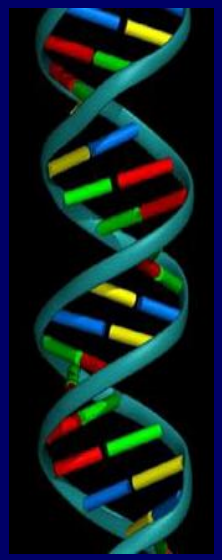
Genetics of Quantitative Phenotypes in ADNI: Candidate Genes, Pathways, and GWAS

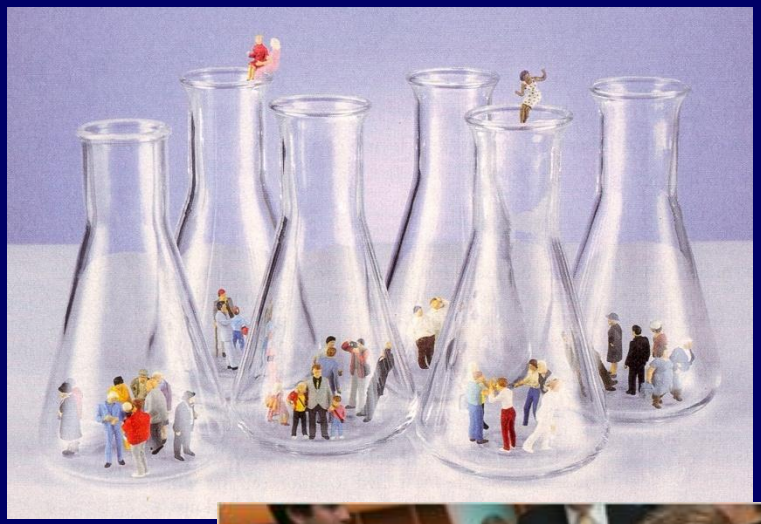


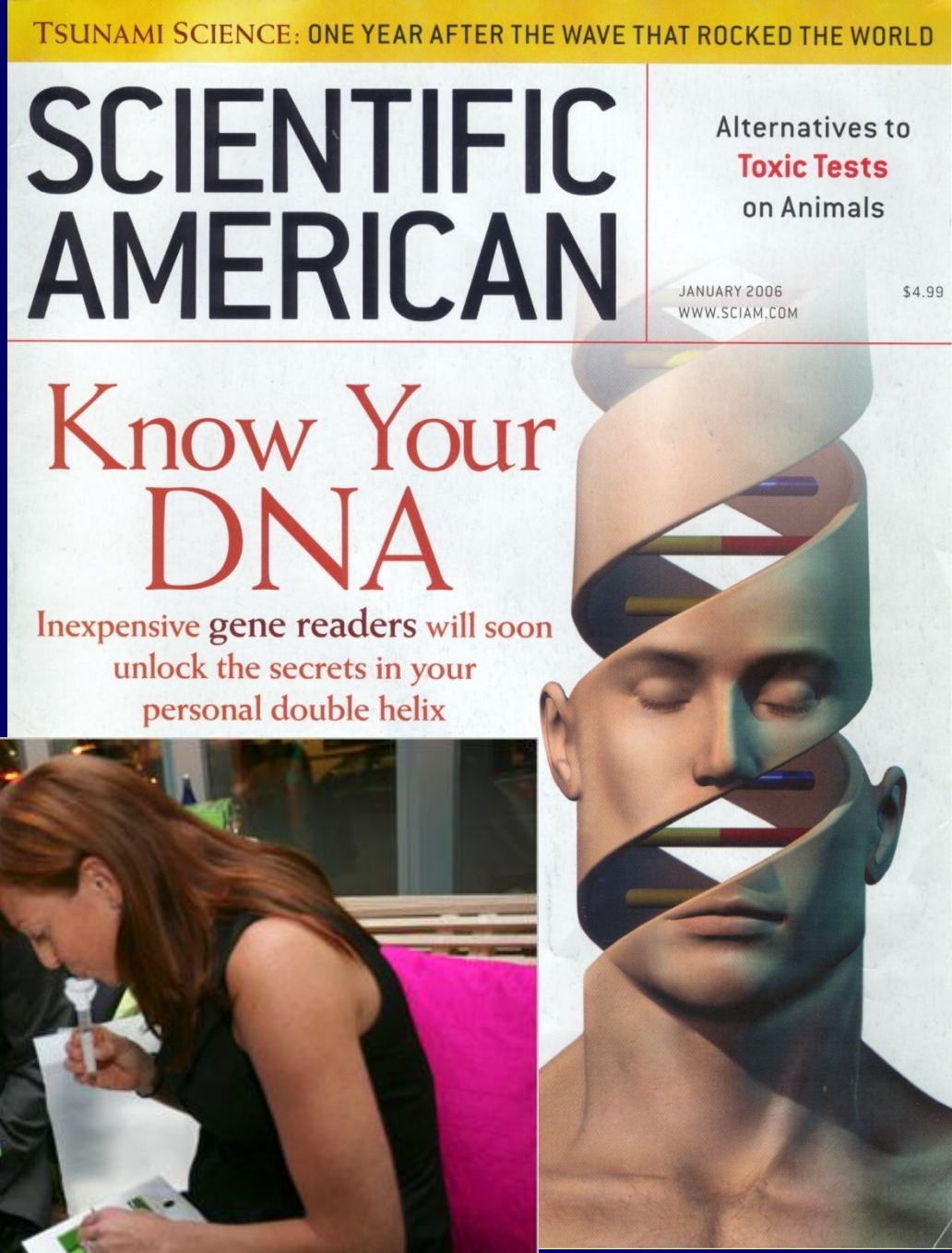
Andrew J. Saykin

Indiana University School of Medicine

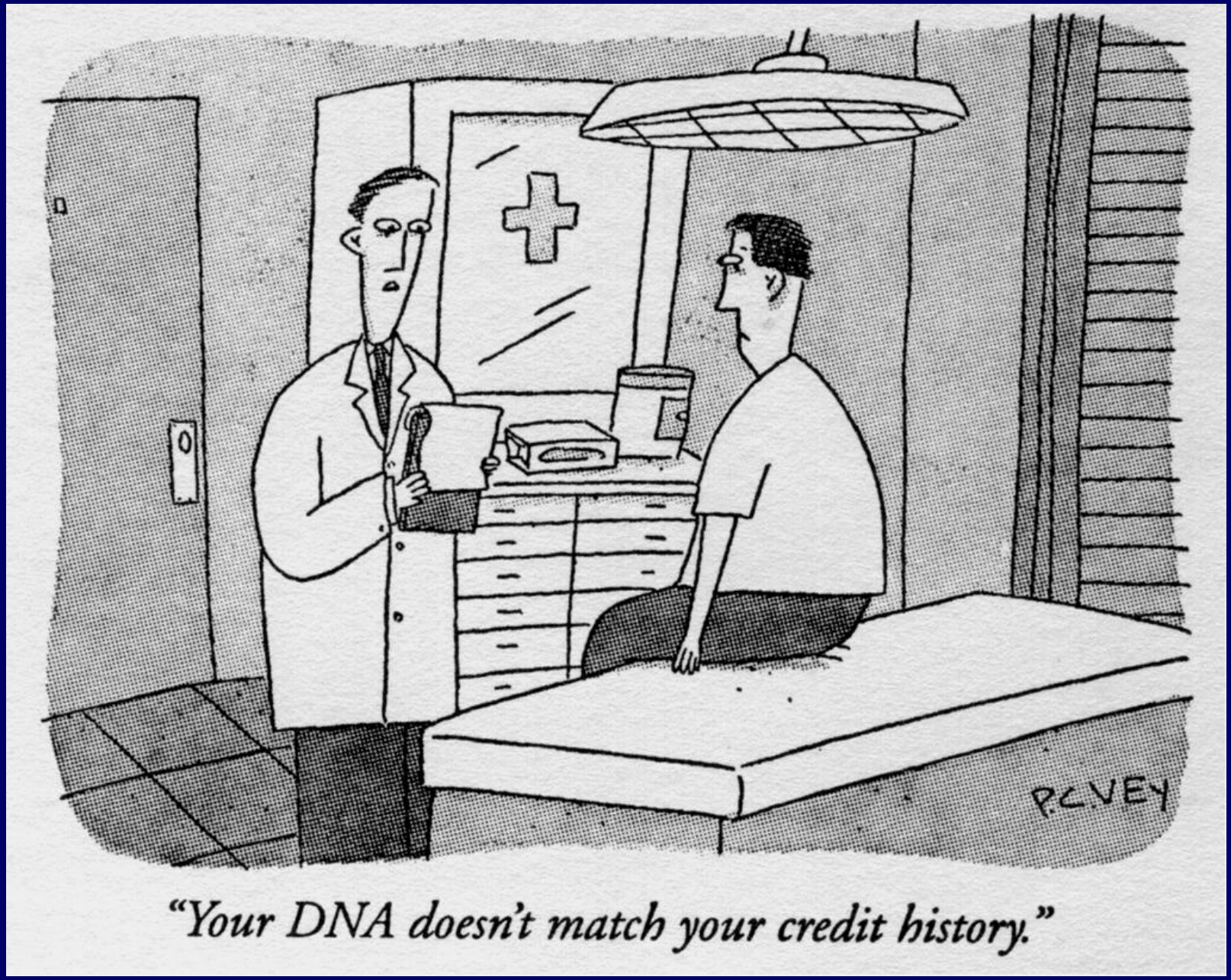
Advanced Psychometrics Methods Workshop
Friday Harbor, WA, June 6-11, 2011

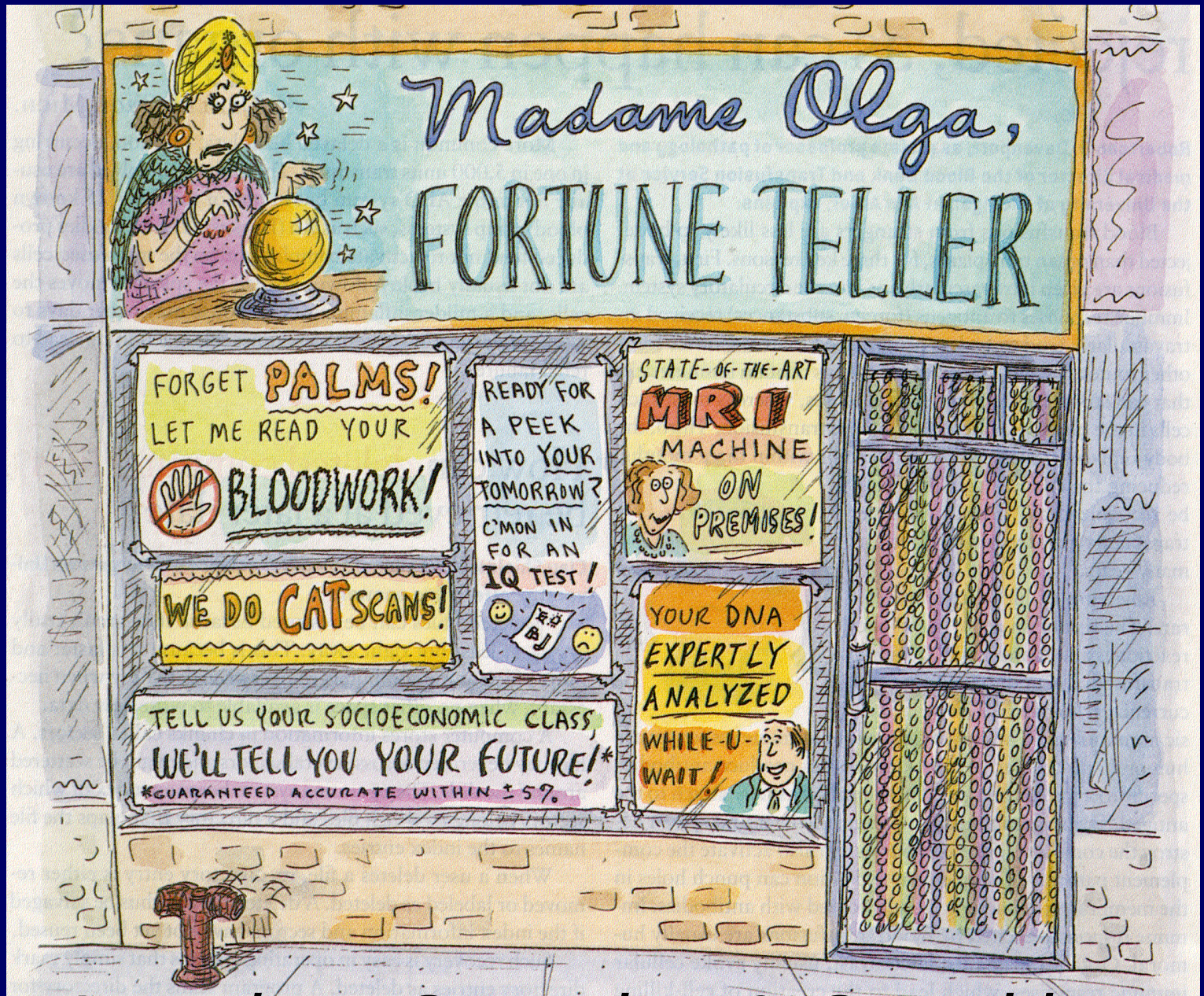
Era of Personal Genomics





And Personalized Medicine ….



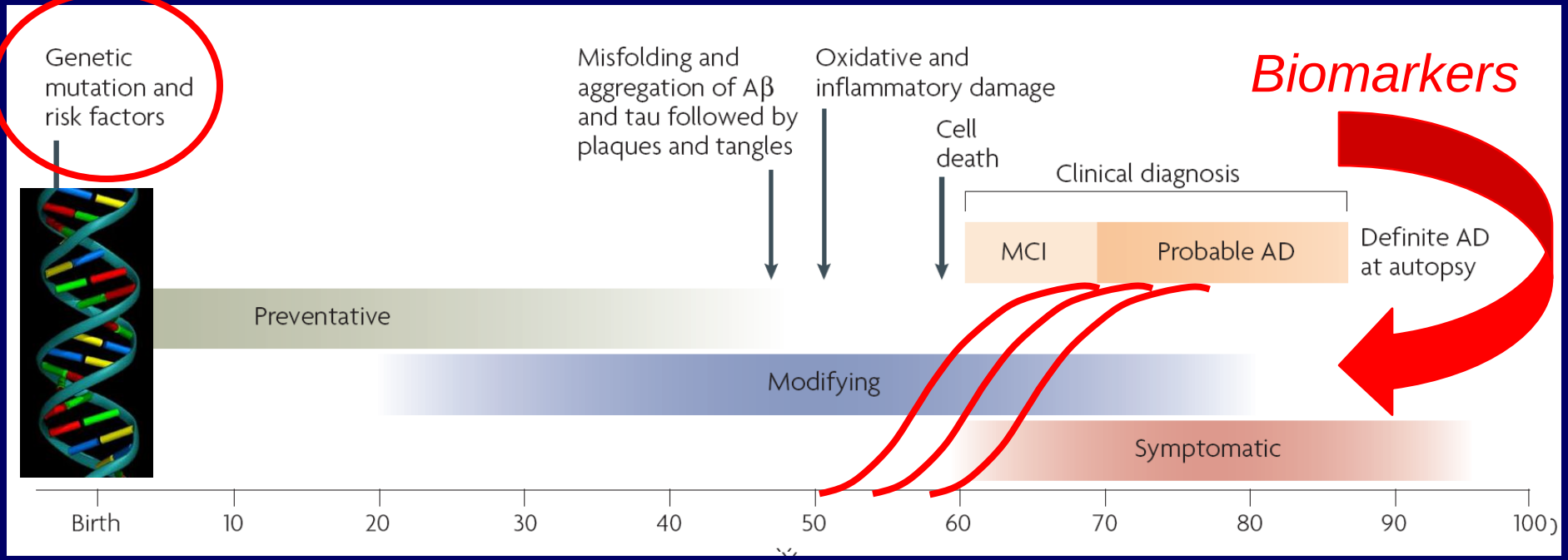


Imaging, Genetics & Cognition

Overview

- Late onset AD (LOAD) genetics
- New developments in GWAS of LOAD
  - ADNI's role; relevance of findings
- Methodological issues in mapping between quantitative phenotypes and genetic data
- Selected results over the past year
  - Genome-wide whole brain analysis
  - GWAS of CSF biomarkers
  - Candidate gene and pathways-based analyses
- Ongoing work and future plans

Timeline for the Onset and Progression of Alzheimer Disease Processes

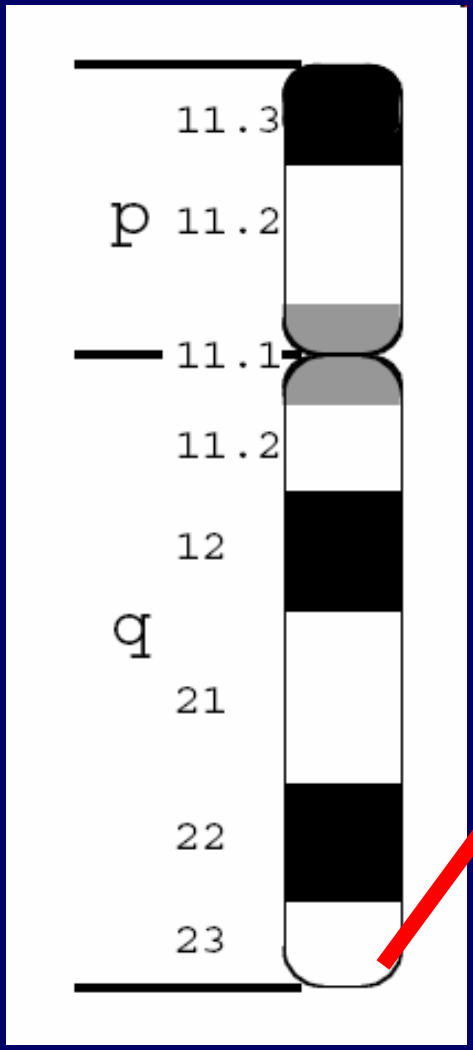


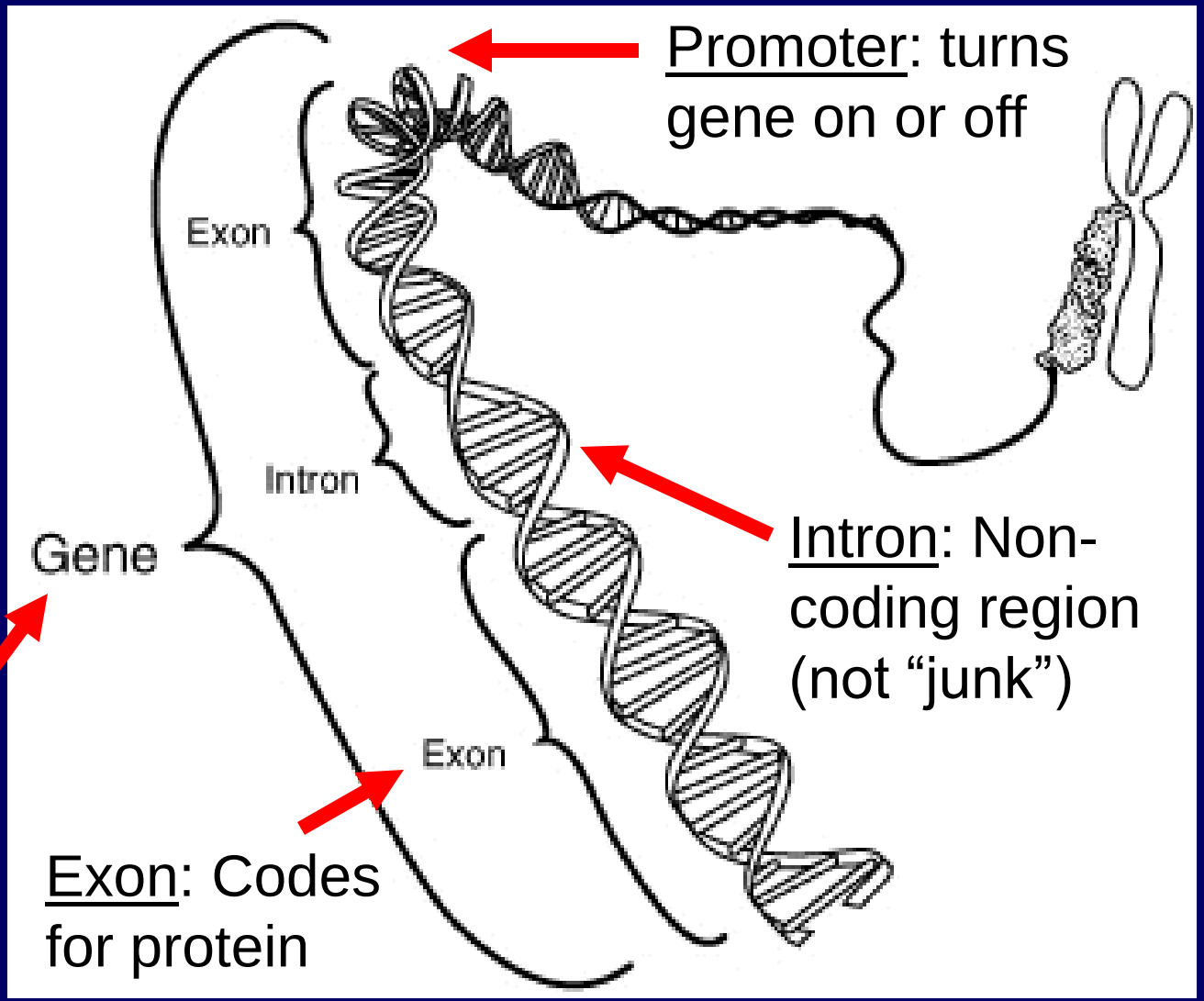
Biomarkers are needed for early diagnosis, to predict transitions from NCI to MCI to AD and clinical trials of disease modifying therapies

Shaw LM, Korecka M, Clark CM, Lee VM.-Y, Trojanowski JQ.
Nat Rev Drug Discovery, 6(4):295-303, 2007.

Gene Regions

- Chromosomes -> DNA -> Gene -> Regions

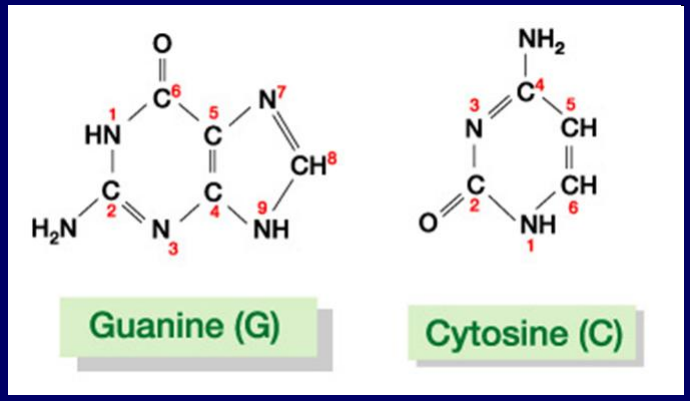


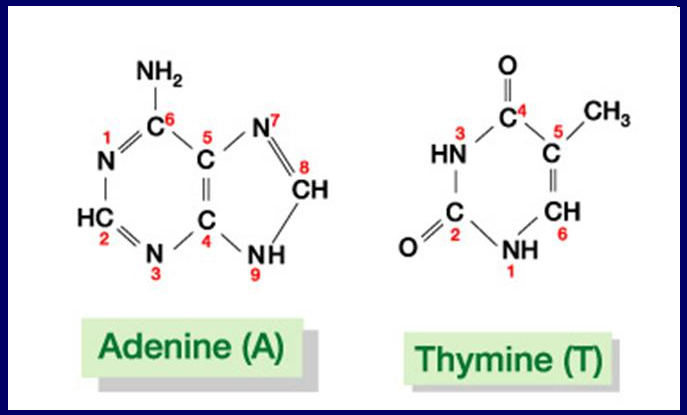


Genetic Code: DNA Letters

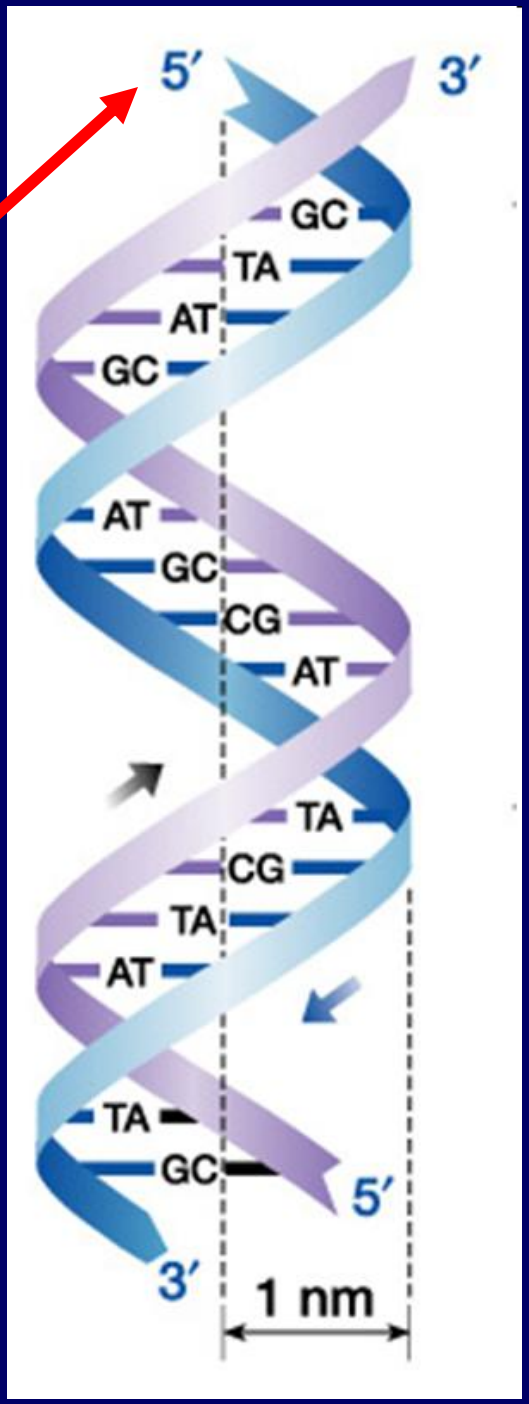
Transcription / translation begins at the 5' end

Base Pairs – "rungs of the DNA ladder"





Note:
In RNA, uracil replaces thymine



From Human Molecular Genetics, 3rd Ed

Code for Making Amino Acids → Protein: 3 Letter Words (from ATGC)

	T	C	A	G
T	TTT Phe (F) TTC " TTA Leu (L) TTG "	TCT Ser (S) TCC " TCA " TCG "	TAT Tyr (Y) TAC TAA **Ter** **TAG Ter**	TGT Cys (C) TGC TGA **Ter** **TGG Trp (W)**
C	CTT Leu (L) CTC " CTA " CTG "	CCT Pro (P) CCC " CCA " CCG "	CAT His (H) CAC " CAA Gln (Q) CAG "	CGT Arg (R) CGC " CGA " CGG "
A	ATT Ile (I) ATC " ATA " **ATG Met (M)**	ACT Thr (T) ACC " ACA " ACG "	AAT Asn (N) AAC " AAA Lys (K) AAG "	AGT Ser (S) AGC " AGA Arg (R) AGG "
G	GTT Val (V) GTC " GTA " GTG "	GCT Ala (A) GCC " GCA " GCG "	GAT Asp (D) GAC " GAA Glu (E) GAG "	GGT Gly (G) GGC " GGA " GGG "

To make a protein, the four nucleotide bases (adenine, thymine, guanine, and cytosine) are combined in various ways to spell out 3-letter "words" (codons) that specify which amino acid is needed at every step.

