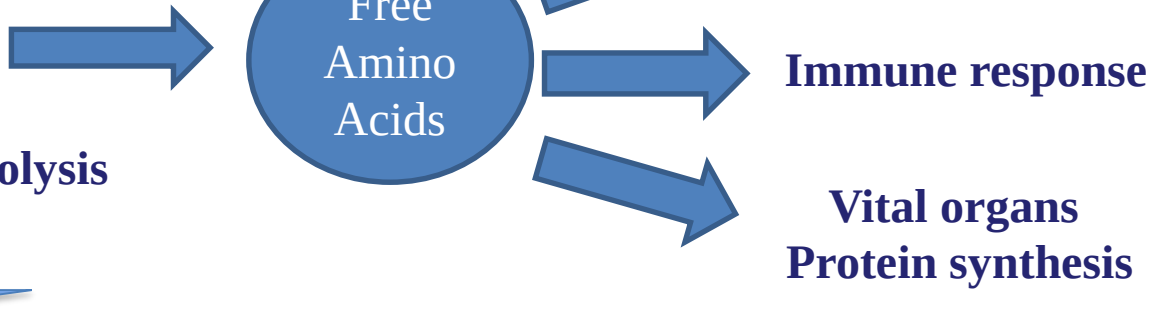
Cancer **Chronic Renal Failure**

Immobilization

Fasting

Diabetes





Reduced efficiency of treatments and of the immune response

General weakness

→ **Increased Morbidity & Mortality** → **High health care costs**

Proteolysis is the main determinant

Proteolytic Systems involved

- ✓ Calpains (cytosolic, Ca^{2+}-dependent)
- ✓ Autophagy (cathepsins, lysosomes, endosomes)
- ✓ Caspases (cytosolic)
- ✓ Proteasome (cytosolic, self-compartmentalized)

UPS: Ubiquitin Proteasome System

Is there an atrophying program including systematically (but not limited to) proteolytic systems?

Why skeletal muscles are so important for cancer patients?

Muscle loss = signal event in cancer cachexia

↓

Reduced quality of life
Increased chemotherapy toxicity

Key points Kenneth Fearon, Jann Arends and Vickie Baracos *Nat. Rev. Clin. Oncol.* 2013

- Cancer cachexia remains an important unmet medical need that affects patients' quality of life and treatment outcomes
- Cachexia can be missed in an ever-increasingly obese population
- Therapy should start early and can run in parallel with antineoplastic therapy
- There is an urgent need to establish best supportive multimodal care for cachexia: beyond good clinical or oncological care, the treatable defects in dietary intake, physical activity and systemic inflammation should be addressed
- Patients with cachexia should be actively considered for entry into clinical trials

Major goal: finding robust biomarkers allowing an early detection of muscle loss

How finding biomarkers that may help fighting against cancer cachexia?

- Specific of muscle atrophy → 2 pathologies with different etiology
- Early detection but sustained modified expression → Both early and late stage disease
- Easy to handle in hospitals and poorly invasive → Proof of concept: muscle biomarkers

Final goal: blood markers

Finding muscle wasting markers following lung cancer in humans: the PROMETHE cohort

- Control patients (CT)
- Cancer patients (pulmonary neoplasia, C)
- Hemodialysis patients (CKD)

→ Muscle biopsies following surgery
Blood samples → Transcriptomic and Proteomic Analysis

	Control patients (n=7)	Lung cancer patients (n=7)	Hemodialysis patients (n=7)	p
Age (years)	71 [60-79]	69 [62-75]	69. [66-77]	> 0.9
M:F	6 :1	6 :1	6 :1	> 0.9
Weight (kg)	76 [73-78][a]	66 [62-72][ab]	64 [59-66][b]	0.05
Height (cm)	170 [174-182][a]	173 [169-176][ab]	165 [160-170][b]	0.03
BMI	24.8 [23.5-25.1]	21.3 [20.2-24.1]	23.4 [22.4-24.4]	0.15
CRP (mg/L)	3.0 [3.0-7.4][a]	9.7 [4.1-37.3][b]	12.8 [4.4-22.5][b]	0.03
Creatinine (µmol/L)	66 [59-81][a]	82 [64-90][a]	535 [494-752][b]	<0.001
GFR mL/min/1.73m^2	88 [85-95]	84. [74-94]	NA	> 0.9

Implication of proteolytic systems on muscle wasting in CKD and cancer patients

	Cancer (n=14)	CKD (n=7)
MAFbx	↗	↗
MuRF1	↗	↗
Mdm2	↗	↗
Nedd4	→	↗
E4B	→	↗
UBE2B	→	→
UBE2D2	→	→

