ERNDIM 2025 SYMPOSIUM

ERNDiM

Thursday 9th
Friday 10th
October 2025
Madrid SPAIN
Crowne Plaza Madrid
Centre Retiro

From Concept to Feasibility: Functional Mitochondrial Diagnostics via a Single Blood Sample

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CENTER FOR
CANCER BIOLOGY

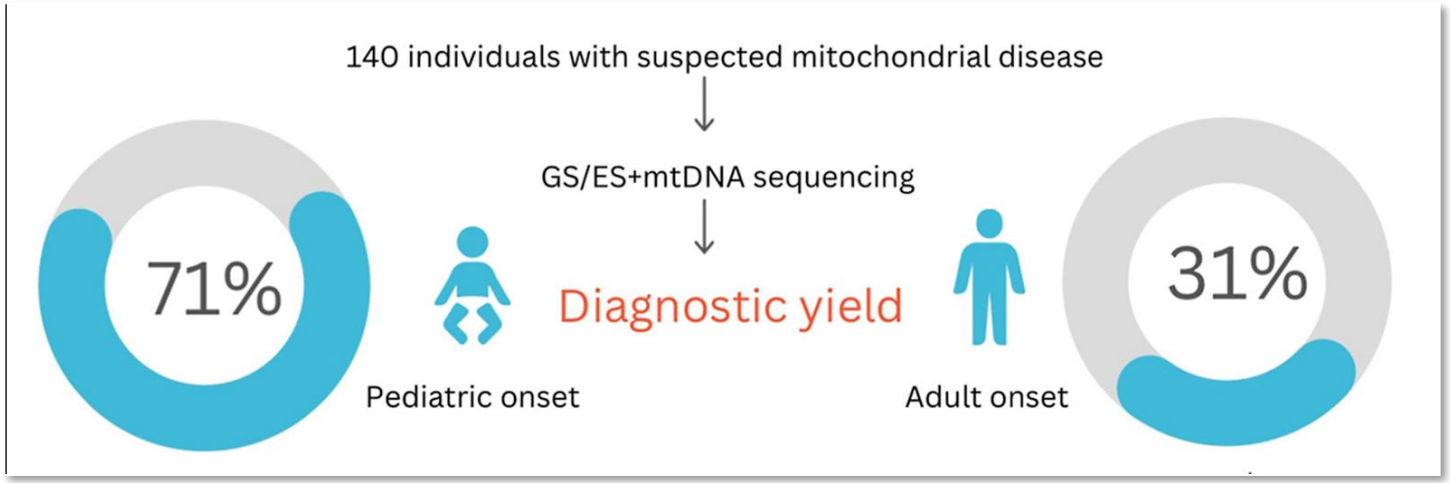
UZ LEUVEN

Conflicts of interests

- No conflicts of interests

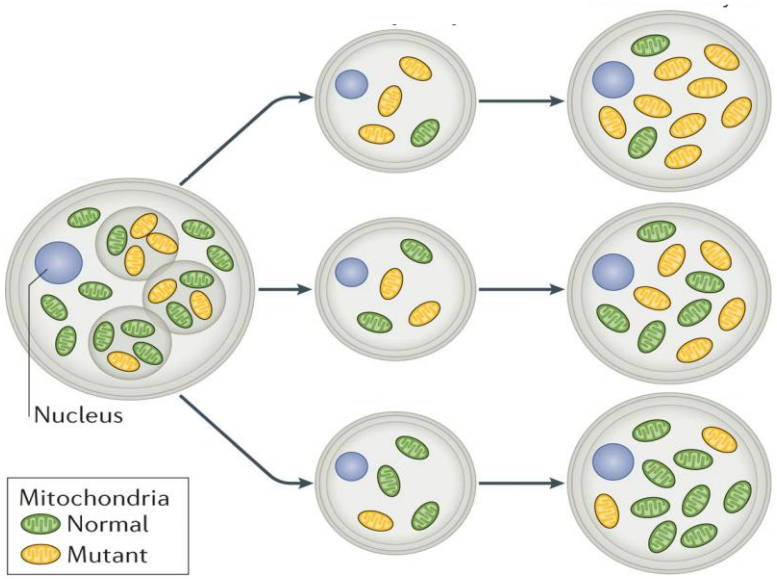
- Flemish Research Foundation (FWO) & UZ Leuven