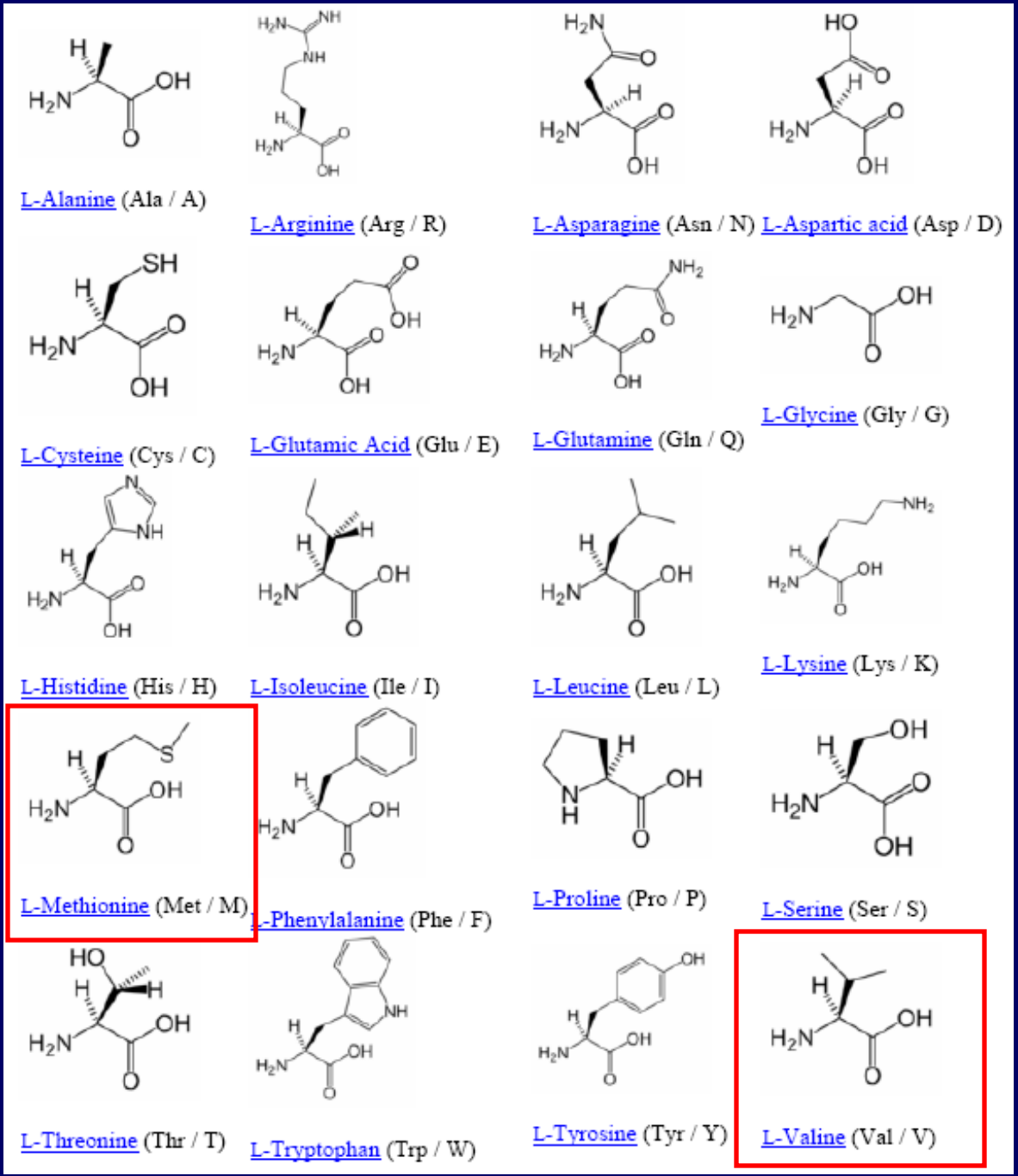
Amino Acids (20)

Small molecules that link together in long chains to form proteins - "building blocks" of proteins.

Met / Val

Methionine

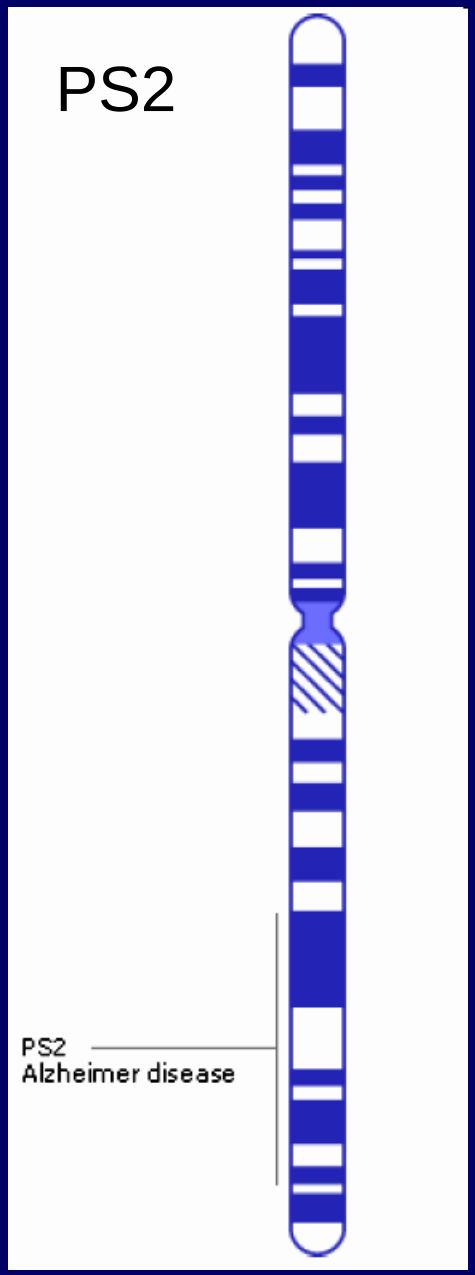
Valine



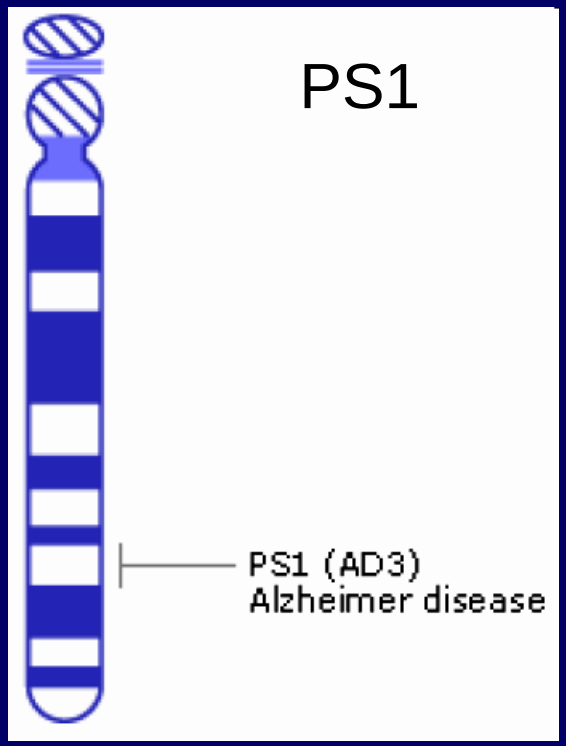
http://en.wikipedia.org/wiki/Amino_acid#List_of_standard_amino_acids

Major Genes: EOAD & LOAD

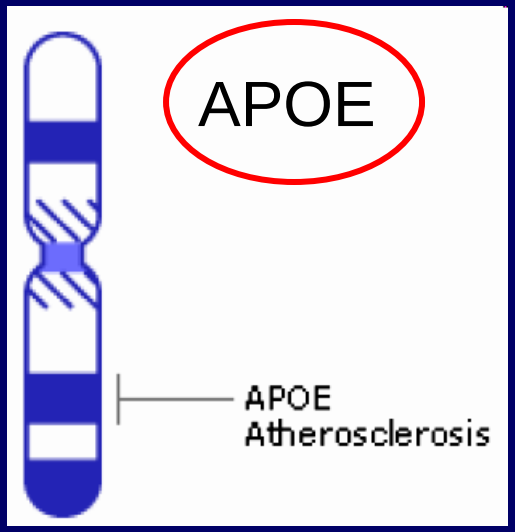
LOAD: genetic factors account for ~60-80% of risk (Gatz et al 2006); APOE accounts for up to 50% (Ashford & Mortimer 2002); so up to 30% remains to be found.



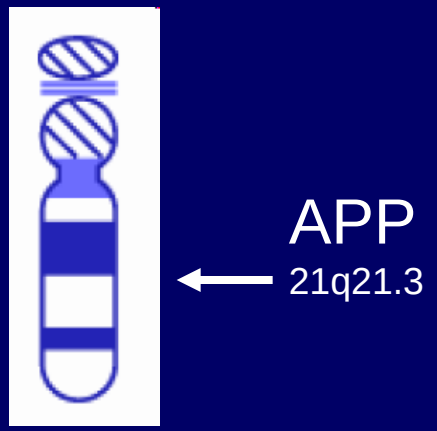
Chromosome 1



Chromosome 14



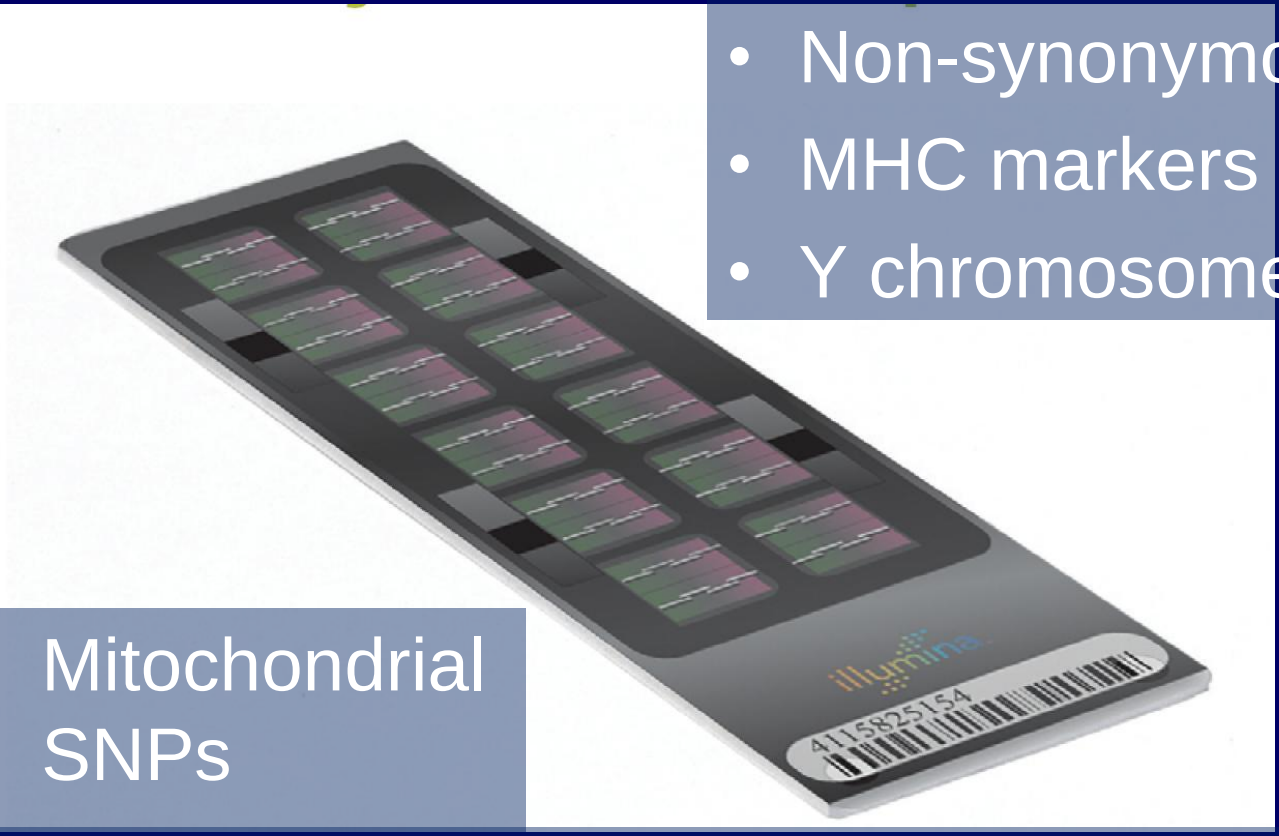
Chromosome 19



Chromosome 21

Genome-Wide Association Studies (GWAS)

"Gene Chip" - Illumina Human 610-Quad



- Non-synonymous SNPs
- MHC markers
- Y chromosome SNPs
- Mitochondrial SNPs

- 620,901 markers (~90% genomic coverage, CEU)
- Single nucleotide polymorphisms (SNPs)
- Copy number variation (CNVs) probes

Harold et al 2009 & Lambert et al 2009 Large Case/Control GWAS in AD

nature genetics

Genome-wide association study identifies variants at *CLU* and *PICALM* associated with Alzheimer's disease

Denise Harold[1,45*], Richard Abraham[1,45], Paul Hollingworth[1,45], Rebecca Sims[1], Amy Gerrish[1], Marian L Hamshere[1], Jaspreet Singh Pahwa[1], Valentina Moskvina[1], Kimberley Dowzell[1], Amy Williams[1], Nicola Jones[1], Charlene Thomas[1], Alexandra Stretton[1], Angharad R Morgan[1], Simon Lovestone[2], John Powell[3], Petroula Proitsi[3], Michelle K Lupton[3], Carol Brayne[4], David C Rubinsztein[5], Michael Gill[6], Brian Lawlor[6], Aoibhinn Lynch[6], Kevin Morgan[7], Kristelle S Brown[7], Peter A Passmore[8], David Craig[8], Bernadette McGuinness[8], Stephen Todd[8], Clive Holmes[9], David Mann[10], A David Smith[11], Seth Love[12], Patrick G Kehoe[12], John Hardy[13], Simon Mead[14], Nick Fox[15], Martin Rossor[15], John Collinge[14], Wolfgang Maier[16], Frank Jessen[16], Britta Schürmann[16], Hendrik van den Bussche[17], Isabella Heuser[18], Johannes Kornhuber[19], Jens Wiltfang[20], Martin Dichgans[21,22], Lutz Frölich[23], Harald Hampel[24,25], Michael Hüll[26], Dan Rujescu[25], Alison M Goate[27], John S K Kauwe[28], Carlos Cruchaga[27], Petra Nowotny[27], John C Morris[27], Kevin Mayo[27], Kristel Sleegers[29,30], Karolien Bettens[29,30], Sebastiaan Engelborghs[30,31], Peter P De Deyn[30,31], Christine Van Broeckhoven[29,30], Gill Livingston[32], Nicholas J Bass[32], Hugh Gurling[32], Andrew McQuillin[32], Rhian Gwilliam[33], Panagiotis Deloukas[33], Ammar Al-Chalabi[34], Christopher E Shaw[34], Magda Tsolaki[35], Andrew B Singleton[36], Rita Guerreiro[36], Thomas W Mühleisen[37,38], Markus M Nöthen[37,38], Susanne Moebus[39], Karl-Heinz Jöckel[39], Norman Klopp[40], H-Erich Wichmann[40–42], Minerva M Carrasquillo[43], V Shane Pankratz[44], Steven G Younkin[43], Peter A Holmans[1], Michael O'Donovan[1], Michael J Owen[1] & Julie Williams[1]

We undertook a two-stage genome-wide association study (GWAS) of Alzheimer's disease (AD) involving over 16,000 individuals, the most powerful AD GWAS to date. In stage 1 (3,941 cases and 7,848 controls), we replicated the established association with the apolipoprotein E (*APOE*) locus (most significant SNP, rs2075650, $P = 1.8 \times 10^{-157}$) and observed genome-wide significant association with SNPs at two loci not previously associated with the disease: at the *CLU* (also known as *APOJ*) gene (rs11136000, $P = 1.4 \times 10^{-9}$) and 5′ to the *PICALM* gene (rs3851179, $P = 1.9 \times 10^{-8}$). These associations were replicated in stage 2 (2,023 cases and 2,340 controls), producing compelling evidence for association with Alzheimer's disease in the combined dataset (rs11136000, $P = 8.5 \times 10^{-10}$, odds ratio = 0.86; rs3851179, $P = 1.3 \times 10^{-9}$, odds ratio = 0.86).

Alzheimer's disease is the most common form of dementia and is highly heritable (with heritability of up to 76%) but genetically complex[1]. Neuropathologically, the disease is characterized by extracellular senile plaques containing β-amyloid (Aβ) and intracellular neurofibrillary tangles containing hyperphosphorylated tau protein[1]. Thus far, four genes have been definitively implicated in the etiology of Alzheimer's disease. Mutations of the genes encoding amyloid precursor protein (*APP*) and presenilin 1 and 2 (*PSEN1*, *PSEN2*) cause rare, mendelian forms of the disease, usually with an early onset. However, in the more common form of the disease, only *APOE* has been established unequivocally as a susceptibility gene[1]. Aiming to identify new Alzheimer's disease loci, several genome-wide association studies (GWAS) have been previously conducted. All have identified strong evidence for Alzheimer's disease risk association to *APOE* but have found less convincing evidence implicating other genes[2–9]. This outcome is consistent with the majority of findings from GWAS of other common disease phenotypes, where susceptibility alleles typically have effect sizes with odds ratios (OR) of 1.5 or less, in contrast to that for *APOE* and Alzheimer's disease (OR ~ 3). Detecting such modest effects requires much larger samples than those used in the GWAS of Alzheimer's disease to date[10], which have all included fewer than 1,100 cases. Based upon the hypothesis that risk alleles for Alzheimer's disease are likely to confer ORs in the range seen with other common diseases, we undertook a more powerful GWAS than has been carried out to date.

We established a collaborative consortium from Europe and the United States from which we were able to draw upon a combined sample of up to 19,000 subjects (before quality control) and conducted a two-stage study. In stage 1, we genotyped 14,639 subjects on Illumina platforms: 5,715 samples were genotyped using the Illumina 610-quad

*A full list of author affiliations appears at the end of the paper.

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nature genetics

Genome-wide association study identifies variants at *CLU* and *CR1* associated with Alzheimer's disease

Jean-Charles Lambert[1–3], Simon Heath[4], Gael Even[1,2], Dominique Campion[5], Kristel Sleegers[6,7], Mikko Hiltunen[8], Onofre Combarros[9], Diana Zelenika[4], Maria J Bullido[10], Béatrice Tavernier[11], Luc Letenneur[12], Karolien Bettens[6,7], Claudine Berr[13], Florence Pasquier[3,14], Nathalie Fiévet[1,2], Pascale Barberger-Gateau[12], Sebastiaan Engelborghs[7,15], Peter De Deyn[7,15], Ignacio Mateo[9], Ana Franck[16], Seppo Helisalmi[8], Elisa Porcellini[17], Olivier Hanon[18], the European Alzheimer's Disease Initiative Investigators[19], Marian M de Pancorbo[20], Corinne Lendon[21], Carole Dufouil[22,23], Céline Jaillard[24], Thierry Leveillard[24], Victoria Alvarez[25], Paolo Bosco[26], Michelangelo Mancuso[27], Francesco Panza[28], Benedetta Nacmias[29], Paola Bossù[30], Paola Piccardi[31], Giorgio Annoni[32], Davide Seripa[33], Daniela Galimberti[34], Didier Hannequin[5], Federico Licastro[17], Hilkka Soininen[8], Karen Ritchie[13], Hélène Blanché[35], Jean-François Dartigues[12], Christophe Tzourio[22,23], Ivo Gut[4], Christine Van Broeckhoven[6,7], Annick Alpérovitch[22,23], Mark Lathrop[4,35] & Philippe Amouyel[1–3,14]

The gene encoding apolipoprotein E (*APOE*) on chromosome 19 is the only confirmed susceptibility locus for late-onset Alzheimer's disease. To identify other risk loci, we conducted a large genome-wide association study of 2,032 individuals from France with Alzheimer's disease (cases) and 5,328 controls. Markers outside *APOE* with suggestive evidence of association ($P < 10^{-5}$) were examined in collections from Belgium, Finland, Italy and Spain totaling 3,978 Alzheimer's disease cases and 3,297 controls. Two loci gave replicated evidence of association: one within *CLU* (also called *APOJ*), encoding clusterin or apolipoprotein J, on chromosome 8 (rs11136000, OR = 0.86, 95% CI 0.81–0.90, $P = 7.5 \times 10^{-9}$ for combined data) and the other within *CR1*, encoding the complement component (3b/4b) receptor 1, on chromosome 1 (rs6656401, OR = 1.21, 95% CI 1.14–1.29, $P = 3.7 \times 10^{-9}$ for combined data). Previous biological studies support roles of *CLU* and *CR1* in the clearance of β amyloid (Aβ) peptide, the principal constituent of amyloid plaques, which are one of the major brain lesions of individuals with Alzheimer's disease.

Alzheimer's disease is a neurological disorder primarily affecting the elderly that manifests through memory disorders, cognitive decline and loss of autonomy. Two principal types of neuropathologic lesions are observed: (i) neurofibrillary degeneration resulting from the intraneuronal accumulation of hyperphosphorylated Tau proteins and (ii) amyloid deposits resulting from the extracellular accumulation of amyloid plaques, which are primarily composed of Aβ peptides. Currently, the processes leading to the formation of these lesions and their combined association with Alzheimer's disease are not adequately understood[1].

Genetic studies have provided significant insights into the molecular basis of Alzheimer's disease. Rare hereditary early-onset forms of the disease have been linked to mutations in three different genes: *APP*, encoding amyloid precursor protein, on chromosome 21; *PS1*, encoding presenilin 1, on chromosome 14; and *PS2*, encoding presenilin 2, on chromosome 1 (ref. 2). These mutations, however, explain less than 1% of all cases of Alzheimer's disease, whereas the vast majority (especially for late-onset forms of the disease) have other, more complex genetic determinants[3].

Genetic studies have led to the consistent identification of the ε4 allele of *APOE* as a susceptibility locus for late-onset Alzheimer's disease[4]. Twin studies suggest that genes may have a role in more than 60% of Alzheimer's disease susceptibility[5] and that *APOE* may account for as much as 50% of this genetic susceptibility[6]. More than 550 other genes have been proposed as candidates for Alzheimer's disease susceptibility, but thus far none have been confirmed to have a role in Alzheimer's disease pathogenesis[7].

As with other multifactorial diseases, this knowledge gap has motivated more comprehensive investigations using genome-wide association studies (GWAS). The first GWAS of case-control Alzheimer's disease data collections have examined a relatively small number of cases (<1,000)[8–12]. Similar to studies done on other multifactorial disorders, these GWAS have shown that, except in the case of *APOE*, larger samples will be necessary to locate common genetic factors of Alzheimer's disease. Here, we report results from a large two-stage GWAS of late-onset Alzheimer's disease.

In the first stage of this study, we undertook a GWA analysis of 537,029 SNPs in 2,032 French Alzheimer's disease cases and 5,328 French controls. Patients with probable Alzheimer's disease were ascertained by neurologists. Individuals without symptoms of

*A full list of authors and affiliations appears at the end of the paper.

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GWAS Developments: Seshadri et al 2010

JAMA®

Online article and related content current as of May 20, 2010.

