Exploring the transcriptome for novel biomarker discovery

AMP-AD Biomarker Pilot Study

Nilüfer Ertekin-Taner, MD, PhD
Professor of Neurology and Neuroscience
Mayo Clinic Florida

Spring ADC Meeting
May 2nd, 2019
AMP-AD Team

Mayo Clinic Florida
Nilüfer Ertekin Taner, MD, PhD
Steven G. Younkin, MD, PhD
Dennis Dickson, MD
Minerva Carrasquillo, PhD
Mariet Allen, PhD
Joseph Reddy, PhD
Xue Wang, PhD
Kim Malphrus
Thuy Nguyen
Sarah Lincoln

University of Florida
Todd Golde, MD, PhD.
Jada Lewis, Ph.D.
Paramita Chakrabarty, PhD
Yona Levites, PhD

Institute for Systems Biology
Nathan Price, PhD
Cory Funk, PhD
Hongdong Li, PhD
Paul Shannon

Banner Sun Health
Tom Beach, MD

New York Genomics Center (WGS)

Mayo Clinic Genomics Core
Bruce Eckloff (RNASeq)
Julie Cunningham, PhD (GWAS)

Mayo Clinic Bioinformatics Core
Asha Nair, PhD

Mayo Clinic Epigenomics Development Laboratory
Tamas Ordog, MD
Jeong-Hong Lee, PhD

Mayo Clinic IT
Andy Cook
Curt Younkin

Mayo Clinic Study of Aging
Ronald Petersen, MD, PhD

*AMP-AD consortium*
Overarching Goal

A System Approach to Targeting Innate Immunity in AD (U01 AG046193)

To identify and validate targets within innate immune signaling, and other pathways that can provide disease-modifying effects in AD using a multifaceted systems level approach.
AMP-AD Phase I

**Original Aim 1:** To detect transcript alterations in innate immunity genes in mice and humans.

- RNAseq human and mice brains.
- Differential expression.
- Protein/Nanostring validation
- Expression quantitative trait loci (eQTL).

**Original Aim 2:** To assess AD risk conferred by variants in innate immunity genes from Aim 1.

- Test eQTL for effects on AD risk
- Functionally annotate AD risk variants for effects on gene expression.
- Expression/Transcription factor networks.

**Original Aim 3:** To manipulate innate immune states in vivo.

- rAAV based genetic manipulation in mice and cells.
- Evaluate Aβ, tau, neurodegeneration in model systems.

**Original Aim 4:** To determine outcome of gene manipulation in wild type mice.

Behavioral studies in nontransgenic mice.
AMP-AD Phase-I: Outcomes

“Immune” co-expression networks enriched for AD risk genes.

- Modulation of innate immunity proteins influences Aβ and tau pathophysiology (Chakrabarty et al., Neuron, 2015; Li et al., FASEB, 2015).

- AD risk genes *PLCG2, ABI3 and TREM2* have higher levels in AD brains, Aβ models and reside in immune networks (Sims et al., Nature Genetics, 2017; Conway et al., Molec Neurodeg, 2018).

- 20 “immune” targets undergoing testing in model systems

AD candidate genes: *CD33, FERMT3*, *HLA-DRB1/6, INPP5D, MS4A, PLD4*, *RIN3, TYROBP, TREM2*
AMP-AD Phase I: Outcomes

Regulatory variant at \textit{TREM} locus.

An intronic variant at the \textit{TREM} locus is associated with higher brain \textit{TREM2} and \textit{TREML1} levels and resides in a TF binding site (Carrasquillo et al., Alzheimer’s and Dementia, 2016). - Omics integration, directionality, mechanism.

Myelin networks in AD.

Conserved brain myelination networks are altered in AD and PSP (Allen et al., Alzheimer’s and Dementia, 2017). - Comparative transcriptomics, novel targets.