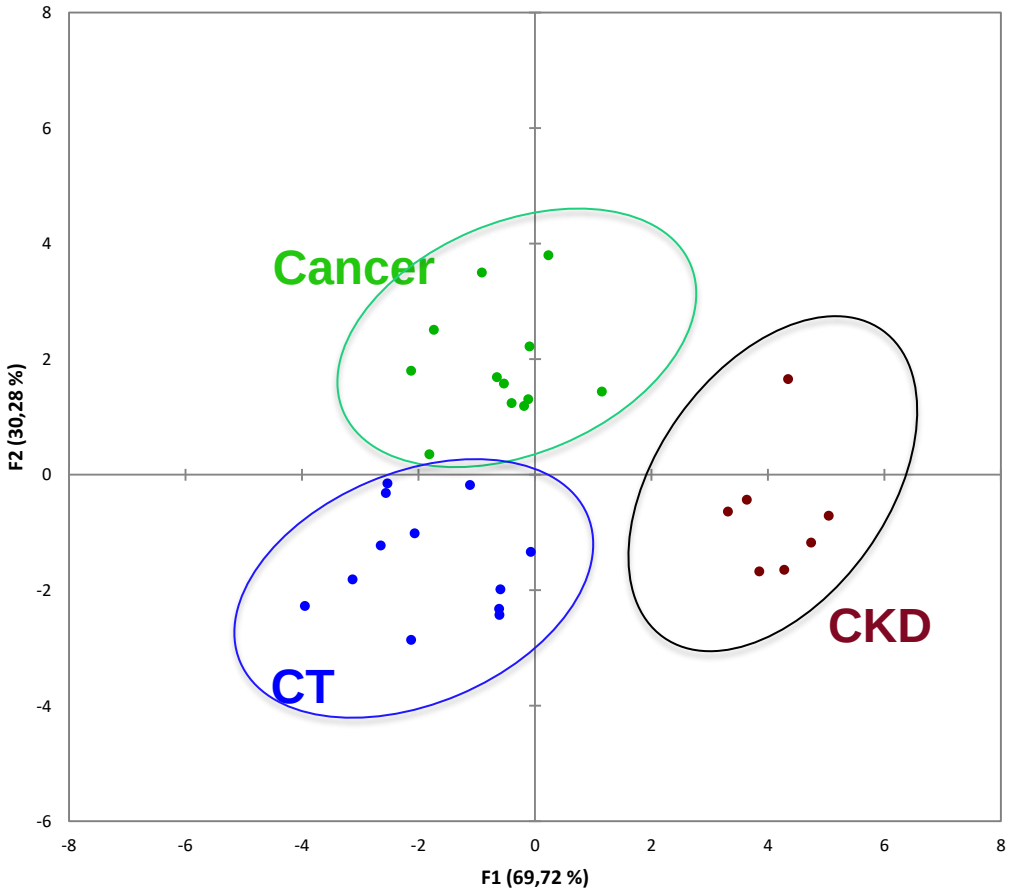
	Cancer (n=14)	CKD (n=7)
C2, C5, C8	→	→
S2	→	↗
S4	→	→
S5a	→	↗
S11	→	↗
S12	→	→
Casp3	→	↗
Casp9	→	↗

So far, only a limited number E3 ligases are good biomarkers of muscle atrophy

Adapted from *Aniort et al. JCSM, 2018*

Transcription analysis of muscles from CKD and cancer patients

mRNA expression of proteolytic enzymes combined and used as two variables for each patient



Adapted from
Aniort et al. JCSM, 2018

Discriminant analysis of mRNA levels from 17 actors of proteolytic systems (UPS and caspases) efficiently separates the 3 populations

Proteomic analysis of muscles from CKD and cancer patients

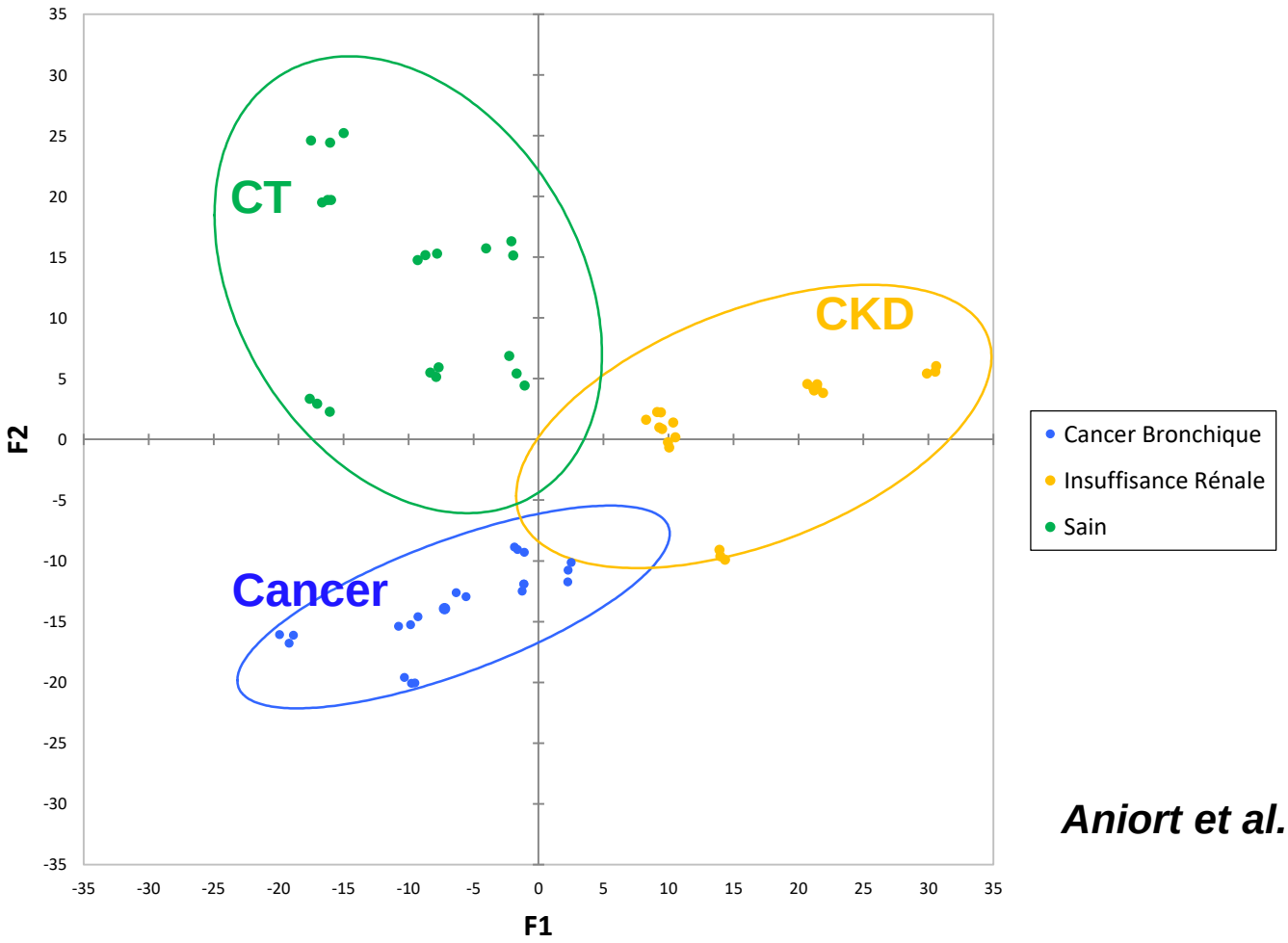
- 21 patients (n = 7 per group)
- **Cytoplasmic**, myofibrillar and UPS substrates
- Shot gun analysis using nanoLC-MS/MS (Ultimate3000 system coupled to an LTQ-Orbitrap Velos mass spectrometer)
- **MaxQuant** (Max Planck Institute) and **Proline** (EDyP-Grenoble, IPBS-Toulouse, IPHC/LSMBO-Strasbourg) analysis

