

Supplementary Material

Urinary Cytokines as Potential Biomarkers of Mild Cognitive Impairment and Alzheimer's Disease: A Pilot Study

Supplementary Table 1A. Multi-group comparison of available clinical and demographic information describing education, gender, and age, SLUMS total scores, CLOXs, MMSEs, and TMTs are provided below with p-values.

	Controls	MCI	AD	p
n	26	25	25	
Age, mean (SD)	80.65 (5.87)	81.24 (4.87)	81.44 (4.56)	0.73 ^a
Gender				
Male	13	11	12	0.84 ^b
Female	13	14	13	
Race				
Caucasian/white	22	20	21	0.47
African American/ Indian American	4	5	4	
MMSE	28.30 (1.32)	22.65 (1.98)	18.88 (5.22)	0.0001
Education	14.68 (2.46)	15.52 (3.86)	14.62 (2.96)	0.39
CLOX Score 1	24.11(4.14)	20.68 (4.78)	13.71(5.86)	0.0054
CLOX Score 2	12.75 (1.89)	12.82 (2.63)	10.86 (2.94)	0.0256
TMT-A	40.57 (31.87)	25.45 (28.68)	11.73 (13.13)	0.0023
TMT-B	51.16 (29.69)	32.02 (26.31)	11.82 (12.75)	0.0018
Slums total score	24.11 (4.14)	20.62 (4.87)	13.71 (5.86)	0.0011

^aOne way ANOVA, ^bChi-square Test

Supplementary Table 1B. Pair-wise comparison of available clinical and demographic information describing education, gender and age, Slums total scores, CLOXs, MMSEs, and TMTs are provided below with p-values.

	Mean (SD)			p		
	Controls	MCI	AD	Control-MCI	MCI-AD	Control-AD
n	26	25	25	0.327	0.883	0.341
Age, mean (SD)	80.65 (5.87)	81.24 (4.87)	81.44 (4.56)			
Gender				0.578	0.482	0.889
Male	13	11	12			
Female	13	14	13			
MMSE	28.30 (1.32)	22.65 (1.98)	18.88 (5.22)	0.0001	0.0001	0.0001
Education	14.68 (2.46)	15.52 (3.86)	14.62 (2.96)	0.38	0.42	0.44
CLOX Score 1	24.11(4.14)	20.68 (4.78)	13.71(5.86)	0.0056	0.0035	0.0047
CLOX Score 2	12.75 (1.89)	12.82 (2.63)	10.86 (2.94)	0.0246	0.0378	0.0182
TMT-A	40.57 (31.87)	25.45 (28.68)	11.73 (13.13)	0.0034	0.0023	0.0038
TMT-B	51.16 (29.69)	32.02 (26.31)	11.82 (12.75)	0.0011	0.0034	0.0023
SLUMS total score	24.11 (4.14)	20.62 (4.87)	13.71 (5.86)	0.0078	0.0089	0.0067

Supplementary Table 1C. Important sociodemographic factors for each participant.

English ^a	Age	Gender	Race	Physician Diagnosis	GDS	STS	MMSE	CLOX Score 1	CLOX Score 2	TMT-A	TMT-B	CDR Score	Hachinski Total Score
Yes	87	F	Black or African American	AD/Vasc mixed	1		15	4	8				
Yes	72	F	Caucasian/White	AD	4	15	28	9	12	3-5	11-18		
Yes	84	F	Black or African American	AD	0	18	21	7	10	<1	<1		
Yes	80	M	Caucasian/White	AD	5	18	23	12	12	2	11-18		
Yes	83	M	Caucasian/White	AD/Vasc mixed	3	11	25	9	10	11-18	19-28		
Yes	78	F	Caucasian/White	AD	3	21	22	11	11	11-18	41-59	1	3
Yes	88	F	Caucasian/White	AD	1	13	19	9	13	11-18	<1	1	2
Yes	82	F	Caucasian/White	AD	1	23	22	15		19-28	11-18	0.5	1
Yes	87	M	Caucasian/White	AD	0	3	11					2	3
Yes	72	F	Caucasian/White	AD	1		15	9	12	<1		1	4
Yes	82	M	Caucasian/White	AD	0	3	8	3	6	<1		2	1
Yes	85	F	American Indian or Alaska Native	AD	2		14	11	11	2	<1	0.5	2
Yes	88	M	Caucasian/White	AD	2	16	22	8	12	<1		0.5	4
Yes	83	F	Caucasian/White	AD	0	18	20	7	12	19-28	3-5	0.5	1
Yes	81	F	Caucasian/White	AD	1		11					1.5	1
Yes	81	M	Caucasian/White	AD	1	9	18	2	2	<1		1	
Yes	79	F	Caucasian/White	AD	5	10	17	10	13	29-40	11-18	1	3
Yes	77	M	Caucasian/White	AD	3	13	23	9	7	11-18			
Yes	76	M	Caucasian/White	AD	0	21	25	11	15	19-28	11-18	0.5	1
Yes	82	M	Caucasian/White	AD	4	15	21	7	14	11-18	19-28	0.5	2