The diagnostic odyssey of mitochondrial diseases

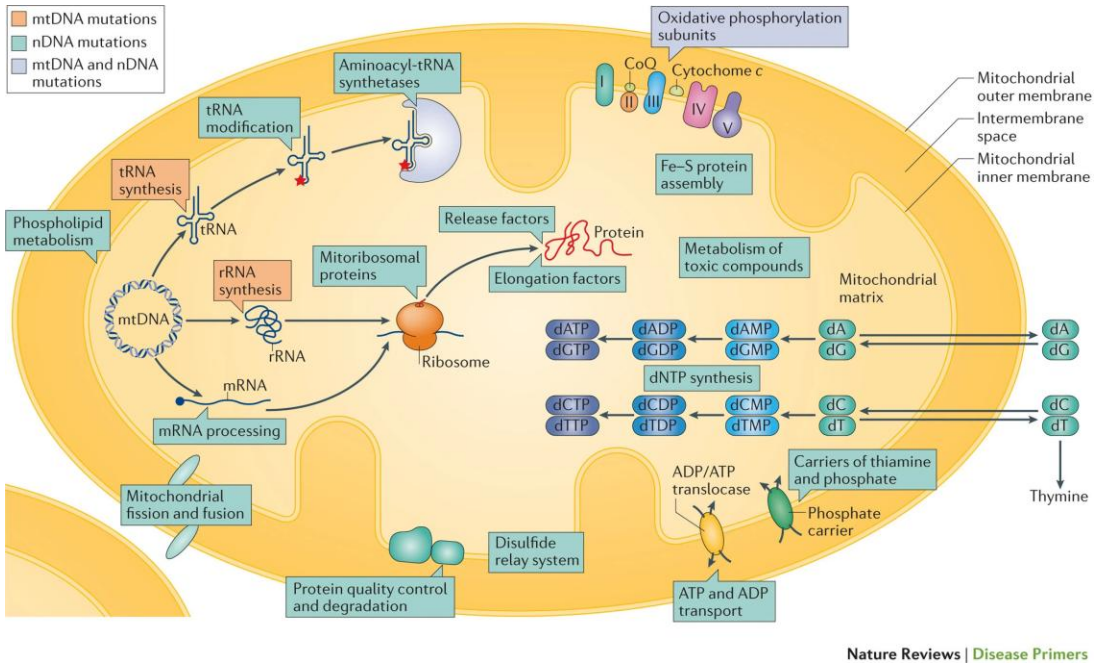


Genetics in Medicine, 2025, Rius *et al.*

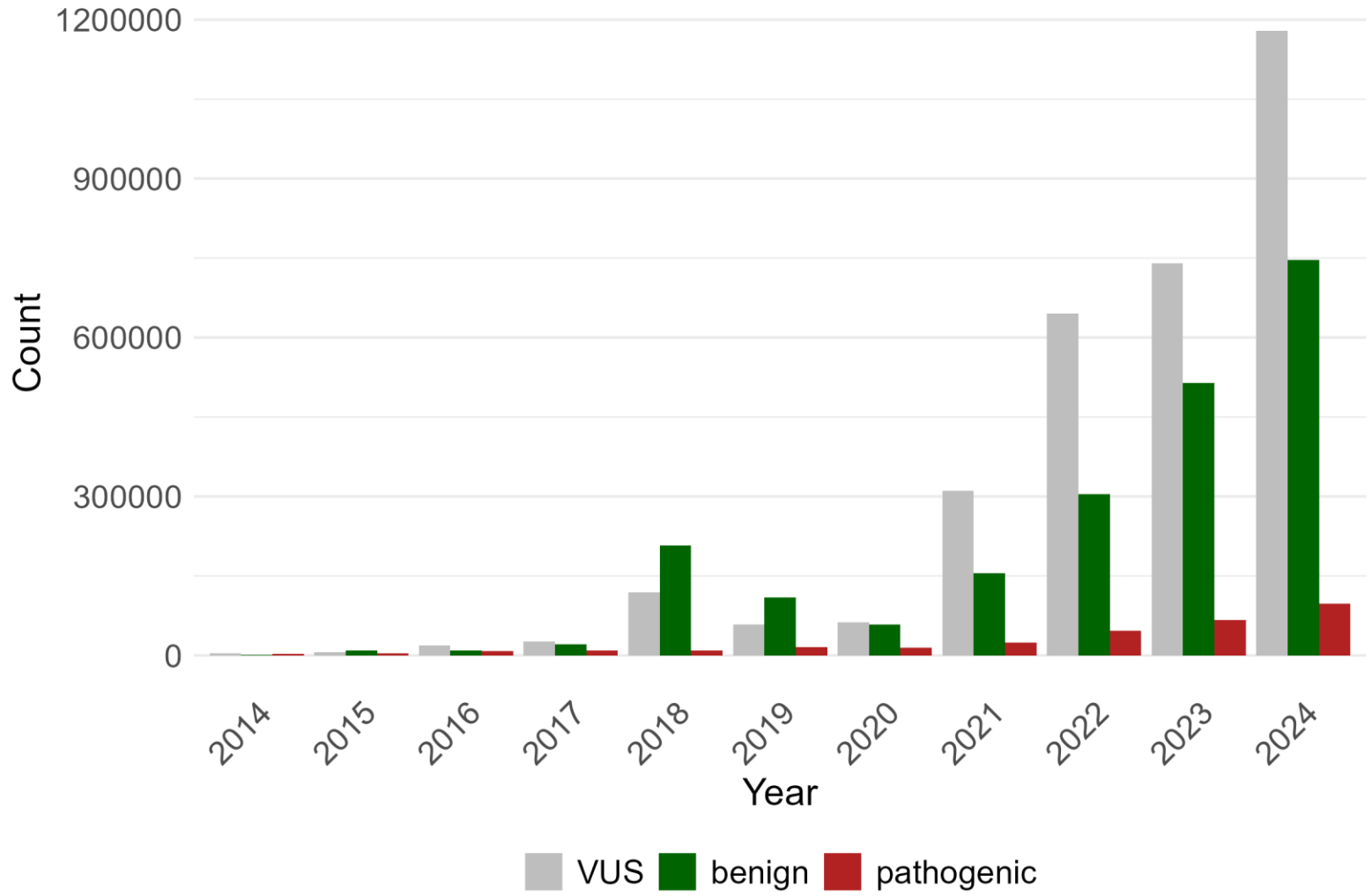
The complexity of mitochondrial diseases



Adapted from Mitochondrial diseases, *Nature Revies Disease primers*, 2016, Gorman *et al.*

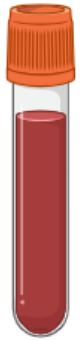


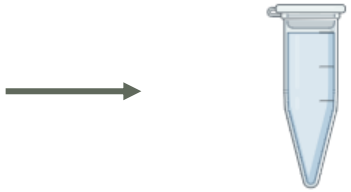
And then there is VUS…

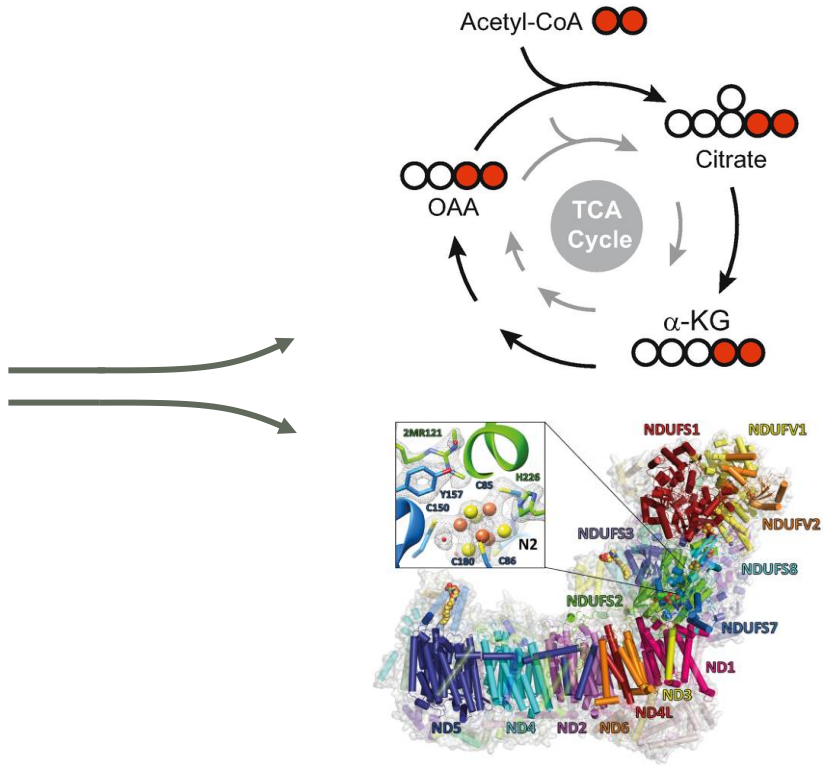


Inspired by work of prof. Vaz FM, EMG Conference, 2025

–omics as answer?



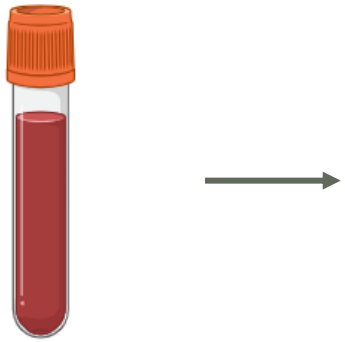


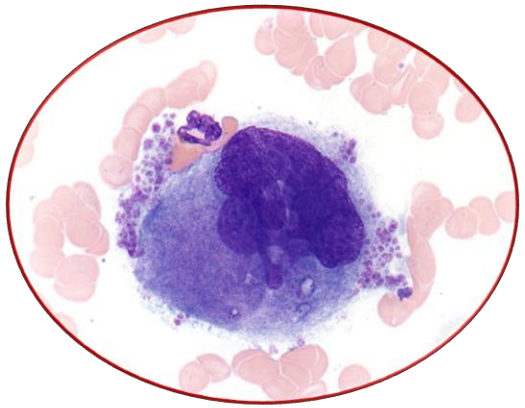


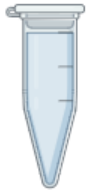
Why -omics? *Phenotyping (VUS)!*

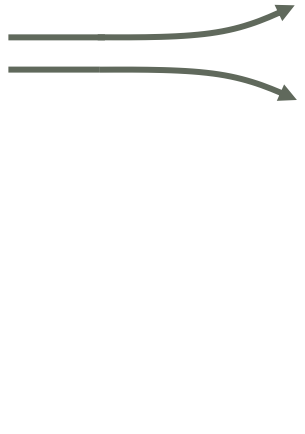
- Met: Δ OXPHOS → Δ labeling
- Prot: Individual protein ↓
  stability complex ↓
  stressmarkers ↑

