Common Genetic Variation Underlying Alzheimer’s Disease and Related MRI and Cognitive Endophenotypes

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Investigator, The Framingham Heart Study
2008
Alzheimer’s Disease
Facts and Figures

10 million U.S. baby boomers will develop Alzheimer’s disease
One in 5 women age 65 will develop AD in her lifetime.

Genetics of AD

• AD is heritable

• Identifying genes helps uncover biology

• Agnostic approaches vs. Candidate Genes

• Endophenotypes: Opportunities and Pitfalls

APP  PSEN1  PSEN2  APOE
Candidate Gene Approach

- **Con:** Limited by our current understanding of biology
- **Pro:** Fewer comparisons, greater power

Candidate gene pathways

??

Amyloid synthesis and removal, metalloproteins

tau phosphorylation, vacuolar sorting proteins

glucose and insulin metabolism, adipokines

nitrous oxide synthesis, oxidative stress, inflammation and lipid pathways

renin-angiotensin, thrombosis and hemostasis endothelial function

Neurotrophic factors
CARe: The NHLBI’s Candidate Gene Association Resource

http://www.broad.mit.edu/gen_analysis/care/index.php/Main_Page
CARe Genotyping Plan

Phase II (Illumina iSelect---IBC Chip)

~49,000 SNPs covering ~2100 candidate genes
typed on ~50,000 persons from all CARe Cohorts
Human genetic variation and its contribution to complex traits

Kelly A. Frazer, Sarah S. Murray, Nicholas J. Schork and Eric J. Topol

3 billion base pairs; 3 million SNPs

Arrays genotype 300,000 to 1 M

We can impute 80-90% of SNPs since we know linkage disequilibrium patterns
Figure 2 | **Insights into the genetic basis of type 2 diabetes (T2D).** Genome-wide association (GWA) studies have identified 18 genomic intervals that confer increased risk to T2D in Caucasians\(^58,59,72-75,123-127\). Four of these contain previously known candidate genes, based on the involvement of rare mutations in monogenic forms of diabetes. However, the remaining 14 intervals contain genes that were previously unsuspected in playing a part in the genetic
Genetics of AD

- AD is heritable

- Identifying genes helps uncover biology

- Agnostic approaches vs. Candidate Genes

- Endophenotypes: Opportunities and Pitfalls
What is an Endophenotype?

- Endophenotypes (or intermediate phenotypes) are heritable traits that reflect the actions of genes predisposing an individual to a disorder.

- Predict risk of incident disease.
- Manifest years before clinical & pathological diagnostic criteria are met.
Problems

• Genetic heterogeneity
• Overwhelming effect of APOE ε4 locus but parental AD impacts cognition in APOE ε4 +ve offspring
• Gene-gene and gene-environment interactions

• Late onset of clinical disease
  – competing risk of mortality
• Selection and survival biases
Solutions

- Genetic heterogeneity
  - Careful phenotypic definition
- Gene-APOE $\varepsilon 4$ interactions
  - Stratified analyses
- Competing risk of mortality
- Gene-environment interactions and selection/survival biases
  - Cohort studies of incident disease in morbid environment
- Collaborative efforts to increase numbers

So we study endophenotypes!

In community-based cohorts
Conceptual Model for Pathways from Genes to Dementia

Risk Factors
Genetic susceptibility

Brain Morphology

Cognitive Function

Clinical MCI & Dementia
Neuropathological AD: Prevalence of Clinical Dementia

Snowdon DA et al., JAMA 1997;277:813-7

Adjusted OR for clinical dementia: 1, 6.7, 20.7
Multiple Axes of Brain Aging Interact

- AD related pathology
  - Amnestic MCI
  - Clinical Dementia/AD

- Vascular pathology
  - Silent infarcts, microhemorrhages, TIA, vMCI
  - Stroke

Pathology

Subclinical Disease

Clinical Disease
Vascular disease may accelerate the pathological processes in AD

- Ischemia induces PS1 and increases APP expression in mouse models of AD

Sadowski et al., Neurochem Res 2004;29:1257-66
AD Endophenotypes

• Two types:
  – Risk Marker (whether & when clinical AD develops)
  – Disease Severity Marker

• Quantitative or Qualitative traits
• With moderate to high heritability
• Reflect pathology
Risk Marker (True) Endophenotypes

- Cognitive Measures
- Volumetric MRI measures
- PET measures of amyloid burden, regional flow
- Functional MRI
- CSF biomarkers
- Circulating and cell expression markers
Quantitative Traits as Disease Severity Markers

