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In Alzheimer's Subjects, Bezisterim Modulates Age-Accelerating Epigenetics in Genes Associated With Sustaining Inflammation via Kinase Cascades, Chromatin Remodeling, and Transcription Factors

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Introduction and Methods

- Bezisterim is an oral anti-inflammatory insulin-sensitizing sterol with an attractive safety profile. We have analyzed DNA methylation changes in mild/moderate AD patients in a recent randomized, placebo-controlled study (NCT04669028). We investigated treatment-related methylation changes in Epigenetic Age Acceleration (EAA) clocks and the promoters of genes associated with aging and Alzheimer's disease (AD) and correlated these changes with changes in clinical measures. We identified 477 genes with significant treatment-associated differences in promoter methylation (FDR < 0.05), which may confer mechanistic benefit in disease according to published reports.
- Working classifications of these genes are described below

Gene Classifications

PBDPM Genes
- Potentially Beneficial Differential Promoter Methylation Genes
- FDR (P<0.05) bezisterim vs placebo average promoter CpG beta values

EAA Clock Genes
- PBDPM Epigenetic Age Acceleration Genes
  - Horvath2.EAA, Hannum.EAA, GrimAge.EAA, DamAge.EAA, IntrinClock.EAA, StocZhang.EAA, StocHorvath.EAA, StocPhenoAge.EAA, RetroClockV1.EAA, RetroClockV2.EAA

Aging and AD Trait Genes
- 13 traits – identified from aging clocks and selected AD covariates
  - EAA, inflammation, cognition, T2D, obesity, myeloid cell polatization, kinase cascades, lipid dysregulation, glycolysis (Warburg effect), phosphoprotein dysregulation, transcription factors , and chromatin remodeling

AD Hub Genes
- PDPBM Alzheimer's disease Hub Genes
  - Genes identified in publications as hub genes in Alzheimer's disease

Results

We have detected significant trends for treatment-associated decreased EAA in 13 biological aging clocks (Table 1). All 13 clocks showed decreased EAA for bezisterim, and increased EAA for placebo. We have identified the potential benefits of bezisterim treatment associated with aging and AD traits (Table 2). Bezisterim anti-inflammatory and age deceleration activities decrease DAMP innate inflammation and EAA, resulting in decreased insulin resistance, blood glucose, and modulated myeloid cell methylome, decreased kinase (e.g., ERK, JNK, p38) , transcription factors (e.g., NFkB, STAT3), chromatin remodeling and cytokines (e.g., TNF), and aging and AD cognitive and functional decline (Alzheimer's Disease Composite Score, Alzheimer's Disease Cooperative Study Activities of Daily Living, Clinical Dementia Rating Sum of Boxes, Alzheimer's Disease Cooperative Study Clinical Global Impression of Change, Alzheimer's Disease Assessment Scale Cognitive Subscale 12, Global Statistical Test, and Neuropsychiatric Index). Table 2 indicates the number of the 447 PBDPM genes associated with the activities, as well as the number of AD hub genes and EAA clock genes, and arrows link figures detailing the interconnections with the specific EAA clock genes in Figures 1-4.

Aging and AD can be viewed in 4 stages:

1. inflammation drives epigenetic age acceleration, leading to
2. alterations in expression of genes involved in metabolic and myeloid inflammatory pathways that trigger
3. kinase and transcription dysregulation which result in
4. altered expression of genes associated with neurologic decline.

All genes depicted in Figures 1-4 are EAA clock genes; the blue symbol indicate EAA clock genes that are also known AD hub genes. Figure 1 indicates that 39 of the 45 EAA clock genes are associated with inflammation, and 19 are AD hub genes. Figure 2 shows 33 EAA clock genes associated with metabolic dysregulation, including myeloid polarization of which, 16 are AD hub genes. Figure 3 details 24 EAA clock genes associated with kinase cascades, transcription and chromatin, including 10 AD hub genes. Figure 4 displays 30 EAA clock genes that are associated with cognition and functional decline in aging and AD; 14 of these are AD hub genes.

Figure 5 resolves genes common to the 4 processes comprised of 9 EAA clock genes (NEAT1 is not depicted, as it is a regulatory long non-coding RNA), of which 7 (including NEAT1) are AD hub genes. The table in Figure 5 identified the EAA clocks associated with CpGs from these genes. Table 3 shows curated aging and AD traits associated with each of the common EAA clock genes in Figure 5.