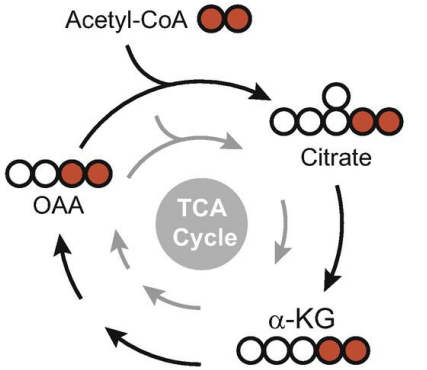
Platelets –omics as answer?

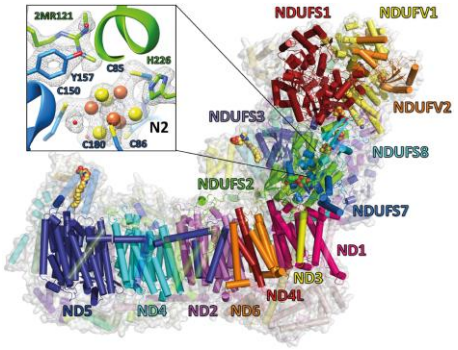












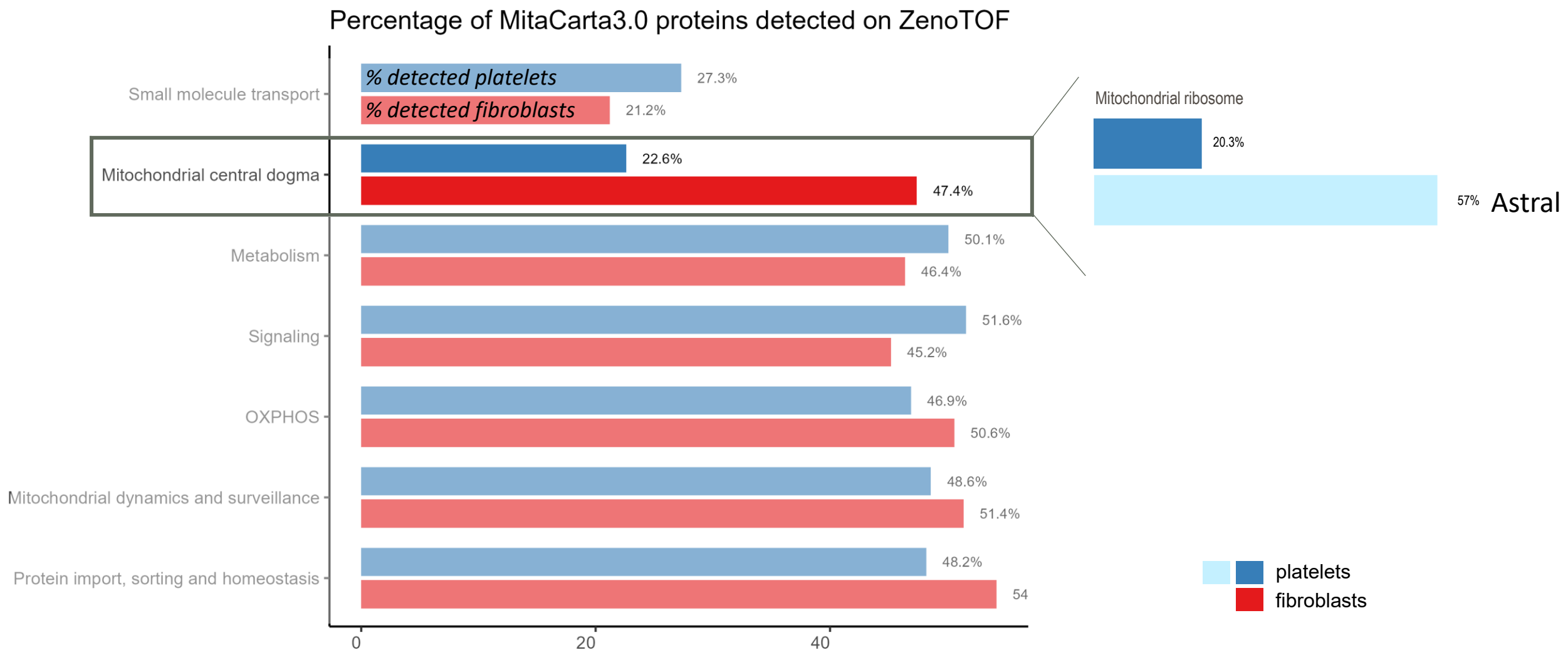
Why Platelets?

- Reliant on OXPHOS
- Non-invasive sampling
- Tissue is not an issue
- Ultra-rapid & high-throughput analysis possible

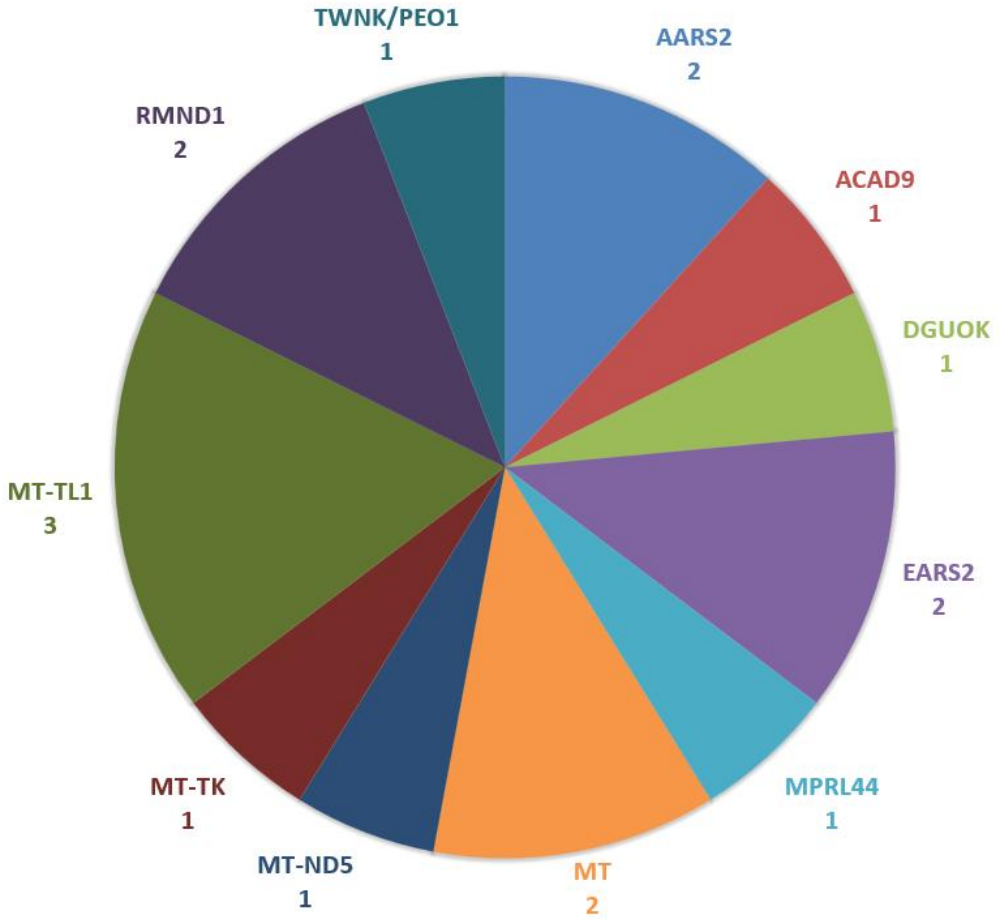
Why -omics? *Phenotyping (VUS)!*

- Met: Δ OXPHOS → Δ labeling
- Prot: Individual protein ↓
  stability complex ↓
  stressmarkers ↑