- **1779 proteins identified**
- **919 differentially expressed proteins**
- **257 proteins either increased or decreased in both Cancer and CKD patients**

Principal Component Analysis (PCA)

Proteomic analysis of muscles from CKD and cancer patients

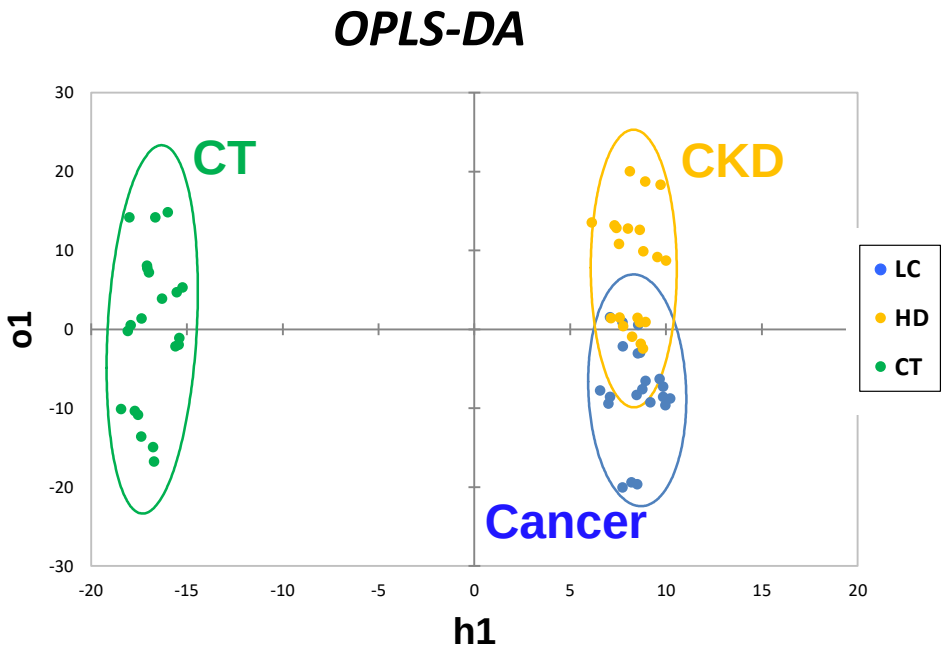
Principal Components Analysis

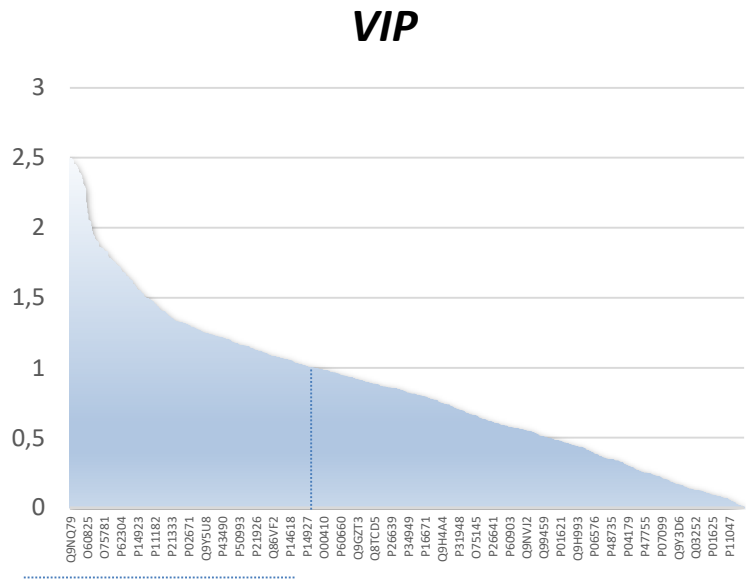


Aniort et al. JCSM, 2018

Discriminant analysis of differentially expressed proteins efficiently separates the 3 populations