Total N > 35,000

8371 AD cases

(*ADNI not included*)

Genome-wide Analysis of Genetic Loci Associated With Alzheimer Disease

Sudha Seshadri, MD; Annette L. Fitzpatrick, PhD; M. Arfan Ikram, MD, PhD; Anita L. DeStefano, PhD; Vilmundur Gudnason, MD, PhD; Merce Boada, MD, PhD; Joshua C. Bis, PhD; Albert V. Smith, PhD; Minerva M. Carrasquillo, PhD; Jean Charles Lambert, PhD; Denise Harold, PhD; Elisabeth M. C. Schrijvers, MD; Reposo Ramirez-Lorca, PhD; Stephanie Debette, MD, PhD; W. T. Longstreth Jr, MD; A. Cecile J. W. Janssens, PhD; V. Shane Pankratz, PhD; Jean François Dartigues, PhD; Paul Hollingworth, PhD; Thor Aspelund, PhD; Isabel Hernandez, MD; Alexa Beiser, PhD; Lewis H. Kuller, MD; Peter J. Koudstaal, MD, PhD; Dennis W. Dickson, MD; Christophe Tzourio, MD; Richard Abraham, PhD; Carmen Antunez, MD; Yangchun Du, PhD; Jerome I. Rotter, MD; Yurii S. Aulchenko, PhD; Tamara B. Harris, MD; Ronald C. Petersen, MD; Claudine Berr, MD, PhD; Michael J. Owen, MB, ChB, PhD; Jesus Lopez-Arrieta, MD; Badri N. Varadarajan, MS; James T. Becker, PhD; Fernando Rivadeneira, MD, PhD; Michael A. Nalls, PhD; Neill R. Graff-Radford, MD; Dominique Campion, MD, PhD; Sanford Auerbach, MD; Kenneth Rice, PhD; Albert Hofman, MD, PhD; Palmi V. Jonsson, MD; Helena Schmidt, MD, PhD; Mark Lathrop, PhD; Thomas H. Mosley, PhD; Rhoda Au, PhD; Bruce M. Psaty, MD, PhD; Andre G. Uitterlinden, PhD; Lindsay A. Farrer, PhD; Thomas Lumley, PhD; Agustin Ruiz, MD, PhD; Julie Williams, PhD; Philippe Amouyel, MD, PhD; Steve G. Younkin, PhD; Philip A.Wolf, MD; Lenore J. Launer, PhD; Oscar L. Lopez, MD; Cornelia M. van Duijn, PhD; Monique M. B. Breteler, MD, PhD for the CHARGE, GERAD1, and EADI1 Consortia

Context Genome-wide association studies (GWAS) have recently identified *CLU*, *PICALM*, and *CR1* as novel genes for late-onset Alzheimer disease (AD).

Objectives To identify and strengthen additional loci associated with AD and confirm these in an independent sample and to examine the contribution of recently identified genes to AD risk prediction in a 3-stage analysis of new and previously published GWAS on more than 35 000 persons (8371 AD cases).

Design, Setting, and Participants In stage 1, we identified strong genetic associations ($P<10^{-3}$) in a sample of 3006 AD cases and 14 642 controls by combining new data from the population-based Cohorts for Heart and Aging Research in Genomic Epidemiology consortium (1367 AD cases [973 incident]) with previously reported results from the Translational Genomics Research Institute and the Mayo AD GWAS. We identified 2708 single-nucleotide polymorphisms (SNPs) with $P<10^{-3}$. In stage 2, we pooled results for these SNPs with the European AD Initiative (2032 cases and 5328 controls) to identify 38 SNPs (10 loci) with $P<10^{-5}$. In stage 3, we combined data for these 10 loci with data from the Genetic and Environmental Risk in AD consortium (3333 cases and 6995 controls) to identify 4 SNPs with $P<1.7\times10^{-8}$. These 4 SNPs were replicated in an independent Spanish sample (1140 AD cases and 1209 controls). Genome-wide association analyses were completed in 2007-2008 and the meta-analyses and replication in 2009.

Main Outcome Measure Presence of Alzheimer disease.

Results Two loci were identified to have genome-wide significance for the first time: rs744373 near *BIN1* (odds ratio [OR],1.13; 95% confidence interval [CI],1.06-1.21 per copy of the minor allele; $P=1.59\times10^{-11}$) and rs597668 near *EXOC3L2/BLOC1S3/MARK4* (OR, 1.18; 95% CI, 1.07-1.29; $P=6.45\times10^{-9}$). Associations of these 2 loci plus the previously identified loci *CLU* and *PICALM* with AD were confirmed in the Spanish sample ($P<.05$). However, although *CLU* and *PICALM* were confirmed to be associated with AD in this independent sample, they did not improve the ability of a model that included age, sex, and *APOE* to predict incident AD (improvement in area under the receiver operating characteristic curve from 0.847 to 0.849 in the Rotterdam Study and 0.702 to 0.705 in the Cardiovascular Health Study).

Conclusions Two genetic loci for AD were found for the first time to reach genome-wide statistical significance. These findings were replicated in an independent population. Two recently reported associations were also confirmed. These loci did not improve AD risk prediction. While not clinically useful, they may implicate biological pathways useful for future research.

JAMA. 2010;303(18):1832-1840 www.jama.com

Seshadri et al. JAMA 303(18):1832-40, 5/12/10

ADGC Replic-ation

Jun et al 2010

7070 AD cases *including ADNI*

8/9/10

ORIGINAL CONTRIBUTION

ONLINE FIRST

Meta-analysis Confirms *CR1*, *CLU*, and *PICALM* as Alzheimer Disease Risk Loci and Reveals Interactions With *APOE* Genotypes

Gyungah Jun, PhD; Adam C. Naj, PhD; Gary W. Beecham, PhD; Li-San Wang, PhD; Jacqueline Buros, BS; Paul J. Gallins, MS; Joseph D. Buxbaum, PhD; Nilufer Ertekin-Taner, MD, PhD; M. Daniele Fallin, PhD; Robert Friedland, MD; Rivka Inzelberg, MD; Patricia Kramer, PhD; Ekaterina Rogaeva, PhD; Peter St. George-Hyslop, MD, FRCP; Alzheimer's Disease Genetics Consortium; Laura B. Cantwell, MPH; Beth A. Dombroski, PhD; Andrew J. Saykin, PsyD; Eric M. Reiman, MD; David A. Bennett, MD; John C. Morris, MD; Kathryn L. Lunetta, PhD; Eden R. Martin, PhD; Thomas J. Montine, MD, PhD; Alison M. Goate, DPhil; Deborah Blacker, MD; Debby W. Tsuang, MD; Duane Beekly, BS; L. Adrienne Cupples, PhD; Hakon Hakonarson, MD, PhD; Walter Kukull, PhD; Tatiana M. Foroud, PhD; Jonathan Haines, PhD; Richard Mayeux, MD; Lindsay A. Farrer, PhD; Margaret A. Pericak-Vance, PhD; Gerard D. Schellenberg, PhD

Objectives: To determine whether genotypes at *CLU*, *PICALM*, and *CR1* confer risk for Alzheimer disease (AD) and whether risk for AD associated with these genes is influenced by apolipoprotein E (*APOE*) genotypes.

Design: Association study of AD and *CLU*, *PICALM*, *CR1*, and *APOE* genotypes.

Setting: Academic research institutions in the United States, Canada, and Israel.

Participants: Seven thousand seventy cases with AD, 3055 with autopsies, and 8169 elderly cognitively normal controls, 1092 with autopsies, from 12 different studies, including white, African American, Israeli-Arab, and Caribbean Hispanic individuals.

Results: Unadjusted, *CLU* (odds ratio [OR], 0.91; 95% confidence interval [CI], 0.85-0.96 for single-nucleotide polymorphism [SNP] rs11136000), *CR1* (OR, 1.14; 95% CI, 1.07-1.22; SNP rs3818361), and *PICALM* (OR, 0.89; 95% CI, 0.84-0.94, SNP rs3851179) were associated with AD in white individuals. None were significantly associated with AD in the other ethnic groups. *APOE* ε4 was significantly associated with AD (ORs, 1.80-9.05) in all but 1 small white cohort and in the Arab cohort. Adjusting for age, sex, and the presence of at least 1 *APOE* ε4 allele greatly reduced evidence for association with *PICALM* but not *CR1* or *CLU*. Models with the main SNP effect, presence or absence of *APOE* ε4, and an interaction term showed significant interaction between presence or absence of *APOE* ε4 and *PICALM*.

Conclusions: We confirm in a completely independent data set that *CR1*, *CLU*, and *PICALM* are AD susceptibility loci in European ancestry populations. Genotypes at *PICALM* confer risk predominantly in *APOE* ε4–positive subjects. Thus, *APOE* and *PICALM* synergistically interact.

Arch Neurol. Published online August 9, 2010.
doi:10.1001/archneurol.2010.201

AlzGene Database: Meta-Analysis of Top Candidate Genes for AD

http://www.alzforum.org/res/com/gen/alzgene/default.asp

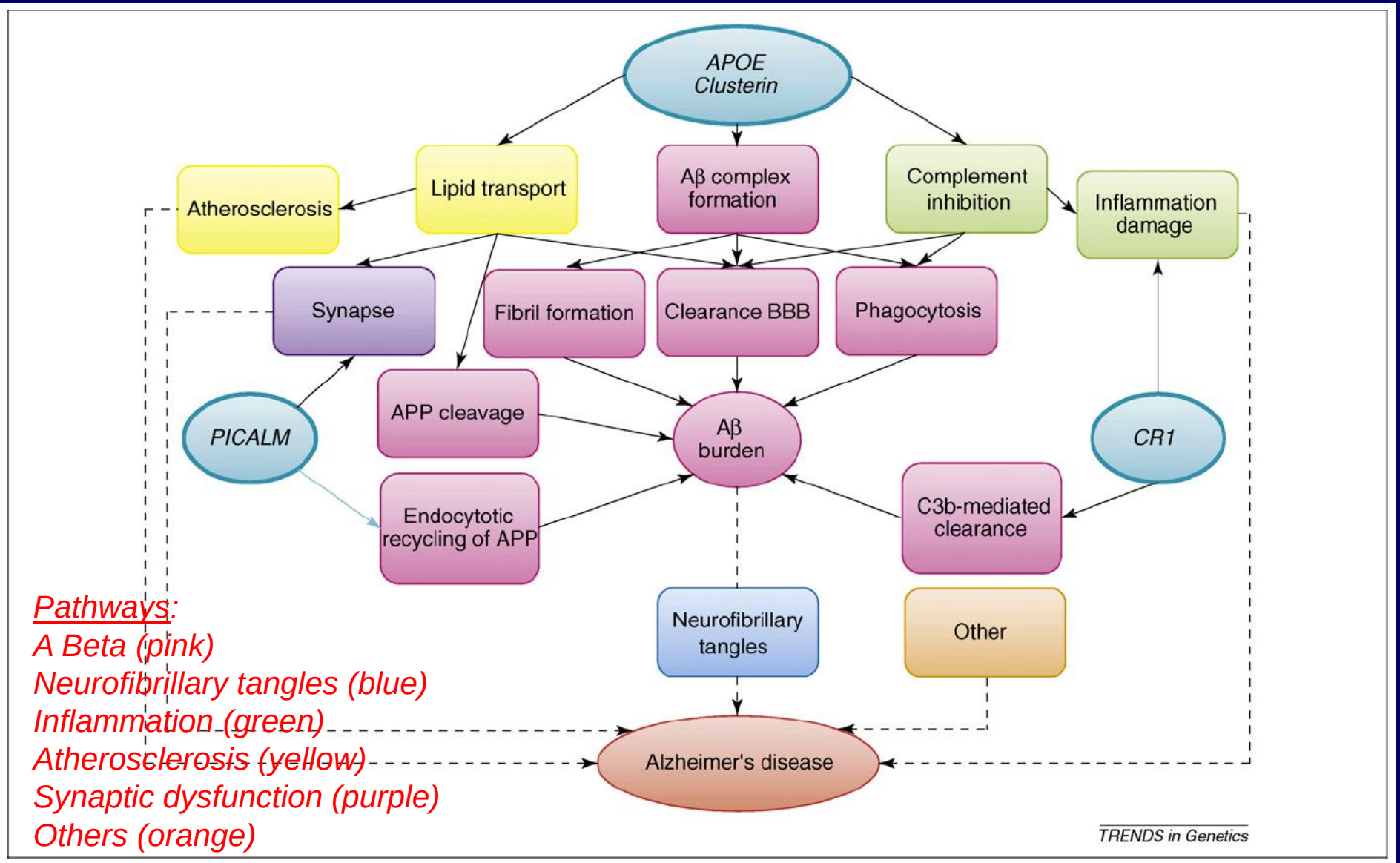
"Top 40": January 25, 2011

AlzGene Top Results

New Top Results Details

1. APOE_e2/3/4
2. CLU
3. PICALM
4. EXOC3L2
5. BIN1
6. CR1
7. SORL1
8. GWA_14q32.13
9. TNK1
10. IL8 [close]
11. LDLR
12. CST3
13. hCG2039140
14. CHRNB2
15. SORCS1
16. TNF
17. CCR2
18. ACE
19. DAPK1
20. GAB2
21. TF
22. PCDH11X
23. MTHFR
24. LOC651924
25. OTC
26. ADAM10
27. NEDD9
28. CH25H
29. IDE
30. LOC439999
31. GRN
32. IL33
33. IL1B
34. PGBD1
35. THRA
36. CALHM1
37. ENTPD7
38. TFAM
39. IL1A
40. ECE1

Gene Discoveries and AD Pathophysiology



Pathways:
A Beta (pink)
Neurofibrillary tangles (blue)
Inflammation (green)
Atherosclerosis (yellow)
Synaptic dysfunction (purple)
Others (orange)

Sleegers, Lambert, Bertram, Cruts, Amouyel & Van Broeckhoven; Trends in Genetics, 2010

Naj et al ADGC GWAS Meta-analysis

nature genetics

(~23K: ADNI AD cases & controls included)

Common variants at *MS4A4/MS4A6E*, *CD2AP*, *CD33* and *EPHA1* are associated with late-onset Alzheimer's disease

The Alzheimer Disease Genetics Consortium (ADGC) performed a genome-wide association study of late-onset Alzheimer disease using a three-stage design consisting of a discovery stage (stage 1) and two replication stages (stages 2 and 3). Both joint analysis and meta-analysis approaches were used. We obtained genome-wide significant results at *MS4A4A* (rs4938933; stages 1 and 2, meta-analysis P (P_M) = 1.7×10^{-9}, joint analysis P (P_J) = 1.7×10^{-9}; stages 1, 2 and 3, $P_M = 8.2 \times 10^{-12}$), *CD2AP* (rs9349407; stages 1, 2 and 3, $P_M = 8.6 \times 10^{-9}$), *EPHA1* (rs11767557; stages 1, 2 and 3, $P_M = 6.0 \times 10^{-10}$) and *CD33* (rs3865444; stages 1, 2 and 3, $P_M = 1.6 \times 10^{-9}$). We also replicated previous associations at *CR1* (rs6701713; $P_M = 4.6 \times 10^{-10}$, $P_J = 5.2 \times 10^{-11}$), *CLU* (rs1532278; $P_M = 8.3 \times 10^{-8}$, $P_J = 1.9 \times 10^{-8}$), *BIN1* (rs7561528; $P_M = 4.0 \times 10^{-14}$, $P_J = 5.2 \times 10^{-14}$) and *PICALM* (rs561655; $P_M = 7.0 \times 10^{-11}$, $P_J = 1.0 \times 10^{-10}$), but not at *EXOC3L2*, to late-onset Alzheimer's disease susceptibility[1–3].

from the association analyses of individual datasets and a joint analysis approach in which genotype data from each study were pooled. The latter method has improved power over the meta-analysis in the absence of between-study heterogeneity[13] and has a more direct correction for confounding sampling bias[14]. We were limited to meta-analysis for stage 3 analyses.

Because the cohorts were genotyped using different platforms, we used imputation to generate a common set of 2,324,889 SNPs. We applied uniform stringent quality control measures to all datasets to remove low-quality and redundant samples and problematic SNPs (**Supplementary Tables 3,4** and Online Methods). We performed an association analysis assuming an additive model on the log odds ratio scale with adjustment for population substructure using logistic regression for case-control data and generalized estimating equations (GEE) with a logistic model for family data. We combined results from individual datasets in the meta-analysis using

published online 3 April 2011; doi:10.1038/ng.801

Hollingworth et al GWAS Meta-analysis

nature genetics *(~ 26K: ADNI AD cases & controls included)*

Common variants at *ABCA7*, *MS4A6A/MS4A4E*, *EPHA1*, *CD33* and *CD2AP* are associated with Alzheimer's disease

We sought to identify new susceptibility loci for Alzheimer's disease through a staged association study (GERAD+) and by testing suggestive loci reported by the Alzheimer's Disease Genetic Consortium (ADGC) in a companion paper. We undertook a combined analysis of four genome-wide association datasets (stage 1) and identified ten newly associated variants with $P \leq 1 \times 10^{-5}$. We tested these variants for association in an independent sample (stage 2). Three SNPs at two loci replicated and showed evidence for association in a further sample (stage 3). Meta-analyses of all data provided compelling evidence that *ABCA7* (rs3764650, meta $P = 4.5 \times 10^{-17}$; including ADGC data, meta $P = 5.0 \times 10^{-21}$) and the *MS4A* gene cluster (rs610932, meta $P = 1.8 \times 10^{-14}$; including ADGC data, meta $P = 1.2 \times 10^{-16}$) are new Alzheimer's disease susceptibility loci. We also found independent evidence for association for three loci reported by the ADGC, which, when combined, showed genome-wide significance: *CD2AP* (GERAD+, $P = 8.0 \times 10^{-4}$; including ADGC data, meta $P = 8.6 \times 10^{-9}$), *CD33* (GERAD+, $P = 2.2 \times 10^{-4}$; including ADGC data, meta $P = 1.6 \times 10^{-9}$) and *EPHA1* (GERAD+, $P = 3.4 \times 10^{-4}$; including ADGC data, meta $P = 6.0 \times 10^{-10}$).

($P = 3 \times 10^{-3}$). A combined analysis of the GERAD1 and EADI1 data yielded highly significant support for all three loci (*CLU* meta $P = 6.7 \times 10^{-16}$; *PICALM* meta $P = 6.3 \times 10^{-9}$; and *CR1* meta $P = 3.2 \times 10^{-12}$). The associations in *CLU*, *PICALM* and *CRI* have since been replicated in several independent datasets[5–8], have shown trends in another dataset[9] and have shown relationships with the neurodegenerative processes underlying disease[10]. In addition, members of this consortium have since reported genome-wide significant association for *BIN1* ($P = 1.6 \times 10^{-11}$) and support for *EPHA1* (encoding ephrin receptor A1) ($P = 1.7 \times 10^{-6}$)[11].

This study sought to identify new common susceptibility variants for Alzheimer's disease by first undertaking a three-stage association study based upon predominantly European samples (GERAD+; **Fig. 1**) and then by testing these samples for loci showing suggestive evidence for association in the ADGC GWAS[12].

The first stage of this study comprised a meta-analysis of four Alzheimer's disease GWAS datasets (6,688 affected individuals (cases) and 13,685 controls) including: the GERAD1 (ref. 3), EADI1 (ref. 4), Translational Genomics Research Institute (TGEN1)[13] and the Alzheimer's Disease Neuroimaging Initiative (ADNI)[14] datasets. SNPs which remained significant at $P \leq 1 \times 10^{-5}$ were then tested for replica-

published online 3 April 2011; doi:10.1038/ng.803

AlzGene Top Ten (4/18/11)

ALZGENE TOP RESULTS

BACK | SEARCH | METHODS | DISCLAIMER | CREDITS

Status: Updated 18 April 2011; ***Please see legend below and consult the "Top Results Methods" for details on new ranking procedure***

RANKING BASED ON HUGENET INTERIM GUIDELINES FOR THE ASSESSMENT OF GENETIC ASSOCIATION STUDIES

#	Gene	Polymorphism	Ethnicity	OR (95% CI)	P-value	N minor (Grade)	I^2 (Grade)	Bias Reason (Grade)	Overall Grade	Bayes Factor (log10)
1	APOE_e2/3/4	APOE_e2/3/4	All	3.685 (3.30-4.12)	<1E-50	4167 (A)	n.a. (A)	(A)	A	>50
2	BIN1	rs744373	All	1.166 (1.13-1.20)	1.59E-26	49650 (A)	n.a. (A)	(A)	A	23.4
3	CLU	rs11136000	Caucasian	0.879 (0.86-0.90)	3.37E-23	72432 (A)	n.a. (A)	(A)	A	20.1
4	ABCA7	rs3764650	All	1.229 (1.18-1.28)	8.17E-22	60569 (A)	n.a. (A)	(A)	A	18.8
5	CR1	rs3818361	Caucasian	1.174 (1.14-1.21)	4.72E-21	47052 (A)	n.a. (A)	(A)	A	18.1
6	PICALM	rs3851179	Caucasian	0.879 (0.86-0.9)	2.85E-20	44358 (A)	n.a. (A)	(A)	A	17.3
7	MS4A6A	rs610932	All	0.904 (0.88-0.93)	1.81E-11	63026 (A)	n.a. (A)	(A)	A	8.7
8	CD33	rs3865444	All	0.893 (0.86-0.93)	2.04E-10	37767 (A)	n.a. (A)	(A)	A	7.7
9	MS4A4E	rs670139	All	1.079 (1.05-1.11)	9.51E-10	64577 (A)	n.a. (A)	(A)	A	6.9
10	CD2AP	rs9349407	All	1.117 (1.08-1.16)	2.75E-09	35840 (A)	n.a. (A)	(A)	A	6.6

NEW: Only meta-analysis results with P-values <0.00001 are displayed in this table. Please contact us to request a list of other at least nominally significant meta-analysis results.

Per gene/locus only one (i.e. the best associated) marker is listed, ranking is based on P-value. All results are assessed for their epidemiological credibility using two methods:

http://www.alzgene.org/TopResults.asp

New Candidate Genes

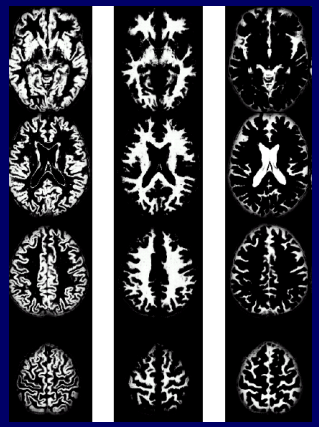
- *ABCA7*: ATP-binding cassette (ABC) transporter; role in lipoprotein particle processing (APOE & CLU)
- *CD33:* sialic-acid-binding IG-like lectins (Siglec) family; role in cell-cell interactions, regulation of immune cell function; role in endocytosis
- *CD2AP:* CD2-associated protein. Scaffold adaptor protein associates with cortactin, a protein involved in regulation of endocytosis
- *EPHA1:* expressed mainly in epithelial tissues; regulates cell morphology and motility; possible role in apoptosis & inflammation
- *MS4A family: (MS4A4, MS4A6E, MS4A6A, MS4A4E):* role in immune function

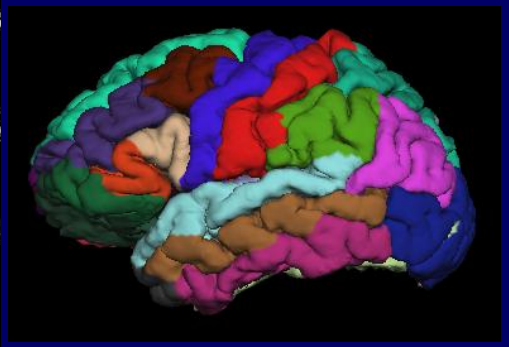
Putative Roles of New Candidate Genes

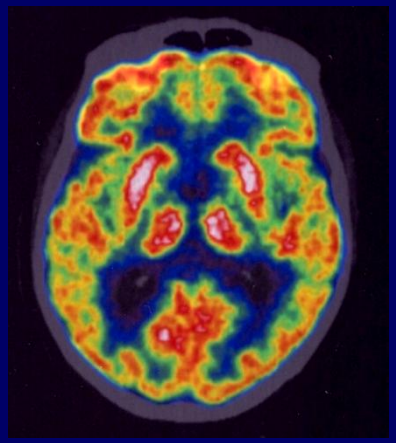
Gene	Lipid Processing	Immune Function	Endocytosis
APOE	X	X	X
ABCA7	X	X	
BIN1			X
CD33		X	X
CD2AP			X
CLU	X	X	
CR1		X	
EPHA1		X	
MS4A family		X	
PICALM			X

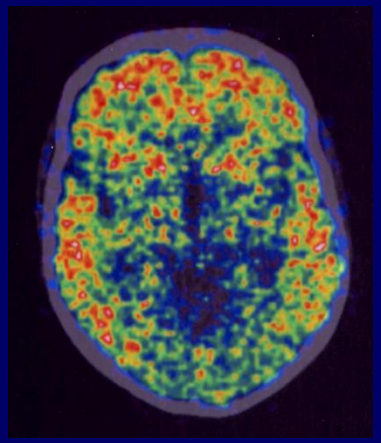
Saykin, AAN, Honolulu, 4/15/11

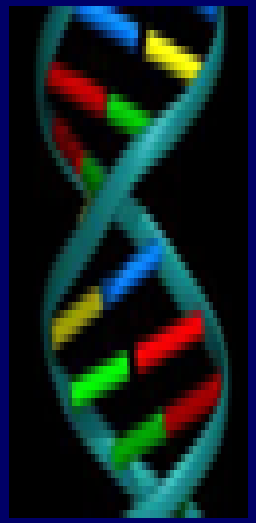
Imaging, Biomarkers & Clinical Endophenotypes