Platelets mito-proteome is comparable to that of fibroblasts



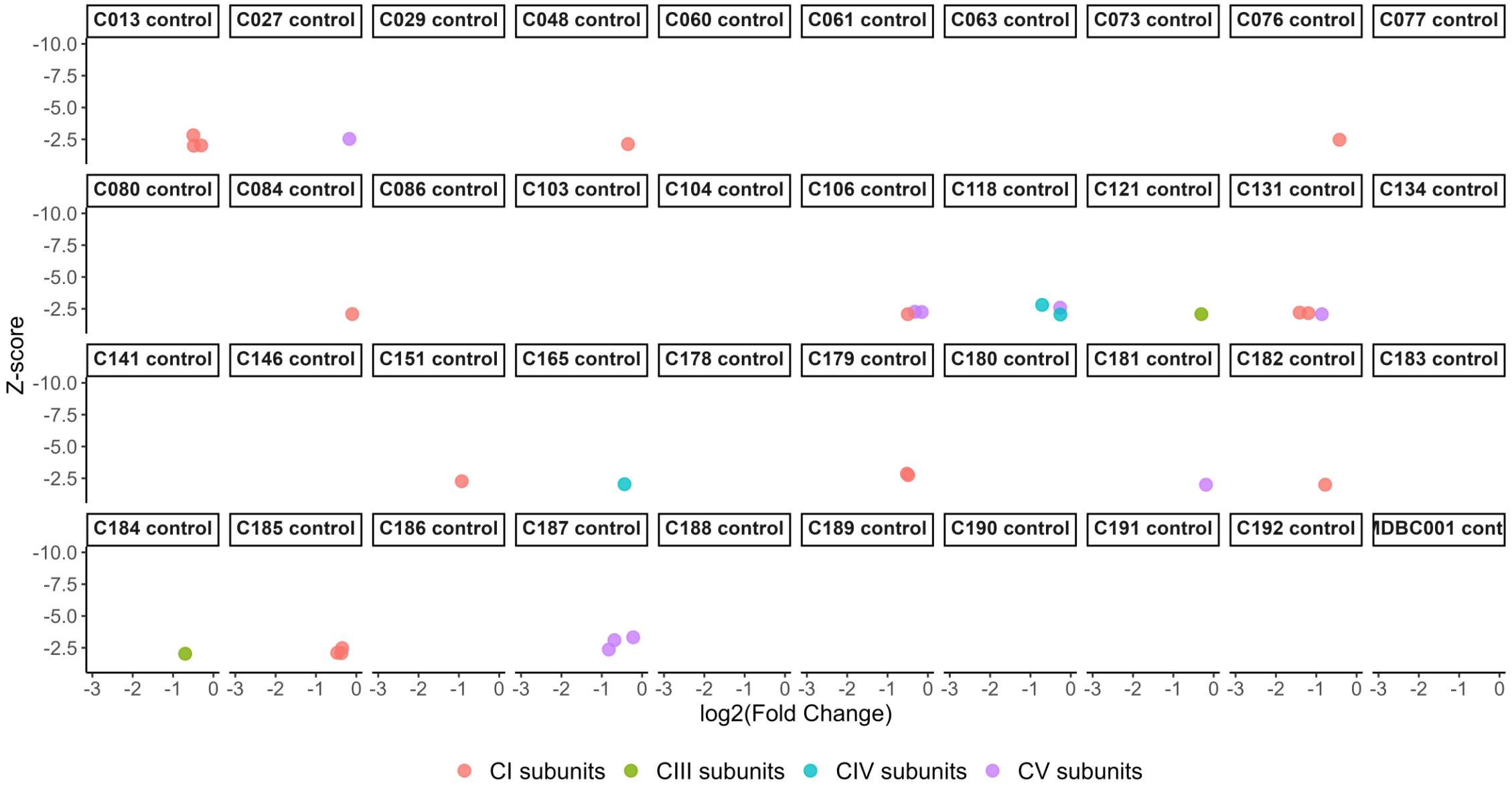
Study Cohort



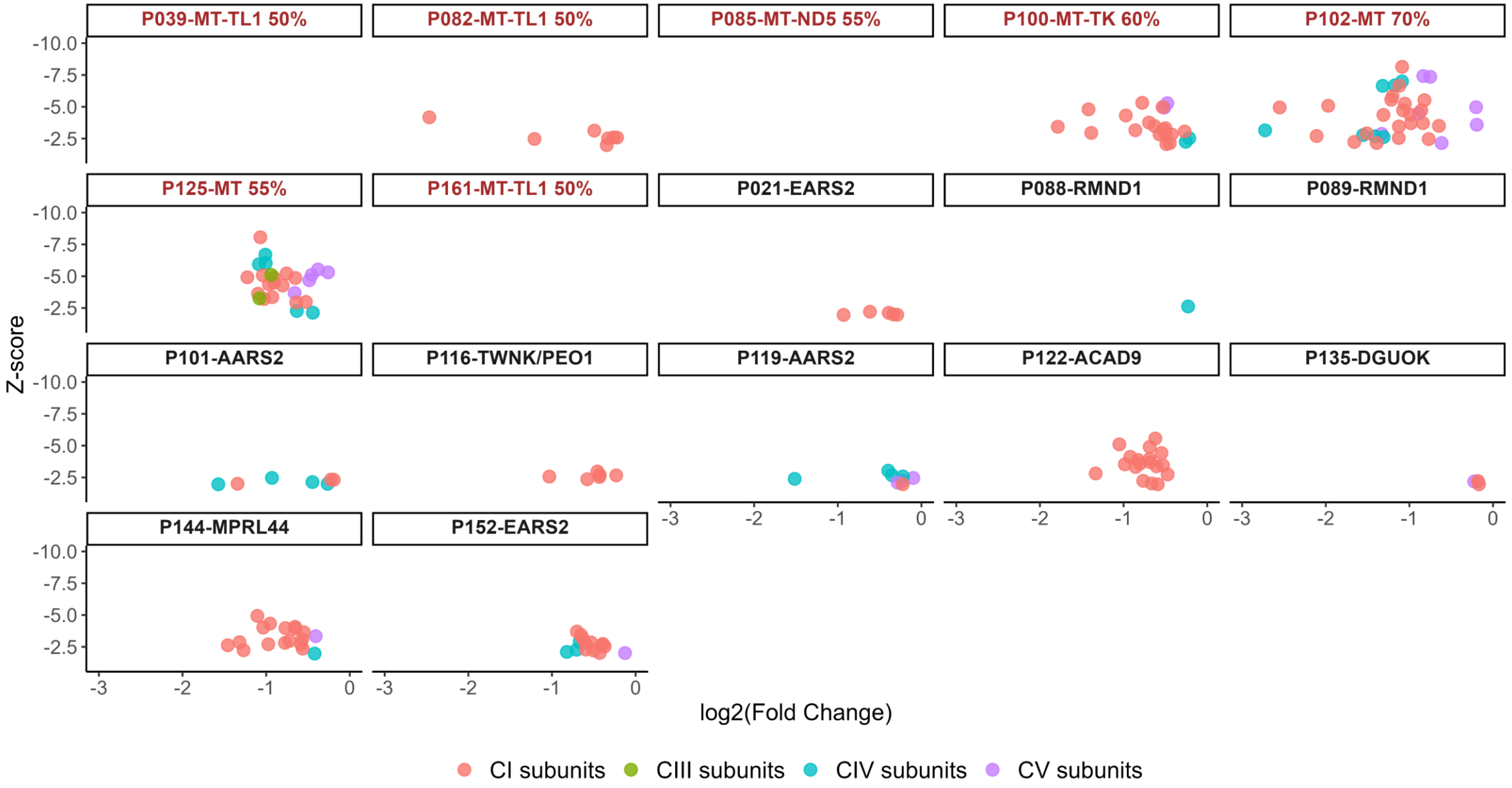
Mito-patients and their affected genes

COHORT	MITOCHONDRIAL	CONTROL
n	17	44
% female	47 %	52 %
Age (years)	30 (± 24)	36 (± 11)
Nuclear mutation	10/17	NA

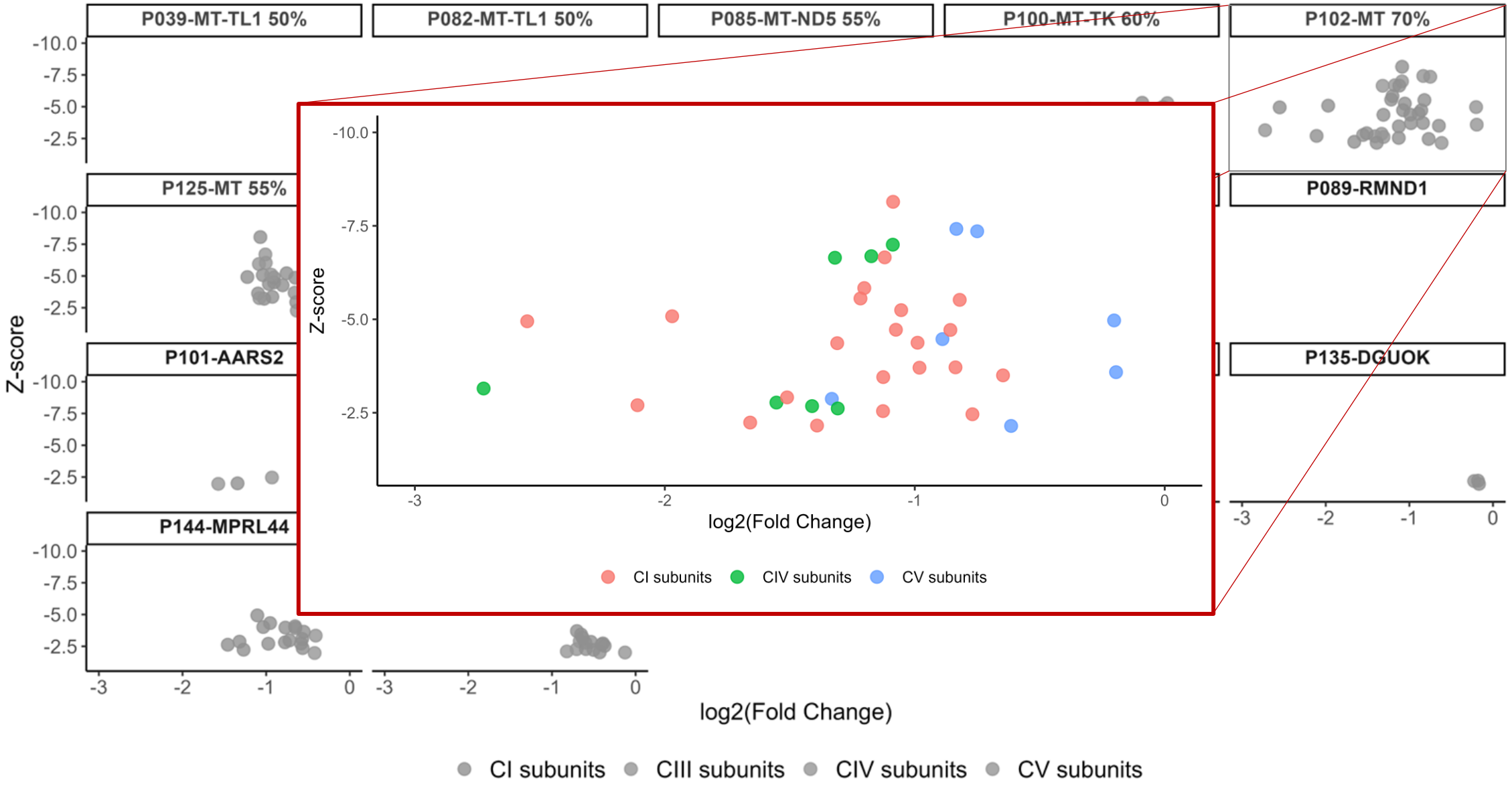
“Controls” show normal OXPHOS expression