- Cognitive Performance
- Neurological Exam Findings
- MRI measures
- Pathological Scores
- Treatment Response

- Disease only sample is adequate

- Pharmacogenomics: Personalized Prevention & Therapy
Framingham Heart Study
Stroke, Dementia and MRI/NP Studies

Original cohort (Gen 1)
Exam 14 (1975) (N=2,842; mean age 67)
Exam 26: 313 Gen 1 survivors MR and NP

Offspring cohort (Gen 2)
Exam 7 (1999-02) MRI & NP (N=2442; mean age 62)
Brain Bank: 1995 (N=700,130 brains)
Exam 8 (2005-08) Repeat: MRI & NP (N=1800; mean age 67)
Framingham Heart Study
Longitudinal Community-Based Family Study

Gen 1 Original cohort

1948 → → → → → → → → → → → → → → → → → → 2008

Gen 2 Offspring cohort

1971 → → → → → → → → → → → → → → → → → → 2008

Gen 3 cohort

2002 → → → 2008

Exam 2 (2009-11) MRI NP & Biomarkers
1076 stroke- and dementia-free subjects underwent neuropsychological testing in 1975. Odds-ratio of developing AD (on 22 year follow-up) increased 31-57% with each SD decrement in baseline performance, after adjusting for age, sex, education, occupation.
Brain Volumes are Endophenotypes

TCBV, HV are associated with verbal memory performance and subsequent risk of AD

**Figure 5. Risk genes and brain structure.** Typical MRI scans are shown from healthy elderly subjects with zero, one, and two $\varepsilon 4$ alleles of the ApoE gene, which confers increased risk for late-onset Alzheimer's disease (data courtesy of Gary Small MD, UCLA Center on Aging). The $\varepsilon 3$ allele is more prevalent, and considered normal. Patients at genetic risk may display metabolic and structural deficits before overt symptoms appear, suggesting that genetic and imaging information may identify candidates for early treatment in dementia (100). Note the hippocampal atrophy (H) and ventricular enlargement (V) in those at risk.
Tracking atrophy progression in familial Alzheimer’s disease: a serial MRI study

Basil H Ridha, Josephine Barnes, Jonathan W Bartlett, Alison Godbolt, Tracey Pepple, Martin N Rossor, Nick CFox

Figure 1. Adjusted total hippocampal volume measurements of mutation carriers (relative to time of clinical diagnosis of AD) and controls (relative to the date of their last scan).

The y-axis scale is logarithmic. AD = Alzheimer’s disease.
Genetic Epidemiology

- Epidemiological correlates of genetic variation: show an association of variant with
  - Brain MRI and Cognitive test of AD risk (intermediate or endophenotypes)
  - Mild Cognitive Impairment (MCI)
  - All dementia
  - Alzheimer’s Disease (AD)
SORL1: New kid on the AD Block

• Strongest phenotype-SNP association
  – On FBAT: SORL1 (rs1131497; p=3.2 x 10^{-6}) and abstract reasoning
  – On GEE: CDH4 (rs1970546; p=3.7 x 10^{-8}) and brain volume
  – In top 25 by GEE and FBAT

• ERBB4, PDLIM5, RFX4 (schizophrenia genes)
  • VIPR2, CTNNB1 (developmental genes)

Rogaeva et al., Nature Genetics, February 2007; pp168-177


This article is available from: http://www.biomedcentral.com/1471-2350/8/S1/S15
Study Sample

• 71,000 SNPs related to
• MRI/Cognitive endophenotypes

• in 705 stroke- and dementia-free FHS Gen 1 and 2 participants
  – Age 62±9 yrs
  – 50% men
### Volumetric Brain MRI

<table>
<thead>
<tr>
<th>Brain Volumes</th>
<th>Total Cerebral (TCBV)</th>
<th>Adjusted for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal (FBV)</td>
<td></td>
<td>Age</td>
</tr>
<tr>
<td>Parietal (PBV)</td>
<td></td>
<td>Current smoking</td>
</tr>
<tr>
<td>Occipital (OBV)</td>
<td></td>
<td>Diabetes</td>
</tr>
<tr>
<td>Temporal (TBV)</td>
<td></td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>Hippocampal (HPV)</td>
<td></td>
<td>Anti-hypertensive drugs</td>
</tr>
<tr>
<td>Ventricular Volumes</td>
<td></td>
<td>Atrial fibrillation</td>
</tr>
<tr>
<td>Lateral (LVV)</td>
<td></td>
<td>EKG-LVH</td>
</tr>
<tr>
<td>Temporal Horn (THV)</td>
<td></td>
<td>Homocysteine</td>
</tr>
<tr>
<td>White Matter Hyperintensity Volume (WMHV)</td>
<td></td>
<td>Homocysteine</td>
</tr>
</tbody>
</table>