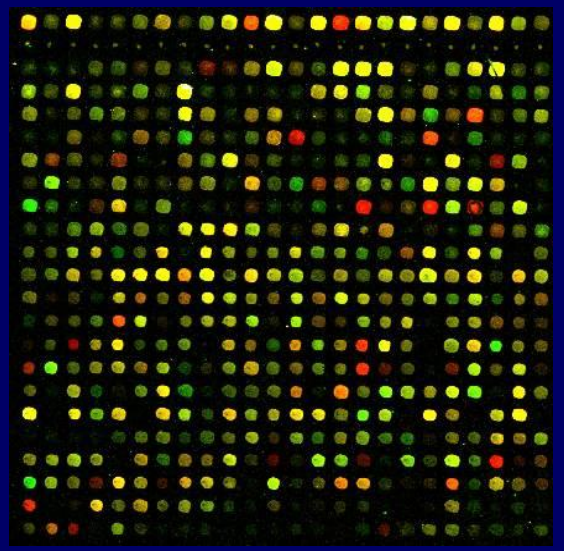








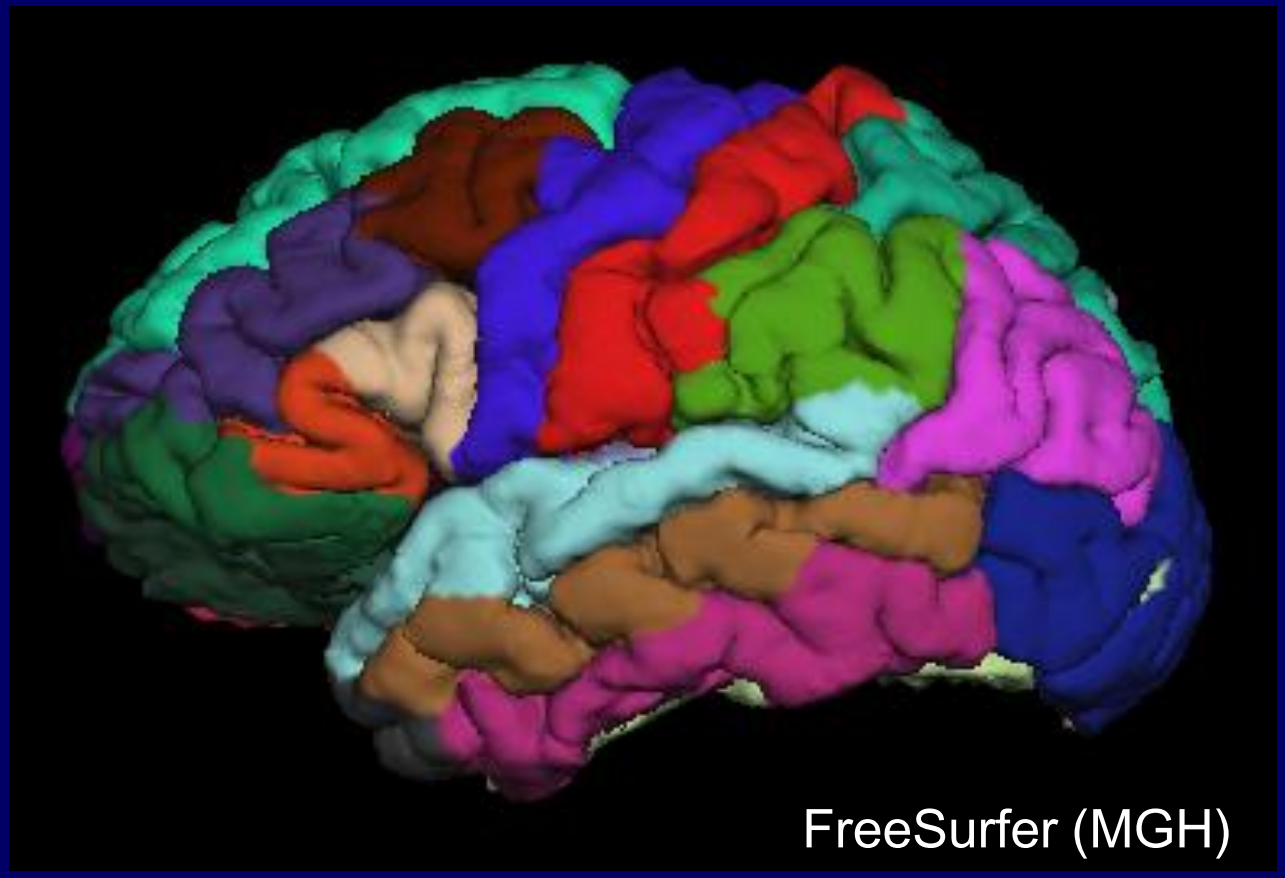




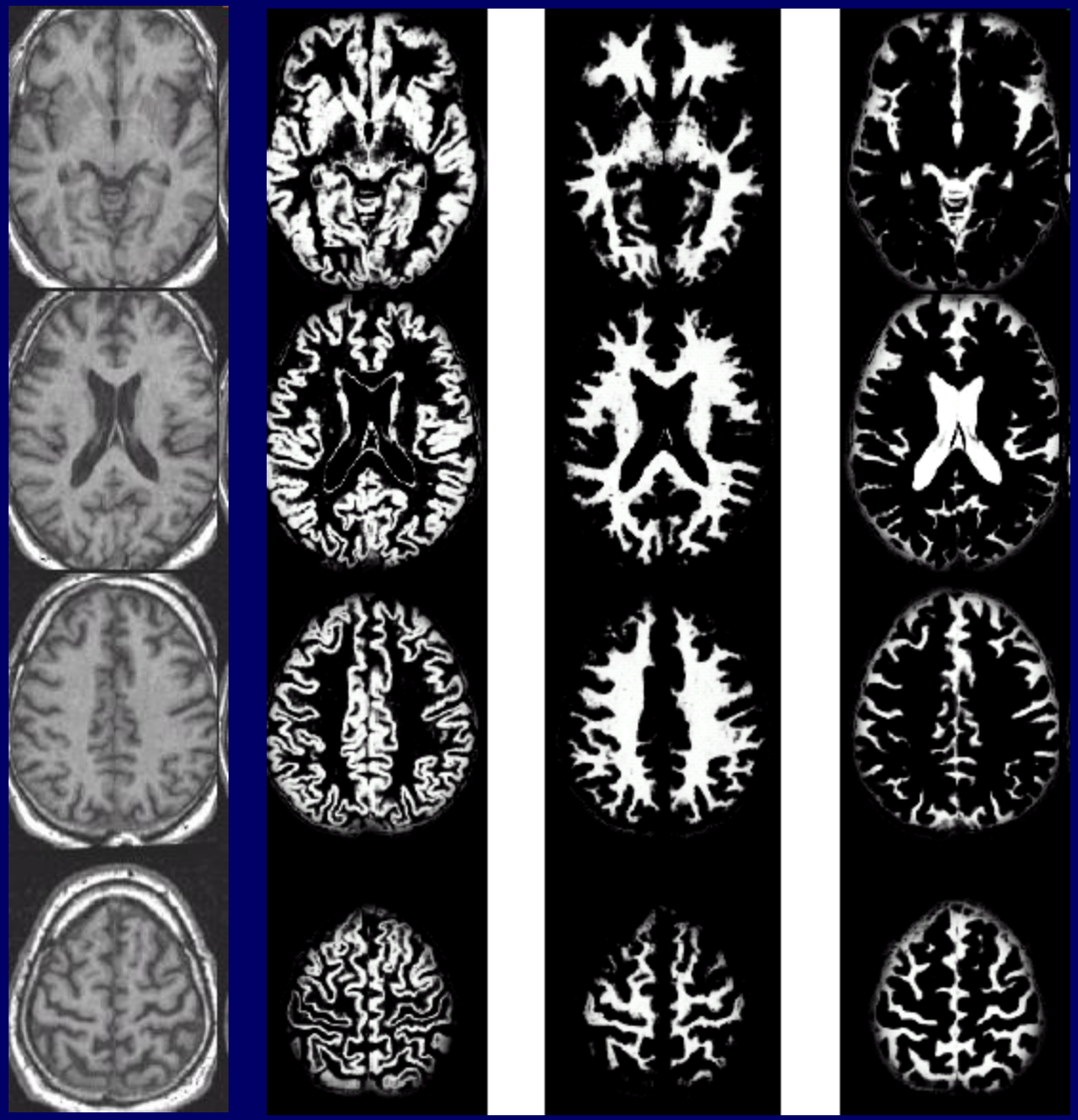
Gene “Chip”

Imaging Biomarkers and Phenotypes:

I. Automated Cortical Parcellation and High Throughput Computation

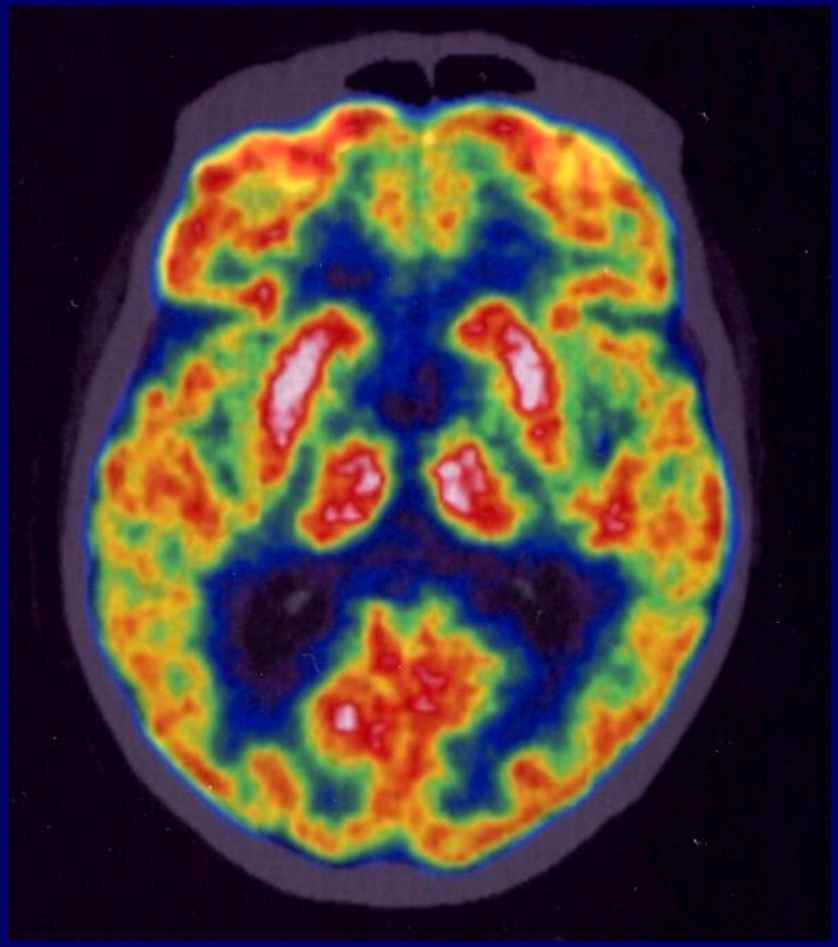


II. Voxel-Based Morphometry (VBM)

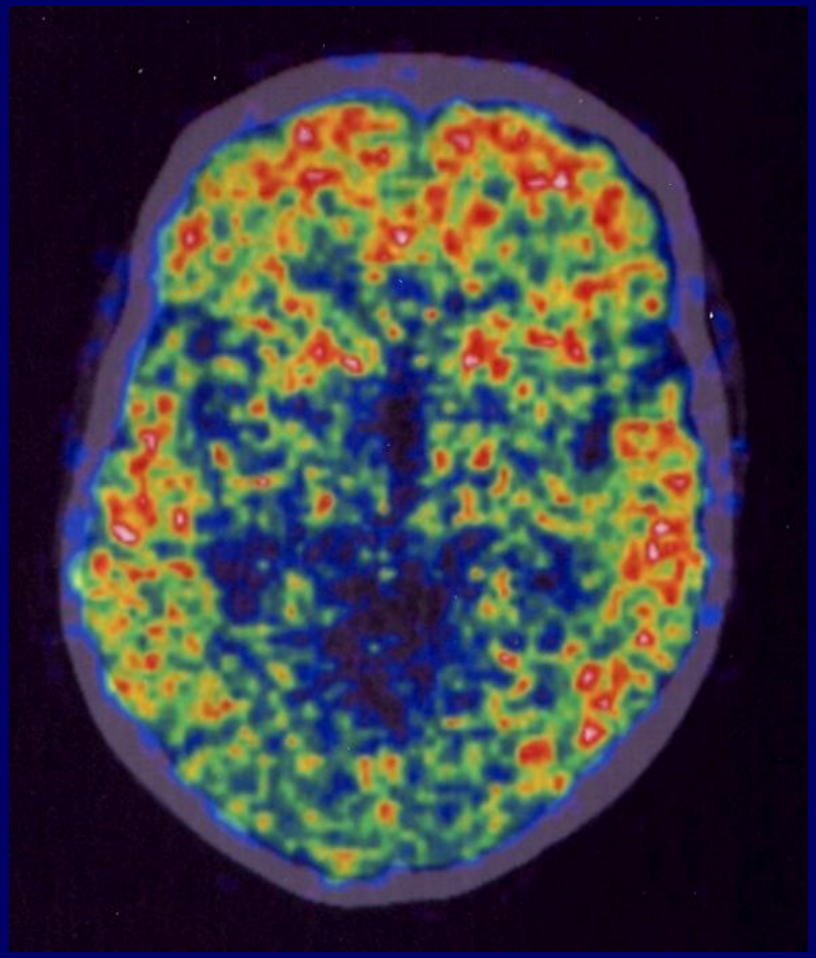


SPM 8 software

III. Molecular Imaging of MCI/AD: Metabolism and Amyloid Deposition



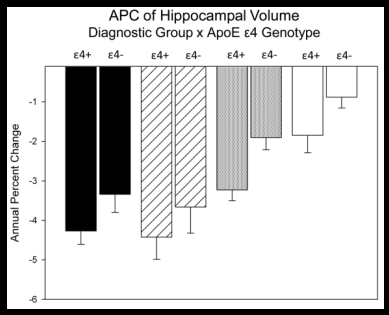
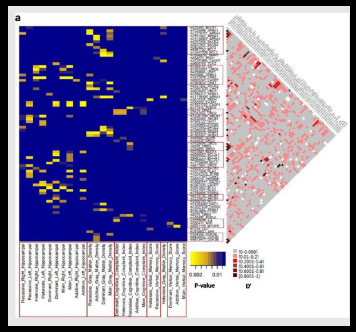
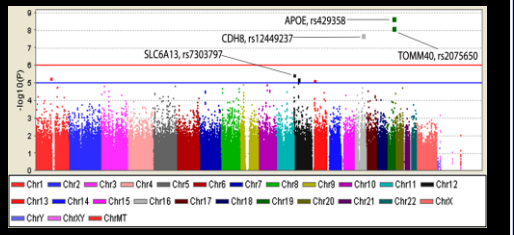
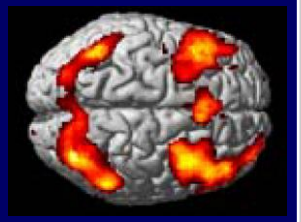
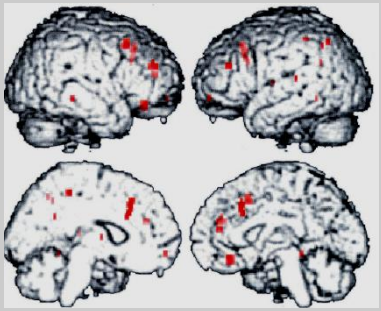
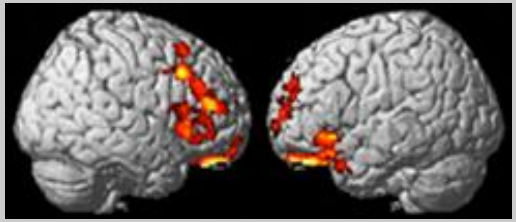
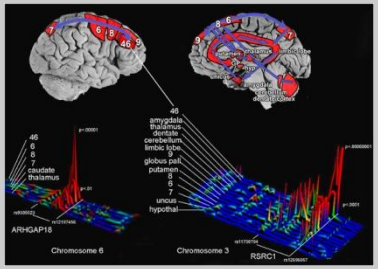
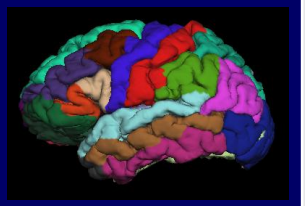
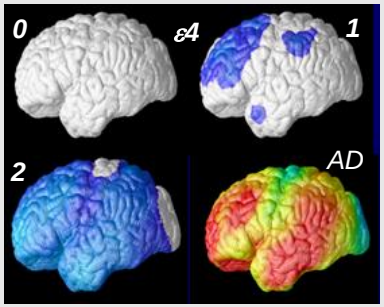
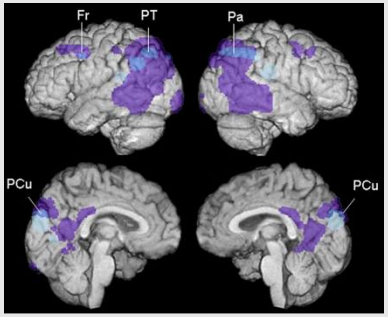
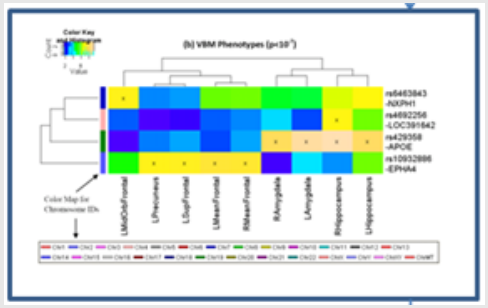
[18F]FDG



[11C]PIB

IUSM 5/07

Brain-Genome Association Strategies

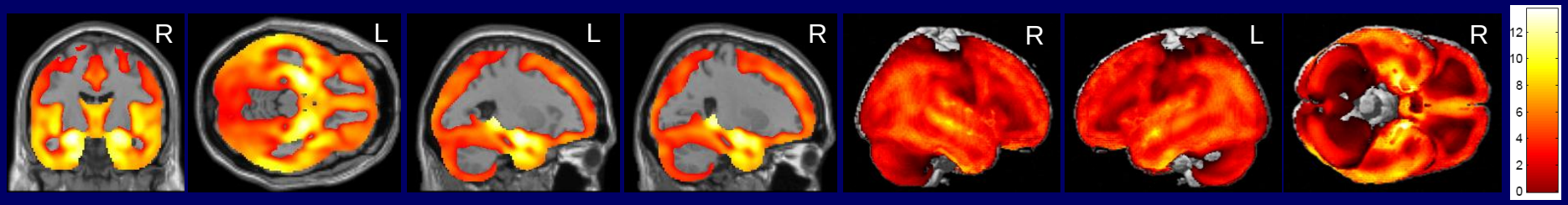
	Candidate Gene/SNP	Biological Pathway	Genome-wide Analysis
ROI		 *Risacher et al 2010*	 *Sloan et al 2010*	 *Potkin et al 2009; Saykin et al 2010*
Circuit		 *Egan et al 2001 COMT*	 *Swaminathan et al 2010 PiB ROIs & amyloid pathway*	 *Potkin et al 2009 Mol Psych schizophrenia study*
Whole Brain		 *Reiman et al PNAS 2009; Also Ho et al 2010 FTO*	 *Reiman et al 2008 cholesterol pathway genes*	 *Shen et al 2010 ROIs; Stein et al 2010 voxels*

Global Grey Matter Density of Patient Groups (AD, MCI-Converter, MCI-Stable) Relative to HC Participants

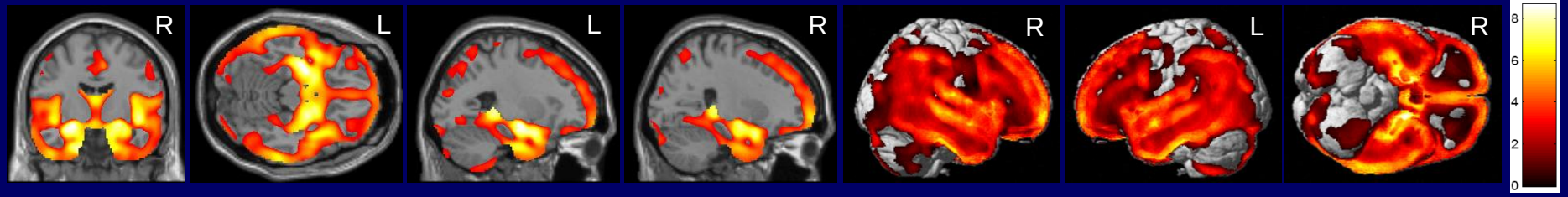
n=693 (148 AD, 62 MCI-C, 277 MCI-S, 206 HC)

Covaried for age, gender, education, handedness and total intracranial volume (ICV)

HC>AD

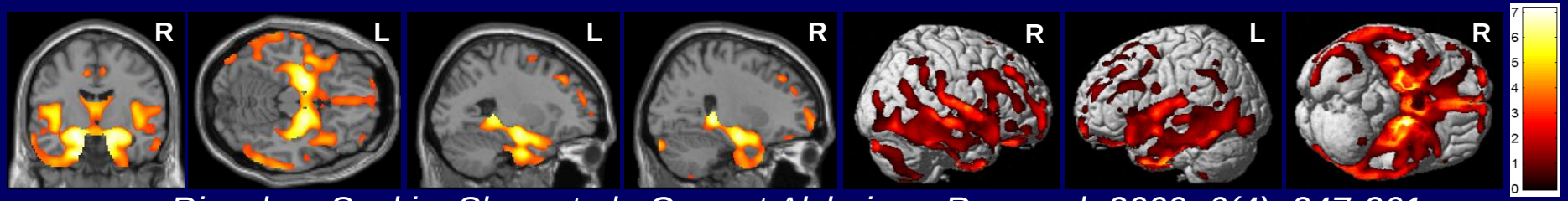


HC>MCI-Converters



HC>MCI-Stable

p<0.005 (FDR), k=27



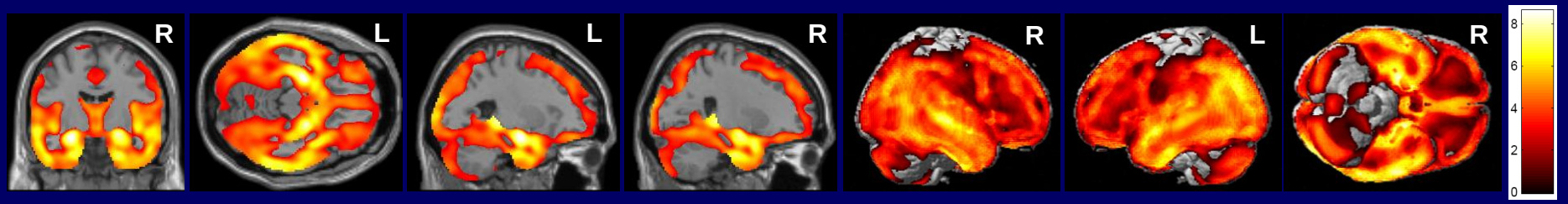
Risacher, Saykin, Shen et al; Current Alzheimer Research 2009; 6(4): 347-361

Relationship of Global Grey Matter Density Among Patient Groups (AD, MCI-Converter, MCI-Stable)

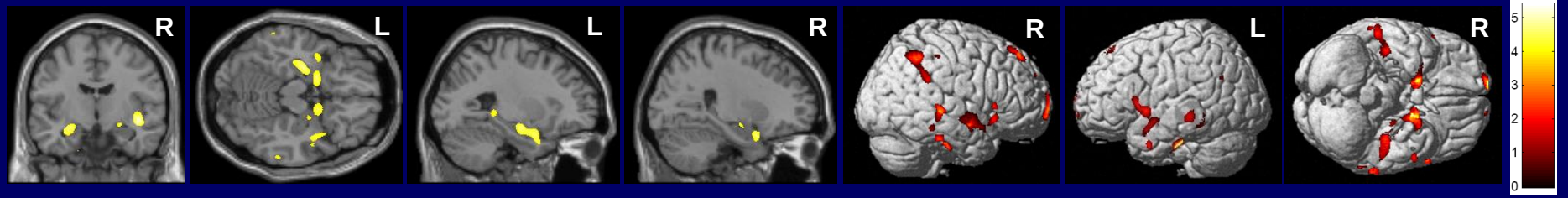
n=693 (148 AD, 62 MCI-C, 277 MCI-S, 206 HC); p<0.005 (FDR), k=27

Covaried for age, gender, education, handedness and total intracranial volume (ICV)

MCI-Stable>AD



MCI-Stable>MCI-Converters

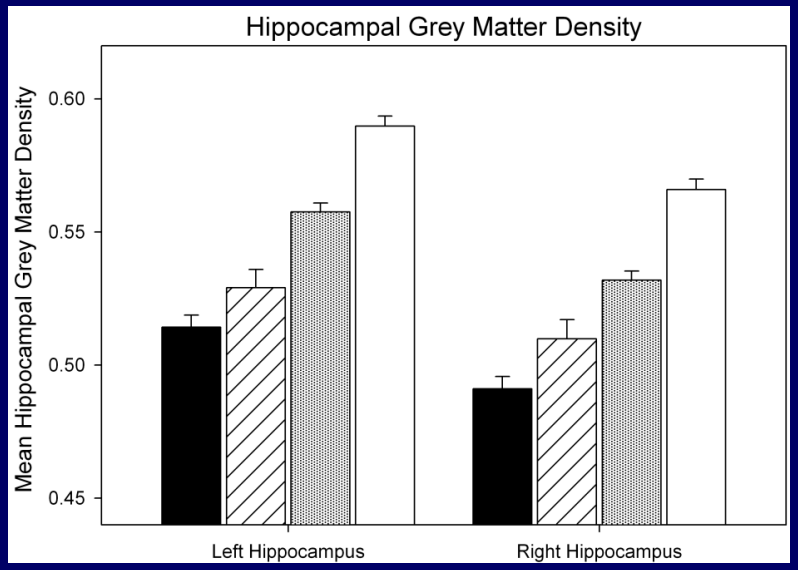


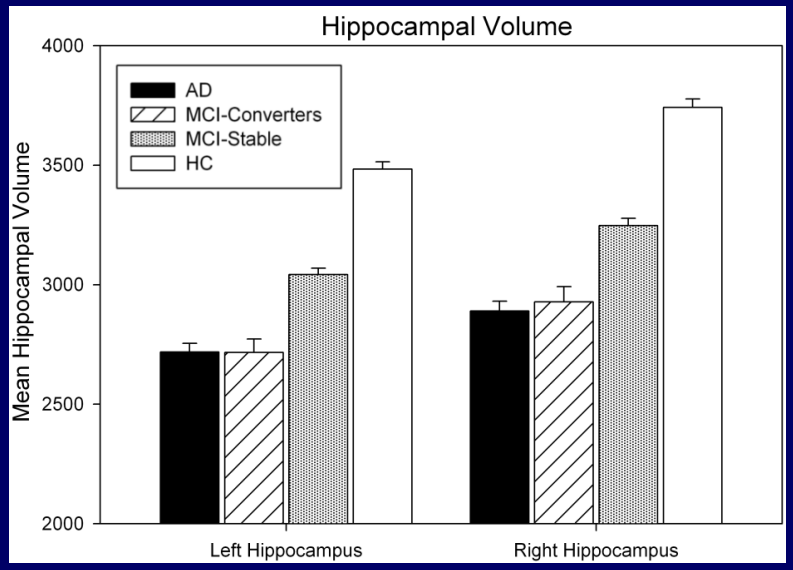
MCI-Converters>AD – No Significantly Different Voxels