Discriminant Analysis on proteins that best characterized atrophying muscles independently of the pathology





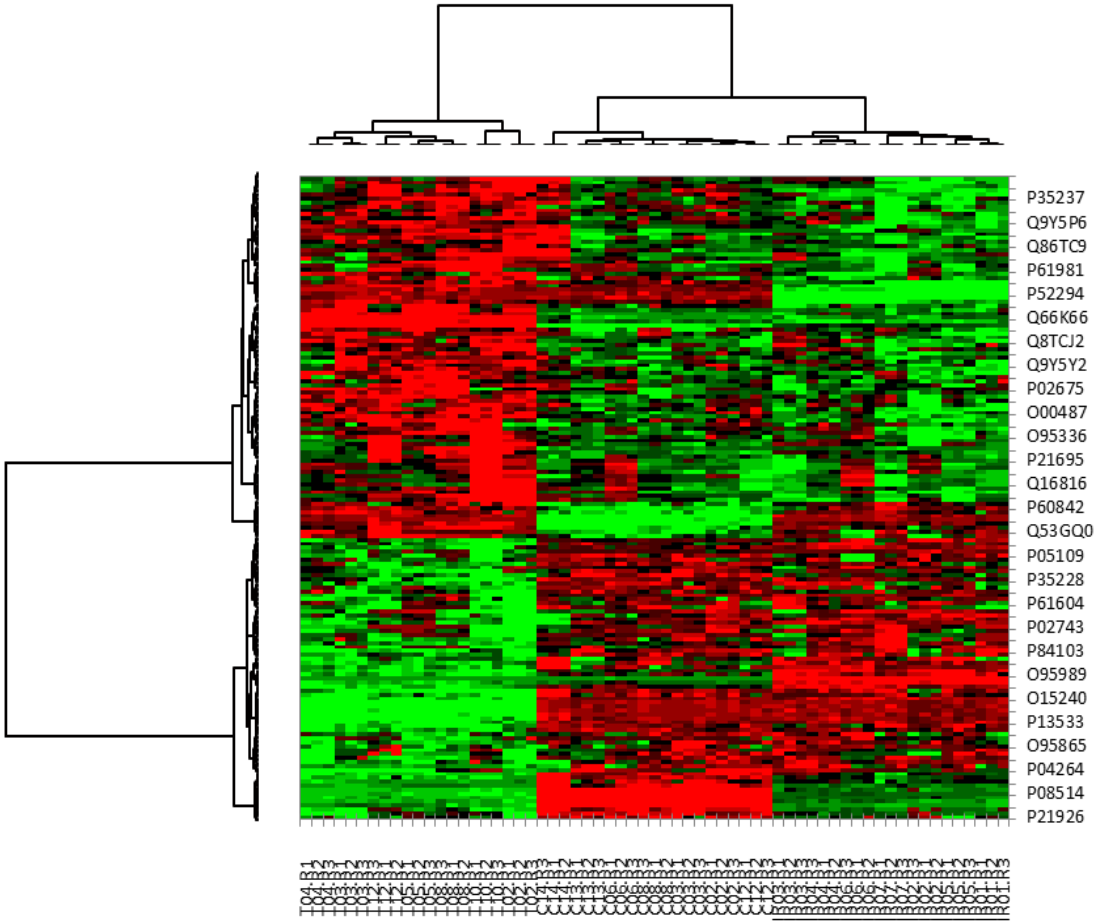
321 proteins VIP > 1 crossed with PCA analysis (**257** proteins)

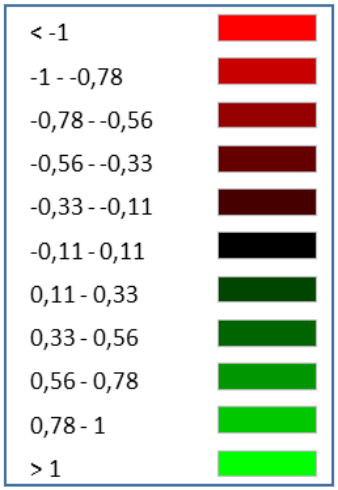
→ **238** significant in both pathologies

Discriminant analysis of differentially expressed proteins efficiently separates the 3 populations

