

MANUSCRIPT DATA IDENTIFICATION FORM

Title: Muscle Transcriptional Networks Underlying Hypertrophic Response Heterogeneity

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Manuscript Table/Figure

Table 1: Participant Characteristics
Table 2: PLIER Latent Variables with relationship to outcomes of interest
Figure 1: Methodological flowchart showing complementary application of WGCNA and PLIER analysis pipelines
Figure 2: Top pathways associated with PLIER latent variables (LVs) for which an acceptably robust (adjusted $P < 0.05$) pathway association was found (16 of 25 LVs).
Figure 3: Heterogeneity in change in thigh muscle area (cm^2) as measured by CT following 14-wk resistance exercise training
Figure 4: Heatmap showing Pearson correlation R values (and associated P-values) for relationship between whole-muscle hypertrophy module eigengenes in WGCNA Prediction analysis
Figure 5: Prediction analysis red module
Figure 6: Prediction analysis magenta module
Figure 7: Representative heatmap for PLIER prediction analysis
Figure 8: Response LVs that changed following RT independently of changes in muscle phenotype.
Figure 9: Normalized relative gene expression for two exercise responsive LVs.

Data in Text (Section/¶)	Program or Chunk Name
METHODS	
Myofiber type and CSA measurements from an average \pm SD of 890 ± 497 myofibers per sample	Chunk 9: bring in histology
Mean \pm SD RIN for samples included in the present analysis was 8.0 ± 0.7 .	Chunk 12: sample RIN summary
Soft thresholding power of 12 was calculated to guide the WGCNA algorithm in network construction (Methods > Computational Pipeline > WGCNA)	Chunk 42: basal approach
...module eigengene change score index was calculated from ME expression within an individual using the formula $1 - \text{pre-RT/post-RT}$ (Methods > Computational Pipeline > WGCNA)	Chunk 97: calculate the change index
Six MetaMEx pathways, two fiber type-specific pathways, and 606 other canonical pathways in public databases (e.g., KEGG, Reactome) were loaded as prior knowledge...	Chunk 50: load pathways
...identified predictive modules maintained an association with the outcome of interest in their absence, albeit slightly weakened (e.g., red module eigengene $P = 2 \times 10^{-6}$ vs. $P = 4 \times 10^{-4}$). (Methods > Computational Pipeline > Sensitivity Analyses)	Chunk 67: test module sensitivity for Prediction modules
RESULTS	
Participant characteristics are presented in Table 1.	Chunk 11: participant table 1
...WGCNA yielded 14 distinct gene modules (Results > Predictive Gene Networks)	Chunk 57: look at basal network
...the red module eigengene was significantly and positively related to the change in skeletal muscle area as measured by CT ($R = 0.7$; $P = 2 \times 10^{-6}$; Figure 5); the magenta module was also positively related to the change in skeletal muscle size as measured by both CT and DEXA at the nominal level ($R = 0.4$; $P = 0.01$); the salmon module eigengene was significantly and negatively related to the change in myofiber CSA ($R = -0.6$; $P = 0.001$) (Results > Predictive Gene Networks)	Chunk 59: prediction heatmap
