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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WAGENINGENUR For quality of life

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.

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



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

- 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



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









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mbol Females symbol Males Coefficient ALS1 ADAM10 IRP8 IRP1 IRP8 IRP1 IRP8 IRP1 IRP8	ene	Top 40	Gene	Top 40	Correlation
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AXIN1VA2VA2CTSDLRRFIP1IPFGF10HPGNASB37ADAMTS1QTNF1AS1AS1PAN14ACAM1SBCD277RARP2IM1LLRLACTSBT1CNFZFSCN1	N		MMP11		
VA2CTSDLHGEF6LRRFIP1IPFGF10HPGNASB37ADAMTS1QTNF1SMARCA4AS1SMARCA4AS1SMAD3PAN14BMPR1AACAM1THBS4SBCD277RADNAJB1RP2GBF1JM1LIL17RDLRLAPTM4BL4CTSBT1DNMT3ATA3SFTPDT1CNTF72FSCN1	C2A3		AXIN1		
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SBCD277RADNAJB1RP2GBF1JM1LIL17RDLRLAPTM4BL4CTSBT1DNMT3ATA3SFTPDT2FSCN1	ACAM1		THBS4		
Z7RADNAJB1RP2GBF1JM1LIL17RDLRLAPTM4BL4PRKNLCTSBT1DNMT3ATA3SFTPDT2FSCN1	RSB		CD27		
RP2GBF1IM1LIL17RDLRLAPTM4BL4PRKNLCTSBT1DNMT3ATA3SFTPDT1CNTF2FSCN1	27RA		DNAJB1		
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CNTF FSCN1	ATA3		SFTPD		
FSCN1	T1		CNTF		
	.2		FSCN1		

Gene	Top 40	Gene	Top 40	Correlation
symbol	Females	symbol	Males	Correlation Coefficient:
CCL21		AREG		0.8
LGALS1		TP63		0.4
ADGRE5		SLAMF6	_	
RPL3		ADAM10		0
HSPB2		EDN1		-0.4
BCR		NUDT1		
PTMA		EED		-0.8
BMP6		IL2RG		
IL20RA		TNFRSF10)B	
KRT18		DBNL		
FGF6		ALDOC		
CCL14		GZMB		
IGFBP5		ANGPT2		
SDC2		LY96		
PTGES		NMT1		
ARF1		FOXO1		
CD14		DHX9		
ANGPTL4		HMGB2		
CYB5R3		CFP		
YWHAQ		NOS3		
ARID5B		FABP3		
TUBB		LAPTM4B		
SCX		PTGS1		
LRP8		FGF10		
GGH		CMA1		
RRAD		FMOD		
SPINT1		LRRFIP1		
ANXA2		FSCN1		
SIRPA		FUCA1		
SULF2		GLI1		
DBNL		PTPN4		
MCM7		TLR2		
NBN		SFRP4		
ADAMTS13		CCND1		
ARHGEF6		SFTPD		
CCT2		IL17RD		
MBP		MAL		
LRPPRC		ULBP2		
DNM1L		NRXN1		
IGLC2		FGL2		

- 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



- 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.
- 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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