BLOOD-BASED BIOMARKERS FOR EARLY DIAGNOSIS OF FRAILTY ARE SEX SPECIFIC: VALIDATION OF A COMBINED IN SILICO PREDICTION AND DATA-DRIVEN APPROACH

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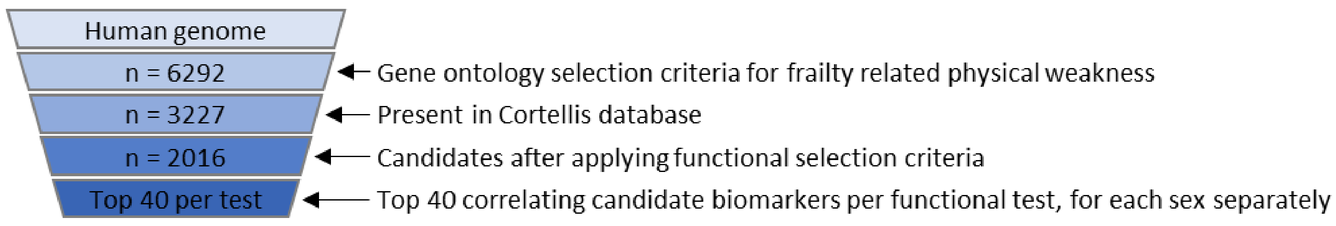
INTRODUCTION AND AIM

- Preferably, frailty is diagnosed at an early stage, however, sensitive tools that can aid to diagnose frailty early are lacking.
- Blood-based biomarkers could be excellent predictors for early-detection of frailty, however, sensitive and accurate biomarkers are lacking and are likely to be sex-specific.
- Therefore, our aim is to identify potential biomarkers for early diagnosis of frailty related physical weakness for each sex separately.

METHODS

- Candidate biomarkers were identified by *a priori* defined frailty-related pathways selected from gene ontology database (db), resulting in an initial set of 6292 genes.
- Further selection was performed by excluding genes that were not present in the Cortellis biomarker db (an extensive biomarker database).
- Using Cortellis, functional selection criteria were applied as well (e.g. candidate must be measurable in human serum and reported to be related to muscle weakness or atrophy).
- Lastly, sex-specific correlation analysis was performed, between:
  - Gene expression levels of the 2016 remaining genes in *vastus lateralis* muscle tissue of fit and pre-frail males (n=28) and females (n=24).
  - Three frailty-related physical parameters (400m walktime, 4m gait speed test and time to perform five chair stands).

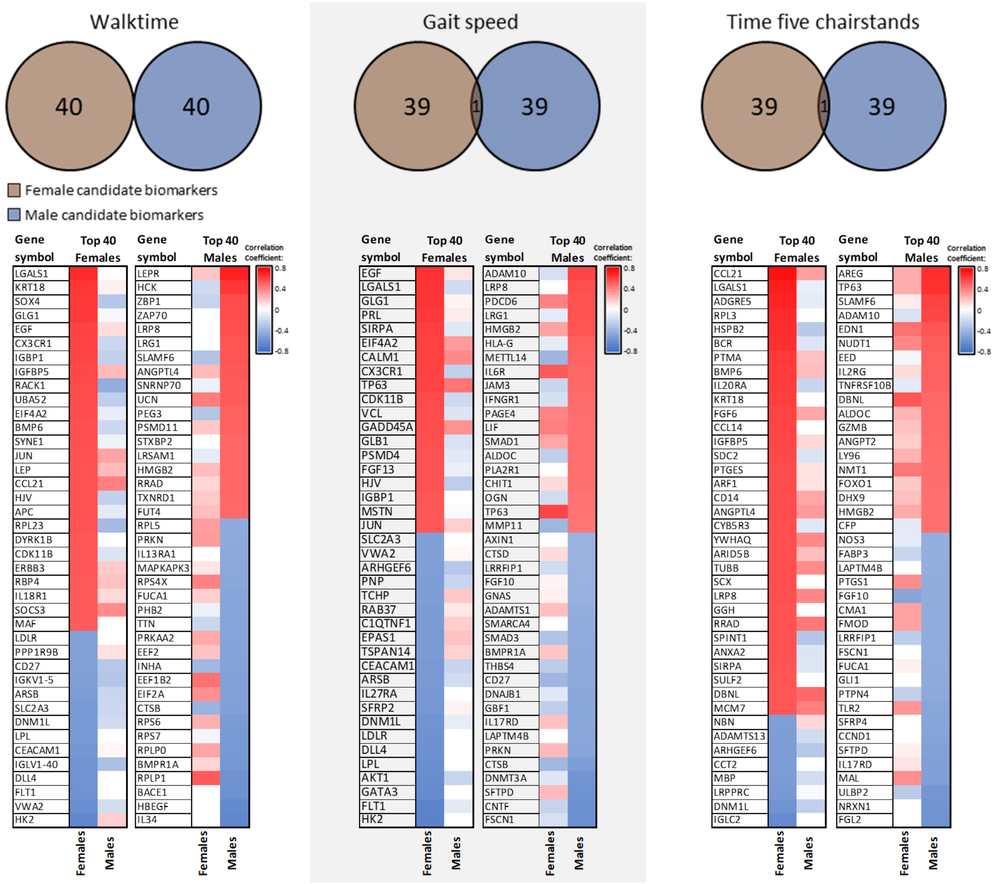
  A dedicated evaluation of the top 40 correlating genes was made based on their role in physical parameters. Serum biomarker concentrations of selected biomarkers were measured using ELISA.