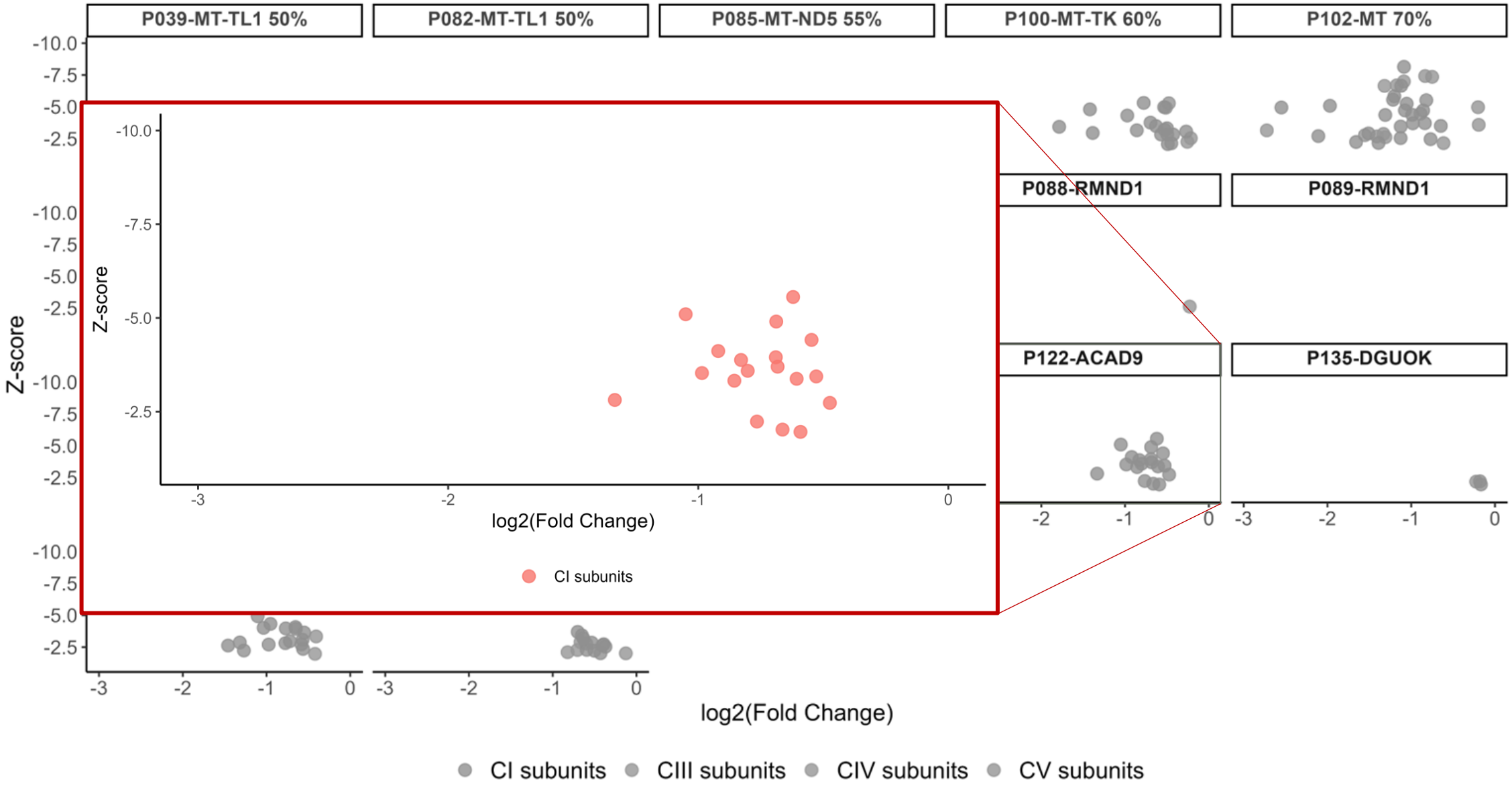
The spectrum of mitochondrial patients:



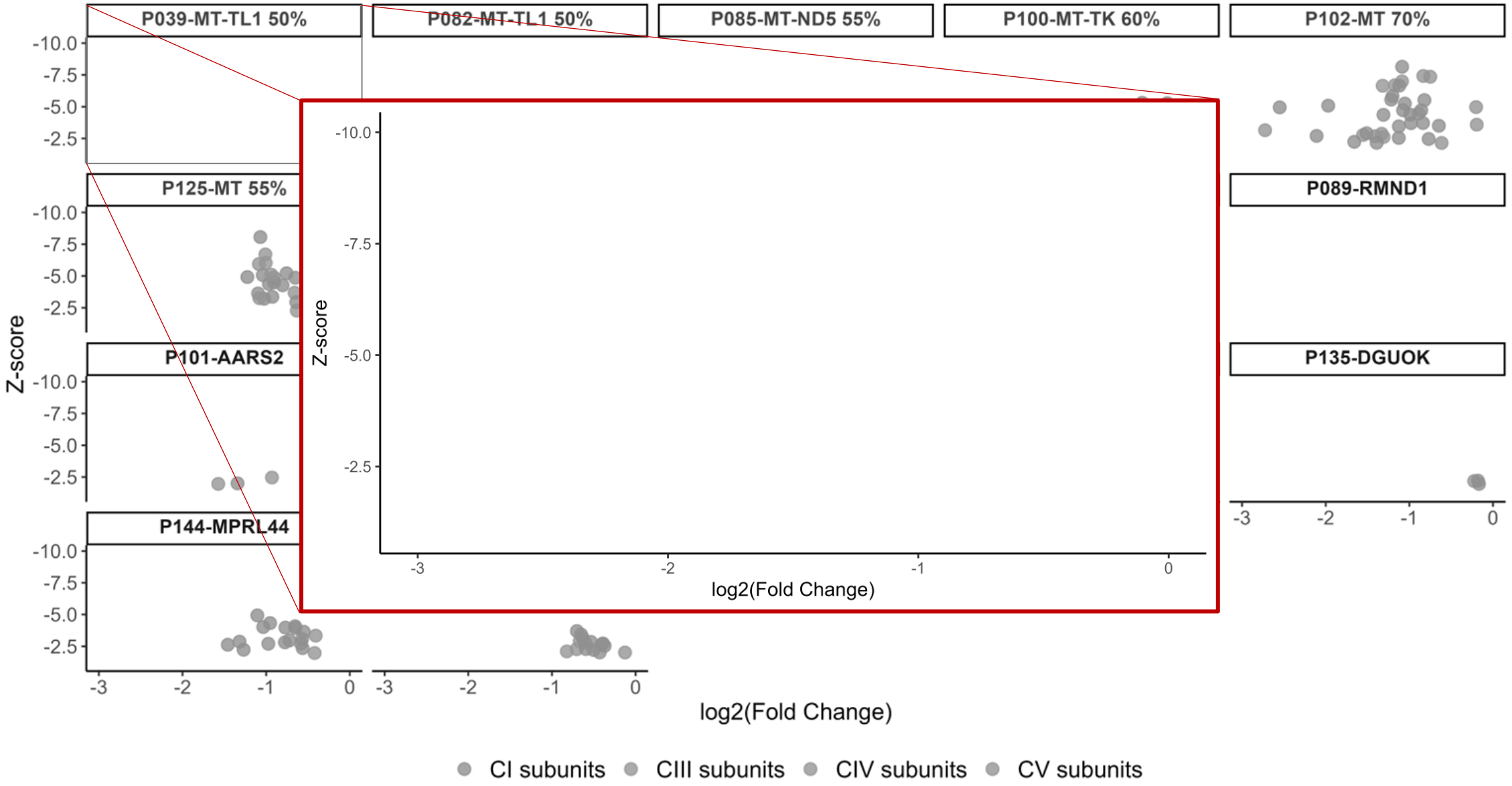
From one look, one diagnosis…



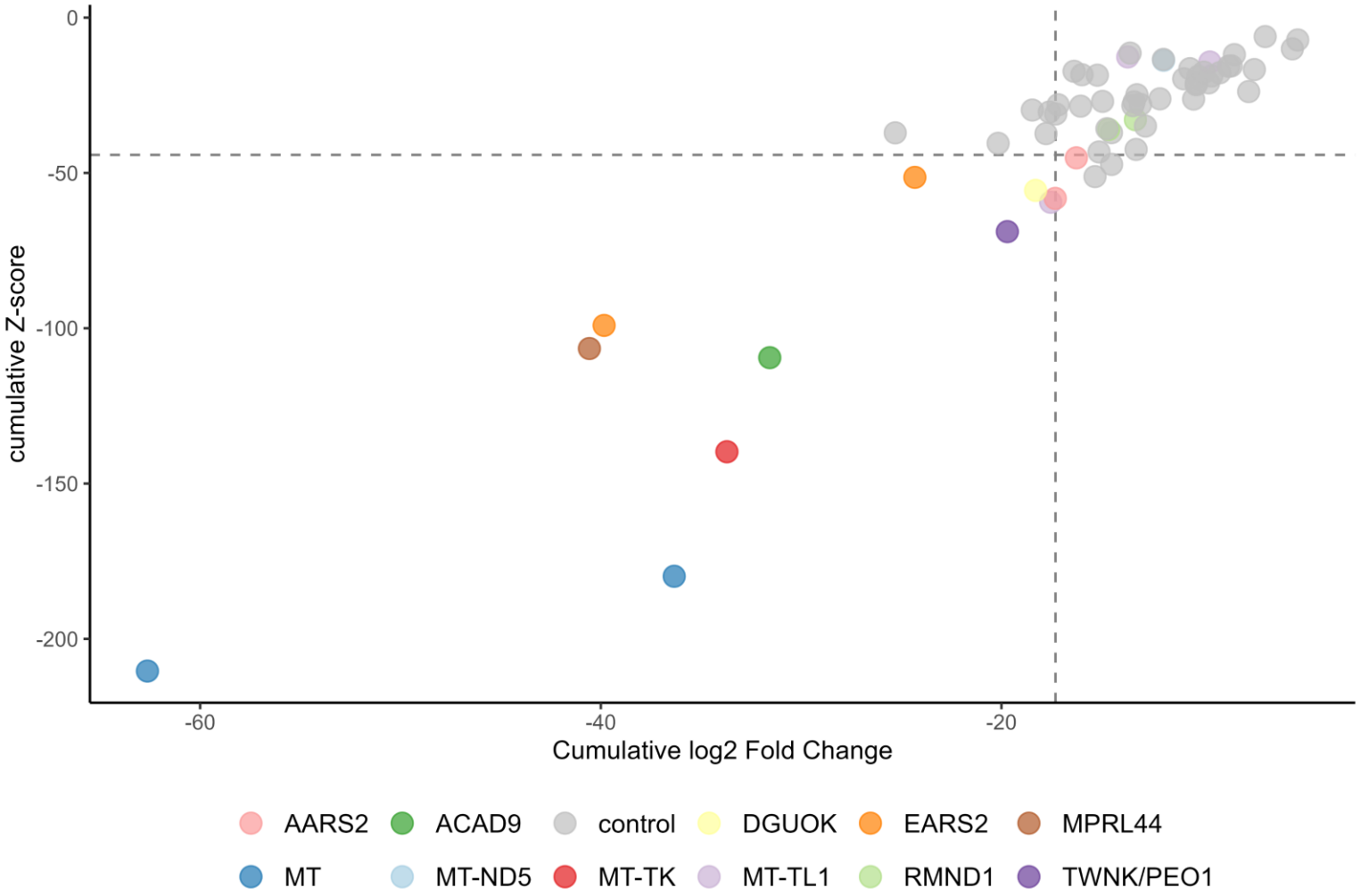
From one look, one diagnosis…



…to no apparent phenotype



Cumulative downregulation of all complexes



Performance “human” pattern recognition

Test	Positive likelihood ratio	Negative likelihood ratio	Sensitivity (%)	Specificity (%)	Accuracy (%)	Optimal cut-off
≥ 1 downregulated protein						
all MitoCarta3.0 proteins						
Z-score	25.9	0.4	59	98	87	-3.4
Log 2 (fold change)	1.9	0.5	65	66	66	-3.2
Pythagorean vector	2.8	0.3	76	73	74	3.6
≥ 1 downregulated protein						
only OXPHOS proteins						
Z-score	3.0	0.2	82	73	75	-2.2
Log 2 (fold change)	2.6	0.4	71	73	72	-2.4
Pythagorean vector	3.6	0.2	82	77	79	2.8
Cumulative complex downregulation						