Aniort et al. JCSM, 2018

Unsupervised hierarchical cluster analysis



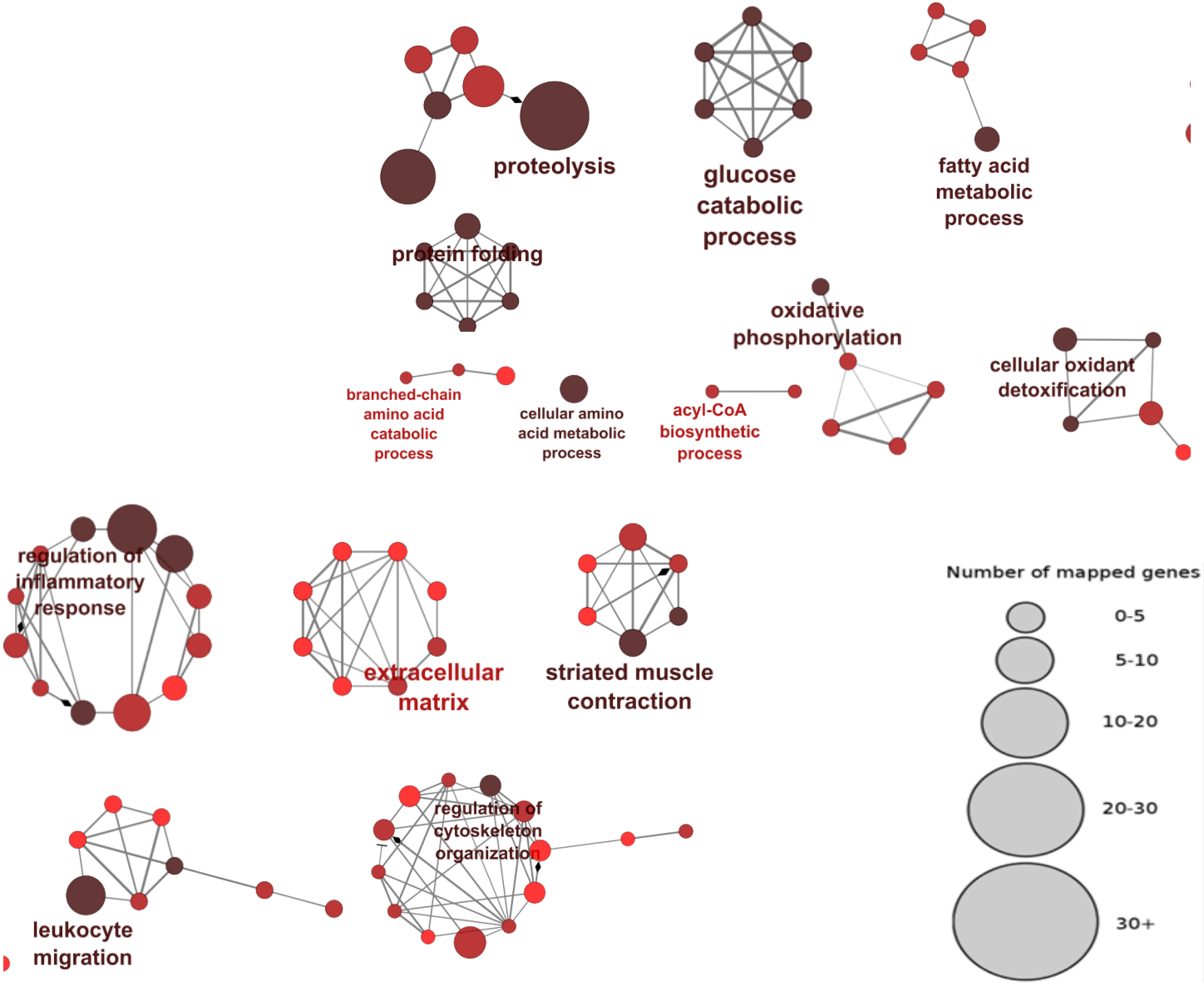


Aniort et al. JCSM, 2018

Most selected proteins (238) are good predictors of muscle atrophy independently of the disease

Biological Processes

Cytoscape software Clue GO application



Conclusions and ongoing experiments

Specific markers of muscle atrophy were found independently of the pathology

- MuRF1 and MAFbx are the best (but not the only) mRNA biomarkers as in animal models
- Both proteolysis-linked mRNAs and specific proteomes discriminate healthy and pathology-developing patients
- Down regulation of several proteins involved in cell growth/proliferation and organization

- Finding biomarkers in more accessible compartments (e.g. blood) that are directly correlated to muscle atrophy markers
  - RNAseq analysis: miRNA and mRNA
    - in progress: > 1500 potential markers
      > 20 blood markers directly witnessing muscle atrophy
- Using more patients/other cohorts for strengthening/validating the conclusions

Acknowledgements

Lab members and students

Daniel Béchet
Agnès Claustre
Lydie Combaret
Cécile Coudy
Christiane Deval
Cécile Polge

