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Use of endophenotypes in the search for genetic risk factors for Alzheimer's disease

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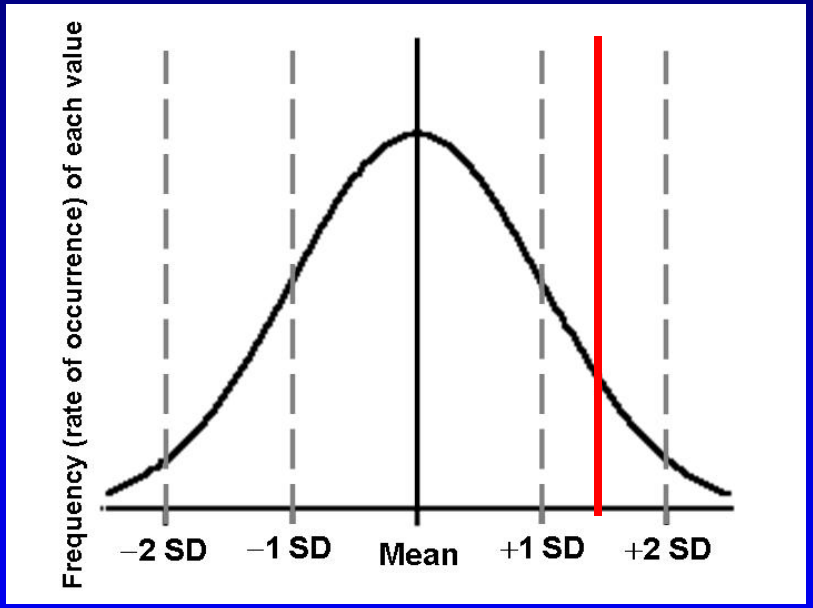
Novel genetic risk factors for Alzheimer's disease

- Common disease, common variant hypothesis
  - Genome-wide association studies
  - *APOE* fits this model but are there others?
- Common disease, many rare variants
  - Large-scale resequencing

Genetic liability

- Dichotomous characters
  - E.g. Presence/absence of a disease
    - Underlying loci are susceptibility genes