Yes	75	F	Caucasian/White	AD	7	5	14	7	12	41-59	1	1	5
Yes	85	M	Caucasian/White	AD	1	15	20	10	10	1		1	
Yes	78	M	Caucasian/White	AD/Vasc mixed	2	19	26	11	12	3-5	1	0.5	1
Yes	85	M	Caucasian/White	AD/Vasc mixed	5	17	22	11	14	<1	<1	1	3
Yes	86	F	Black or African American	AD	5	5	10					2	2
Yes	86	M	Black or African American	Normal cognition	1	15	25	11	10	6-10.	1		
Yes	73	F	Caucasian/White	Normal cognition	5	26	29	11	13	19-28	29-40	0	0
Yes	82	F	Caucasian/White	Normal cognition	2	22	28	6	12	11-18	60-71	0	1
Yes	78	F	Caucasian/White	Normal cognition	1	15	28	6	7	3-5.	<1	0	0
Yes	80	M	American Indian or Alaska Native	Normal cognition	13	19	27	12	13	11-18	19-28	0	2
Yes	85	F	Caucasian/White	Normal cognition	1	30	29	15	15	29-40		0	0
Yes	83	M	American Indian or Alaska Native	Normal cognition	4	20	28	10	11	3-5.	1	0	1
Yes	82	M	Caucasian/White	Normal cognition	5	27	30	11	14	11-18	29-40	0	1
Yes	87	M	Caucasian/White	Normal cognition	0	18	28	8	14	82-89	19-28	0	1
Yes	82	F	Black or African American	Normal cognition	4	20	29	12	13	6-10	<1	0	7
Yes	84	F	Caucasian/White	Normal cognition	0	24	30	15		6-10	41-59	0	1
Yes	73	M	Caucasian/White	Normal cognition	0	29	30	15	15	6-10	41-59	0	2
Yes	73	F	Caucasian/White	Normal cognition	0	26	27	15		29-40	72-81	0	1
Yes	87	F	Caucasian/White	Normal cognition	0	26	29	11	14	11-18	41-59	0	1
Yes	79	F	Caucasian/White	Normal cognition	0	26	30	11	14	19-28	41-59	0	1
Yes	71	M	Caucasian/White	Normal cognition	2	28	29	14		29-40	41-59	0	
Yes	73	M	Caucasian/White	Normal cognition	1	21	27	11	11	41-59	72-81	0.5	6