A total of 307 genes were assigned to the red module: from these, the majority fell into PLIER-identified latent variables 2, 9, 18, and 17 (Results > Predictive Gene Networks)	Chunk 57: look at basal network & Chunk 81: mod LV check
Separately, PLIER's algorithm identified three Prediction LVs (7, 16, 23) associated with RT-induced changes in mid-thigh muscle CSA, one LV (15) associated with RT-induced change in normalized bilateral thigh lean mass. (Results > Predictive Gene Networks)	Chunk 73: prediction effect lm & Chunk 79: sensitivity checks for plier prediction
...LV 7 did not have a pathway association based on the prior knowledge loaded into PLIER but still exhibited a strong relationship with the change in thigh muscle area as measured by CT (Results > Predictive Gene Networks)	Chunk 78: plot prediction LVs & Chunk 79: sensitivity checks for plier prediction
...none of the genes in LV7 or other PLIER-constructed LVs overlapped with genes in the red or magenta modules from WGCNA that were significantly related to change in muscle thigh area. (Results > Predictive Gene Networks)	Chunk 83: bring in CT LV files and compare to red magenta genes
PLIER found six LVs that were significantly changed following 14 weeks of RT (Results > Exercise Responsive Gene Networks)	Chunk 86: exercise effect for sensitivity check
Follow-up analysis showed that most of these genes increased following resistance training in the MetaMEx database. This was true across all modules of interest (Table S15). (Results > Exercise Responsive Gene Networks)	Chunk 119: use idoverlaps & Chunk 122: connections to mods of interest for AT
In the Plasticity analysis, WGCNA yielded 12 distinct gene modules from 44 paired samples (Results > Plastic Gene Networks)	Chunk 93: look at paired network
The pink module eigengene change index was significantly and positively related to the change in myofiber CSA ($R = 0.6$; $P = 0.001$) (Results > Plastic Gene Networks)	Chunk 102: plasticity heatmap
Only one PLIER-identified LV (LV 20, Figure S4) was robust to removal of extreme data points (Table 2). (Results > Plastic Gene Networks)	Chunk 113: sensitivity test for plier cbi lvs
FIGURE CAPTIONS	
Figure 5A: relationship between mean baseline expression of red module genes and RT-induced change in thigh muscle area as measured by CT in each individual ($R^2 = 0.5375$; $P = 1.6 \times 10^{-6}$). B: Association for all red module genes between module membership and relationship to associated trait $R = 0.56$, $P = 9.7 \times 10^{-27}$.	Chunk 66: calculate R value for red magenta salmon & Chunk 68: intramodular scatterplot
Figure 6A: relationship between mean baseline expression of magenta module genes and RT-induced change in thigh muscle area as measured by CT in each individual ($R^2 = 0.1404$; $P = 0.02$). B: Association for all magenta module genes between module membership and relationship to associated trait $R = 0.6$; $P = 1.7 \times 10^{-23}$.	Chunk 66: calculate R value for red magenta salmon & Chunk 68: intramodular scatterplot