Selection tree, numbers represent amount of genes remaining after each exclusion step.

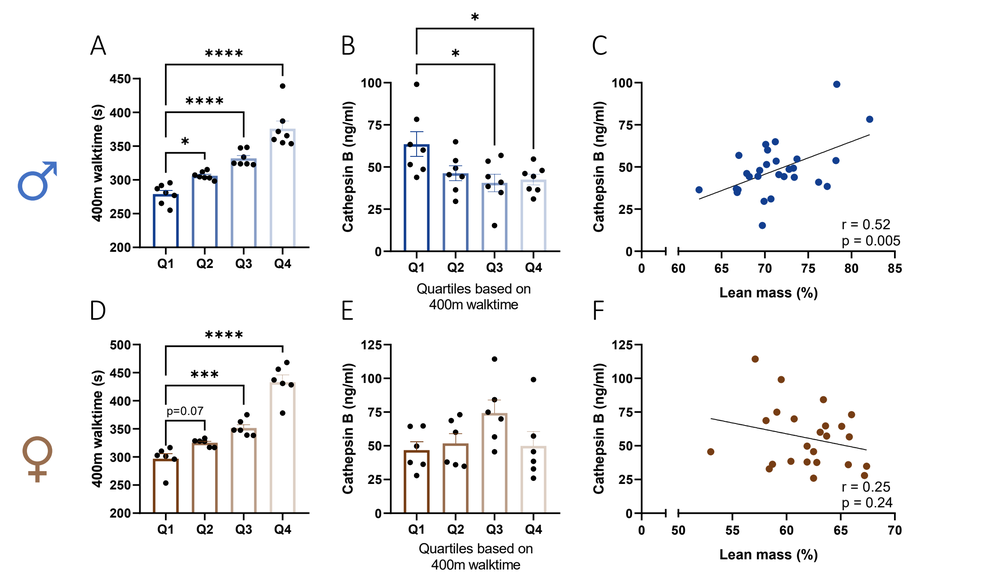
COMBINED IN SILICO PREDICTION AND DATA DRIVEN APPROACH PREDICT HIGHLY SEX-SPECIFIC BIOMARKERS

Top 40 correlating candidate biomarkers per physical parameter For each sex separately



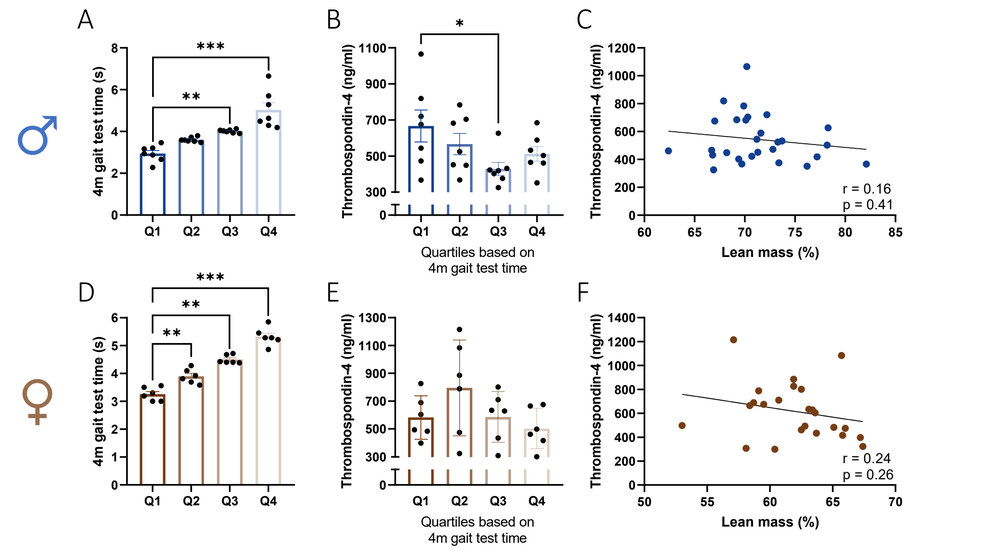
- Venn diagrams (upper panels) display number of top 40 correlating candidates that were either shared between the sexes or unique for one sex. Strikingly, nearly all top candidate biomarkers were sex-specific.
- Heatmaps (lower panels) display the correlation coefficient (r) of the top 40 correlating candidate biomarkers. Correlations were calculated between the frailty related physical parameters and RNA-seq derived gene expression. Interestingly, distinct correlations in the male and female groups were observed.