Yes	87	M	Caucasian/White	Normal cognition	0	29	30	14		60-71	82-89	0	1
Yes	64	F	Caucasian/White	Normal cognition	2	28	30	12	12	95-97	60-71	0	
Yes	77	F	Caucasian/White	Normal cognition	8	22	28	12	13	72-81	60-71	0	2
Yes	80	M	Caucasian/White	Normal cognition	1	26	27	13	14	90-94	95-97	0	
Yes	77	M	Caucasian/White	Normal cognition	1	27	29	15		41-59	90-94	0.5	2
Yes	82	F	Caucasian/White	Normal cognition	4	27	27	15		72-81	72-81	0	2
Yes	88	M	Caucasian/White	Normal cognition	4	25	26	12	15	82-89	72-81	0	3
Yes	85	F	Caucasian/White	Normal cognition	0	27	27	13	13	95-97	90-94	0	
Yes	82	M	Caucasian/White	Normal cognition	1	24	29	9	12	29-40	41-59	0	4
Yes	91	M	Black or African American	MCI	2	22	28	13	12	19-28	11-18		
Yes	79	M	Caucasian/White	MCI	1	11	25	9	10	11-18	11-18		
Yes	87	F	Caucasian/White	MCI	8	23	29	10		41-59	41-59		
Yes	90	M	Caucasian/White	MCI	3	24	28	11	12				
Yes	82	F	Caucasian/White	MCI	7	12	26	11	10	11-18	11-18		
Yes	84	M	Caucasian/White	MCI	0	23	28	12	12	82-89	98		1
Yes	84	F	Caucasian/White	MCI	1	10	23	9	9	11-18		0.5	2
Yes	74	M	Caucasian/White	MCI	4	21	24	11	13	6-10	2	0.5	3
Yes	79	F	Caucasian/White	MCI	0	22	29	14	15	11-18	41-59	0.5	1
Yes	77	F	Caucasian/White	MCI	0	28	27	13	12	3-5	11-18	0.5	2
Yes	83	F	Black or African American	MCI	6	21	25	12	13	2	1	0.5	3
Yes	75	F	Caucasian/White	MCI	1	13	24	6	14	41-59	29-40	0.5	2
Yes	82	F	Caucasian/White	MCI	1	16	23	10	13	2	11-18	0.5	2

Yes	85	M	Caucasian/White	MCI	0	26	25	13	15	95-97	95-97	0.5	2
Yes	81	F	Caucasian/White	MCI	1	21	29	11	14	2	<1	0.5	1
Yes	72	F	Caucasian/White	Mild MCI	8	23	27	13	13	29-40	29-40	0	1
Yes	78	F	Caucasian/White	MCI	0	23	27	11	12	11-18	11-18	0.5	1
Yes	83	F	Caucasian/White	MCI	0	29	28	13	11	69-71	98	0.5	2
Yes	82	F	Black or African American	MCI	11	19	25	10	11	72-81	29-40	0.5	1
Yes	81	M	Caucasian/White	MCI	7	23	28	15		6-10	<1	0.5	2
Yes	77	F	Caucasian/White	MCI	0	21	28	12	13	11-18	1	1	2
Yes	87	M	Black or African American	MCI	2	22	29	7	12	<1	<1	0.5	6
Yes	78	M	Black or African American	MCI	5	19	24	13	14	6-10	1	0.5	6
Yes	86	F	Caucasian/White	MCI	0	22	29	10	12	2	<1	0.5	
Yes	74	M	Caucasian/White	MCI	3	23	26	13	12	6-10	2	0.5	3

^aProficiency with written and spoken English

GDS, Geriatric depression score; SLUMS, Saint Louis University Mental Status total score; AD, Alzheimer's disease; MCI, mild cognitive impairment

Supplementary Table 2. 1. Raw data of cytokines analyzed; 2. Average, p-value, and SD for each cytokines; 3. Measurement sensitivities for each cytokines. See Excel file.

Supplementary Table 3. Enrichment analysis on differentially expressed cytokines using the FunRich tool reveals several targets along with their corresponding p-value.

A. Cytokine targeting LINC RNA

LINC RNA	p-value	Cytokine targeting LINC RNA
LINC01937	0.00001269	Pentraxin-3, MMP-2, MMP-3, IL-11
LINC02376	0.00001269	IL-8, MMP-1, MMP-2, IL-11
LINC02090	0.0004032	IL-10, IL-35, IFN- γ
LINC01104	0.0004032	IL-10, IL-35, IFN- γ
LINC02665	0.0004032	IL-26, MMP-3, IL-11
LINC01920	0.0004032	IL-26, Pentraxin-3, MMP-2
LINC00687	0.0004032	MMP-1, MMP-2, MMP-3, IL-26
LINC00944	0.0004032	IL-35, IFN- γ , IL-32
LINC01501	0.009203	IL-10, IL-35
LINC01054	0.009203	IL-10, IL-35