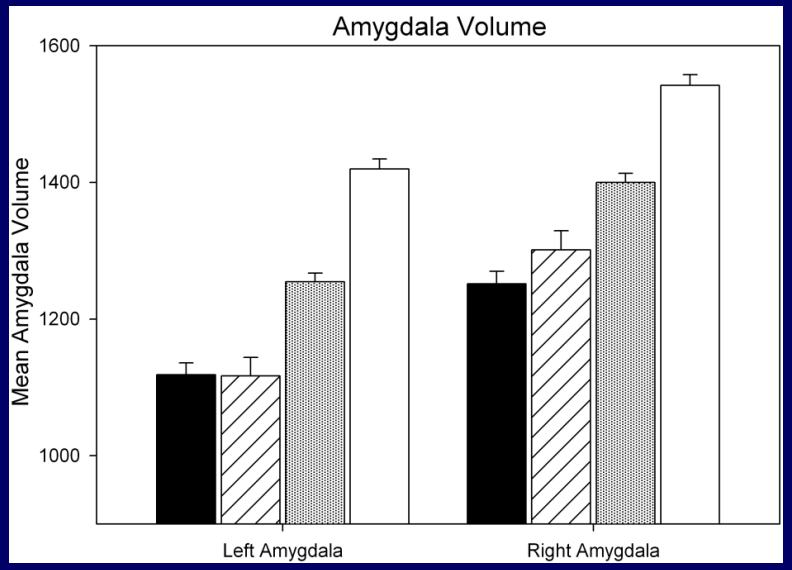
Risacher, Saykin, Shen et al; Current Alzheimer Research 2009; 6(4): 347-361

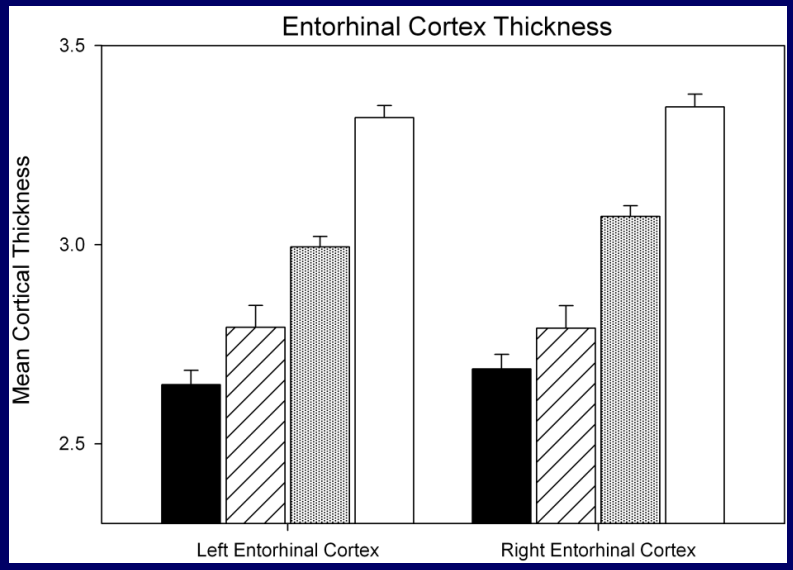
AD Phenotype: MTL Grey Matter Density, Volume, and Cortical Thickness in the ADNI Sample at Baseline

N=693 (148 AD, 62 MCI-C, 277 MCI-S, 206 HC); p<0.005 (FDR)



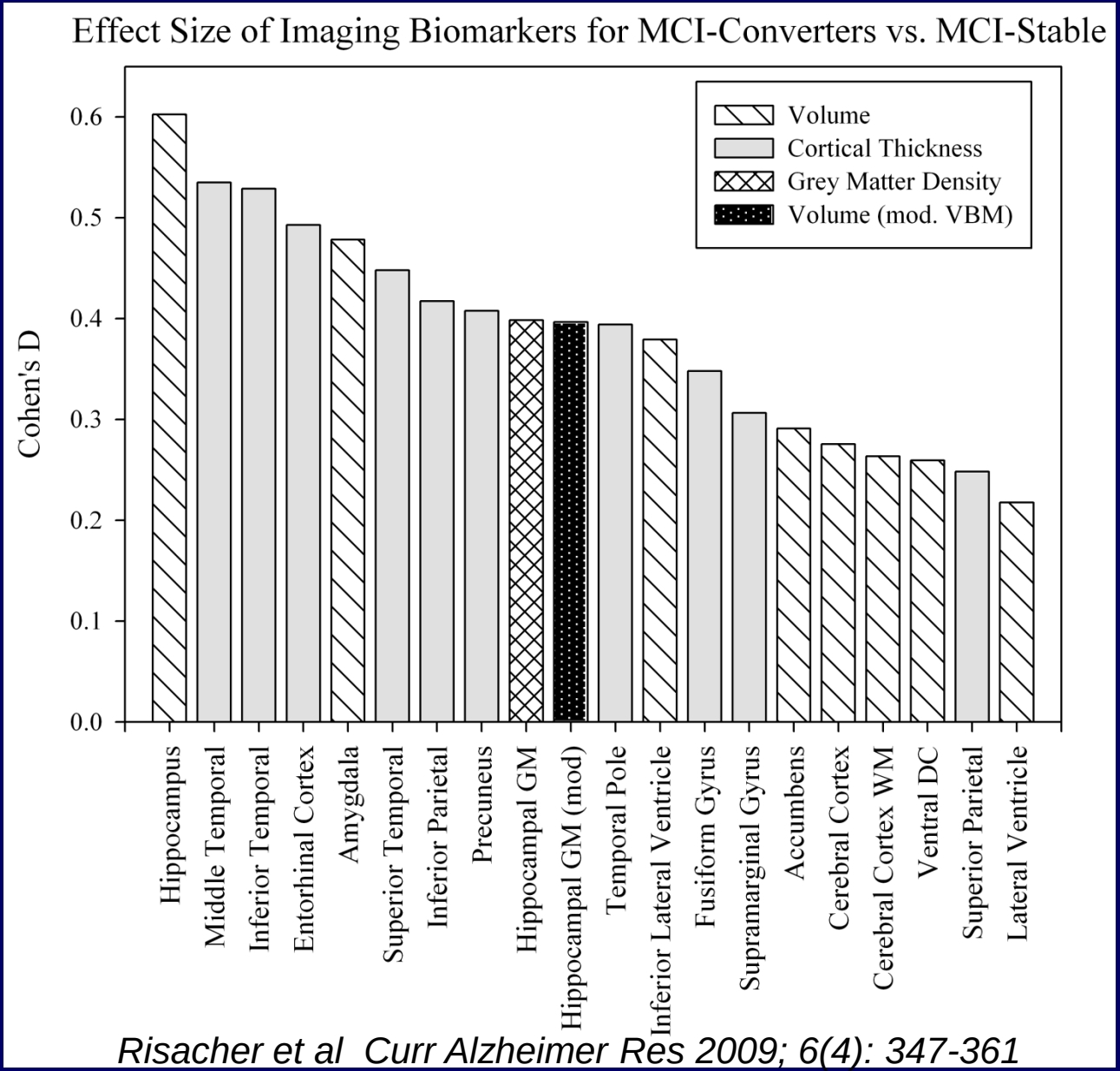






Risacher, Saykin, Shen et al; Current Alzheimer Research 2009; 6(4): 347-361

Regions Showing the Greatest Effect Sizes when Comparing MCI-Converter and MCI-Stable Participants at Baseline



Risacher et al Curr Alzheimer Res 2009; 6(4): 347-361

Overview of ADNI Genetics

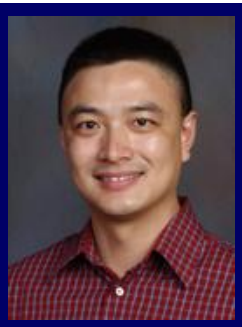
ELSEVIER

Alzheimer's & Dementia 6 (2010) 265–273

Alzheimer's & Dementia

Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: Genetics core aims, progress, and plans

Andrew J. Saykin[a,b,*], Li Shen[a,c], Tatiana M. Foroud[b], Steven G. Potkin[d], Shanker Swaminathan[a,b], Sungeun Kim[a,c], Shannon L. Risacher[a], Kwangsik Nho[a,e], Matthew J. Huentelman[f], David W. Craig[f], Paul M. Thompson[g], Jason L. Stein[g], Jason H. Moore[h,i], Lindsay A. Farrer[j], Robert C. Green[j], Lars Bertram[k], Clifford R. Jack, Jr.[l], Michael W. Weiner[m,n,o,p]; and the Alzheimer's Disease Neuroimaging Initiative






Saykin et al (2010) Alzheimer's & Dementia

Publications using ADNI GWAS data (partial): Spring 2011

1. Biffi, A., et al., *Genetic Variation and Neuroimaging Measures in Alzheimer Disease.* Arch Neurol, 2010. 67(6): p. 677-685.
2. Cruchaga, C., et al., *SNPs associated with cerebrospinal fluid phospho-tau levels influence rate of decline in Alzheimer's disease.* PLoS Genet, 2010. 6(9).
3. Furney, S.J., et al., *Genome wide association with MRI atrophy measures as a quantitative trait locus for Alzheimer's disease.* Molecular Psychiatry, 2010. in press.
4. Hibar, D.P., et al., *Voxelwise gene-wide association study (vGeneWAS): multivariate gene-based association testing in 731 elderly subjects.* NeuroImage, 2011 in press.
5. Ho, A.J., et al., *A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly.* Proceedings of the National Academy of Sciences, 2010. 107(18): p. 8404-8409.
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8. Kauwe, J.S.K., et al., *Validating Predicted Biological Effects of Alzheimer's Disease Associated SNPs Using CSF Biomarker Levels.* Journal of Alzheimer's Disease, 2010.
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11. Naj, A.C., et al., *Dementia revealed: novel chromosome 6 locus for late-onset Alzheimer disease provides genetic evidence for folate-pathway abnormalities.* PLoS Genet, 2010. 6(9).
12. Naj, A., et al., *Common variants in MS4A4/MS4A6E, CD2AP, CD33, and EPHA1 are associated with late-onset Alzheimer's disease.* Nature Genetics, 2011 in press.
13. Potkin, S.G., et al., *Hippocampal Atrophy as a Quantitative Trait in a Genome-Wide Association Study Identifying Novel Susceptibility Genes for Alzheimer's Disease.* PLoS ONE, 2009. 4(8): p. e6501.
14. Rimol, L.M., et al., *Sex-dependent association of common variants of microcephaly genes with brain structure.* Proceedings of the National Academy of Sciences, 2010. 107(1): p. 384-388.
15. Saykin, A.J., et al., *Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: Genetics core aims, progress, and plans.* Alzheimer's and Dementia, 2010. 6(3): p. 265-273.
16. Shen, L., et al., *Whole genome association study of brain-wide imaging phenotypes for identifying quantitative trait loci in MCI and AD: A study of the ADNI cohort.* NeuroImage, 2010. 53(3): p. 1051-1063.
17. Stein, J.L., et al., *Voxelwise genome-wide association study (vGWAS).* NeuroImage, 2010. 53(3): p. 1160-1174.
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Initial ADNI GWAS Report

Potkin et al 8/09

OPEN ACCESS Freely available online

PLoS one

Hippocampal Atrophy as a Quantitative Trait in a Genome-Wide Association Study Identifying Novel Susceptibility Genes for Alzheimer's Disease

Steven G. Potkin[1]*, Guia Guffanti[1,2], Anita Lakatos[1], Jessica A. Turner[1], Frithjof Kruggel[3], James H. Fallon[1], Andrew J. Saykin[4], Alessandro Orro[5], Sara Lupoli[6], Erika Salvi[2], Michael Weiner[7], Fabio Macciardi[1,2] for the Alzheimer's Disease Neuroimaging Initiative

1 Department of Psychiatry and Human Behavior, University of California Irvine, Irvine, California, United States of America, **2** Department of Sciences and Biomedical Technologies, University of Milan, Segrate (MI), Italy, **3** Department of Biomedical Engineering, University of California Irvine, Irvine, California, United States of America, **4** Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, Indiana, United States of America, **5** Istituto di Tecnologie Biomediche, Consiglio Nazionale delle Ricerche, Segrate (MI), Italy, **6** Universita' Vita e Salute, Department of Neurology and Neuroscience, Milan, Italy, **7** Department of Radiology, Psychiatry and Neurology, University of California San Francisco, San Francisco, California, United States of America

Abstract

Background: With the exception of APOE ε4 allele, the common genetic risk factors for sporadic Alzheimer's Disease (AD) are unknown.

Methods and Findings: We completed a genome-wide association study on 381 participants in the ADNI (Alzheimer's Disease Neuroimaging Initiative) study. Samples were genotyped using the Illumina Human610-Quad BeadChip. 516,645 unique Single Nucleotide Polymorphisms (SNPs) were included in the analysis following quality control measures. The genotype data and raw genetic data are freely available for download (LONI, http://www.loni.ucla.edu/ADNI/Data/). Two analyses were completed: a standard case-control analysis, and a novel approach using hippocampal atrophy measured on MRI as an objectively defined, quantitative phenotype. A General Linear Model was applied to identify SNPs for which there was an interaction between the genotype and diagnosis on the quantitative trait. The case-control analysis identified APOE and a new risk gene, TOMM40 (translocase of outer mitochondrial membrane 40), at a genome-wide significance level of $\leq 10^{-6}$ (10^{-11} for a haplotype). TOMM40 risk alleles were approximately twice as frequent in AD subjects as controls. The quantitative trait analysis identified 21 genes or chromosomal areas with at least one SNP with a p-value$\leq 10^{-6}$, which can be considered potential "new" candidate loci to explore in the etiology of sporadic AD. These candidates included EFNA5, CAND1, MAGI2, ARSB, and PRUNE2, genes involved in the regulation of protein degradation, apoptosis, neuronal loss and neurodevelopment. Thus, we identified common genetic variants associated with the increased risk of developing AD in the ADNI cohort, and present publicly available genome-wide data. Supportive evidence based on case-control studies and biological plausibility by gene annotation is provided. Currently no available sample with both imaging and genetic data is available for replication.

Conclusions: Using hippocampal atrophy as a quantitative phenotype in a genome-wide scan, we have identified candidate risk genes for sporadic Alzheimer's disease that merit further investigation.

Citation: Potkin SG, Guffanti G, Lakatos A, Turner JA, Kruggel F, et al. (2009) Hippocampal Atrophy as a Quantitative Trait in a Genome-Wide Association Study Identifying Novel Susceptibility Genes for Alzheimer's Disease. PLoS ONE 4(8): e6501. doi:10.1371/journal.pone.0006501

Translocase of Outer Mitochondrial Membrane 40 homolog (TOMM40) 523 PolyT Assay: Collaboration with Roses et al to replicate & extend

TPJ Open

The Pharmacogenomics Journal (2009), 1–10

www.nature.com/tpj

ORIGINAL ARTICLE

A *TOMM40* variable-length polymorphism predicts the age of late-onset Alzheimer's disease

AD Roses[1,2], MW Lutz[1,2], H Amrine-Madsen[3], AM Saunders[1,2], DG Crenshaw[1,2], SS Sundseth[1,2], MJ Huentelman[4], KA Welsh-Bohmer[1,5] and EM Reiman[4,6,7]

[1]*Department of Medicine, Duke University, Durham, NC, USA;* [2]*Deane Drug Discovery Institute, Durham, NC, USA;* [3]*GlaxoSmithKline, Research Triangle Park, NC, USA;* [4]*Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ, USA;* [5]*Alzheimer's Disease Clinical Center, Durham, NC, USA;* [6]*Banner Alzheimer's Institute, Phoenix, AZ, USA and* [7]*Department of Psychiatry, University of Arizona, Phoenix, AZ, USA*

Correspondence:
Dr AD Roses, Deane Drug Discovery Institute, School of Medicine and Fuqua School of Business, Duke University, R. David Thomas Executive Training Center, One Science Drive, Suite 342, Box 90344, Durham, NC 27708-0120, USA.
E-mail: allen.roses@duke.edu

The ε4 allele of the apolipoprotein E (*APOE*) gene is currently the strongest and most highly replicated genetic factor for risk and age of onset of late-onset Alzheimer's disease (LOAD). Using phylogenetic analysis, we have identified a polymorphic poly-T variant, rs10524523, in the translocase of outer mitochondrial membrane 40 homolog (*TOMM40*) gene that provides greatly increased precision in the estimation of age of LOAD onset for *APOE* ε3 carriers. In two independent clinical cohorts, longer lengths of rs10524523 are associated with a higher risk for LOAD. For *APOE* ε3/4 patients who developed LOAD after 60 years of age, individuals with long poly-T repeats linked to *APOE* ε3 develop LOAD on an average of 7 years earlier than individuals with shorter poly-T repeats linked to *APOE* ε3 (70.5 ± 1.2 years versus 77.6 ± 2.1 years, $P = 0.02$, $n = 34$). Independent mutation events at rs10524523 that occurred during Caucasian evolution have given rise to multiple categories of poly-T length variants at this locus. On replication, these results will have clinical utility for predictive risk estimates for LOAD and for enabling clinical disease prevention studies. In addition, these results show the effective use of a phylogenetic approach for analysis of haplotypes of polymorphisms, including structural polymorphisms, which contribute to complex diseases.
The Pharmacogenomics Journal advance online publication, 22 December 2009; doi:10.1038/tpj.2009.69

Keywords: AD genetics; phylogenetic analysis; *TOMM40*; *APOE*; poly-T variants

Whole Brain & Genome-wide Analysis

ROI-based

Contents lists available at ScienceDirect

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Whole genome association study of brain-wide imaging phenotypes for identifying quantitative trait loci in MCI and AD: A study of the ADNI cohort

Li Shen [a,b,*], Sungeun Kim [a,b], Shannon L. Risacher [a], Kwangsik Nho [a,c], Shanker Swaminathan [a,d], John D. West [a], Tatiana Foroud [d], Nathan Pankratz [d], Jason H. Moore [e], Chantel D. Sloan [e], Matthew J. Huentelman [f], David W. Craig [f], Bryan M. DeChairo [g], Steven G. Potkin [h], Clifford R. Jack Jr [i], Michael W. Weiner [j,k], Andrew J. Saykin [a,d,*] and the Alzheimer's Disease Neuroimaging Initiative [1]

[a] Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine, 950 West Walnut Street R2 E124, Indianapolis, IN 46202, USA
[b] Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, 410 West 10th Street, Suite 5000, Indianapolis, IN 46202, USA
[c] Regenstrief Institute, 410 West 10th Street, Suite 2000, Indianapolis, IN 46202, USA
[d] Department of Medical and Molecular Genetics, Indiana University School of Medicine, 975 West Walnut Street, Indianapolis, IN 46202, USA
[e] Computational Genetics Laboratory, Departments of Genetics and Community and Family Medicine, Dartmouth Medical School, Lebanon, NH 03756, USA
[f] The Translational Genomics Research Institute, 445 N. Fifth St., Phoenix, AZ 85004, USA
[g] Neuroscience, Molecular Medicine, Pfizer Global R&D, New London, CT 06320, USA
[h] Department of Psychiatry and Human Behavior, University of California, Irvine, Irvine, CA 92697, USA
[i] Mayo Clinic, Rochester, MN 55905, USA
[j] Departments of Radiology, Medicine and Psychiatry, UC San Francisco, San Francisco, CA 94143, USA
[k] Department of Veterans Affairs Medical Center, San Francisco, CA 94121, USA

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ABSTRACT

A genome-wide, whole brain approach to investigate genetic effects on neuroimaging phenotypes for identifying quantitative trait loci is described. The Alzheimer's Disease Neuroimaging Initiative 1.5 T MRI and genetic dataset was investigated using voxel-based morphometry (VBM) and FreeSurfer parcellation followed by genome-wide association studies (GWAS). One hundred forty-two measures of grey matter (GM) density, volume, and cortical thickness were extracted from baseline scans. GWAS, using PLINK, were performed on each phenotype using quality-controlled genotype and scan data including 530,992 of 620,903 single nucleotide polymorphisms (SNPs) and 733 of 818 participants (175 AD, 354 amnestic mild cognitive impairment, MCI, and 204 healthy controls, HC). Hierarchical clustering and heat maps were used to analyze the GWAS results and associations are reported at two significance thresholds ($p<10^{-7}$ and $p<10^{-6}$). As expected, SNPs in the APOE and TOMM40 genes were confirmed as markers strongly associated with multiple brain regions. Other top SNPs were proximal to the EPHA4, TP63 and NXPH1 genes. Detailed image analyses of rs6463843 (flanking NXPH1) revealed reduced global and regional GM density across diagnostic groups in TT relative to GG homozygotes. Interaction analysis indicated that AD patients homozygous for the T allele showed differential vulnerability to right hippocampal GM density loss. NXPH1 codes for a protein implicated in promotion of adhesion between dendrites and axons, a key factor in synaptic integrity, the loss of which is a hallmark of AD. A genome-wide, whole brain search strategy has the potential to reveal novel candidate genes and loci warranting further investigation and replication.

Shen et al NeuroImage 53 (2010) 1051–1063

Voxel-based

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Voxelwise genome-wide association study (vGWAS)

Jason L. Stein [a], Xue Hua [a], Suh Lee [a], April J. Ho [a], Alex D. Leow [a,b], Arthur W. Toga [a], Andrew J. Saykin [c,d], Li Shen [c], Tatiana Foroud [d], Nathan Pankratz [d], Matthew J. Huentelman [e], David W. Craig [e], Jill D. Gerber [e], April N. Allen [e], Jason J. Corneveaux [e], Bryan M. DeChairo [f], Steven G. Potkin [g], Michael W. Weiner [h,i], Paul M. Thompson [a,*] and the Alzheimer's Disease Neuroimaging Initiative [1]

[a] Laboratory of Neuro Imaging, Department of Neurology, University of California, Los Angeles School of Medicine, Neuroscience Research Building 225E, 635 Charles Young Drive, Los Angeles, CA 90095-1769, USA
[b] University of Illinois School of Medicine, Chicago, IL USA, and Community Psychiatry Associates, Sacramento, CA, USA
[c] Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN, USA
[d] Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA
[e] The Translational Genomics Research Institute, Phoenix, AZ, USA
[f] Neuroscience, Molecular Medicine, Pfizer Global Research and Development, New London, CT, USA
[g] Department of Psychiatry and Human Behavior, University of California, Irvine, Irvine, CA, USA
[h] Departments of Radiology, Medicine and Psychiatry, University of California, San Francisco, San Francisco, CA, USA
[i] Department of Veterans Affairs Medical Center, San Francisco, CA, USA

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ABSTRACT

The structure of the human brain is highly heritable, and is thought to be influenced by many common genetic variants, many of which are currently unknown. Recent advances in neuroimaging and genetics have allowed collection of both highly detailed structural brain scans and genome-wide genotype information. This wealth of information presents a new opportunity to find the genes influencing brain structure. Here we explore the relation between 448,293 single nucleotide polymorphisms in each of 31,622 voxels of the entire brain across 740 elderly subjects (mean age ± s.d.: 75.52 ± 6.82 years; 438 male) including subjects with Alzheimer's disease, Mild Cognitive Impairment, and healthy elderly controls from the Alzheimer's Disease Neuroimaging Initiative (ADNI). We used tensor-based morphometry to measure individual differences in brain structure at the voxel level relative to a study-specific template based on healthy elderly subjects. We then conducted a genome-wide association at each voxel to identify genetic variants of interest. By studying only the most associated variant at each voxel, we developed a novel method to address the multiple comparisons problem and computational burden associated with the unprecedented amount of data. No variant survived the strict significance criterion, but several genes worthy of further exploration were identified, including *CSMD2* and *CADPS2*. These genes have high relevance to brain structure. This is the first voxelwise genome wide association study to our knowledge, and offers a novel method to discover genetic influences on brain structure.