- Disseminated rAAV vectors to the AMP-AD research community
- Many AD candidate risk genes have strong eQTL and/or differential expression in brain (Allen et al, Neurology Genetics 2015, 2017; Ridge et al., Genome Medicine, 2017; Mukherjee et al., Alzheimer’s and Dementia, 2017).
**Hypothesis**: Brain transcriptional changes that occur in AD may be reflected in the periphery (plasma or blood).

- Genetic/epigenetic risk factors **upstream** of disease neuropathology may lead to perturbations in gene expression levels/splicing. *E.g.* Transcriptional changes as a consequence of regulatory disease variants.

- Gene expression changes **downstream** of disease neuropathology may be detectable in both brain and periphery. *E.g.* Transcriptional changes reflecting neuronal loss, microglial activation etc.

**Goal**: Establish and validate a scalable and reliable approach to identify AD biomarkers leveraging brain and plasma transcriptome from well-characterized cohorts.
Approach

Gene Expression Profiling (GEP) utilizing RNAseq on plasma samples

**Analysis 1:** GEP Baseline, Decliners vs Non-decliners

- **Time point A (Baseline):** All participants are clinically normal (CN).
- **Time point B:** Decliners-Incident MCI/AD vs. Non-decliners-CN

**Analysis 2:** GEP Time point A to B, Decliners vs Non-Decliners

- **Plasma, CN:** PET- Aβ- (N=44)
- **Plasma, CN:** PET- Aβ- (N=51)
- **Plasma, CN:** (PET- Aβ+ where available) (N=42)

**Analysis 3:** GEP Decliners vs Non-decliners

- **Plasma, MCI/AD** (N=50)

- **Analysis 1:** Preclinical biomarker, differences between decliners and non-decliners prior to clinical conversion to MCI/AD.
- **Analysis 2:** Biomarkers of rate of decline, plasma expression changes over time in decliners vs. non-decliners.
- **Analysis 3:** Biomarkers of clinical impairment, differences between decliners and non-decliners, for comparison with brain GEP results from AMP-AD.
Cohort

Data points and sample sizes collected for MCSA.
To date there are 4,877 study subjects, most with extensive phenotypic information relevant to aging and dementia.
## Cohort Demographics

### Demographics

<table>
<thead>
<tr>
<th>Plasma RNAseq Cohort (n = 104)</th>
<th>Decliners (n = 51)</th>
<th>Non-Decliners (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% APOE e4+</td>
<td>39.2%</td>
<td>35.8%</td>
</tr>
<tr>
<td>% Female</td>
<td>40.0%</td>
<td>43.4%</td>
</tr>
<tr>
<td>Mean Age at Time Point A (SD)</td>
<td>79.6 (6.7)</td>
<td>79.1 (6.3)</td>
</tr>
<tr>
<td>Mean Age at Time Point B (SD)</td>
<td>82.1 (6.8)</td>
<td>81.6 (6.5)</td>
</tr>
<tr>
<td>Mean Age difference Time Point B-A (SD)</td>
<td>2.5 (0.5)</td>
<td>2.5 (0.6)</td>
</tr>
<tr>
<td>Date Range Time Point B</td>
<td>10/2008-12/2017</td>
<td>8/2008-11/2017</td>
</tr>
</tbody>
</table>

- All 104 participants are part of the Mayo Clinic Study of Aging.
- Decliners and non-decliners are matched for ages, sex, dates of collection.
- Plasma for Time Point A was available for 42 decliners and 44 non-decliners.
- Plasma for Time Point B was available for 50 decliners and 51 non-decliners.
- RNA was extracted from a total of 187 unique plasma samples (961 aliquots).
Plasma cell-free mRNA and miRNA expression

| MCSA Frozen plasma | Total RNA extraction (Qiagen miRNeasy Serum/Plasma Kit) + DNase I treatment + Spike-in control: miR-39 | NEXTflex Small RNA-Seq Kit v3 (Bio Scientific) for miRNAseq
| Draw years: 2005-2017 | | SMARTer Pico RNA-Seq (Clontech) for mRNAseq
| N=104 participants, 187 plasma samples, 961 aliquots | | Human Neuropathology custom panel (NanoString) for targeted analysis (760 genes)