(Nephrology Department)
Julien Aniort
C. Philipponnet
A. Poyet

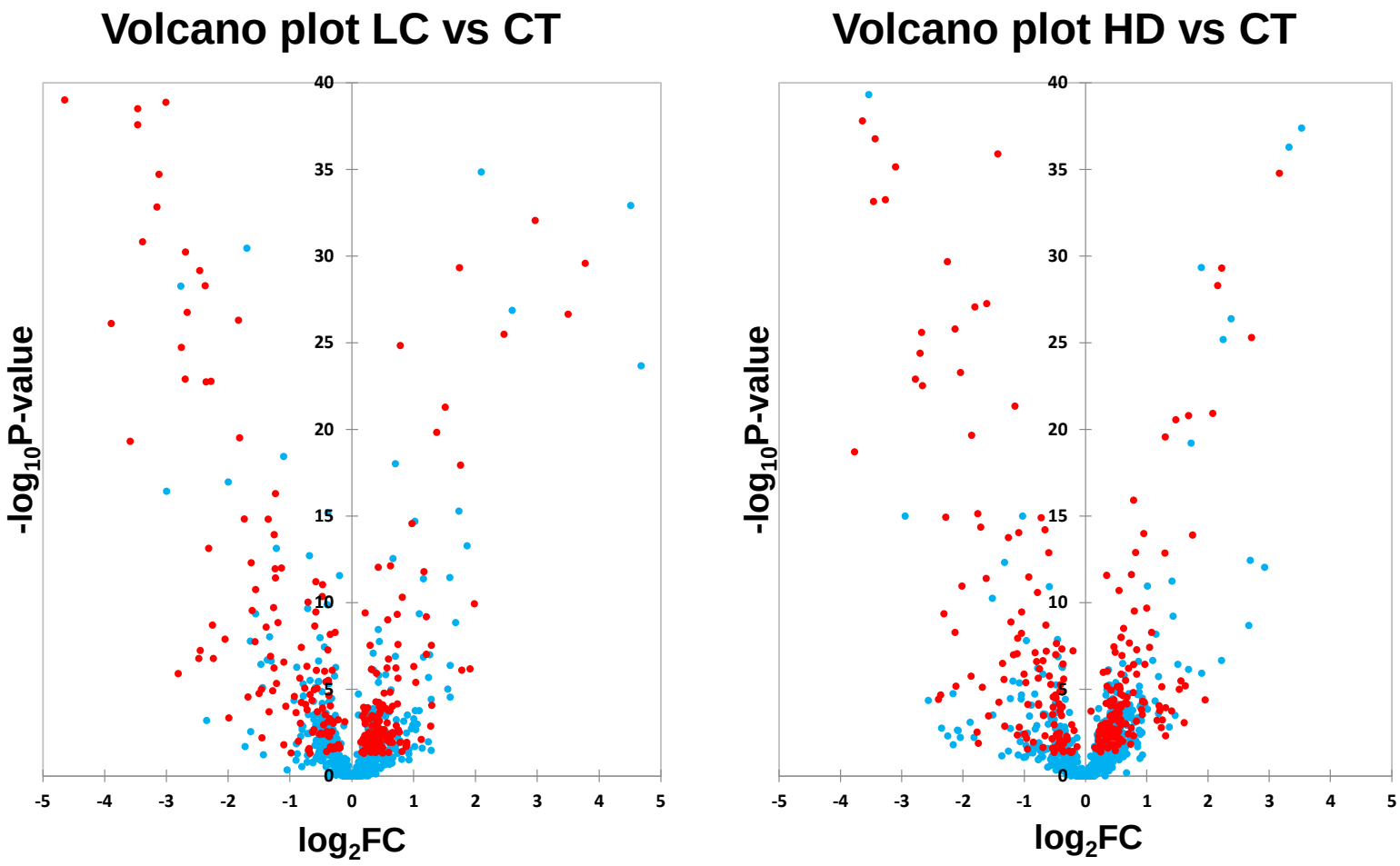
Anne-Elisabeth Heng (Nephrology Department)
Daniel Taillandier

Collaborators

S. Boisgard (Orthopedic Surgery Department)
E. Rosset (Vascular Surgery Department)
M. Filaire (Thoracic Surgery Department)