B. miRNAs targeting cytokines

miRNA	p-value	miRNAs targeting cytokines
hsa-miR-7152-3p	0.01584	IL6ST
hsa-miR-27a-3p	0.001849	IL-10, Osteopontin
hsa-miR-142-3p	0.02012	IL6ST
miR-224	0.02154	Pentraxin-3,
hsa-miR-503-3p	0.002382	IL-2, IL-10
hsa-miR-130a-3p	0.02579	IL6ST
hsa-miR-299-5p	0.003753	TSLP, Osteopontin
hsa-miR-4661-5p	0.02862	IL6ST
hsa-miR-125b-1-3p	0.03144	Osteocalcin
hsa-let-7a-5p	0.03565	IL-10

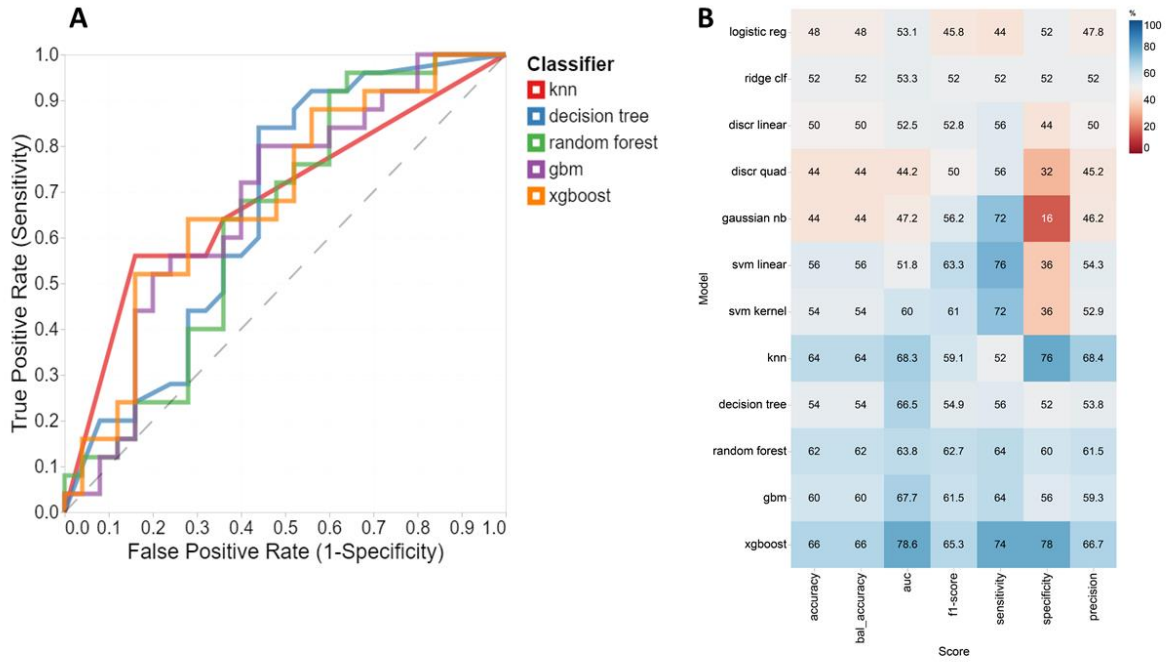
C. miRNA regulating cytokines

miRNA	p-value	miRNA regulating cytokines
miR-1945	0.03051	IL-1, IL-12(P40)
hsa-miR-551a	0.07726	IL6ST, IL-11
hsa-miR-551b	0.07726	IL6ST, IL-11
hsa-miR-4278	0.02724	IL6ST, IL-11, TSLP, IL-10, IL-22, MMP-2
hsa-miR-3181	0.1505	IL-27, MMP-2
hsa-miR-572	0.1550	IL-34, MMP-2,
hsa-miR-18-5p	0.09458	IL6ST, IL-11, IL-19, IL-29, MMP-2
hsa-miR-337-5p	0.2420	IL-11, IL-19, IL-29, MMP-2
hsa-miR-638	0.1089	IL-11, IFN- γ , MMP-1, TNFSF14