Stein et al NeuroImage 53 (2010) 1160–1174

INDIANA UNIVERSITY
SCHOOL OF MEDICINE

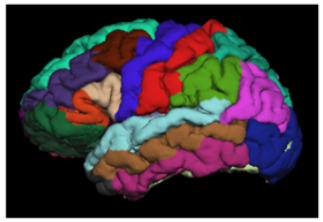
Methodological Overview

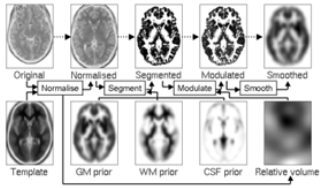
Indiana University Center for Neuroimaging CfN

Shen et al 2010 [Epub] ,NeuroImage

Baseline MRI Scans

FreeSurfer: 56 volume or cortical thickness measures





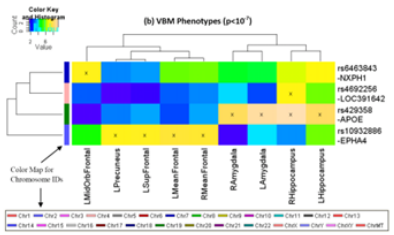
VBM: 86 GM density measures

142 QTs

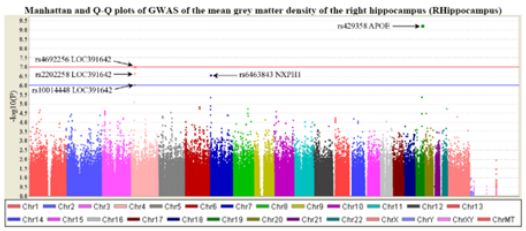
QC'ed genotyping data

530,992 SNPs

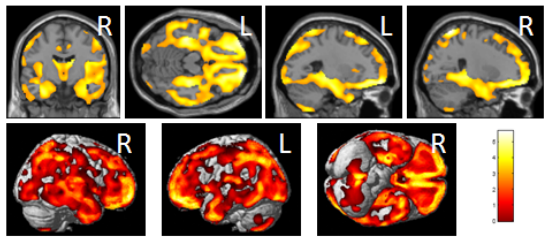
GWAS of Imaging Phenotypes



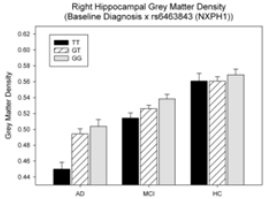
Strong associations represented by heat maps



GWAS of candidate QT

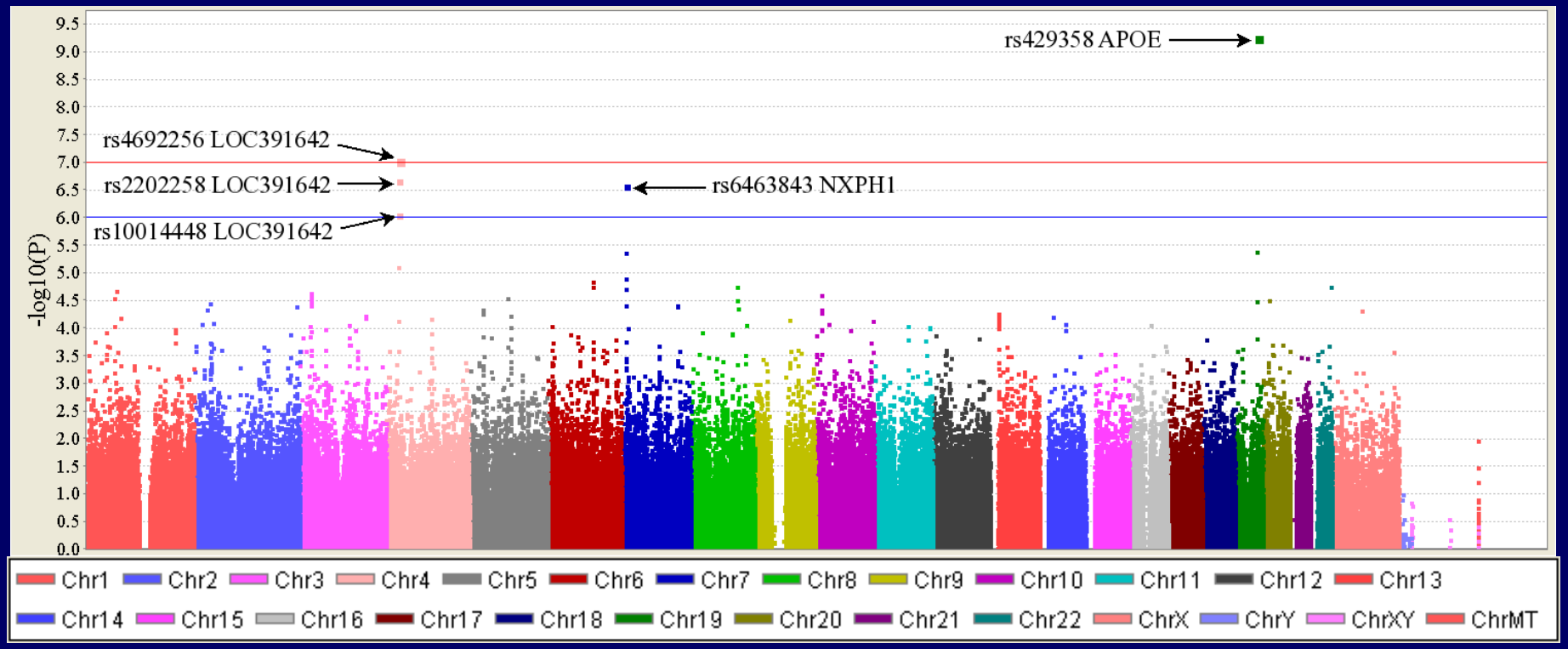


VBM of candidate SNP



Refined modeling of candidate association

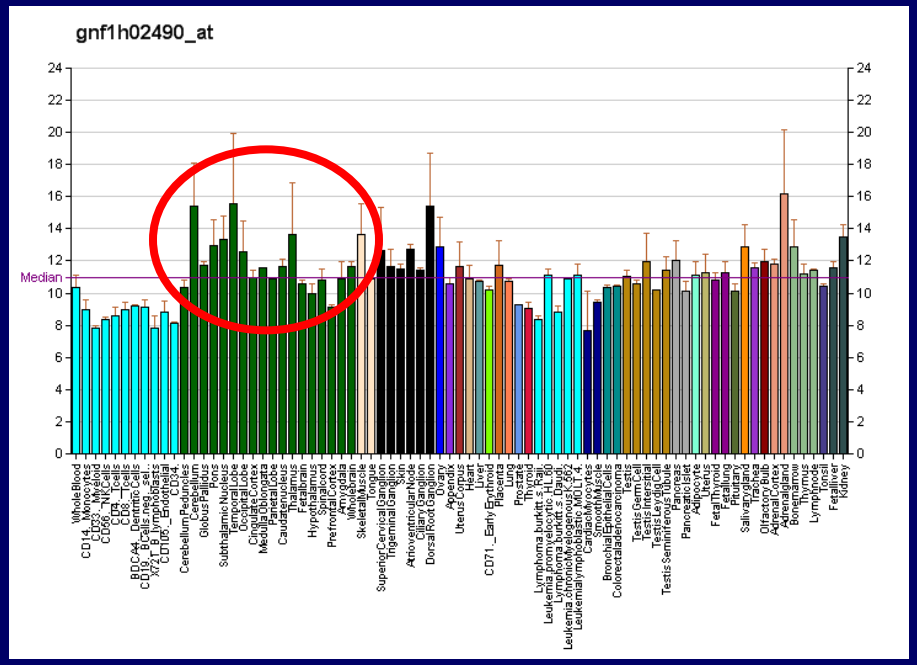
GWAS of Mean Grey Matter Density: Right Hippocampus (Manhattan Plot)



Shen et al 2010 NeuroImage

NXPH1: Novel Candidate Gene for AD that codes for neurexophilin-1 protein

Expressed in Brain

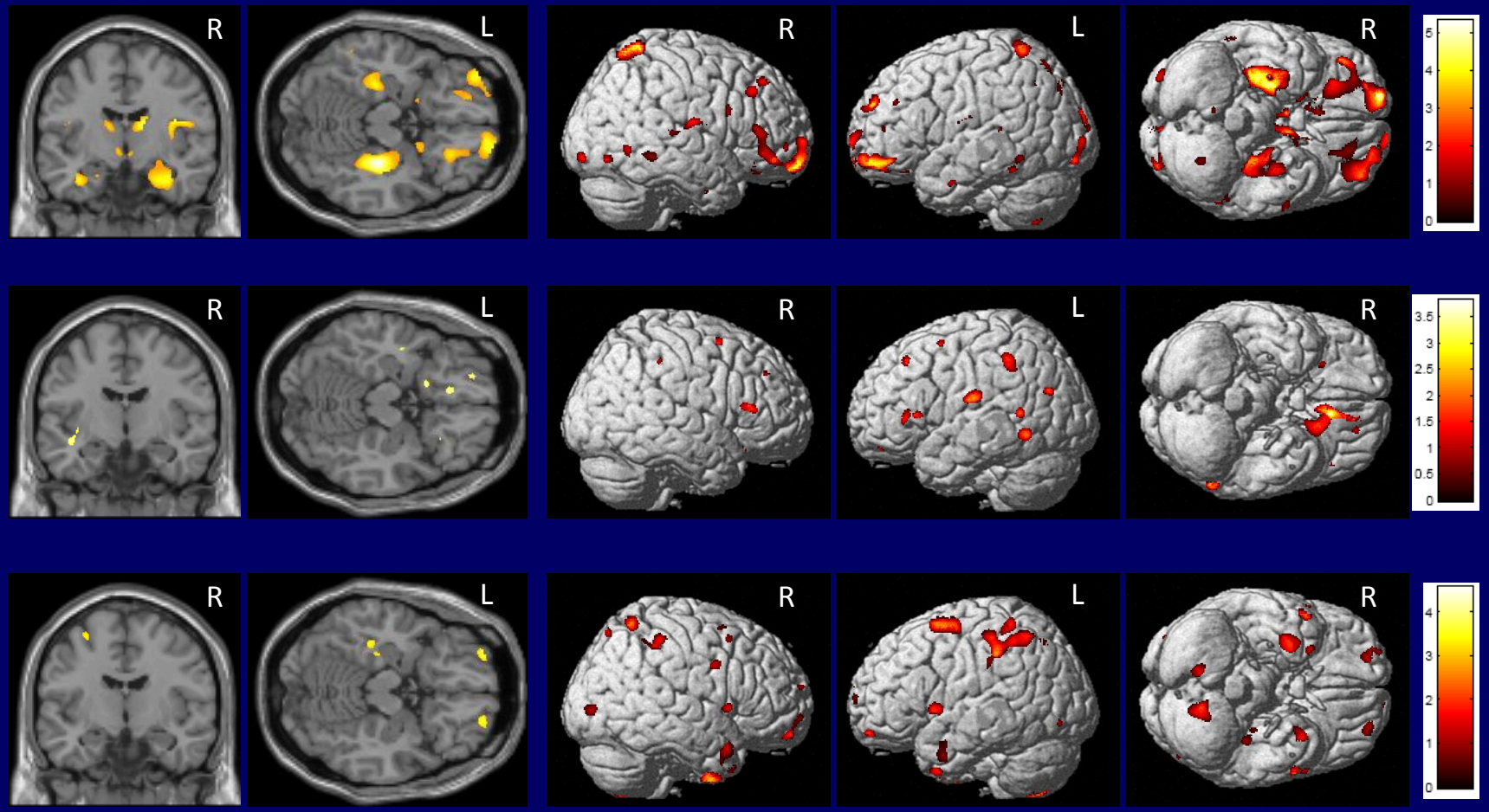


<u>Neuroexphilin-1's likely role in synaptogenesis</u>:

Protein forms a very tight complex with *alpha neurexins*, a group of proteins that promote adhesion between dendrites and axons (EntrezGene).

VBM Analysis of NXPH1

Baseline Diagnosis x SNP for rs6463843: GG > TT



n=715

166 AD (44 TT, 78 GT, 44 GG); 346 MCI (82 TT, 170 GT, 94 GG); 203 HC (35 TT, 105 GT, 63 GG)

p<0.001 (unc.), k=27

Covaried for age, gender, education, handedness and baseline ICV

Shen et al 2010 NeuroImage

vGWAS, ROIs and Candidate Genes (UCLA)

Voxelwise GWAS: Ran genome-wide association for a quarter of a million points across 700 subjects - new gene discovery method; many new SNPs; power calculations for replication (Stein et al, NeuroImage, 2010a)

GRIN2b, a common glutamate receptor genetic variant, is associated with greater temporal lobe atrophy and with AD; NMDA-receptor is a target for memantine therapy (Stein et al, NeuroImage, 2010b)

FTO, an obesity risk gene carried by 46% of Europeans, is associated with 10-15% frontal and occipital atrophy, and with a ~1.7kg weight gain, on average (April Ho et al, PNAS, 2010)

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[a] Laboratory of Neuro Imaging, Department of Neurology, UCLA School of Medicine, Neuroscience Research Building 225E635 Charles Young Drive, Los Angeles, CA 90095-1769, USA
[b] Resnick Neuropsychiatric Hospital at UCLA, Los Angeles, CA, USA
[c] Center for Neuroimaging, Department of Radiology, Indiana University School of Medicine, Indianapolis, IN, USA
[d] Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA
[e] The Translational Genomics Research Institute, Phoenix, AZ, USA
[f] Neuroscience, Molecular Medicine, Pfizer Global R and D, New London, CT, USA
[g] Department of Psychiatry and Human Behavior, University of California, Irvine, Irvine, CA, USA
[h] Departments of Radiology, Medicine and Psychiatry, UC San Francisco, San Francisco, CA, USA
[i] Department of Veterans Affairs Medical Center, San Francisco, CA, USA

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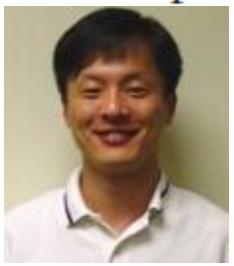
ABSTRACT

The structure of the human brain is highly heritable, and is thought to be influenced by many common genetic variants, many of which are currently unknown. Recent advances in neuroimaging and genetics have allowed collection of both highly detailed structural brain scans and genome-wide genotype information. This wealth of information presents a new opportunity to find the genes influencing brain structure. Here we explore the relation between 448,293 single nucleotide polymorphisms in each of 31,622 voxels of the entire brain across 740 elderly subjects (mean age ± s.d.: 75.52 ± 6.82 years; 438 male) including subjects with Alzheimer's disease, Mild Cognitive Impairment, and healthy elderly controls from the Alzheimer's Disease Neuroimaging Initiative (ADNI). We used tensor-based morphometry to measure individual differences in brain structure at the voxel level relative to a study-specific template based on healthy elderly subjects. We then conducted a genome-wide association at each voxel to identify genetic variants of interest. By studying only the most associated variant at each voxel, we developed a novel method to address the multiple comparisons problem and computational burden associated with the unprecedented amount of data. No variant survived the strict significance criterion, but several genes worthy of further exploration were identified, including *CSMD2* and *CADPS2*. These genes have high relevance to brain structure. This is the first voxelwise genome wide association study to our knowledge, and offers a novel method to discover genetic influences on brain structure.

NeuroImage 51 (2010) 542–554

Genome-wide association study of CSF biomarkers $A\beta_{1\text{-}42}$, t-tau, and p-tau_{181p} in the ADNI cohort



S. Kim, PhD*
S. Swaminathan, BTech*
L. Shen, PhD
S.L. Risacher, BS
K. Nho, PhD
T. Foroud, PhD
L.M. Shaw, PhD
J.Q. Trojanowski, MD, PhD
S.G. Potkin, MD
M.J. Huentelman, PhD
D.W. Craig, PhD
B.M. DeChairo, PhD
P.S. Aisen, MD
R.C. Petersen, MD
M.W. Weiner, MD
A.J. Saykin, PsyD
For the Alzheimer's Disease Neuroimaging Initiative

ABSTRACT

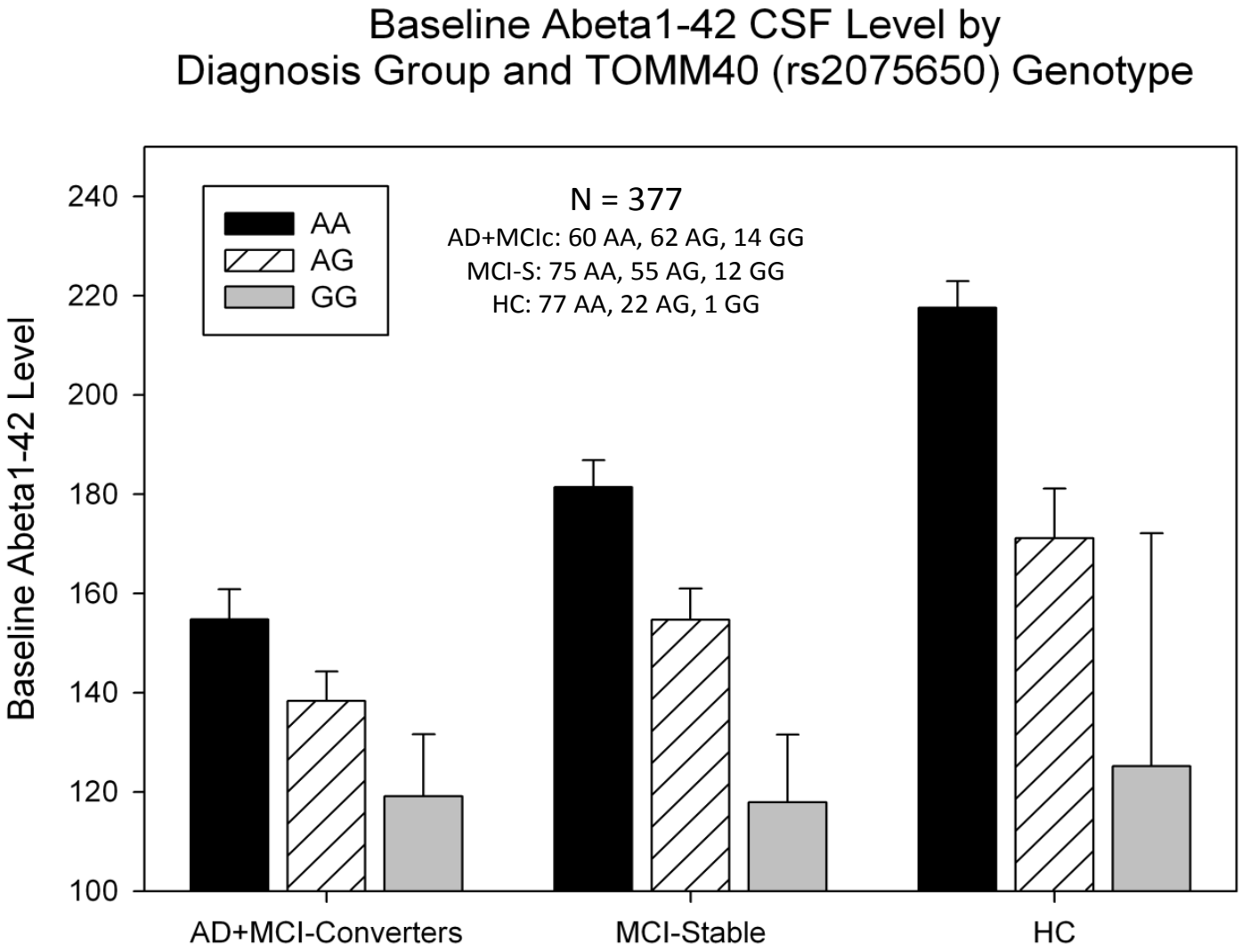
Objectives: CSF levels of $A\beta_{1\text{-}42}$, t-tau, and p-tau_{181p} are potential early diagnostic markers for probable Alzheimer disease (AD). The influence of genetic variation on these markers has been investigated for candidate genes but not on a genome-wide basis. We report a genome-wide association study (GWAS) of CSF biomarkers ($A\beta_{1\text{-}42}$, t-tau, p-tau_{181p}, p-tau_{181p}/$A\beta_{1\text{-}42}$, and t-tau/$A\beta_{1\text{-}42}$).

Methods: A total of 374 non-Hispanic Caucasian participants in the Alzheimer's Disease Neuroimaging Initiative cohort with quality-controlled CSF and genotype data were included in this analysis. The main effect of single nucleotide polymorphisms (SNPs) under an additive genetic model was assessed on each of 5 CSF biomarkers. *p* Values of all SNPs for each CSF biomarker were adjusted for multiple comparisons by the Bonferroni method. We focused on SNPs with corrected $p < 0.01$ (uncorrected $p < 3.10 \times 10^{-8}$) and secondarily examined SNPs with uncorrected *p* values less than 10^{-5} to identify potential candidates.

Results: Four SNPs in the regions of the *APOE*, *LOC100129500*, *TOMM40*, and *EPC2* genes reached genome-wide significance for associations with one or more CSF biomarkers. SNPs in *CCDC134*, *ABCG2*, *SREBF2*, and *NFATC4*, although not reaching genome-wide significance, were identified as potential candidates.

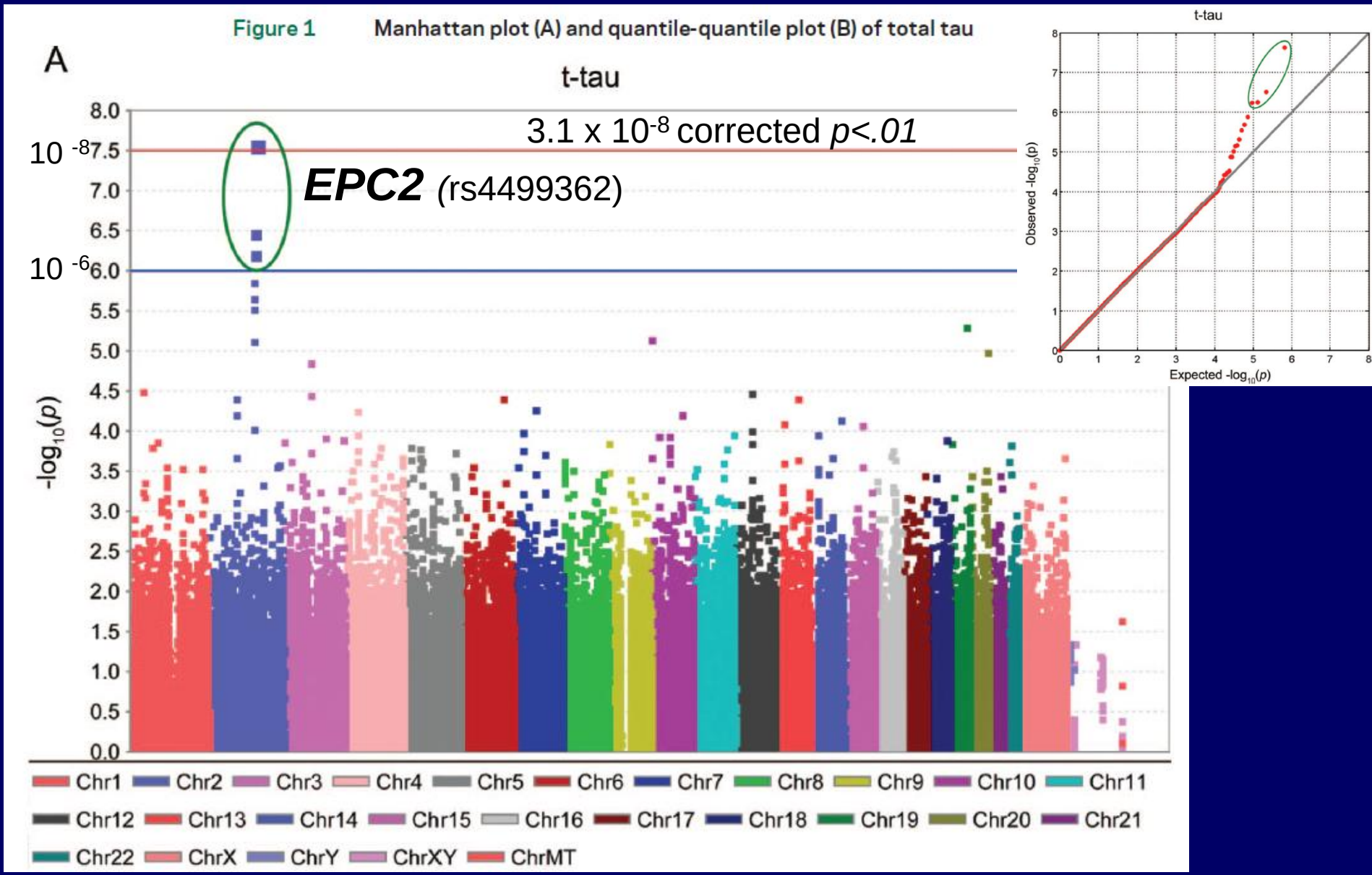
Conclusions: In addition to known candidate genes, *APOE* and *TOMM40* and one hypothetical gene *LOC100129500* partially overlapping *APOE*; one novel gene, *EPC2*; and several other interesting genes were associated with CSF biomarkers that are related to AD. These findings, especially the new *EPC2* results, require replication in independent cohorts. **Neurology® 2011;76:1–1**