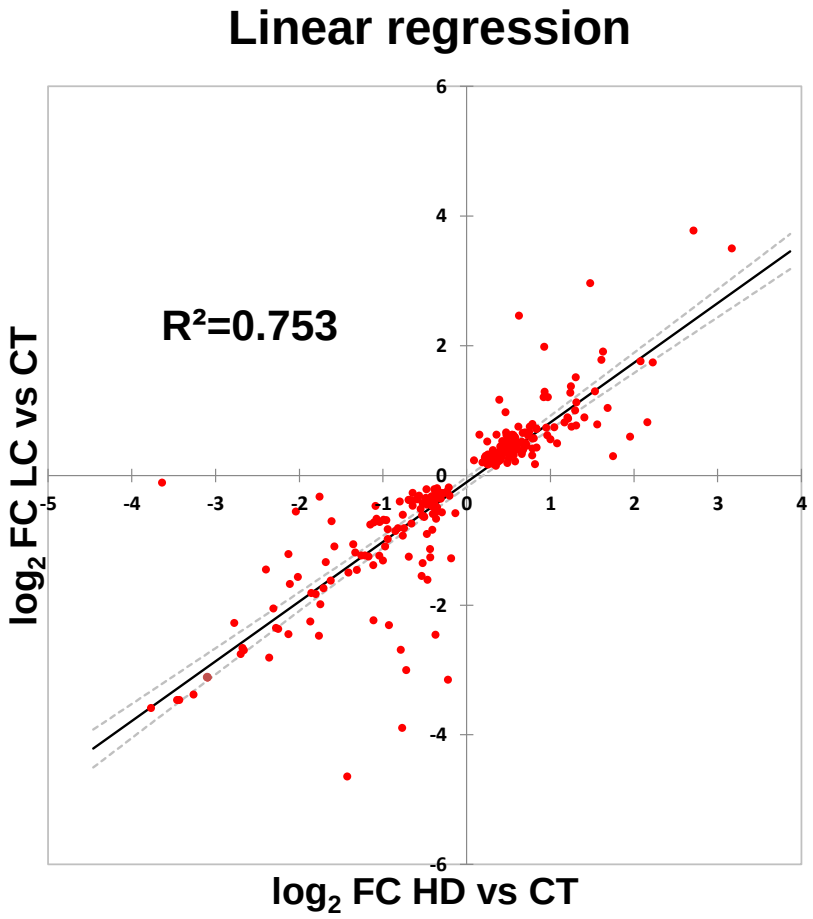
Odile Schiltz
Alexandre Stella

Financial support



- Proteins significantly increased or decreased both in LC and HD patients

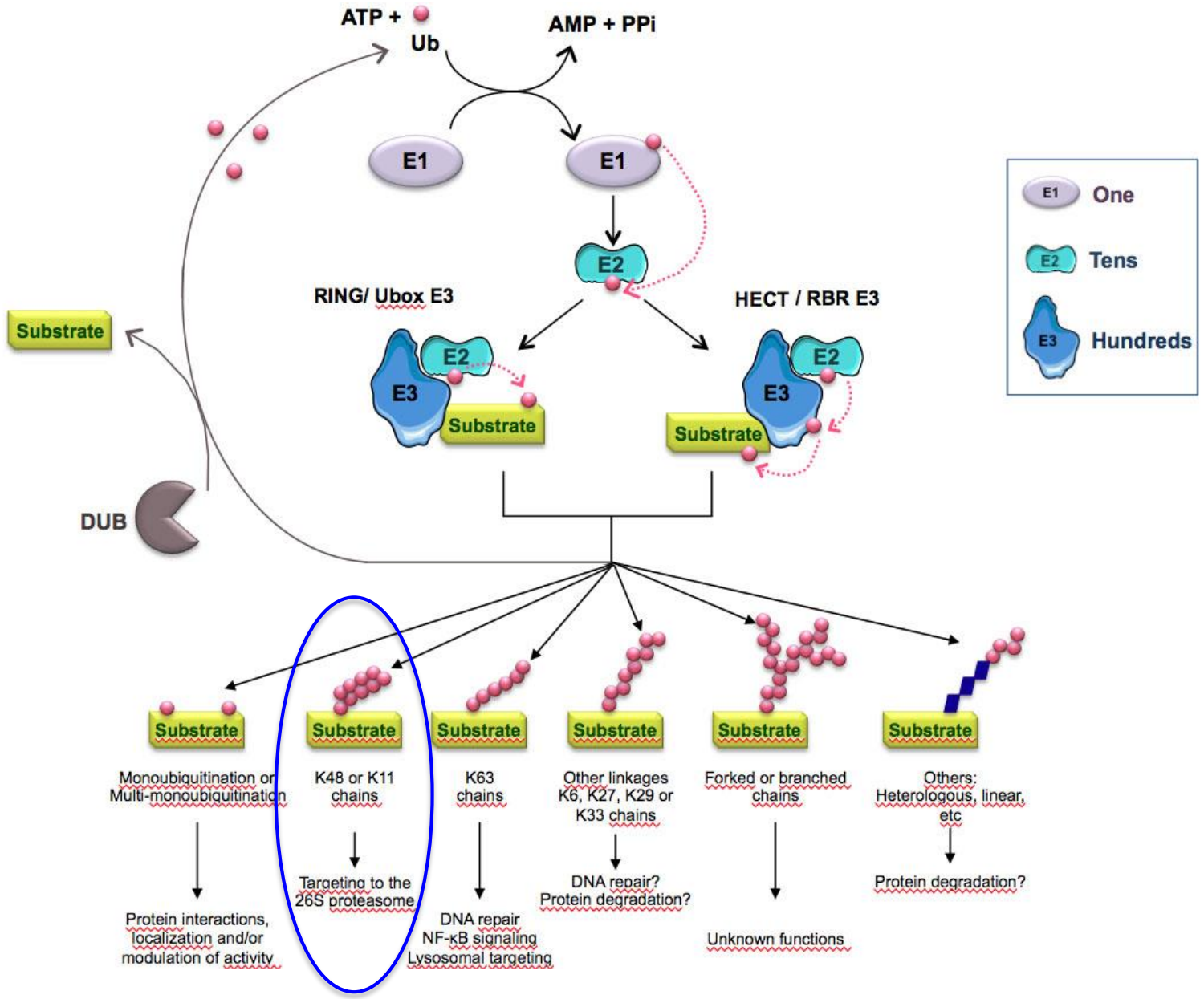
Differentially-expressed proteins exhibit highly similar variation levels independently of the disease



Fold change in protein expression in LC vs. HD patients relative to CT patients

CT, healthy; HD, haemodialysis; LC, lung cancer

Ubiquitin Proteasome System (UPS)