Table 1. Bezisterim Decreased Epigenetic Age Acceleration

Clock	Bezisterim mean change	Placebo mean change	t-test P (Welch Correction)
PCGrimAge.EAA	-0.94	+1.00	0.058
PCHannum.EAA	-1.10	+1.18	0.058
PCHorvath2.EAA	-0.74	+0.78	0.087
IntrinClock.EAA	-2.12	+2.25	0.025
DamAge.EAA	-1.90	+2.01	0.063
StocPhenoAge.EAA	-1.85	+1.97	0.0078
StocZhang.EAA	-2.57	+2.73	0.015
StocHorvath1.EAA	-1.66	+1.78	0.049
RetroClock.EAA	-1.40	1.49	0.0087
RetroClockV2.EAA	-1.56	+1.66	0.0077
SystemsAge.EAA	-1.93	+2.05	0.012
Heart.EAA	-1.98	+2.10	0.014
Lung.EAA	-1.45	+1.54	0.081

Figure 1. Inflammation EAA Clock PBDPM Genes

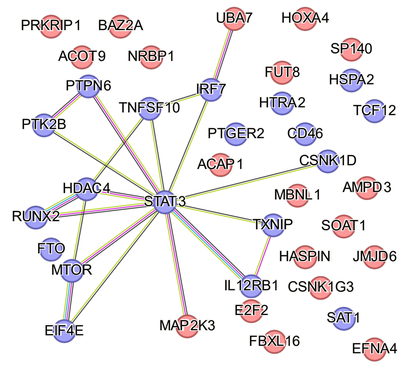


Figure 2. Metabolic Inflammation EAA Clock PBDPM Genes

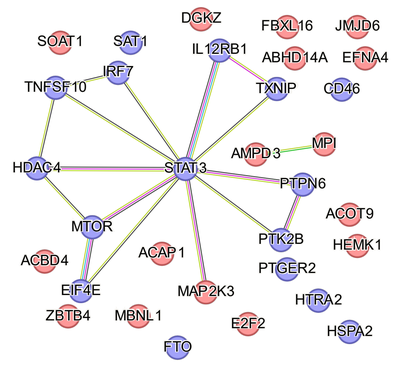


Figure 3. Kinase & Transcription EAA Clock PBDPM Genes

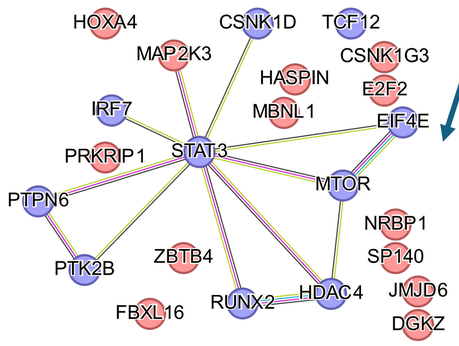


Figure 4. Cognition & Neurologic Assessment Genes

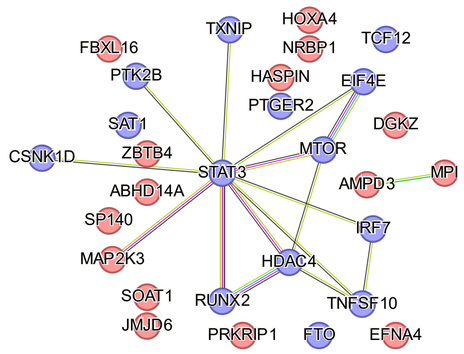
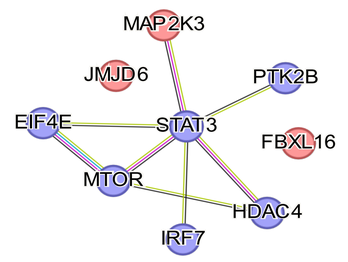


Table 2 Bezisterim Modulates Promoter Methylation of AD and Aging G

Damage-Associated Molecular Patterns (DAMP)	Bezisterim	EAA Clock Genes	AD Hub Genes	PBDPM Genes
Innate Immune Inflammation Epigenetic Age Acceleration	Anti-inflammatory Decreases epigenetic age acceleration	39 45	148	353
Metabolic Dysregulation Myeloid Cell Polarization	Insulin sensitizer Modulates Myeloid Methylome	33	117	279
Kinase Cascades Transcription Factors Chromatin Remodeling	Modulates ERK Modulates NFκB Modulates TNF	24	125	242
Cognitive decline Alzheimer's disease	Directional improvement in AD neurologic assessments	30	90	189

