DNA methylation differences across multiple brain regions implicate *ANKRD30B* in Alzheimer's disease

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Introduction

- DNA methylation (DNAm) is an epigenetic mark that can regulate gene expression.
- Recent studies found specific differences in DNAm associated with Alzheimer's disease (AD). [1-5]
- Without studying gene expression it is difficult to conclude which gene is involved.

Aim: Integrate DNAm and gene expression profiling to discover novel genes involved in Alzheimer's disease.

Methods

Data Collection

- DNA methylation (DNAm) and gene expression of homogenate postmortem tissue was profiled.
- Four brain regions:
  - Cerebellum (CRB)
  - Dorsolateral prefrontal cortex (DLPFC)
  - Entorhinal cortex (ERC)
  - Hippocampus (HIPPO)
- DNAm measured with Human Methylation 450k BeadChip.
- Gene expression quantified via RNA-sequencing.

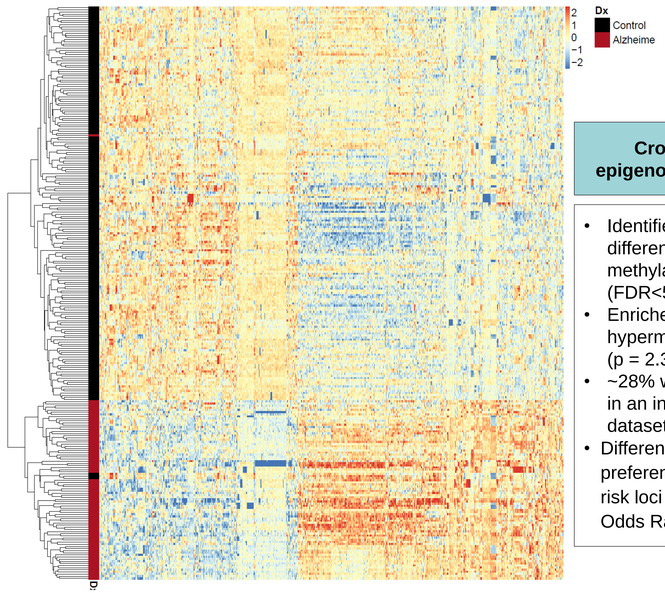
Characteristics of Samples

	CRB		DLPFC		HIPPO		ERC	
	Control	AD	Control	AD	Control	AD	Control	AD
N	43	24	47	21	48	17	49	20
Mean Age (SD)	60.63 (7.04)	79.47 (10.02)	61.54 (7.71)	79.95 (9.46)	61.71 (7.66)	81.54 (9.16)	61.62 (7.61)	79.69 (9.64)
Caucasian (%)	17 (39.5)	21 (87.5)	18 (38.3)	18 (85.7)	19 (39.6)	14 (82.4)	20 (40.8)	17 (85.0)
Male (%)	24 (55.8)	11 (45.8)	28 (59.6)	11 (52.4)	29 (60.4)	8 (47.1)	29 (59.2)	9 (45.0)
APOE4 (%)								
0	35 (87.5)	8 (33.3)	38 (86.4)	7 (33.3)	39 (86.7)	5 (29.4)	40 (87.0)	7 (35.0)
1	4 (10.0)	12 (50.0)	5 (11.4)	11 (52.4)	5 (11.1)	10 (58.8)	5 (10.9)	10 (50.0)
2	1 (2.5)	4 (16.7)	1 (2.3)	3 (14.3)	1 (2.2)	2 (11.8)	1 (2.2)	3 (15.0)