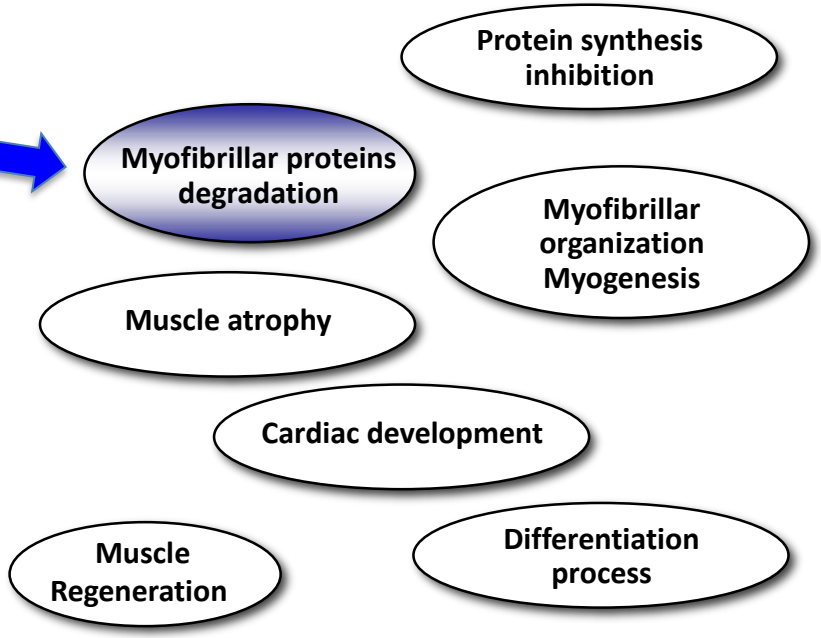


Ubiquitination in various human diseases

E3 ligase	Targeted protein or pathway	Ubiquitin chain type	Disease
FANCL	FANCD2, FANC1	monoUb	Fanconi anemia
DDB2	Chromatin	monoUb	Xeroderma pigmentosum
Cbl family	RTKs	monoUb	Cancer
Nedd4	PTEN, α-synuclein	monoUb, Lys63, polyUb	Cowden syndrome, Parkinson
Rabex-5	Ras	monoUb	Cancer
HDM2	p53	Lys48	Cancer
APC/C10	Cyclin–CDK	Lys48	Genomic instability
SOCS1/3	IRS2	Lys48	Metabolic syndrome
MG53	IR, IRS1	Lys48	Metabolic syndrome
pVHL15	HIF	Lys48	Von Hippel Lindau
IAPs	NIK	Lys48	Multiple myeloma
Rnf168	Histones	Lys63, polyUb	Cancer, RIDDLE syndrome
TRAF6	TRAF6, NEMO, huntingtin	Lys63, polyUb	Inflammatory disease, Huntington's disease
Itch, IAPs	RIP2	Lys63, polyUb	Crohn's disease
Parkin	Mitochondrial outer membrane proteins	Lys63, polyUb	Parkinson's disease
CHIP, Parkin	Huntingtin, β-amyloid, tau	Lys63, polyUb	Huntington's disease, Alzheimer's disease
BRCA1	BRCA1	Atypical polyUb	Breast and ovarian cancer
LUBAC	NEMO	Linear polyUb	Autoinflammation, muscular amylopectinosis, bacterial infections

E3 ligases impact muscle protein homeostasis

- **Muscle-specific E3s**
  - MAFbx
  - MuRF1
  - ASB2
  - Ozz
  - E4b/UFD2a
  - Fbxo30/MUSA1
  - SMART
- **Ubiquitous E3s**
  - Trim32
  - Nedd4



The FASEB Journal article fj.11-180968. Published online August 2, 2011.

The FASEB Journal • Research Communication

Muscle actin is polyubiquitinylated *in vitro* and *in vivo* and targeted for breakdown by the E3 ligase MuRF1

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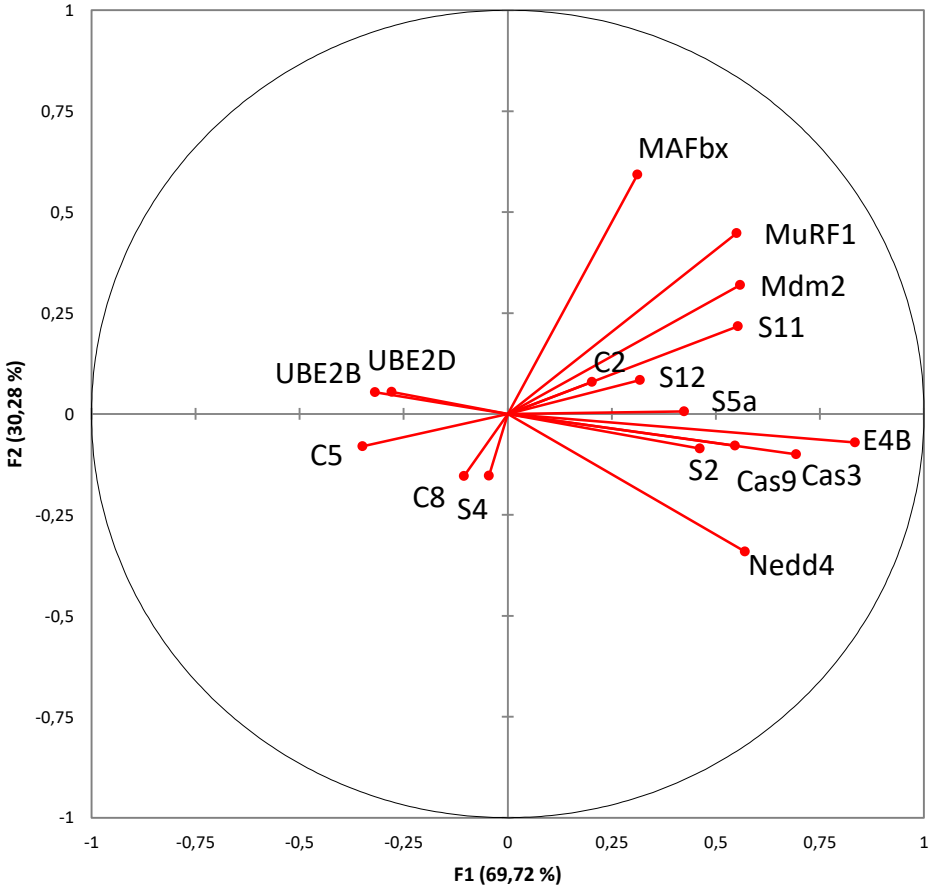
Transcription analysis of muscles from CKD and cancer patients

Catabolic/control expression ratio of proteolytic enzymes



Discriminant analysis of mRNA levels confirm that some E3 ligases (MuRF1, MAFbx and Mdm2) are the best predictors for muscle atrophy in CKD and cancer patients