Figure 5. Common EAA Clock PBDPM Genes



Gene	Name	EAA Clocks
EIF4E	eukaryotic translation initiation factor 4E	StocPhenoAge.EAA
FBXL16	F-box and leucine rich repeat protein 16	PCHorvath2.EAA, StocZhang.EA
HDAC4	histone deacetylase 4	PCHorvath2.EAA
IRF7	interferon regulatory factor 7	DAMAge.EAA
JMJD6	jumonji domain containing 6, arginine demethylase and lysine hydroxylase	IntrinClock.EAA
MAP2K3	mitogen-activated protein kinase kinase 3	IntrinClock.EAA, StocPhenoAge.EAA
MTOR	serine/threonine-protein kinase mTOR	IntrinClock.EAA
NEAT1	nuclear paraspeckle assembly transcript 1	StocZhang.EAA, RetroClock V1/2
PTK2B	protein-tyrosine kinase 2-beta	DAMAge.EAA
STAT3	signal transducer and activator of transcription 2	DAMAge.EAA

Table 3. Common EAA Clock PBDMP Gene Traits

Gene	Traits
EIF4E	Dementia, Phosphoprotein, Microglial Neuroinflammation, M1 polarization, Obesity, Lipids
FBXL16	APP, Neuroinflammation, Cognition, pTau, T2D, Warburg, M1 polarization, NIA Nominated Aging and AD Target
HDAC4	Cognition, Microglial neuroinflammation, T2D, Obesity, Warburg, M1 polarization, Chromatin, AD, PD expression
IRF7	Microglial neuroinflammation, synapse loss, Abeta, Cognition, T2D, M1 polarization, Warburg, Obesity, Kinase, Phosphoprotein, Lipids
JMJD6	AD Demethylation Epigenetic Regulation, Cognition, pTau, Inflammation, TRAF6, NFkB, Obesity, Lipids, Histone Modification
MAP2K3	(p38 MAPK) Dementia, Cognition, Aging, Microglial neuroinflammation
MTOR	(Molecular Target Of Rapamycin) pTau, Abeta, Cognition, ROS, Microglial neuroinflammation, Warburg, Obesity, T2D
NEAT1	AD, pTau, Warburg, Microglial neuroinflammation, NFkB, M1 polarization, Lipids, T2D, Obesity, Cognition
PTK2B	Abeta, pTau, Cognition, Warburg, M1 polarization, Obesity, T2D, NIA Nominated Aging and AD Target
STAT3	Transcription, Chromatin, Neuroinflammation, Abeta, Cognition, M1 polarization, Warburg, T2D, Obesity, NIA Nominated aging and AD target

Conclusions

- In this exploratory analysis, bezisterim was associated with decreased EAA in 13 biological aging clocks, with significant potentially beneficial modulation of promoter methylation of 477 genes associated with aging and AD, including 45 EAA clock genes and 172 AD hub genes. These genes wer broadly represented in 13 covariate traits.
- Changes in cognitive and functional neurologic traits were associated with 189 PBDPM genes, including 30 EAA clock genes and 14 AD hub genes.
- There were 10 EAA clock genes with significant treatment-associated modulation of promoter methylation common to the 4 classifications in Figures 1-4, 7 of which were AD hub genes (Figure 5). These 10 genes include 3 genes nominated by NIA as AD Aging and AD Targets: the AD demethylation gene *JMJD6*, the molecular target of the anti-aging drug Rapamycin (MTOR), kinases (p38 MAPK and PTK2B), and transcriptional and chromatin remodeling genes STAT3 and HDAC4. Bezisterim may modulate a tightly coupled stress-immune-epigenetic-translation axis that converts transient danger signals into persistent inflammatory cell states that contribute to AD disease progression.
- We previously reported that bezisterim treatment (n = 24) vs placebo (n = 26) of AD participants was associated with directional improvements in all 8 neurologic assessments [1], unexpected by chance (Fisher Exact Test P = 1.6e4), with magnitudes comparable to the 2 approved anti-amyloid antibodies at that time. DNA methylation data was available for 17 bezisterim and 16 placebo participants. Confirmation of bezisterim clinical activity in AD awaits replication in a well-powered study.
- We are currently analyzing bezisterim promoter methylation changes in PD (NCT06757010) and LC (NCT0684719) clinical studies prospectively for modulation of PBDPM genes that may provide important biomarkers to support drug approval and orthogonal measures of efficacy as well as increasing our understanding of the etiopathogenesis of neuroinflammatory disease that may give insight into epigenetic drivers of these diseases for therapeutic intervention.

References

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