Data Analysis

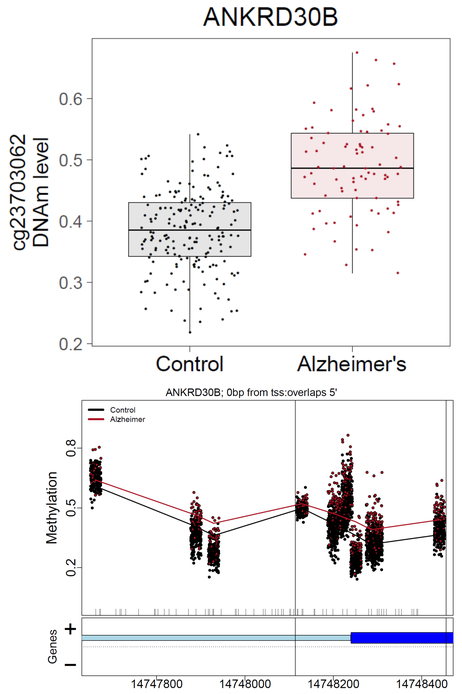
- "Cross-region" linear model to test association between DNAm and Alzheimer's disease (420,852 sites).
- Adjusted for age, sex, ancestry, and the first two principal components of negative control probes.
- Tested genes within 10kb of differential methylation for differential expression and association with DNAm.

Results



Cross-region epigenome-wide scan

- Identified 858 differentially methylated sites (FDR<5%)
- Enriched for hypermethylation in AD ($p = 2.30 \times 10^{-5}$)
- ~28% were consistent in an independent dataset [2]
- Differences are preferentially within AD risk loci (p = 0.00655, Odds Ratio = 4.37)



Hypermethylated site within *ANKRD30B*

- A CpG site within *ANKRD30B* is more methylated in AD than controls ($p = 6.49 \times 10^{-12}$, $\Delta = 0.104$)
- This association does not appear to be driven by:
  - Cell-type composition
  - APOE4 dosage
  - Age
- Replicated in Lunnon et al. [2] (p = 0.00035, $\Delta = 0.029$)

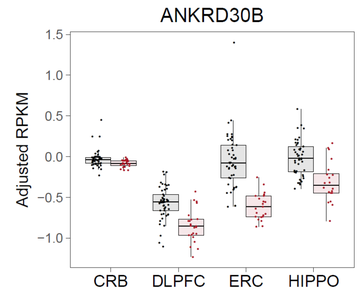
Hypermethylated region overlapping *ANKRD30B*

- 'Bumphunting' approach jointly tests adjacent probes for differential methylation
- A region overlapping the transcript start site of *ANKRD30B* is more methylated in AD than controls (FWER=0.027, 511 bp, 9 probes)

Results

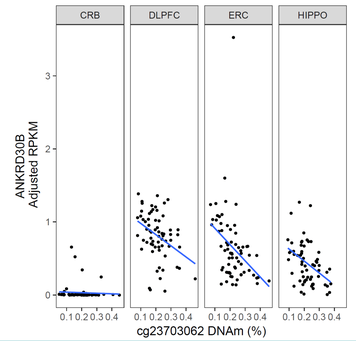
ANKRD30B is less expressed in Alzheimer's disease

- *ANKRD30B* is differentially expressed in:
  - Entorhinal cortex ($p = 3.71 \times 10^{-5}$, $\log_2$ fold change = -1.50)
  - Hippocampus (p = 0.00242, $\log_2$ fold change = -1.70)



Hypermethylation associates with reduced _ANKRD30B_ expression

- DNAm at cg23703062 correlates inversely with *ANKRD30B* expression in ERC:
  - p = 0.0233
  - β = -0.792



Discussion

Limitations

- Cell-type heterogeneity.
- Epiphenomena and secondary disease processes.

Conclusions

- Epigenetic changes are responsible for *ANKRD30B* dysregulation in Alzheimer's disease.

Future Directions

- Apply single-cell approaches to DNAm and gene expression profiling.

References

1. De Jager et al. (2014). Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. *Nature Neuroscience*.

2. Lunnon et al. (2014). Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. *Nature Neuroscience*.

3. Smith et al. (2018). Elevated DNA methylation across a 48-kb region spanning the HOXA gene cluster is associated with Alzheimer's disease neuropathology. *Alzheimer's & Dementia*.

4. Sanchez-Mut et al. (2014). Promoter Hypermethylation of the Phosphatase DUSP22 Mediates PKA-Dependent TAU Phosphorylation and CREB Activation in Alzheimer's Disease. *Hippocampus*.

5. Sanchez-Mut et al. (2018). PM20D1 is a quantitative trait locus associated with Alzheimer's disease. *Nature Medicine*.