only OXPHOS proteins						
Z-score	15.5	0.3	71	95	89	-44.1
Log 2 (fold change)	5.7	0.4	65	89	82	-17.3
Pythagorean vector	∞	0.4	65	100	90	52.4

Performance

Test	Positive likelihood ratio	Negative likelihood ratio	Sensitivity (%)	Specificity (%)	Accuracy (%)	Optimal cut-off
≥ 1 downregulated protein						
all MitoCarta3.0 proteins						
Z-score	6.0	0.4	59	98	87	-2.2
Log 2 (fold change)	4.9	0.4	65	93	86	-0.4
Pythagorean vector	2.8	0.3	76	73	74	3.6
≥ 1 downregulated protein						
only OXPHOS proteins						
Z-score	3.0	0.2	82	73	75	-2.2
Log 2 (fold change)	2.9	0.2	82	74	76	-0.4
Pythagorean vector	3.6	0.2	82	77	79	2.8
Cumulative complex downregulation						
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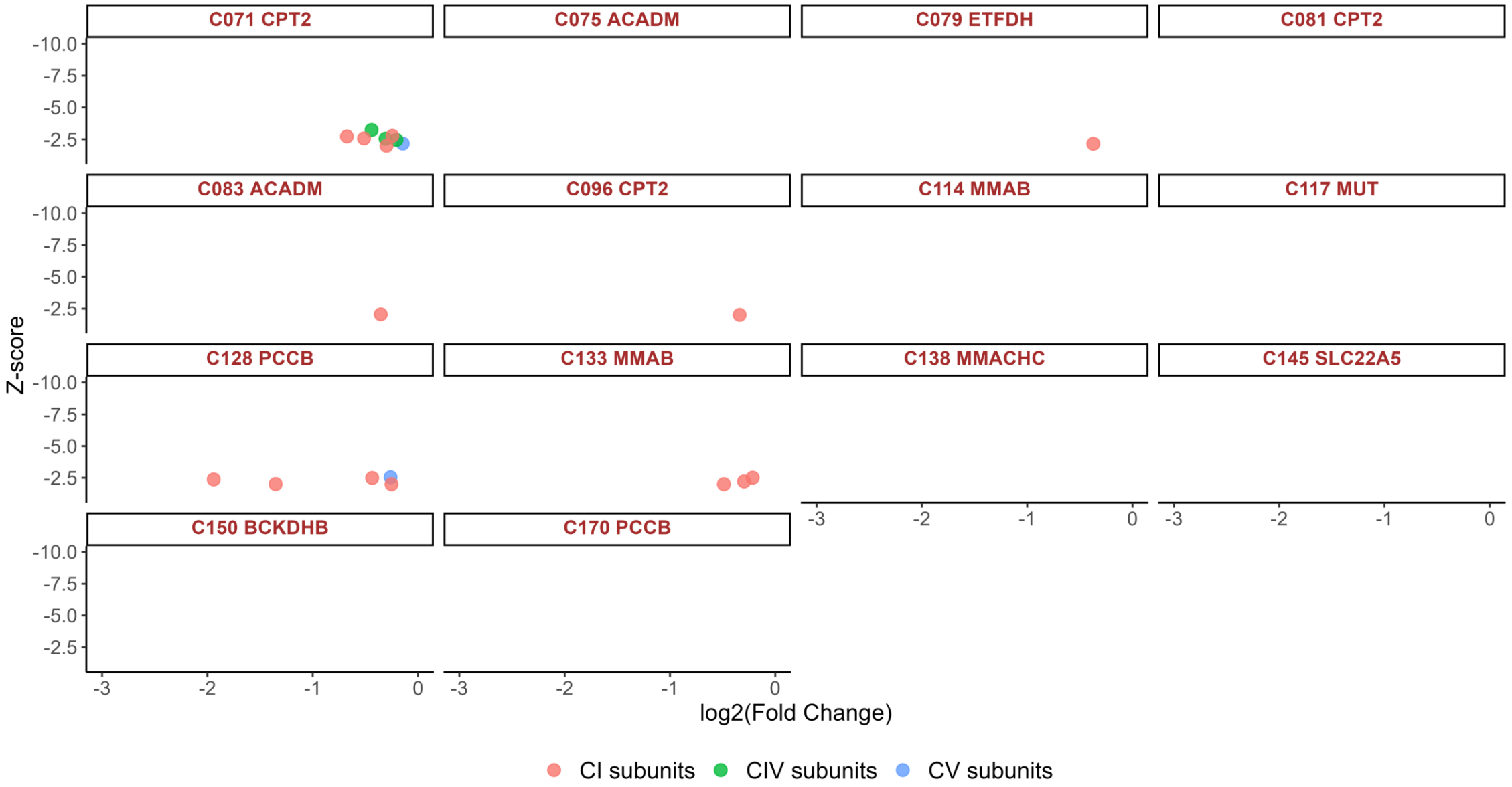
Pythagorean vector	∞	0.4	65	100	90	52.4