CSF Biomarkers: Aβ1-42 & *TOMM40*



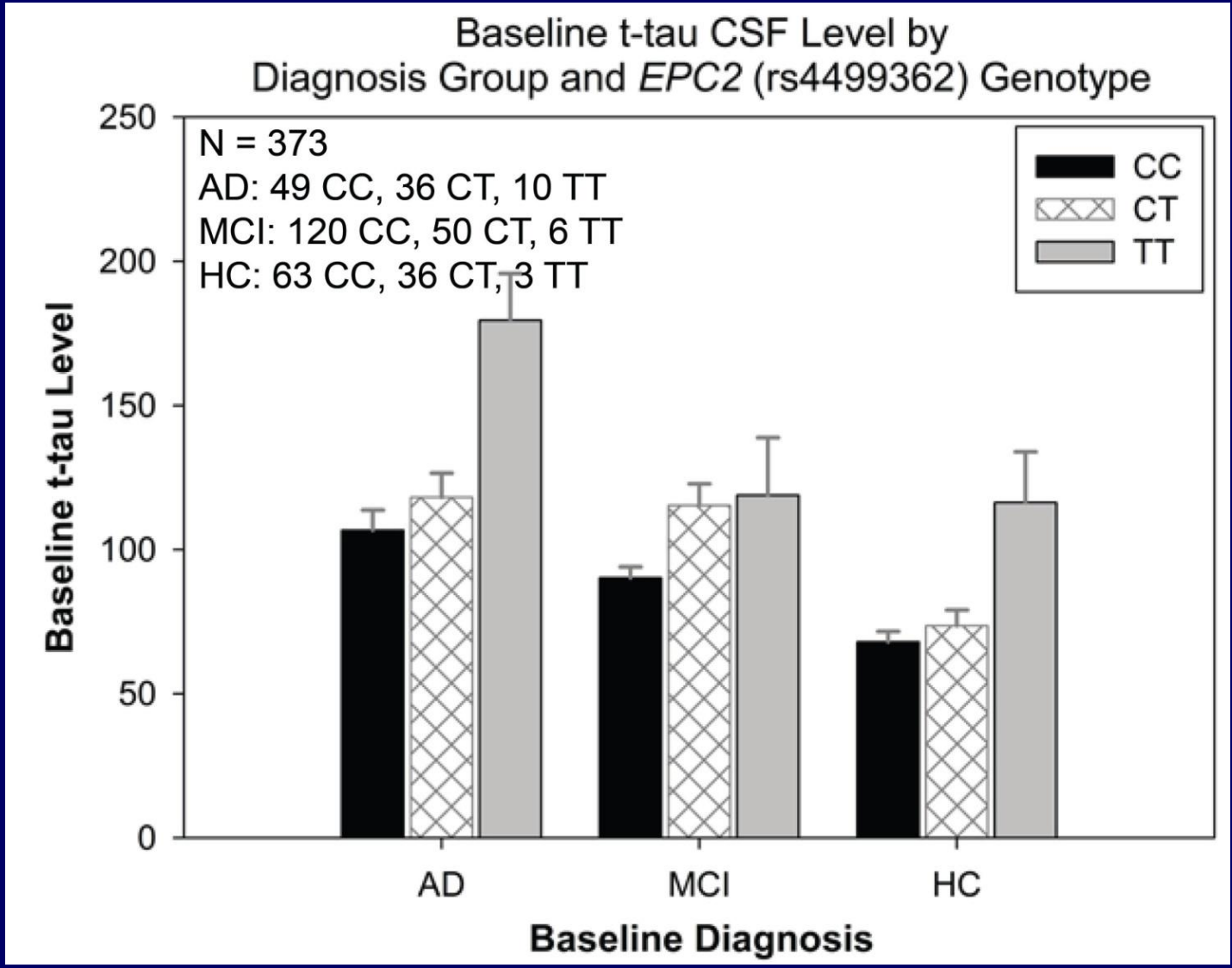
Kim, Swaminathan, et al Neurology (Jan 4 2011)

CSF Biomarkers: Total Tau



Kim, Swaminathan, et al Neurology (Jan 4 2011)

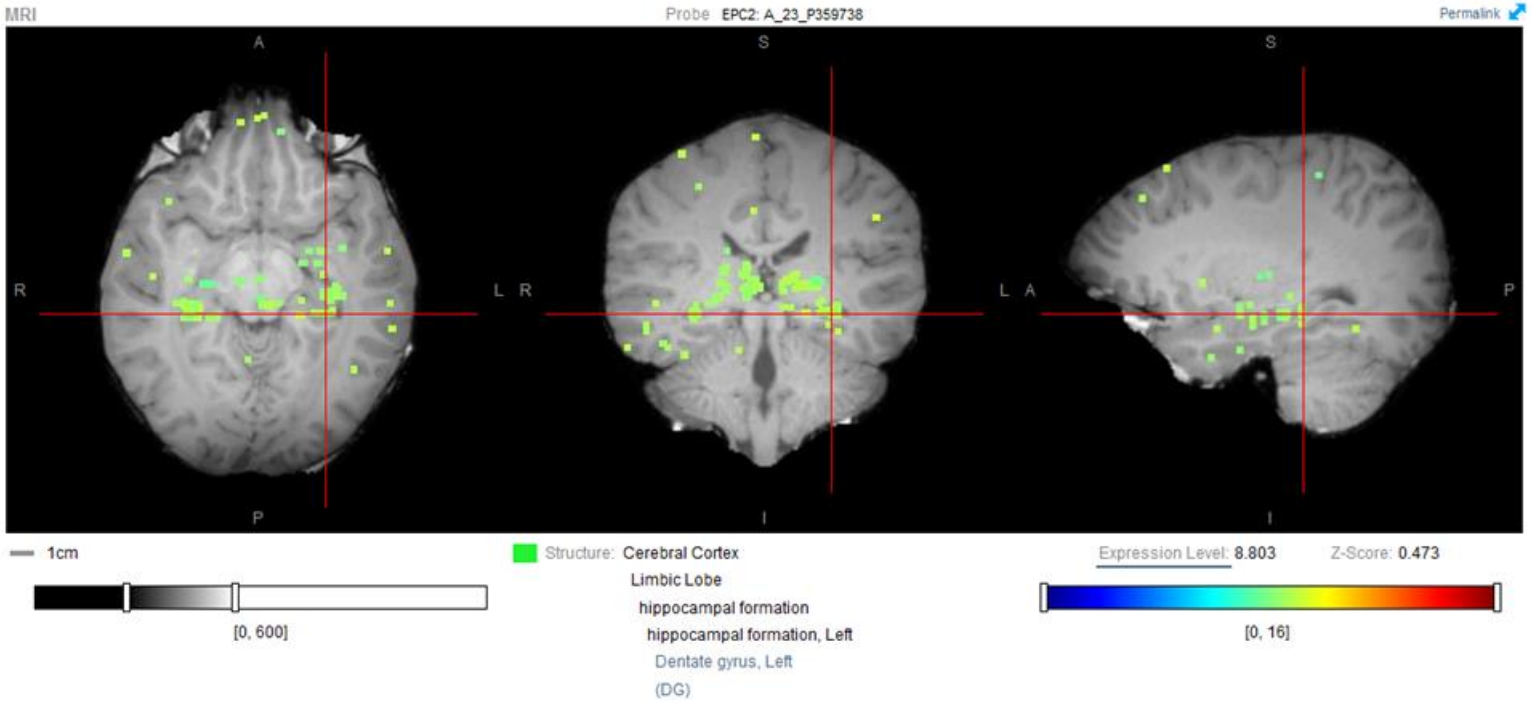
New finding from CSF t-tau GWAS: Enhancer of polycomb homolog 2 (*EPC2*)



Kim, Swaminathan, et al Neurology (Jan 4 2011)

Enhancer of polycomb homolog 2 (*EPC2*)

- Multiple SNPs were associated with t-tau at $p<10^{-6}$
- Involved in formation of heterochromatin (Doyon et al. 2004) & Microdeletion syndrome of 2q23.1 (mental retardation, short stature & epilepsy (van Bon et al. 2010)



Raw Expression Level of *EPC2* in Hippocampus

Allen Institute for Brain Science (http://human.brain-map.org/mri_viewer.html?probes=1045018,1045019)

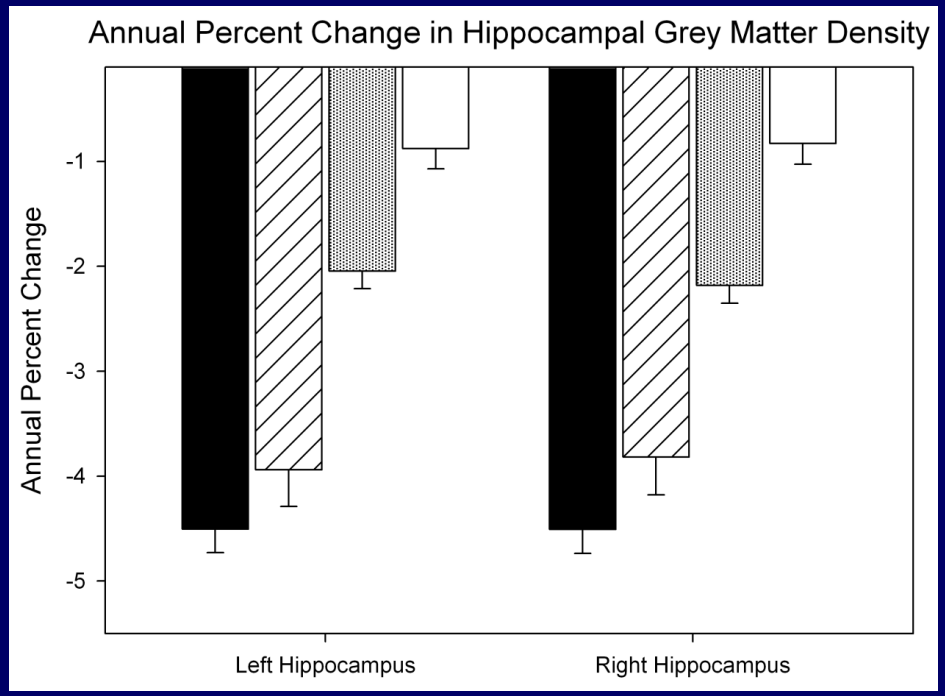
Genome Wide Association Study (GWAS) on Annual Percent Change of 1.5T MRI: Initial Data

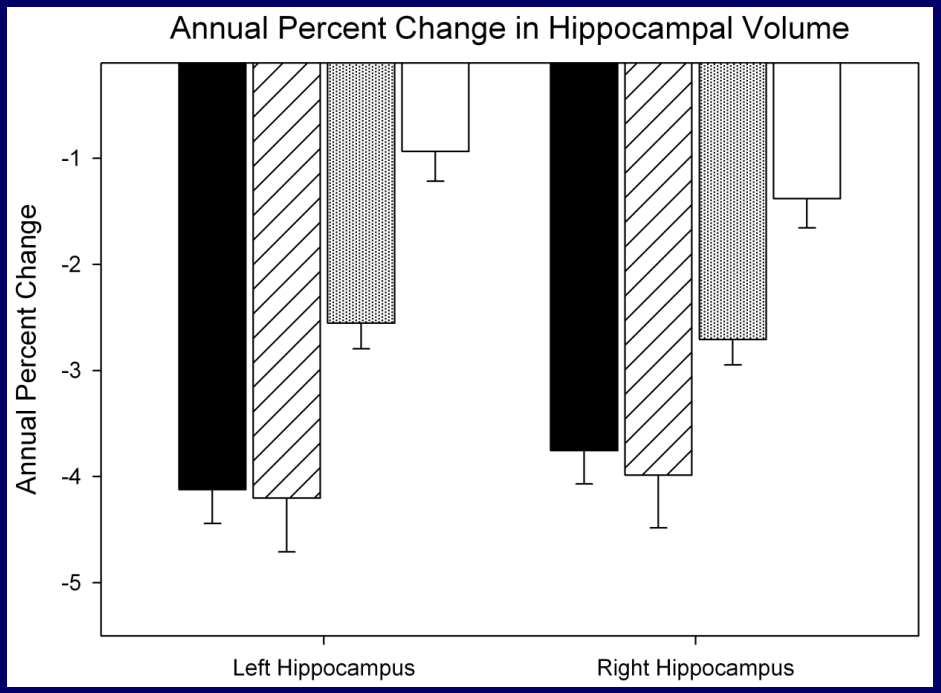
- 818 ADNI Subjects
  - 589 cases (MCI or AD), 229 controls
  - 476 males, 342 females
- 620901 +2 Markers
  - 620901 from Illumina 610 Quad array
  - 2 APOE SNPs
- Extensive QC protocol

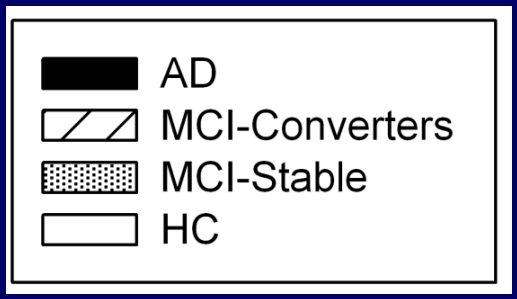
Saykin et al 2010 Alz Dementia

Annual Percent Change in Hippocampal Volume and Grey Matter Density (ADNI Cohort)

Covaried for baseline age, sex, education, handedness & ICV



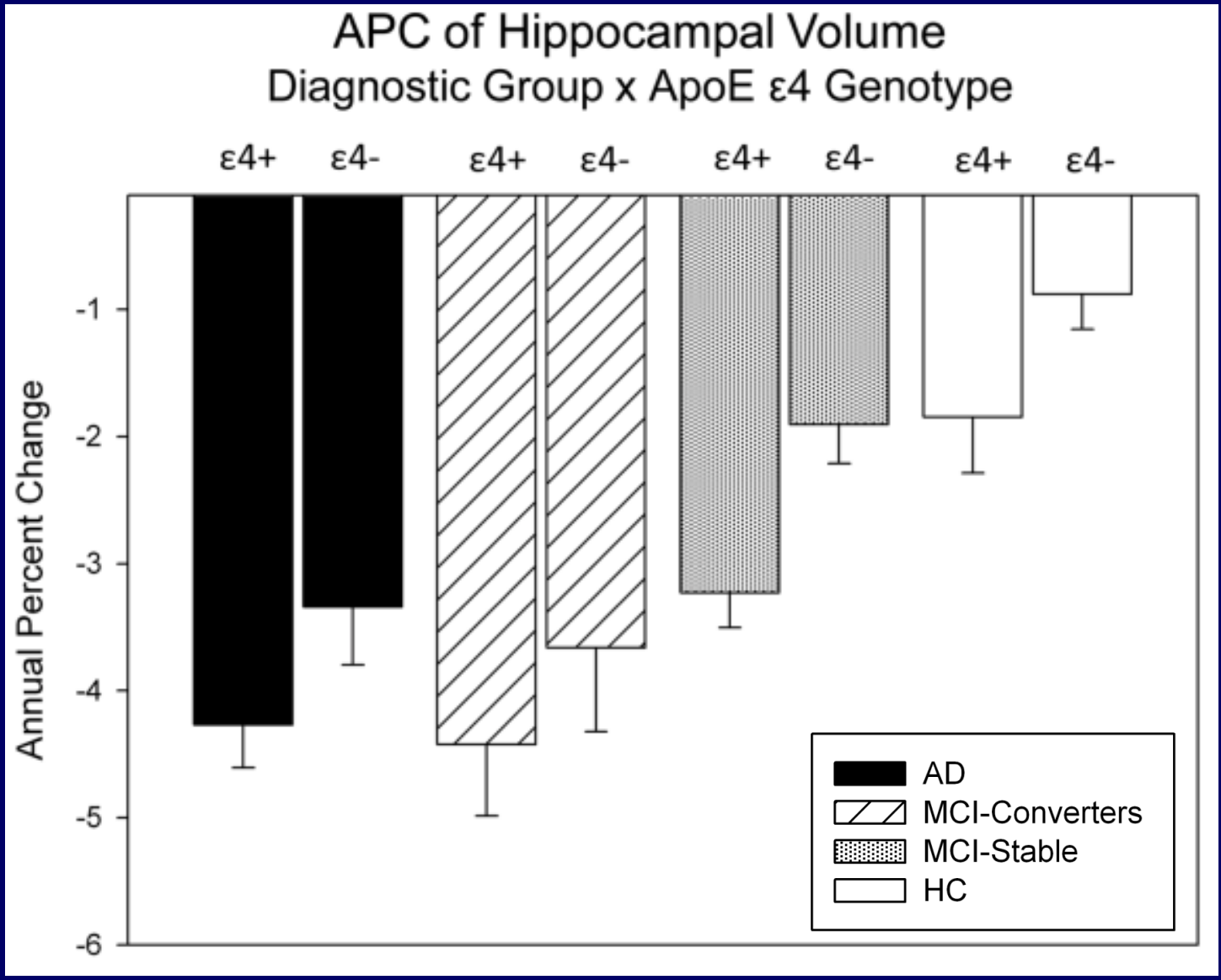




Risacher, Saykin et al
Neurobiol Aging (2010)

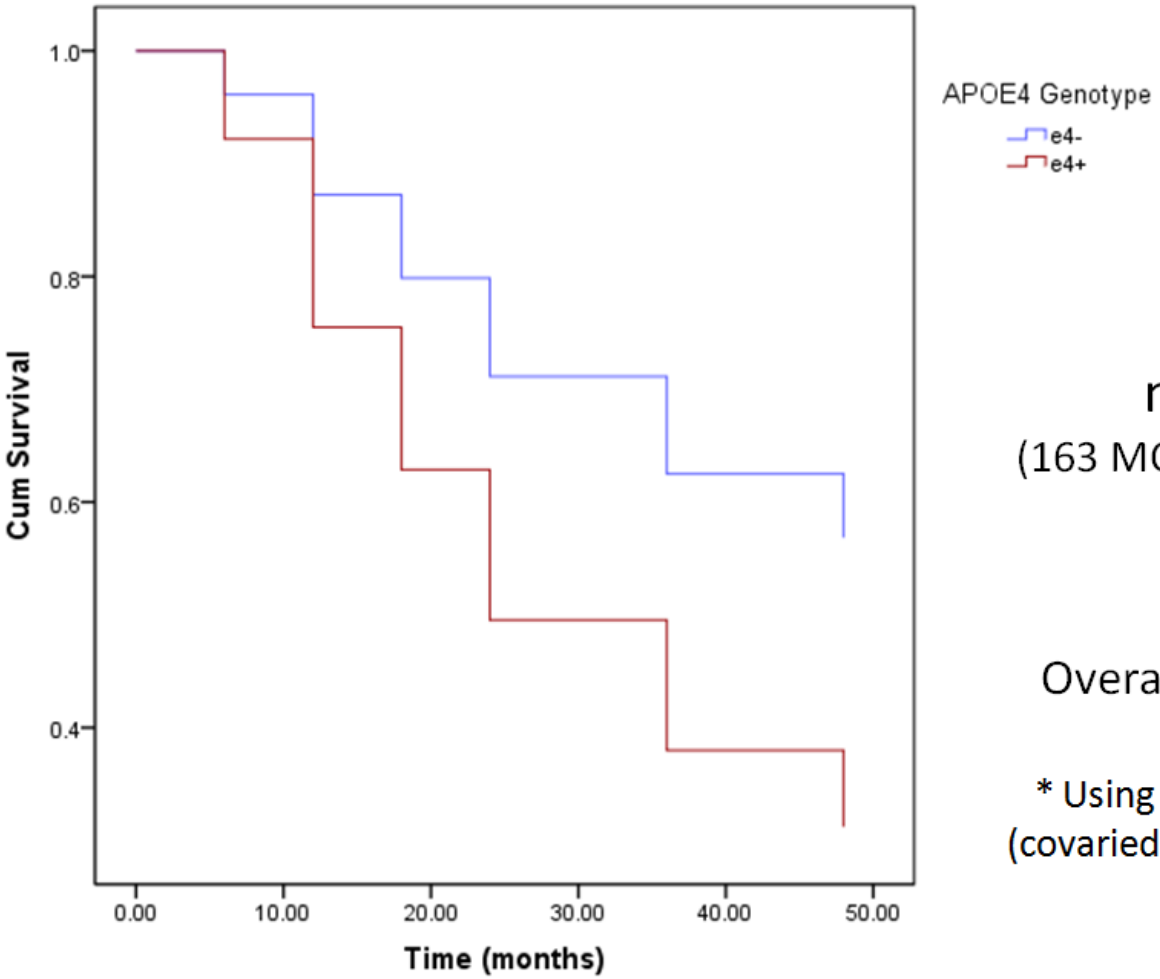
Rate of Change: Role of *APOE*

Main effect versus Interaction



Risacher et al Neurobiology of Aging (2010); 31:1401-1418

Survival Plot for MCI to AD Conversion by APOE Genotype (comparing ε4 negative to ε4 positive)



n = 363
(163 MCI-C, 200 MCI-S)

Overall: p < 0.001*

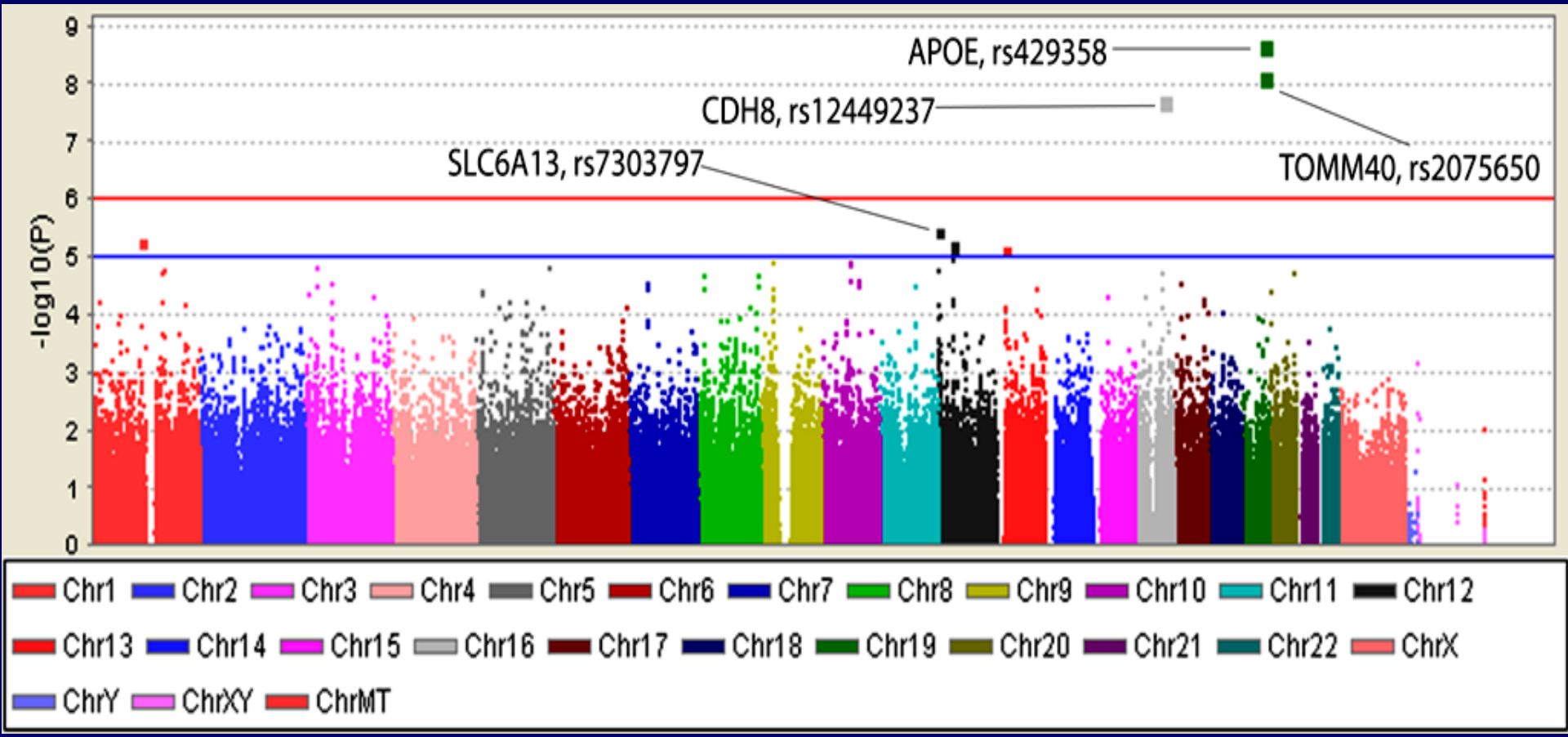
* Using Cox Regression
(covaried for baseline age)

163 MCI-C (50 ε4 negative, 113 ε4 positive)

200 MCI-S (112 ε4 negative, 88 ε4 positive)

Updated as of 3/29/11, S. Risacher

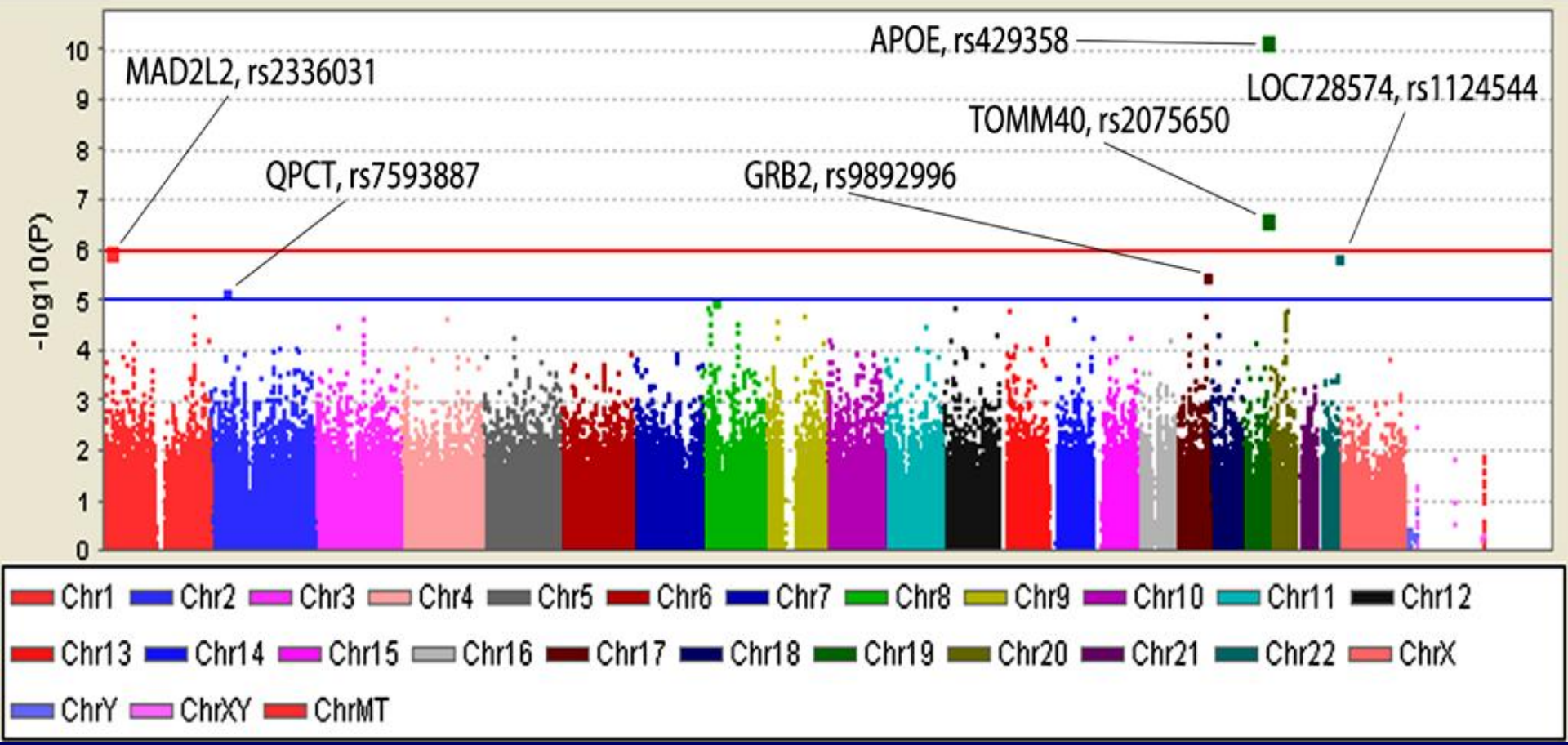
Adjusted Annual Percent Change in Hippocampal Volume