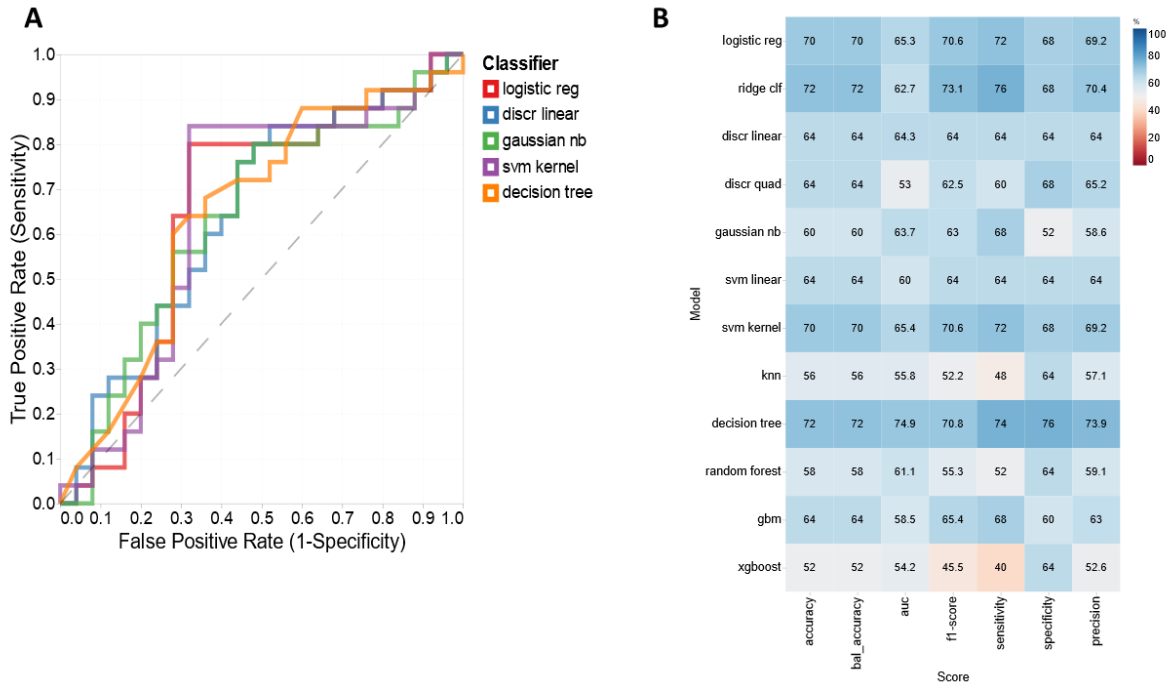
D. TFs regulated by cytokines

Name	p-value	TFs regulated by cytokines
FLI1	1.08E-05	IL-8, Osteopontin, MMP-2, IL6ST, IL-32, Pentraxin-3
ATF6	0.000132	MMP-3, Osteopontin, IL-11, IFN- γ
TWIST2	0.000201	MMP-3, IL-8, Pentraxin-3, MMP-1
TP53	0.000906	MMP-3, IL-8, MMP-1
ZXDC	8.3E-05	IL-8, Osteopontin, MMP-1, CHI3L1, CD163
IRF5	8.93E-05	IL-8, IL-32, IFN α 2, IL-27, IFN- γ
IRF4	0.000287	IL-27, IL-22, IL-10, IL-2
MECOM	0.000292	IL-22, IL-10, TNFSF14
HNF4A	0.000312	MMP-3, IL-8, Osteopontin, IL-11, IL-12 (p-40)