- Sensitivity: 60-80% (- likelihoodratio :0.4-0.2)
- Specificity: 66-100% (+ likelihoodratio:1.9-∞)

Secondary mitochondrial dysfunction?



Performance

Test	Positive likelihood ratio	Negative likelihood ratio	Sensitivity (%)	Specificity (%)	Accuracy (%)	Optimal cut-off
≥ 1 downregulated protein						
all MitoCarta3.0 proteins						
Z-score	3.0	0.4	59	98	87	-3.3
Log 2 (fold change)	1.9	0.4	59	96	86	-1.2
Pythagorean vector	2.8	0.3	76	73	74	3.6
≥ 1 downregulated protein						
only OXPHOS proteins						
Z-score	3.0	0.2	82	73	75	-2.2
Log 2 (fold change)	1.9	0.2	82	77	79	-1.4
Pythagorean vector	3.6	0.2	82	77	79	2.8
Cumulative complex downregulation						
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Z-score	3.0	0.3	71	95	89	-44.1
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Pythagorean vector	∞	0.4	65	100	90	52.4

- Sensitivity: 60-80% (- likelihoodratio : 0.4-0.2)
- Specificity: 66-100% (+ likelihoodratio:1.9-∞)

→ Could AI help?

Selecting a model

Criteria

- **Supervised** machine learning models
- **Binary** classification
- High dimensional data (Induce feature sparsity)
- Low sample numbers
- Not prone to overfitting

Models

- GLMnet
- sPLS-DA
- Random Forest

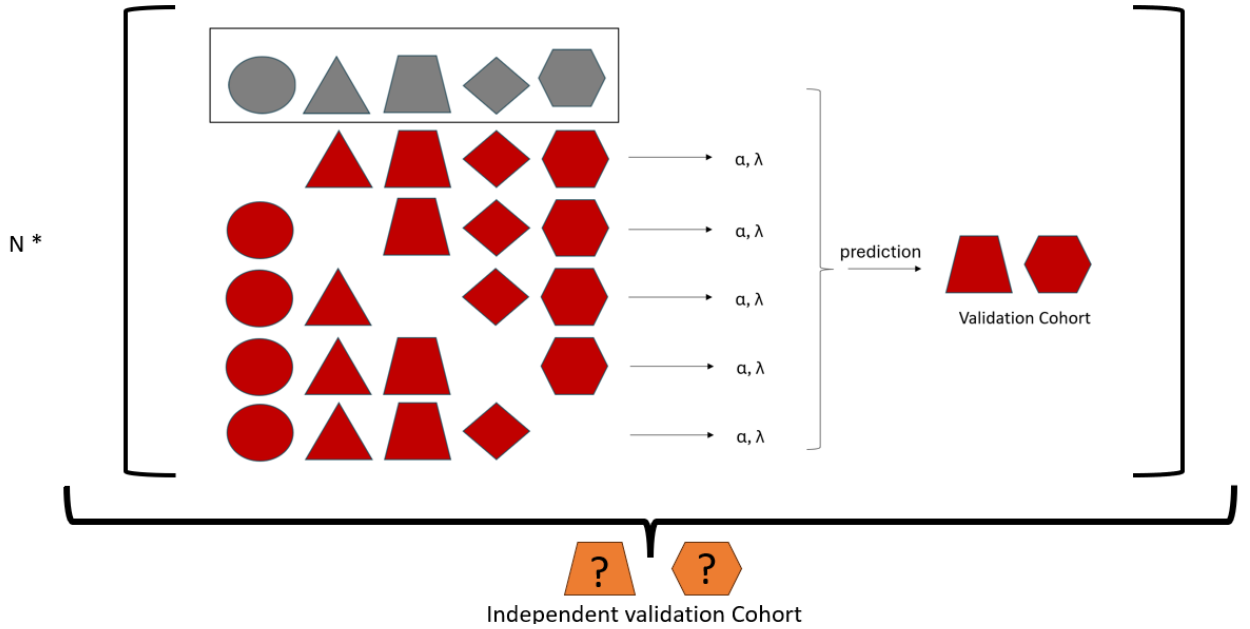
Reducing chances of overfitting

Prior feature selection

- Biological
- Statistical: point-biserial correlation

Nested cross loop validation

- Monte Carlo



Could AI help?

Test	Positive likelihood ratio	Negative likelihood ratio	Sensitivity (%)	Specificity (%)	Accuracy (%)	Mean accuracy cross validation %(SD)	$N_{features}$ final model
Proteomics							
all MitoCarta3.0 proteins							
Glmnet	∞	0.25	75	100	93.3	90.9 (16)	135
sPLS-DA	8.3	0.28	75	91	86.7	81.6 (11)	50
Random Forest	∞	0.25	75	100	93.3	87.1 (12)	193
Metabolomics							
Corrected isotopologue fraction							
Glmnet	∞	0.5	50	100	85.7	58.9 (14)	11
sPLS-DA	7.5	0.28	75	90	85.7	68 (15)	40
Random Forest	7.5	0.28	75	90	85.7	75 (14)	50
Metabolomics							
Isotopologue concentration							
Glmnet	∞	0.25	75	100	92.9	72.2 (15)	5
sPLS-DA	∞	0.5	50	100	85.7	84 (12)	5
Random Forest	∞	0.5	50	100	85.7	82 (12)	50

Could AI help?

Independent cohort: 4 patients, 11 controls

- Specificity: 90-100% (+ likelihoodratio: 7.5-∞)
- Sensitivity: 50-75% (- likelihoodratio : 0.25-0.75)
  - Sample bias?

Take home message

-omics in platelets are a promising diagnostic tool

- More is yet to come: “high-sentive proteomics” by Astral

Use of AI allows to detect & combine mild patterns that enhance diagnosis.

Futher –prospective– validation is needed to determine phenotype specific accuracy.

Acknowledgement

- **Prof. Pieter Vermeersch**
- **Prof. David Cassiman**
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- Prof. Daisy Rymen
- Prof. Wouter Meersseman
- Dr. Lode Van Overbeke

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- **Pedro Magalhães, PhD**
- & LAMA's team

Collaborators

- **Johan Van Hove**
- Robbin Bouwmeester
- Saskia Wortmann

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Thank you for your attention

Questions?

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