- Quantitative or continuous traits
  - E.g. Height, weight, blood pressure
    - Underlying loci are quantitative trait loci



Endophenotypes

- A heritable quantitative trait that is correlated with disease but measurable in all individuals regardless of disease status
- Should have simpler genetic architecture so that SNPs in candidate genes may directly lead to changes in the endophenotype
- Can provide a biological model of disease

Current Sample

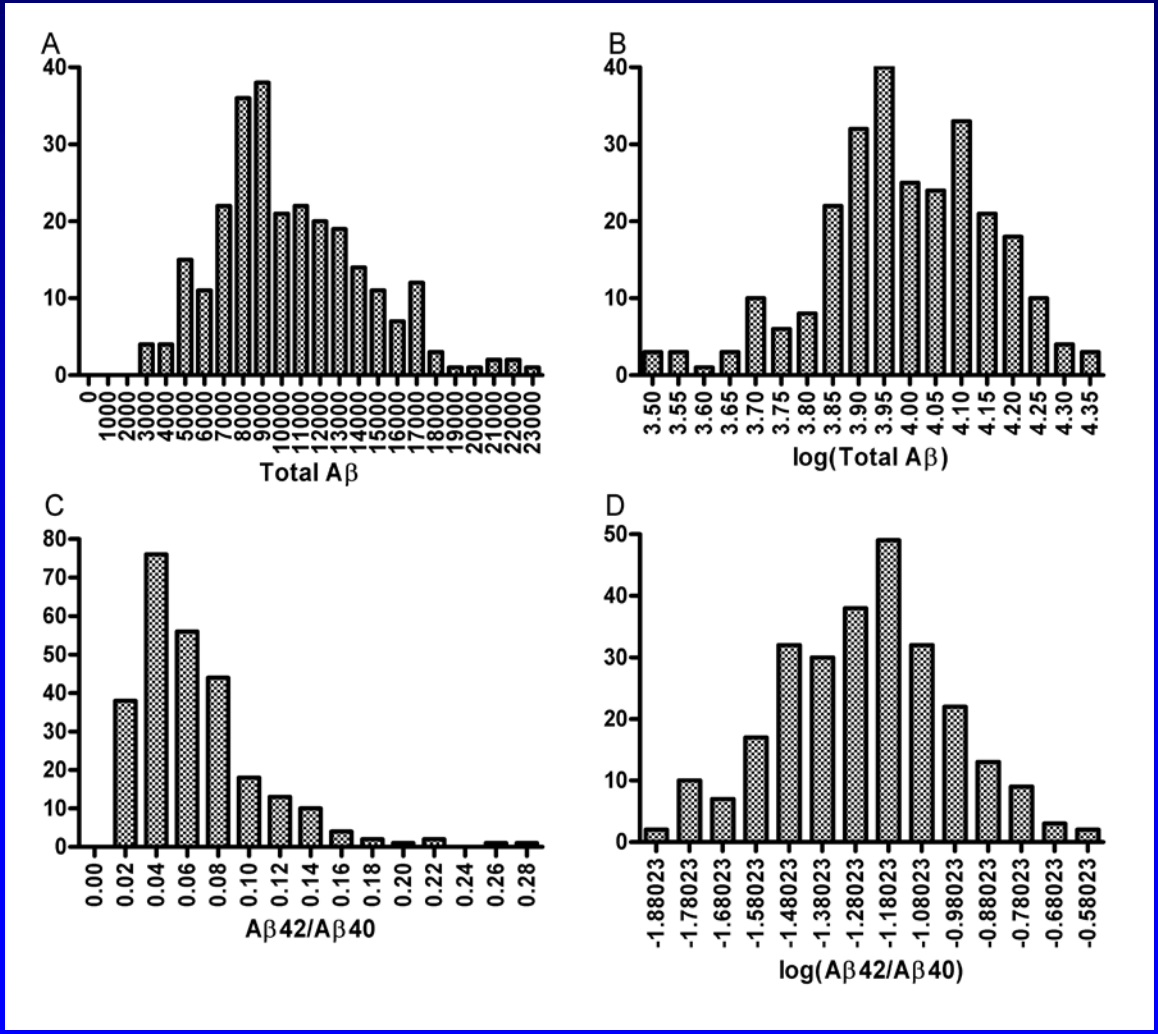
	Sample
N	313
Age in years mean (range, SD)	67.5 (43-95,11.5)
%female	63%
%APOE4+	42%
CDR	0=72% 0.5=20% 1=8%