CATHEPSIN-B IS A MALE SPECIFIC EARLY BIOMARKER FOR DECREASED WALKING SPEED



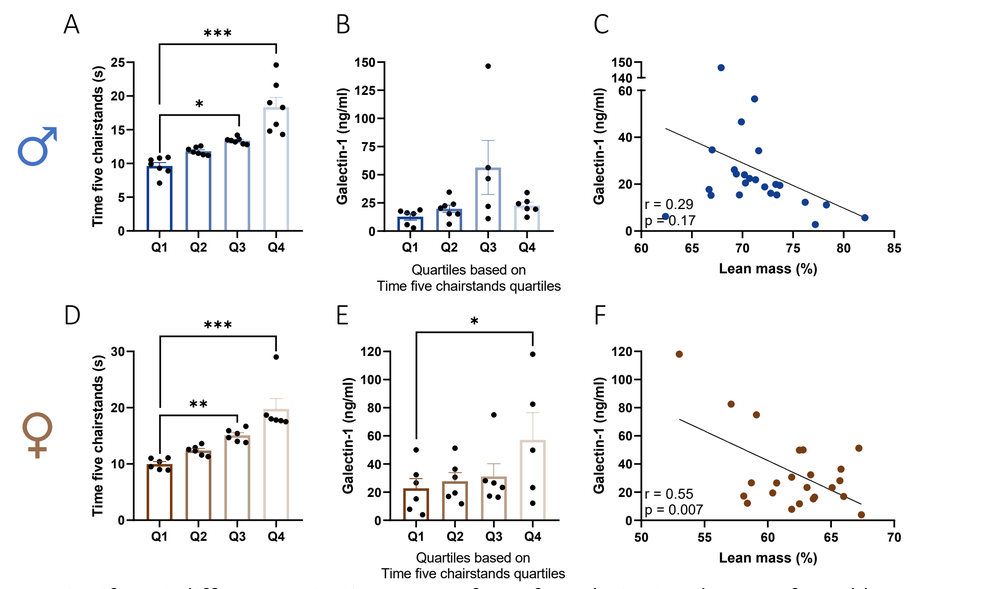
- Significant differences in walking speed were found between quartiles (A&D).
- Serum concentrations of cathepsin B was decreased in slower walking males, but not in slower walking females compared to faster walking participants (B&E).
- Cathepsin B serum concentration correlated significantly with %lean mass in males but not in females (C&F).

THROMBOSPONDIN-4 IS A MALE SPECIFIC EARLY BIOMARKER FOR DECREASED 4M GAIT SPEED



- Significant differences in 4m test time were found between quartiles (A&D).
- Serum concentrations of thrombospondin-4 were significantly decreased in the third quartiles Q3 compared to Q1 males, but not in slower walking females compared to faster walking females (B&E).
- No correlations between thrombospondin-4 and %lean mass were found in both males and females (C&F).

GALECTIN-1 IS A FEMALE SPECIFIC EARLY BIOMARKER FOR INCREASED TIME TO PERFORM FIVE CHAIR STANDS



- Significant differences in time to perform five chair stands were found between the quartiles (A&D).
- Galectin-1 serum concentrations were significantly increased in slower females compared to faster females, but no such differences were found in males (B&E).
- Galectin-1 levels correlated significantly with %lean mass in females but not in males (C&F).

CONCLUSIONS

We conclude that biomarkers for frailty related physical weakness are highly likely to be sex-specific. In males, Cathepsin-B and Thrombospondin-4 were biomarkers of interest, particularly since serum levels correlated with decreased walking function in a population including only fit and pre-frail older adults, indicating their potential to be early biomarkers for frailty related physical weakness. In females, so far we showed Galectin-1 to be a biomarker of interest. Measurements are ongoing for additional biomarkers, which together might serve the opportunity to be used as a biomarker panel for early frailty diagnosis.

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