**Neuropsychology measures**: memory, attention executive function, language, visuospatial skills
**Neuroimaging**: amyloid PiB-PET, FDG-PET
**Clinical history**: smoking, obesity, hypertension, type-2 diabetes
**Clinical diagnosis**: AD, amnestic MCI, normal

Data Analysis
RNA extraction from plasma: Sample QC

- Nanodrop Spectroscopic absorbance at 415nm for detection of low level hemolysis ($A_{415}$ readings >0.2 classified as being hemolyzed): **961 plasma aliquots extracted/QC’ed.**

  

- Agilent Bioanalyzer Total RNA 6000 Pico used to determine cfRNA concentration. (Absence of ribosomal bands indicates no cellular RNA contamination).

- Taqman qPCR for detection of expressed genes HIPK3, FLI1 and Cel_miR-39 Spike in control (Qiagen)

  

Concentration (pg/ul): mean = 159; min = 14, max = 857
RNA extraction from plasma: Concentrations

For the 187 unique plasma samples that will undergo RNAseq, RNA was extracted from a total of 961 plasma aliquots in order to obtain sufficient RNA.

- Median RNA concentration = 134 pg/ul, mean±standard deviation = 159±121.9, range 14-857 pg/ul.
Plasma miRNAseq pilot: Bioinformatics pipeline

CAP-miRSeq

Raw Reads (fastq/fastq.gz)

Cutadapt

Adapter Trimmed Reads (fastq)

Bowtie

miRDeep2 mapper

Ref Genome

miRBase

Aligned BAM

IGV

miRNA coding region variants

GATK

HTSeq

Summary report

Known/Novel miRNAs

Other RNAs

dgeR

Differential miRNAs

A. Read pre-processing
B. Core module for known and novel miRNA detection
C. Differential miRNA expression
D. Read visualization, SNV detection, all RNA quantification

CAP-miRSeq: a comprehensive analysis pipeline for microRNA sequencing data

Zhifu Sun, Jared Evans, Aditya Bhagwate, Sumit Middha, Matthew Bockol, Huihuang Yan, and Jean-Pierre Kocher


PMCID: PMC4070549
### Plasma miRNAseq pilot preliminary results

<table>
<thead>
<tr>
<th>Dataset</th>
<th>miRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSE105052</td>
<td>285</td>
</tr>
<tr>
<td>GSE53451</td>
<td>268</td>
</tr>
<tr>
<td>Mayo8</td>
<td>160</td>
</tr>
<tr>
<td>Genboree8</td>
<td>141</td>
</tr>
</tbody>
</table>

**GSE105052**: Plasma miRNA profiles of 25 Friedreich's ataxia patients and 17 healthy subjects, by deep sequencing using Illumina HiScan SQ.

**GSE53451**: Plasma exosomes miRNA sequenced from 7 humans

- CAP-miR >Genboree for miRNA detection
- Mayo Clinic plasma samples have ~50% overlap with public databases
MAP-RSeq: Mayo Analysis Pipeline for RNA sequencing

Kalari et al. BMC Bioinformatics 2014, 15:224

Plasma mRNAseq pilot: Bioinformatics pipeline
Plasma mRNAseq pilot: Preliminary results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total counts</th>
<th>Counts in Genes</th>
<th>Intergenic</th>
<th>Intronic</th>
<th>Globins</th>
<th>Ribo</th>
<th>UTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>s_65289_RNA_Plasma</td>
<td>10894248</td>
<td>2557724 (23.5)</td>
<td>15%</td>
<td>14%</td>
<td>27 (0%)</td>
<td>136 (0%)</td>
<td>25%</td>
</tr>
<tr>
<td>s_66883_RNA_Plasma</td>
<td>8283524</td>
<td>1598128 (19.3)</td>
<td>20%</td>
<td>22%</td>
<td>63 (0%)</td>
<td>82 (0%)</td>
<td>22%</td>
</tr>
<tr>
<td>s_66959_RNA_Plasma</td>
<td>16646080</td>
<td>5135575 (30.9)</td>
<td>7%</td>
<td>7%</td>
<td>66 (0%)</td>
<td>104 (0%)</td>
<td>30%</td>
</tr>
</tbody>
</table>