Summary of Sample Characteristics. Sample size (N), age, percent of females, percent of ApoE4 allele carriers, and Clinical dementia rating (CDR) for the sample.

CSF Aß levels as an endophenotype for LOAD risk

- A quantitative trait measurable in all individuals regardless of disease status
- Correlated with disease
  - FAD mutations increase Aß42/Aß40 ratio
  - CSF Aß42 levels decrease in AD
  - CSF Aß42 levels correlate with Aß deposition as detected by PET imaging with PIB
- Likely to have simpler genetic architecture than LOAD risk
- Can provide a biological model of disease

Distribution of CSF levels of total Aß and Aß42/Aß40 ratio

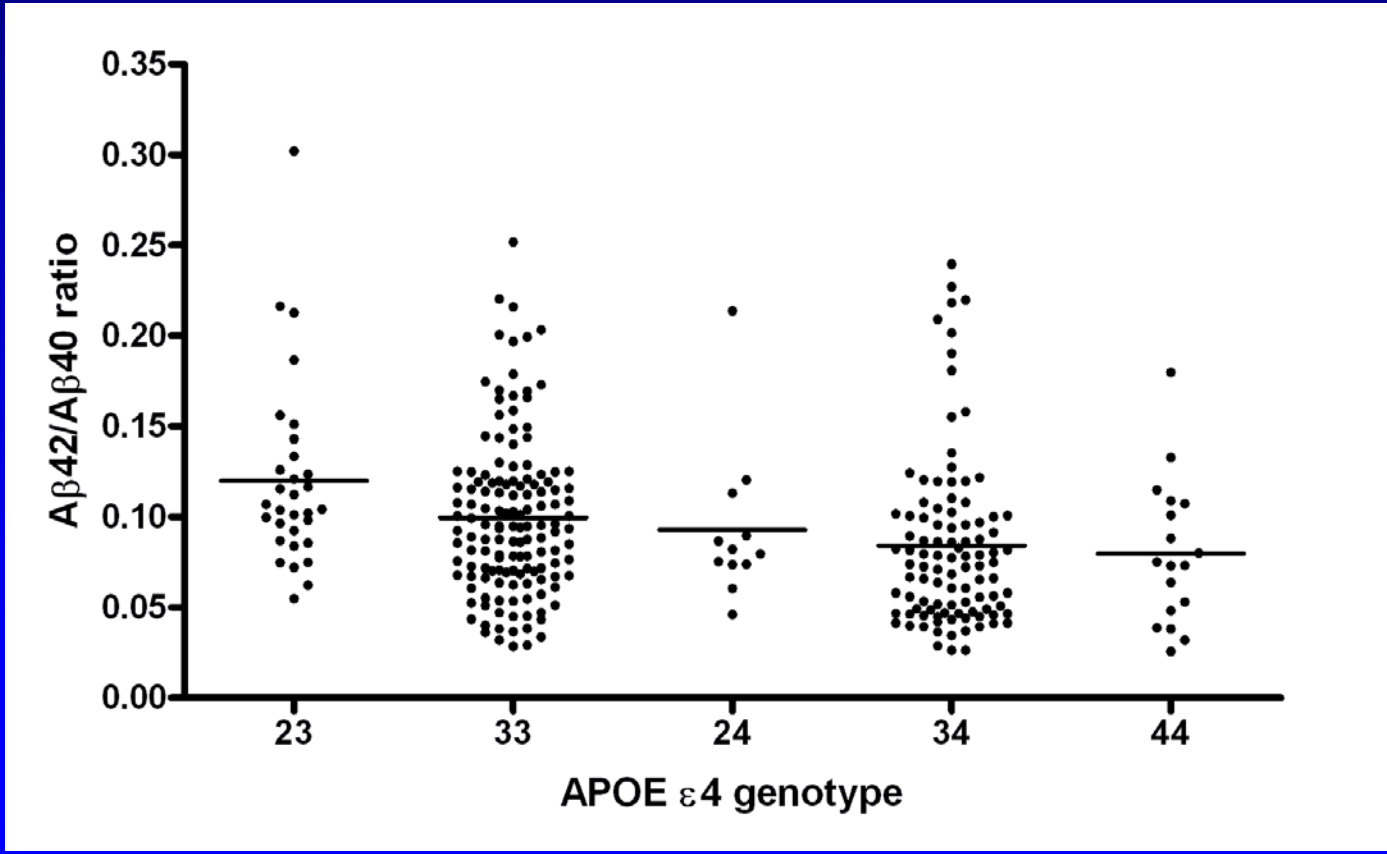


CDR, APOE, Age and Gender modify Aß42 levels

	CDR	Age	apoenum	Gender
log(CSF A§40)	0.9159	0.4998	0.17	0.0606
Estimate	-0.00797	0.00132	-0.05015	0.08692
log(CSF A§42)	***<0.0001***	***0.0056***	***<0.0001***	***0.023***
Estimate	-0.48487	-0.00649	-0.22423	0.12566
log(CSF A§ ratio)	***<0.0001***	***0.0003***	***<0.0001***	0.4461
Estimate	-0.4769	-0.00781	-0.17407	0.03873
log(CSF total A§)	0.5592	0.7339	0.0745	***0.0432***
Estimate	-0.04274	0.000645	-0.06332	-0.09093

P-values and parameter estimates from linear regression contrasting log-transformed CSF A§ levels and Clinical Dementia Rating (CDR), age, the number of *APOE* ε4 alleles (apoenum), and gender. Results are from 298 individuals.

CSF Aß42/Aß40 ratio is associated with *APOE* genotype



Aß related phenotypes but not tau phenotypes are strongly associated with *APOE4*

		Case-control Status	PIB	CSF Aß42 levels	CSF ptau181 levels
# APOE ε4 alleles					
	0	Reference	Reference	Reference	Reference
	1	1.33 (0.55-3.27)	5.39 (2.39-12.1)	13.4 (3.93-45.6)	1.50 (0.47-4.82)
	2	5.80 (0.93-36.2)	35.77 (7.05-181)	44.95 (13.1-154)	1.94 (0.63-6.02)