APOE (Chr 19): rs429358 is the epsilon 4 allele marker
TOMM40 (Chr 19): translocase of outer mitochondrial membrane 40 homolog (LD with APOE)
CADH8 (Chr 16): cadherin 8, type 2; synaptic adhesion, axonal growth/guidance (no data in AD)

Saykin et al Alzheimer's & Dementia (2010); 6:265–273

Adjusted Annual Percent Change in Hippocampal Gray Matter Density



APOE (Chr 19): rs429358 is the epsilon 4 allele marker / TOMM40 in LD with APOE
MAD2L2 (Chr 1) mitotic arrest deficient-like 2 (mitotic spindle assembly)
LOC728574 (Chr 22): similar to retinitis pigmentosa GTPase regulator isoform C

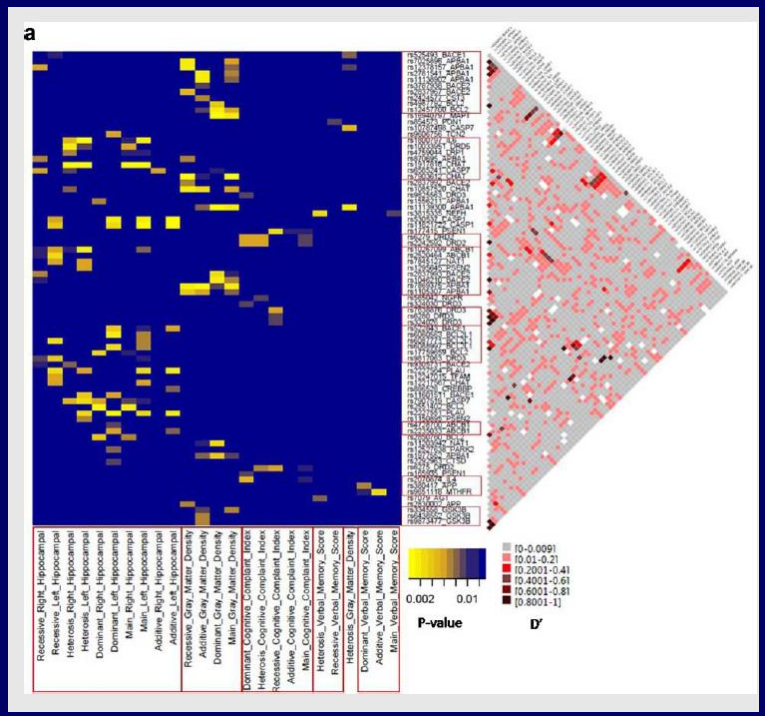
Saykin et al Alzheimer's & Dementia (2010); 6:265–273

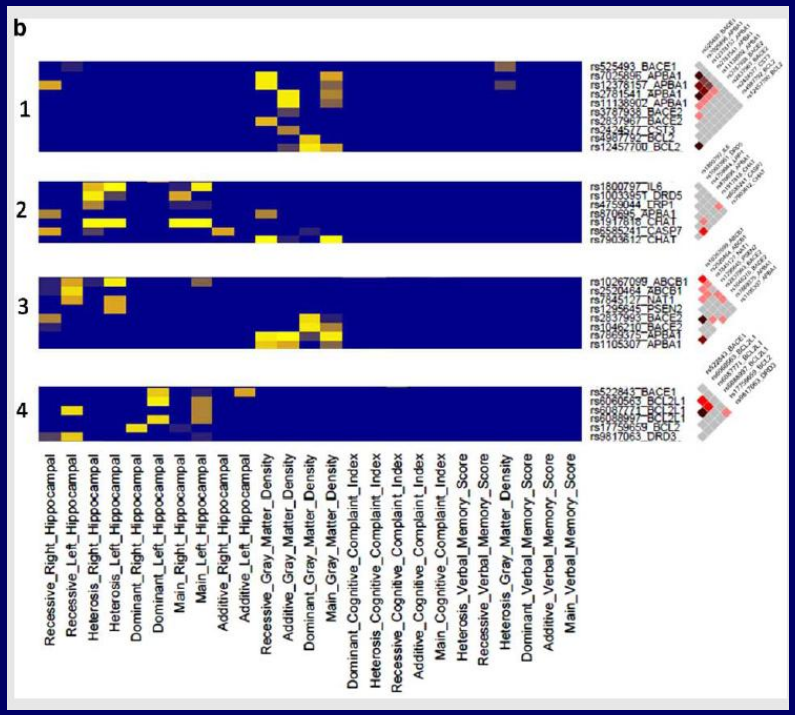
RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Genetic Pathway-Based Hierarchical Clustering Analysis of Older Adults With Cognitive Complaints and Amnestic Mild Cognitive Impairment Using Clinical and Neuroimaging Phenotypes

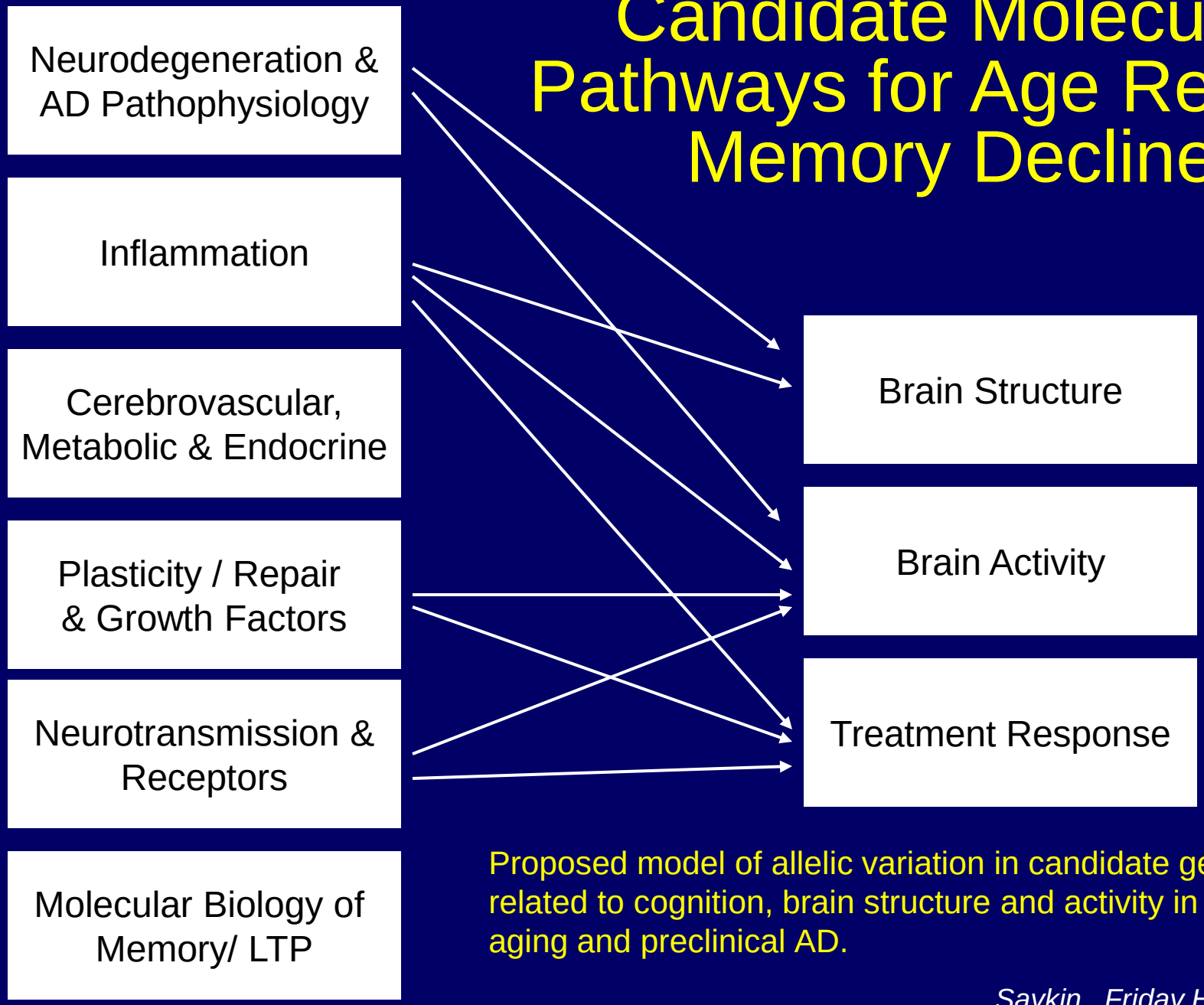
Chantel D. Sloan,[1] Li Shen,[2,3] John D. West,[2] Heather A. Wishart,[4] Laura A. Flashman,[4] Laura A. Rabin,[4] Robert B. Santulli,[4] Stephen J. Guerin,[4] C. Harker Rhodes,[5] Gregory J. Tsongalis,[5] Thomas W. McAllister,[4] Tim A. Ahles,[6] Stephen L. Lee,[7] Jason H. Moore,[1,8,9,10] and Andrew J. Saykin[2,4,11]*





Sloan et al 2010 [Epub], Am J Med Genet, DOI 10.1002/ajmg.b.31078

Candidate Molecular Pathways for Age Related Memory Decline



Proposed model of allelic variation in candidate genes related to cognition, brain structure and activity in healthy aging and preclinical AD.

Saykin , Friday Harbor 2011

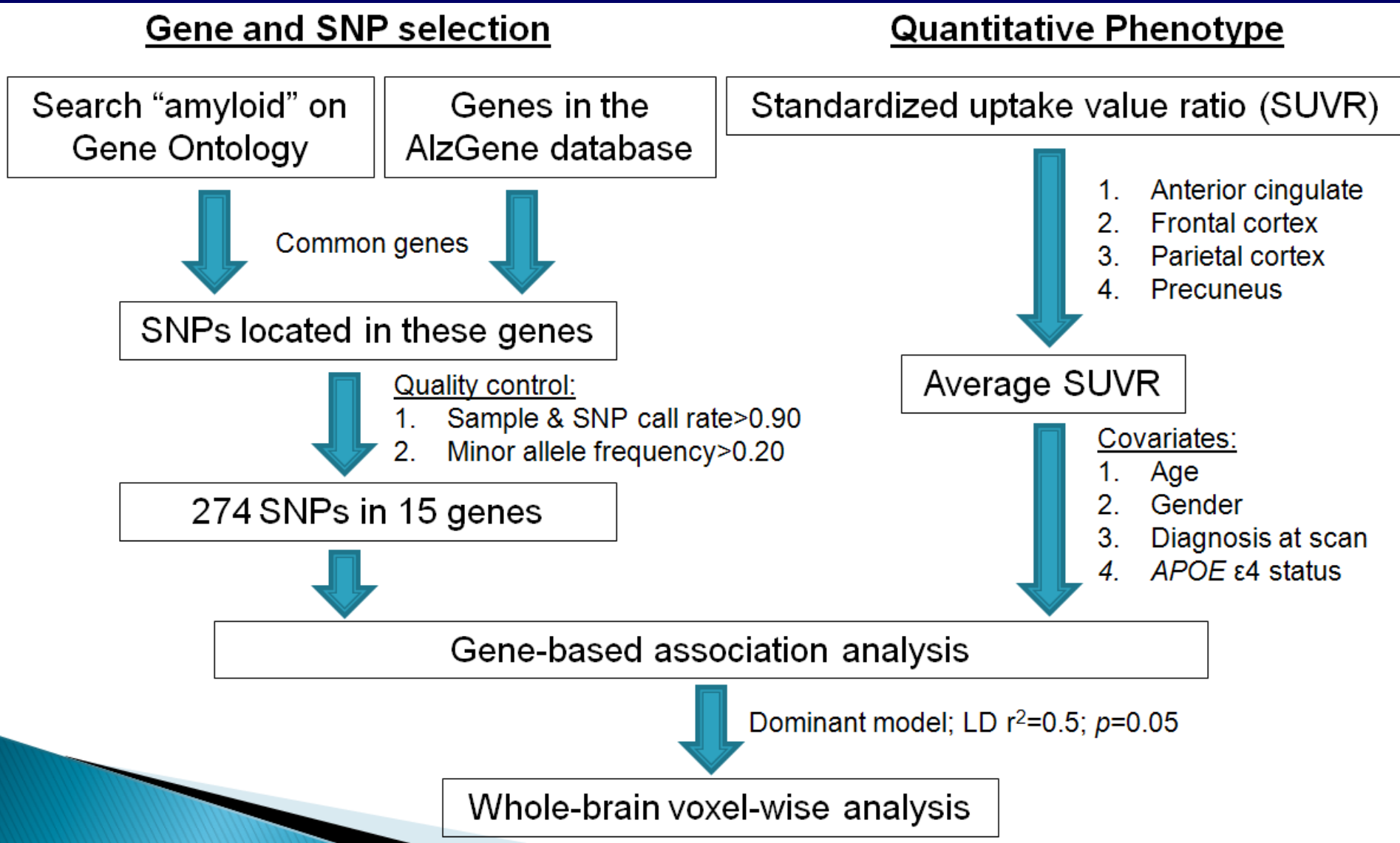
Cluster Results: Role of Genes

TABLE II. The Hypothesized Role of Each Gene Found in One of the Three Clusters on AD and MCI Based on a Literature Search With Sample References

Gene (alias)	Suggested role in AD/MCI
ABCB1	Regulates beta-amyloid levels [Lam et al., 2001; Kuhnke et al., 2007]
APBA1 (MINT1, X11)	Binds APP and affects cleavage and translocation [Miller et al., 2006; Ho et al., 2008; Saito et al., 2008] (also known as MINT1, X11)
BACE1	Cleaves APP [Haass, 2004; McConlogue et al., 2007; Willem et al., 2009]
BACE2	Cleaves APP, a BACE1 homolog [Stockley and O'Neill, 2007], increases IL-1R2 secretion [Kuhn et al., 2007]
BCL2	Induces apoptosis [Lu et al., 2005]
BCL2L1 (BCL-X)	Anti-apoptotic signaling [Lukiw and Bazan, 2006; Shimohama, 2009]
CASP7	Apoptosis regulator, neuron loss in AD [Pompl et al., 2003; Matsui et al., 2006]
CHAT	Synthesizes acetylcholine, which is depleted in AD [Burgess et al., 2009], ChAT fibers increasingly immunoreactive in AD, and MCI [Cuello et al., 2007]
CST3	Studies show mixed results, colocalizes with beta-amyloid [Lin et al., 2003; Monastero et al., 2005; Nacmias et al., 2006]
DRD3	Associated with depression symptoms that co-occur with AD associated [Serretti et al., 2007]
DRD5	Connection to AD uncertain, normally functions as dopaminergic receptor [Cosentino et al., 2009]
IL6	Inflammatory response, tau phosphorylation [Papassotiropoulos et al., 2001; Quintanilla et al., 2004]
LRP1	Involved in APP processing and trafficking [Waldron et al., 2008; Yamada et al., 2008]
NAT1	Folate metabolism [Johnson et al., 2004]
PSEN2	Gamma-secretase complex formation with PSEN1, well-established AD susceptibility gene [Bertram and Tanzi, 2008; Bertram, 2009; Marcon et al., 2009]

Sloan et al 2010 [Epub], Am J Med Genet, DOI 10.1002/ajmg.b.31078

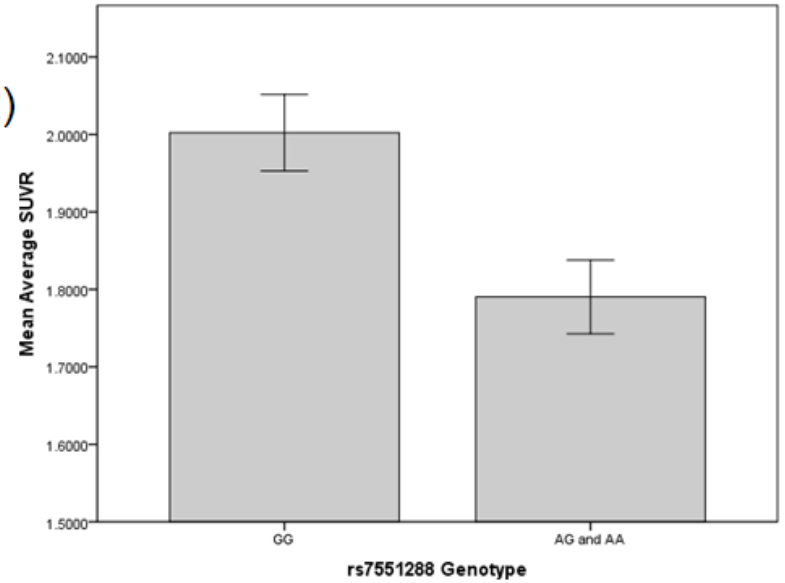
Amyloid Pathway PET Study: [11C]PiB



Swaminathan et al, ASHG, Wash DC, Nov 2010 & submitted

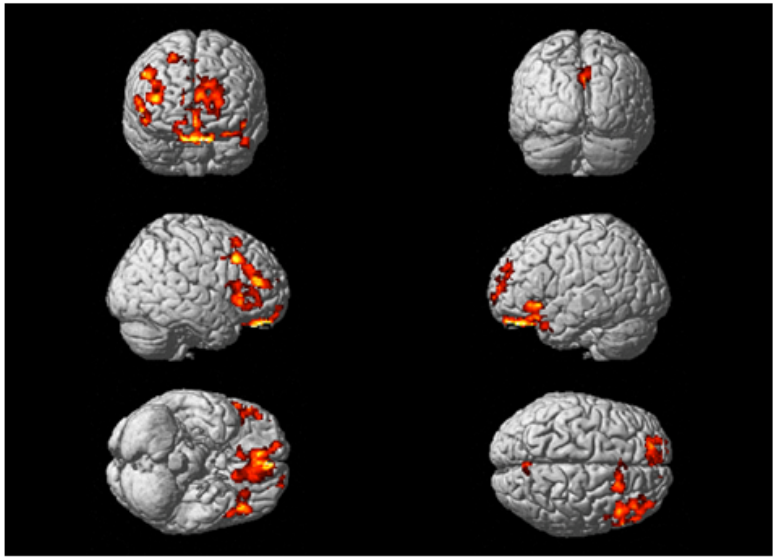
Amyloid Gene Pathway-PiB: Preliminary Results

- Gene-based association analysis:
  - *DHCR24* significantly associated with AVG (p=0.012)
  - One SNP (rs7551288) in gene found to be significant
  - Dominant effect of minor allele



- Whole-brain voxel-wise-analysis:
  - Increased PiB uptake in frontal regions
  - Frontal cortex known to have increased PiB uptake in AD patients

- *DHCR24* gene:
  - 24-dehydrocholesterol reductase - enzyme that synthesizes cholesterol from desmosterol (Peri et al. *J Mol Endocrinol*, 2008)
  - AKA: *seladin-1* or *selective AD indicator-1*
  - Reduced expression in temporal cortex in AD
  - Neuroprotective role
    - Confers resistance against Aβ and oxidative stress-induced apoptosis
    - Possible mediator of neuroprotective effects of estrogens/SERMs.



GG vs. AG & AA (unc. p<0.005, k=200)

Swaminathan et al, ASHG, Wash DC, Nov 2010 & submitted

ADNI Genetics: Next Steps

- ADNI-GO/2
  - Ongoing DNA, RNA, cell line sample collection
  - Planning for genotyping of new samples
- ADNI-1 data analysis
  - Baseline and rate of change
  - Copy number variation
  - Candidate genes & pathways, GWAS approaches
  - Associations with PET & CSF/plasma biomarkers
  - Collaborative projects, replication, other cohorts
- Future:
  - Targeted DNA and RNA resequencing – identify key regions for intensive scrutiny
  - Epistasis, Transcriptomics/expression, microRNA
  - Epigenomics (DNA methylation, etc)

ADNI Genotyping Working Group

Indiana University

- Imaging Genomics Lab
  - Andrew J. Saykin
  - Li Shen
  - Mark Inlow
  - Sungeun Kim
  - Kwangsik Nho
  - Shannon Risacher
  - Shanker Swaminathan
- NCRAD & Genetics
  - Tatiana M. Foroud
  - Kelley M. Faber
  - Nathan Pankratz
- Pfizer Genetics
  - Bryan DeChairo
  - Elyse Katz

- TGen
  - Eric Reiman
  - David W. Craig
  - Matt J. Huentelman

- UC Irvine:
  - Steven G. Potkin
  - Guia Guffanti
  - Anita Lakatos

- Core Consultants (ADNI-2):
  - Lars Bertram (Max Planck)
  - Lindsay Farrer (BU)
  - Robert Green (BU)
  - Jason Moore (Dartmouth)
  - Paul Thompson (UCLA)

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- Alzheimer's Association
- Indiana Economic Development Corporation (IEDC)

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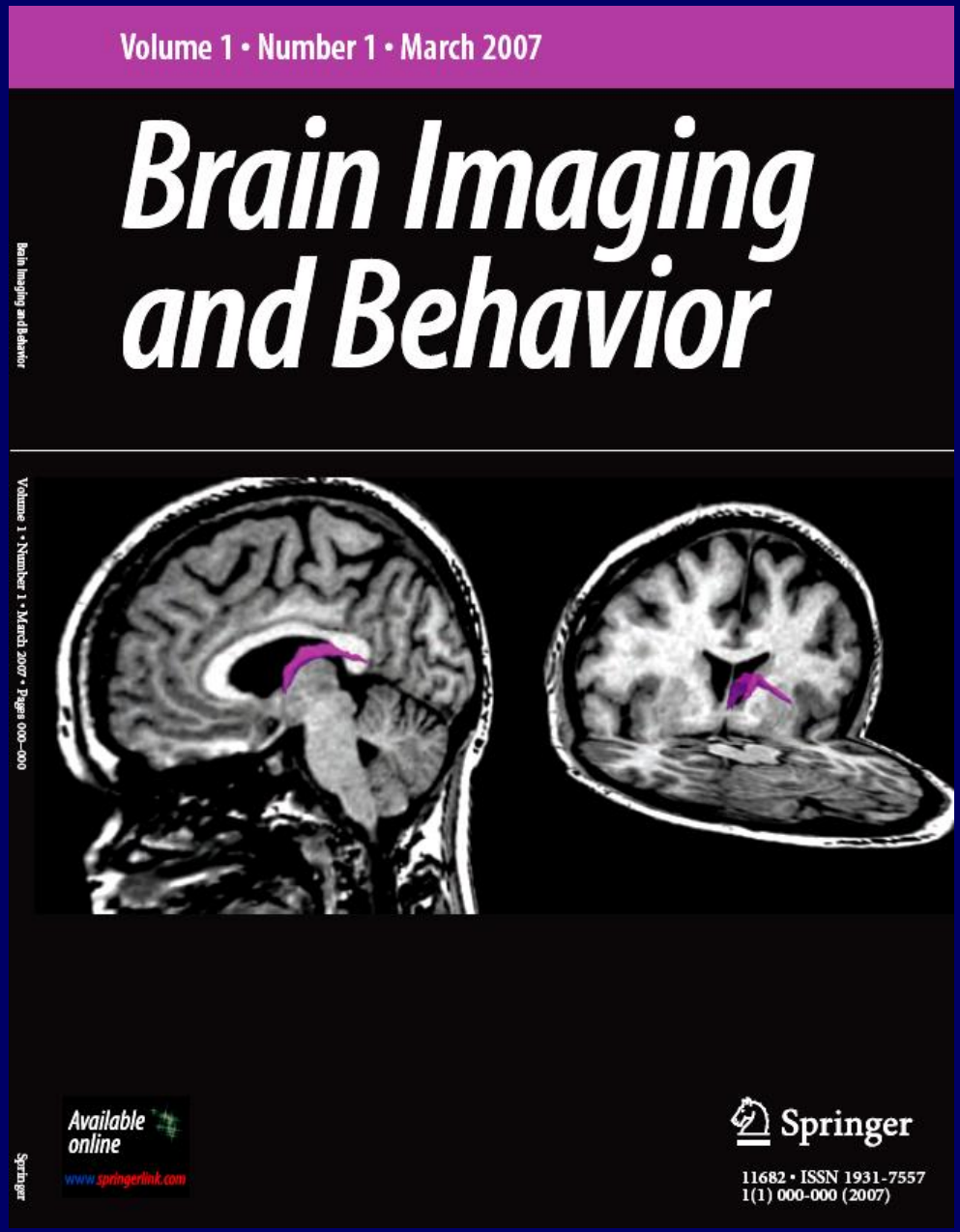
Special Issue including Friday Harbor papers on ADNI-related neuroimaging, cognition, biomarker & genetics studies



URL: www.springer.com/journal/11682