- ~11,000 genes with >10 raw reads (~10,000 protein coding).
- Negligible counts for globin and ribosomal genes.
Plasma mRNA Expression: Targeted Panel

<table>
<thead>
<tr>
<th>Official Symbol</th>
<th>Accession</th>
<th>Alias / Previous Symbol</th>
<th>Official Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE</td>
<td>NM_000412</td>
<td>AD2</td>
<td>apolipoprotein E</td>
</tr>
<tr>
<td>APP</td>
<td>NM_00484.3</td>
<td>AD1</td>
<td>amyloid beta precursor protein</td>
</tr>
<tr>
<td>BDNF</td>
<td>NM_170732.4</td>
<td></td>
<td>brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>CD14</td>
<td>NM_000591.2</td>
<td></td>
<td>CD14 molecule</td>
</tr>
<tr>
<td>CD33</td>
<td>NM_01177608.1</td>
<td>SIGLEC3, SIGLEC-3, p67, FLJ00391</td>
<td>CD33 molecule</td>
</tr>
<tr>
<td>CLU</td>
<td>NM_203339.2</td>
<td>CLI, APOJ, SGP-2, SP-40, TRPM-2, KUB1, CLU1, CLU2</td>
<td>clusterin</td>
</tr>
<tr>
<td>CR1</td>
<td>NM_000573.3</td>
<td>CD35, KN</td>
<td>complement component 3b/4b receptor 1 (Knops blood group)</td>
</tr>
<tr>
<td>CRH</td>
<td>NM_000756.1</td>
<td>CRF, CRH1</td>
<td>corticotropin releasing hormone</td>
</tr>
<tr>
<td>CSF1R</td>
<td>NM_005211.2</td>
<td>FMS, C-FMS, CSFR, CD115</td>
<td>colony stimulating factor 1 receptor</td>
</tr>
<tr>
<td>CX3CR1</td>
<td>NM_001337.3</td>
<td>GPR13, CMKBR1, CMKDR1, V28, CCR1</td>
<td>chemokine (C-X3-C motif) receptor 1</td>
</tr>
<tr>
<td>CXCL16</td>
<td>NM_01100812.1</td>
<td>SR-PSOX, CXCL16, SRSPOX</td>
<td>chemokine (C-X-C motif) ligand 16</td>
</tr>
<tr>
<td>HDAC1</td>
<td>NM_004964.2</td>
<td>RPD3L1, HD1, GON-10</td>
<td>histone deacetylase 1</td>
</tr>
<tr>
<td>HDAC2</td>
<td>NM_001527.1</td>
<td>RPD3, YAF1</td>
<td>histone deacetylase 2</td>
</tr>
<tr>
<td>HDAC6</td>
<td>NM_00132225.1</td>
<td>KIAA0901, JM21, HD6, FLJ16239, PPP1R90</td>
<td>histone deacetylase 6</td>
</tr>
<tr>
<td>HDAC7</td>
<td>NM_001098416.2</td>