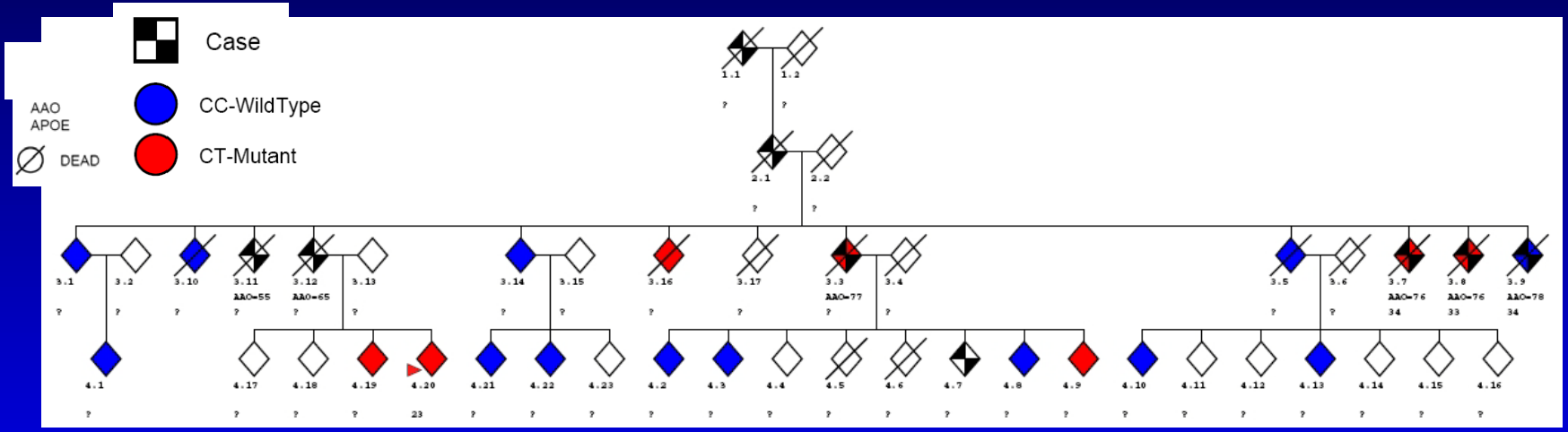
Summary

- Aß phenotypes show interindividual variation
- CSF Aß42 and PIB but not CSF tau are strongly associated with *APOE* genotype
- Odds ratios for *APOE* genotypes are much higher for Aß phenotypes than case control status or tau
- The Aß phenotypes closely reflect the molecular mechanism of *APOE4* risk

Is rare variation in FAD genes associated with extreme Aß levels?

- Rare variation with high penetrance
  - Top and bottom 5% from the distributions of Aβ40, Aβ42, and 42:40 residuals for each gender were identified
  - *PSEN1*, *APP*, *PSEN2* and *APOE* sequenced in 42 individuals to look for rare coding variants that explain the extreme Aß levels

PSEN1 Exon 4 A79V



- Known FAD mutation (Onset 50-62 in four previously reported families)
- Carrier is a 54 year old non-demented individual from a family with late onset AD
  - 5th highest adjusted Aβ42 value and the 4th highest 42:40 value in our sample
  - Mutation segregates with AD in the family
  - Mean AAO in family is 69yrs but varies widely (55-78yrs)

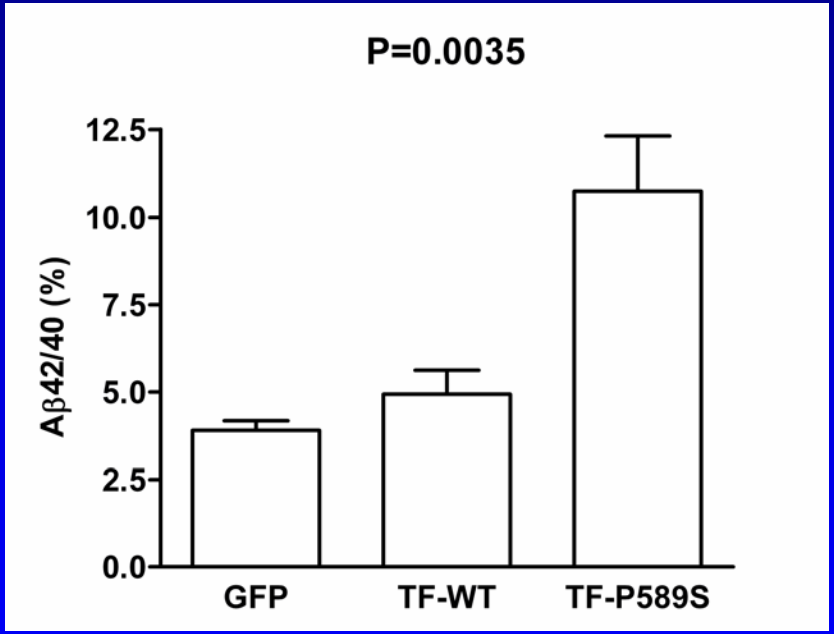
CSF biomarker levels and common genetic variation

- Alzgene
  - Test SNPs which show association with AD in the Alzgene.org meta-analyses (4 independent samples) for association with CSF Aβ
  - Specific prior hypothesis based on case control data