*All analyses were run between 06/29/20 and 10/31/20. Revisions were performed between 01/18/2021 and 02/11/21.

Newly-Derived Variables

The following variables are generated by running the pipeline.

a	cts_paired.mat	magenta gene table	PlierBox
AA_explore	cts.updated	magenta LV associations	plier_cbi
AA_genes	current_genes	magenta RT_down	plier_cbi_1
AA_geneset	customplotTopZ	magenta RT_up	plier_cbi_3
AA_processed_full	customplotTopZcontinuous	MASplierResult	plotWGCNA
above0	deltas	MASplier_updated	predict
adj.pheno.long	DEXA	MASTERS CT	prediction_sens_check
adj.pheno.paired.ME	df_id	MASTERSpheno	p.threshold
adj.sampleTree.ecl	dge	mergedColors	r
adjvar	DXA_LVs	MEs	red_AT_down
adj.vars.of.interest	ensembl_to_gene	MEs_basal	red_AT_up
A.ecl	examine_exercise	MEs_paired	red_CT
AEmagenta.fit	exercise	MetaMEx_matrix	red_gene_table
AEred.fit	exp.outlier	microarray_genes	red_magenta
AEsalmon.fit	expression_data	MMPvalue	red_RT_down
all_CT_genes	FiberType_matrix	modColors_basal	red_RT_up
all_genes	filt_basal	modColors_paired	res
allPaths	filtered	modLabels_basal	resid.pheno.paired
AR_geneset	filt_paired	modLabels_paired	resid.pheno.paired.ME
AT_common	final_rnaseq_phenotype	mod_LV_check	resid.pheno.wide
AT_down	find.outliers	modNames	RINscores
AT_down_genes	fit	mods_of_interest	RT_common
AT_gene_data	fit1	mods_of_interest_AE	RT_down
AT_geneset	fit2	mods_of_interest_plastic	RT_down_genes
AT_up	fit3	moduleGenes	RT_gene_data
AT_up_genes	for_tree	ModuleTable	RT_geneset
avg_expr	full_expr	moduleTraitCor_basal	RT_up
b	gene	moduleTraitCor_paired	RT_up_genes
basal_avg_expr	gene_df	moduleTraitPvalue	salmon_AT_down
basal_combat_adj	geneModuleMembership	moduleTraitPvalue_paired	salmon_AT_up
basal_cts.adj	genesums	name	salmon_fCSA
basal_expr	geneTree_basal	NetworkExam	salmon_gene_table
basal_mod_table	geneTree_paired	newyN	salmon_LV_associations
basal_network	getCytoscape	nGenes	salmon_RT_down
basal_red	getSize	nominal.mods	salmon_RT_up
basal_set	gsg	nSamples	sample.mat
batch	hcd_pheno	numeric_cols	sampleTree
batch.basal	hcd_pheno_adj	outlier_check	sampleTree.ecl
batch.paired	hex_codes1	outlier_check_plasticity	show
blindvst	HT_geneset	outlierColor.ecl	signifTable_plasticity_updated
bloodCellMarkersIRISDMP	i	overlaps	signifTable_predict_updated
c	IDmods	paired_CBI	signifTable_updated
calc_CBI	IDoverlaps	paired_CBI_sens_check	simpledf
canonicalPathways	ihc	paired_change	simpledf1
CBI	ihc.seq	paired_combat_adj	subject_data
CBI_df	ihc.summary	paired_cts.adj	subjecttable
CBI_long	IHCtemp	paired_expr	table_1
CBI_long2	j	pairedkey	textMatrix
CBIpink.fit	k.ecl	paired_mod_table	threshold
CBI_plot	keep	paired_network	thresholdZ.k.ecl
CBIpurple.fit	key	paired_set	TMgenes
CBIred.fit	LV13	pathdata_updated	to_bind
Chat	LV16	pathdata_updated_plasticity	to_mutate
clean_path	LV19	pathdata_updated_predict	traits_of_interest
cm.genes	LV23	pathdata_updated.RT	transposed
column	LV7	pDat.clean_noNA	tscale
columns	lv7fit	perc_75th	ttest
combat_adj	LV_sensitivity_check	perc_80th	updated_red_LV
continuousdf	LV_sensitivity_check_plasticity	pheno1	variable
covar.mod	LV_sensitivity_check_response	pheno3	vars.of.interest
covar.mod.basal	LVs_of_interest_exercise_updated	pheno.long	vstExpr0

covar.mod.paired	LVs_of_interest_plastic_updated	pheno.long.paired	working.pheno.long
createPlotfor	LVs_of_interest_prediction_updated	pheno.paired.ME	x
createTablefor	LVs_plasticity	pheno.wide	y
CT.clean	LVs_prediction	pheno.wide.fiber	yN
CT_geneset	LV_Table2_plasticity	pink_AT_down	yN.svd
cts	LV_Table2_prediction	pink_AT_up	z
cts.adj	LV_Table2_response	pinkgenes	Z.k.ecl
cts_basal	LVtablemaker	pink_modmem	z.means
cts_basal.mat	magenta_AT_down	pinkPLIER	
cts.mat	magenta_AT_up	pink_RT_down	
cts_paired	magenta_CT	pink_RT_up	

Special Files: Generated Outside Main Program**Program Name**

Subject Characteristics (Table 1)	Microsoft Word
Computational Pipeline (Figure 1)	Microsoft PowerPoint
Fiber type-specific Gene Marker/Pathway Association Matrix	R
MetaMEx Gene / Pathway Association Matrix	R
Network Maps (Prediction: red, magenta)	Cytoscape
Table S15: directional changes in MetaMEx database	Microsoft Excel
Number of genes in PLIER-identified LVs	Manually assessed