<td>HDAC7A, DKKP5860917</td>
<td>histone deacetylase 7</td>
</tr>
<tr>
<td>IL10</td>
<td>NM_000572.2</td>
<td>CSIF, TGIF, IL10A, IL-10</td>
<td>interleukin 10</td>
</tr>
<tr>
<td>IL1R</td>
<td>NM_000576.2</td>
<td>IL1F2, IL-1B, IL1-BETA</td>
<td>interleukin 1 beta</td>
</tr>
<tr>
<td>IL1R1</td>
<td>NM_001320984.1</td>
<td>IL1R, IL1RA, D2S1473, CD121A</td>
<td>interleukin 1 receptor, type I</td>
</tr>
<tr>
<td>IL6</td>
<td>NM_000600.3</td>
<td>IFNB2, IL-6, BSF2, HGF, HSF</td>
<td>interleukin 6</td>
</tr>
<tr>
<td>NINJ2</td>
<td>NM_016533.4</td>
<td></td>
<td>ninjurin 2</td>
</tr>
<tr>
<td>NTRK1</td>
<td>NM_001012331.1</td>
<td>TRK, TRKA, MTC</td>
<td>neurotrophic tyrosine kinase, receptor, type 1</td>
</tr>
<tr>
<td>SORL1</td>
<td>NM_003105.5</td>
<td>C11orf32, gp250, LR11, LRP9, SorLA, SorLA-1</td>
<td>sortilin-related receptor, L(DLR class) A repeats containing</td>
</tr>
<tr>
<td>SP1</td>
<td>NM_003109.1</td>
<td></td>
<td>Spi1 transcription factor</td>
</tr>
<tr>
<td>SPI1</td>
<td>NM_003120.1</td>
<td>PU.1, SPI-A, OF, SPFI1, SPI-1</td>
<td>Spi-1 proto-oncogene</td>
</tr>
<tr>
<td>STAT1</td>
<td>NM_139266.1</td>
<td>STAT91, ISGF-3</td>
<td>signal transducer and activator of transcription 1</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>NM_000660.4</td>
<td>TGFβ, PD1, CED, TGFbeta</td>
<td>transforming growth factor beta 1</td>
</tr>
<tr>
<td>TLR4</td>
<td>NM_138554.2</td>
<td>hToll, CD284, TLR-4, ARMD10</td>
<td>toll like receptor 4</td>
</tr>
<tr>
<td>TNF</td>
<td>NM_000594.2</td>
<td>TNFA, TNFSF2, DIF, TNF-alpha</td>
<td>tumor necrosis factor</td>
</tr>
<tr>
<td>TNFRSF1A</td>
<td>NM_001065.2</td>
<td>TNFR1, TNF-R, TNFAR, TNFRI, CD120a, TNF-R55</td>
<td>tumor necrosis factor receptor superfamily member 1A</td>
</tr>
<tr>
<td>TREM2</td>
<td>NM_018965.3</td>
<td>TREM-2, Trem2a, Trem2b, Trem2c</td>
<td>triggering receptor expressed on myeloid cells 2</td>
</tr>
</tbody>
</table>