Alzgene SNPs in *ACE, APOE, DAPK1* and *TF* affect CSF Aß levels

Polymorphism	Gene	MAF	Function	Aß42/Aß40	Total Aß	AD allele (OR)*
rs1800764	*ACE*	0.49	.	***0.0141†***	0.8112†	C (0.83)
ApoE4	*APOE*	0.2	NONSYN	***0.0001***	0.1614	E4 (3.68)
rs405509	*APOE*	0.48	LOCUS	0.0937	***0.0297***	G (0.75)
rs4877365	*DAPK1*	0.27	INTRON	0.2329†	***0.02†***	A (0.8)
rs4878104	*DAPK1*	0.35	INTRON	0.4165‡	***0.0059‡***	A (0.87)
rs1049296	*TF*	0.16	NONSYN	***0.0297†***	0.1663†	T (1.22)

Transferrin variant P589S modifies Aß42/40 ratios *in vitro*



Summary

- Evidence for association of AD risk polymorphisms in *ACE*, *APOE*, *DAPK1*, and *TF* with CSF Aβ levels
- Indicates a possible Aβ related mechanism for AD risk

MAPT, AD and CSF tau

- Many neurodegenerative diseases are characterized by tangles (tauopathy)
- Tau and p181tau are elevated in AD
- Mutations in *MAPT* cause tauopathy
- Polymorphisms in *MAPT* associated with PSP
  - Some evidence SNPs also associated with AD and CSF tau

CSF tau levels

	CDR	Age	Apoenum	Gender
log(log CSF tau)	***<0.0001***	***<0.0001***	***0.05***	0.2206
Estimate	0.11332	0.00255	0.01653	0.01303
log(log CSF ptau181)	***<0.0001***	***<0.0001***	***0.0045***	0.2243
Estimate	0.08561	0.00267	0.02741	0.01473

P-values and parameter estimates from linear regression contrasting log-log transformed CSF tau and ptau181 (ptau) levels and Clinical Dementia Rating (CDR), Age, the number of ApoE4 alleles (Apoenum), and Gender. Results are from 301 individuals.

CSF biomarker levels and common genetic variation

- Tau metabolism candidate genes
  - Kinases
  - Phosphatases
  - O-glcNAcylation
  - Degradation

CSF tau and p-tau associated with regulatory and catalytic subunit genes for protein phosphatase 3

		WU Series		ADNI series		Combined Series	
gene	rs	tau	ptau181	tau	ptau181	tau	ptau181
PPP3R1	rs1868402	**2.25×10^{-05}**	**0.001**	0.096	**0.031**	**1.72×10^{-05}**	**6.28×10^{-05}**
PPP3CA	rs17030739	0.006	**0.001**	0.064	**0.049**	**9.26×10^{-04}**	**2.05×10^{-04}**
CTSD	rs1317356	0.075	**0.016**	0.851	0.642	0.217	0.138
F2	rs2070852	**0.002**	**0.008**	0.602	0.948	0.007	0.051
FAS	rs1800682	**0.008**	0.065	0.526	0.819	0.110	0.209
FYN	rs927010	**5.00×10^{-04}**	**5.00×10^{-04}**	0.606	0.424	0.006	0.073
GSK3B	rs7431209	**0.007**	**0.005**	0.692	0.143	0.017	0.002
MGEA5	rs2305192	**7.00×10^{-04}**	**0.008**	0.860	0.803	0.010	0.037
PSEN1	rs1800587	**3.70×10^{-05}**	**1.00×10^{-04}**	0.587	**0.030**	**4.42×10^{-04}**	**1.41×10^{-05}**

Table 5. P-values from ANCOVA analyses of polymorphisms which show association with tau and/or ptau181 CSF levels. For each SNP the gene, rs number, p-value for tau and ptau181 in the WU, ADNI and combine series is shown.

Minor allele of rs1868402 associated with AD

					Minor Allele frequency			
	Series	**Cases**	**Controls**	**Minor Allele**	**Cases**	**Controls**	**p-value**	**OR**
rs1868402	MRC	662	801	C	0.31	0.28	0.06	
	ADNI	230	204	C	0.28	0.26	0.03	
	ADRC	312	222	C	0.30	0.28	0.14	
	Total	1204	1227	C	0.30	0.28	**0.017**	**1.19 (1.01 - 1.39)**

Summary

- Variants in *PPP3R1* and *PPP3CA1* influence CSF tau and ptau levels
- *PPP3R1* variants influence risk for AD

Acknowledgements

Collaborators
David Holtzman
Anne Fagan
John C. Morris
Mark Mintun

Funding Sources
NIA, AHAF,
AstraZeneca,
Alzheimer's Assn

AD group: Jun Wang, Sumi Chakraverty, Petra Nowotny, Keoni Kauwe, Kevin Mayo, Carlos Cruchaga, Joanne Norton, Denise Levitch, Jan Konrad