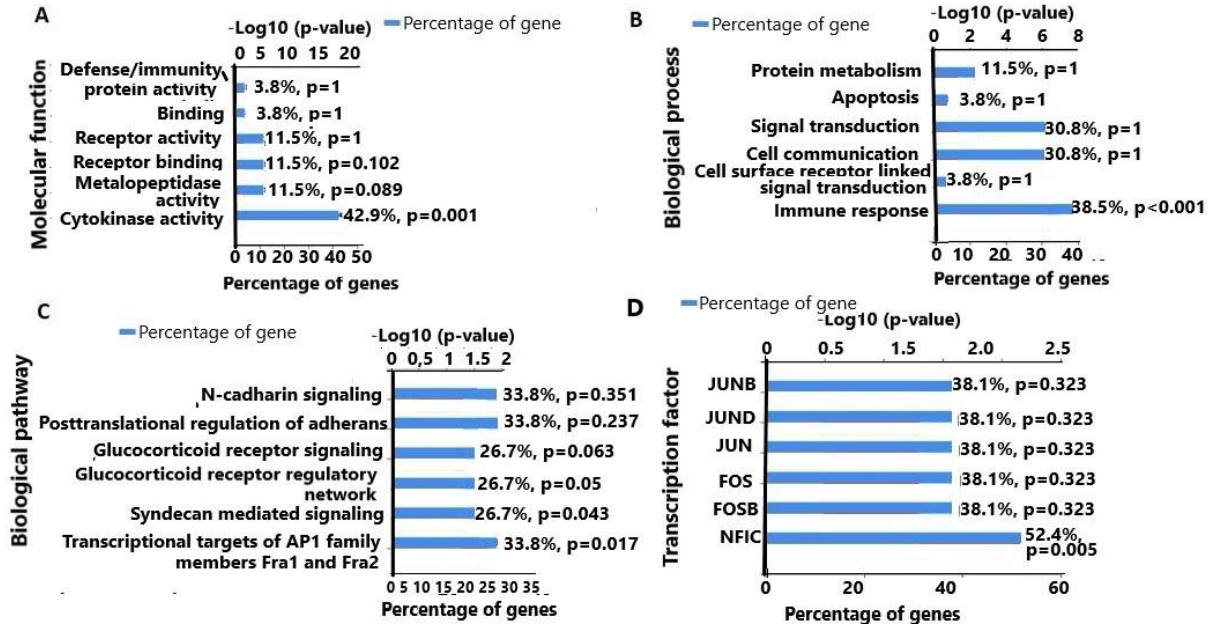
Supplementary Figure 1. Performance evaluation metrics for each ML-based model to include sensitivity, specificity, accuracy, and AUC using the RFE variable selection algorithm for distinguishing MCI from NC and corresponding AUC curves for the top five models.



Supplementary Figure 2. Performance evaluation metrics for each ML-based model to include sensitivity, specificity, accuracy, and AUC using the RFE variable selection algorithm for distinguishing MCI sufferers from AD and corresponding AUC curves for the top five models.



Supplementary Figure 3. Cytokines involved in various biological between AD and controls. The pathways affected include (A) molecular function; (B) biological processes; (C) biological pathways; (D) transcription factors.



Supplementary Figure 4. A) Clustergram of LINC RNA with cytokines: (1) LINC01937, (2) LINC02376, (3) LINC02090, (4) LINC01104, (5) LINC02665, (6) LINC01920, (7) LINC00687, (8) LINC00944, (9) LINC01501, and (10) LINC 01054. B) Clustergram of miRNA with cytokines: (1) miR-1934, (2) hsa-miR-27a-3p, (3) hsa-miR-142a-3p, (4) miR-224-5p, (5) hsa-miR-503-3p, (6) hsa-miR-130a-3p, (7) hsa-miR-299-5p, (8) hsa-miR-466-1-5p, (9) hsa-miR-125b-1-3p and (10) hsa-let-7e-5p. C) Clustergram of miRNA with cytokines. (1) miR-1945, (2) hsa-miR-55-1a, (3) hsa-miR-551b, (4) hsa-miR-4278, (5) hsa-miR-3181, (6) hsa-miR-572, (7) hsa-miR-18b, (8) hsa-miR-18a, (9) hsa-miR-337-5p, and (10) hsa-miR-638. D) Clustergram of TFs (Transcriptional factors) affected by these cytokines. (1) FLI1 1598 UP, (2) (2) FLI1 1597 UP, (3) ATF6, (4) TWIST2, (5) TP53, (6) 9ZXDC, (7) IRF5, (8) IRF4, (9) MECOM, (10) HNF4A