Table 1. Subset of the 760 gene transcripts that can be tested with the Nanostring neuropathy panel.
Plasma mRNA Targeted Panel: Results

- 384/760 genes from the NanoString Neuropathology panel were detected above background (>26 counts).
- Median raw counts for detectable genes ~27-12,000.
Plasma mRNA Expression: Mayo Clinic Custom Panel

- **NanoString nCounter™ Mayo Clinic Custom CodeSet Design:**
  - 50 genes enriched for those implicated in inflammation and/or vascular disease.
  - 49 transcripts already detected in plasma by either RNAseq or the NanoString Human Neuropathology panel pilots.

- Florida Consortium for African-American AD Studies (FCA³DS)* case-control cohort will be evaluated with this panel for 442 plasma RNA samples.
  - Mayo Clinic Florida (N. Ertekin-Taner/N. Graff-Radford).
  - Miami Mount Sinai (M. Greig-Custo/R. Duara)
  - University of Florida (M. Wicklund)

- Differential gene expression and eQTL analyses will be performed.

<table>
<thead>
<tr>
<th>Gene 1</th>
<th>Gene 2</th>
<th>Gene 3</th>
<th>Gene 4</th>
<th>Gene 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2M</td>
<td>CCL2</td>
<td>HDAC1</td>
<td>IL4</td>
<td>PICALM</td>
</tr>
<tr>
<td>ABCA7</td>
<td>CD14</td>
<td>HDAC2</td>
<td>ITGAL</td>
<td>SORL1</td>
</tr>
<tr>
<td>ACTB</td>
<td>CD33</td>
<td>HDAC6</td>
<td>ITGB2</td>
<td>SP1</td>
</tr>
<tr>
<td>AIF1</td>
<td>CLU</td>
<td>HDAC7</td>
<td>LRP1</td>
<td>SPI1</td>
</tr>
<tr>
<td>AKAP9</td>
<td>CR1</td>
<td>ICAM1</td>
<td>LY86</td>
<td>STAB1</td>
</tr>
<tr>
<td>AOAH</td>
<td>CRH</td>
<td>IL16</td>
<td>LY96</td>
<td>STAT1</td>
</tr>
<tr>
<td>APOE</td>
<td>CSF1R</td>
<td>IL18</td>
<td>LYZ</td>
<td>TGFβ1</td>
</tr>
<tr>
<td>APP</td>
<td>CX3CR1</td>
<td>IL1B</td>
<td>MS4A6A</td>
<td>TLR4</td>
</tr>
<tr>
<td>BIN1</td>
<td>CXCL16</td>
<td>IL1RN</td>
<td>NINJ2</td>
<td>TNFRSF1A</td>
</tr>
<tr>
<td>BLNK</td>
<td>HCK</td>
<td>IL33</td>
<td>OAZ1</td>
<td>TNFRSF1B</td>
</tr>
</tbody>
</table>

*Funded by Florida Department of Health Ed and Ethel Moore Alzheimer’s Disease Grant
Summary of Upcoming Biomarker Datasets

- Plasma RNAseq – AMP-AD U01 biomarker pilot
- Plasma miRNAseq – AMP-AD U01 biomarker pilot
- Plasma Nanostring (African-Americans) – FCA³DS
- PAXgene RNAseq – M²OVE AD RF1

AMP-AD U01 supplement:

Analysis 1: GEP Baseline, Decliners vs Non-decliners

Analysis 2: GEP Time point A to B, Decliners vs Non-Decliners

Analysis 3: GEP Decliners vs Non-decliners
Expected Outcomes

• Establish **scalable methodology** for measurement of cell-free transcriptome in archived plasma samples.

• Discovery of transcripts and transcriptional networks from plasma:
  • That are altered prior to clinical MCI/AD: Candidate **pre-clinical biomarkers**.
  • That have differential rate of change in decliners vs. non-decliners: Candidate **biomarkers of rate of decline**.
  • That are altered in incident MCI/AD: Candidate **biomarkers of clinical impairment**.

• Cross-comparisons with brain transcripts and transcriptional networks that are altered in AD to identify **CNS transcriptional changes that are reflected in the periphery**.

• Identification of **eQTL that may drive transcript biomarkers**.
Future Implications

• To follow progression of specific aspects of the disease pathophysiology as reflected in peripheral transcripts and transcriptional networks (e.g. inflammation, synaptic physiology, lipoproteins).

• Plasma transcriptional biomarkers that have strong correlations with AD pathology may be utilized in the stratification and enrichment of subjects for clinical trials.

• These biomarkers can be used to follow response to therapy for those drugs that target specific transcripts/proteins or their networks.
### Other blood gene expression studies

All study samples come from PAXgene blood from the Mayo Clinic Study of Aging

<table>
<thead>
<tr>
<th>WG-DASL</th>
<th>NanoString: Transcripts</th>
<th>NanoString: Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GHR Foundation</strong></td>
<td><strong>Mayo Clinic ADRC</strong></td>
<td><strong>Mayo Clinic CIM</strong></td>
</tr>
</tbody>
</table>

**WG-DASL**
- N: 44 AD, 56 aMCI, 82 CN-PiB+, 61 CN-PiB-
- 29,377 probes (> 20,000 expressed in > 50% of samples).
- DEG analysis to identify early (CN-PiB+ vs CN-PiB-), disease relevant (AD vs CN-PiB-) changes.
- Future directions: Co-expression network analysis, PiB correlations and eQTL.

**NanoString: Transcripts**
- N: WG-DASL cohort (and 8 internal replicates)
- 78 targets + housekeeping and cell type markers.
- AD candidate genes: overall expression and alternatively spliced exons.
- DEG analysis between all groups.
- Future directions: Exon eQTL and PiB correlations.

**NanoString: Biomarker**
- Validation: WG-DASL cohort
- Replication: 116 CN PiB+, 209 CN PiB- (and 8 internal replicates)
- 47 targets (WG-DASL DEG’s) + housekeeping and cell type markers.
- Future Directions: Validation of WG-DASL measures and DEG replication analysis is ongoing.
NanoString Biomarker

**Approach**

**Hypothesis:** Disease relevant gene expression changes can be detected in blood. If true, these may be used as a biomarker to predict disease risk and/or identify subsets of individuals that may benefit from targeted therapies aimed at prevention or treatment.

**Discovery:** Illumina WG-DASL microarray; >17,000 genes

**RNA isolated from whole blood (PAXgene)**

**Replication:** Nanostring nCounter, 46 genes

**Validation:** Nanostring nCounter, 46 genes

**Phenotypes include:** Neurocognitive measures (Memory, Attention executive function, Language, Visuospatial skills); Neuroimaging (Amyloid PiB-PET, FDG-PET); Clinical history (smoking, obesity, hypertension, type-2 diabetes); Clinical diagnosis (AD, amnestic MCI, clinically normal)

**Target Gene Selection**

**DEG, N=28 genes**

**Extreme, N=9 genes**

**KCNH7 Expression**

**Replication**

25/46 genes measured reliably with NanoString; 17/25 consistent direction

**Samples**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Diagnosis</th>
<th>N</th>
<th>Mean Age (SD)</th>
<th>Females (%)</th>
<th>Mean RIN (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery</td>
<td>AD</td>
<td>44</td>
<td>87.39 (5.6)</td>
<td>24 (55%)</td>
<td>9.62 (0.44)</td>
</tr>
<tr>
<td>Discovery</td>
<td>aMCI</td>
<td>56</td>
<td>82.82 (5.5)</td>
<td>25 (45%)</td>
<td>9.66 (0.31)</td>
</tr>
<tr>
<td>Discovery</td>
<td>CN-PiB+</td>
<td>82</td>
<td>81.41 (5.7)</td>
<td>32 (39%)</td>
<td>9.58 (0.41)</td>
</tr>
<tr>
<td>Discovery</td>
<td>CN-PiB-</td>
<td>61</td>
<td>78.48 (4.4)</td>
<td>29 (48%)</td>
<td>9.68 (0.32)</td>
</tr>
<tr>
<td>Replication</td>
<td>CN-PiB+</td>
<td>116</td>
<td>74.11 (7.7)</td>
<td>58 (50%)</td>
<td>9.48 (0.49)</td>
</tr>
<tr>
<td>Replication</td>
<td>CN-PiB-</td>
<td>209</td>
<td>71.08 (7.5)</td>
<td>100 (48%)</td>
<td>9.35 (0.54)</td>
</tr>
</tbody>
</table>

**Results: Validation**

- **AD Candidate:** 5 ABCA7, CLU, APP, PSEN1, PSEN2
- **Housekeeping:** 4 ACTB, TXNL1, GAPDH, EIF4A2
- **Cell Type:** 4 CD3G, CD8B1, CD20, NAMPT

©2016 MFMER | slide-26