All volumes were expressed as a ratio of total intracranial volume (TCV).
## Cognitive Measures

<table>
<thead>
<tr>
<th>Factor 1: Verbal Memory: LM</th>
<th>Adjusted for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor 2: Visual Memory and Organization: VM, HVOT</td>
<td>Birth cohort by decade Education</td>
</tr>
<tr>
<td>Factor 3: New Learning: PAS</td>
<td>Framingham Stroke Risk Profile score</td>
</tr>
<tr>
<td>Factor 4: Attention and Executive Function: Trails A and B</td>
<td>ApoE genotype (ε4 +/-)</td>
</tr>
<tr>
<td>Boston Naming Test</td>
<td></td>
</tr>
<tr>
<td>Abstract Reasoning: Similarities</td>
<td></td>
</tr>
<tr>
<td>Reading and Vocabulary: Wide-Range Achievement Test (WRAT)</td>
<td></td>
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</tbody>
</table>
Results: Unbiased Analyses

- Strongest SNP-trait association
  - On FBAT: **SORL1** (rs1131497; $p=3.2 \times 10^{-6}$) and abstract reasoning
SORL1 and AD related endophenotypes

- Another 100K SNP on SORL1 (rs726601) that was in LD (D'=1, r^2 >0.8) with flanking SNPs (rs2282649, rs1010159) associated with AD in all Caucasian samples

  Rogaeva et al., Nature Genetics, February 2007; pp168-177

- Was also associated with abstract reasoning, (FBAT p=8.2 x 10^{-4})
Association of Distinct Variants in SORL1 With Cerebrovascular and Neurodegenerative Changes Related to Alzheimer Disease

Karen T. Cuenco, PhD; Kathryn L. Lunetta, PhD; Clinton T. Baldwin, PhD; Ann C. McKee, MD; Jianping Guo, MS; L. Adrienne Cupples, PhD; Robert C. Green, MD, MPH; Peter H. St. George-Hyslop, MD; Helena Chui, MD; Charles DeCarli, MD; Lindsay A. Farrer, PhD; for the MIRAGE Study Group

Arch Neurol. 2008;65(12):1640-1648

Table 3. SORL1 SNPs Showing Association With at Least 1 MRI Trait in the MIRAGE White Families

<table>
<thead>
<tr>
<th>SNP</th>
<th>WMH</th>
<th>CVR</th>
<th>CA</th>
<th>MTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.053 (73)</td>
<td>.046 (73)</td>
<td>.43 (73)</td>
<td>.21 (T^b/73)</td>
</tr>
<tr>
<td>6</td>
<td>.03 (66)</td>
<td>.16 (66)</td>
<td>.65 (66)</td>
<td>.18 (T^b/66)</td>
</tr>
<tr>
<td>8</td>
<td>.001 (81)</td>
<td>.006 (81)</td>
<td>.35 (81)</td>
<td>.34 (C^b/81)</td>
</tr>
<tr>
<td>9</td>
<td>&lt;.001 (76)</td>
<td>.002 (76)</td>
<td>.44 (76)</td>
<td>.29 (G^b/76)</td>
</tr>
<tr>
<td>10</td>
<td>.006 (78)</td>
<td>.02 (78)</td>
<td>.94 (78)</td>
<td>.16 (C^b/78)</td>
</tr>
<tr>
<td>11</td>
<td>.08 (76)</td>
<td>.42 (76)</td>
<td>.57 (T^b/76)</td>
<td>.050 (T^b/76)</td>
</tr>
<tr>
<td>15</td>
<td>.04 (G^b/80)</td>
<td>.47 (80)</td>
<td>.42 (G^b/80)</td>
<td>.12 (80)</td>
</tr>
<tr>
<td>16</td>
<td>.33 (A^b/31)</td>
<td>.21 (A^b/31)</td>
<td>.004 (31)</td>
<td>.36 (A^b/31)</td>
</tr>
<tr>
<td>18</td>
<td>.15 (29)</td>
<td>.03 (29)</td>
<td>.45 (29)</td>
<td>.98 (29)</td>
</tr>
<tr>
<td>21</td>
<td>.38 (G^b/38)</td>
<td>.35 (G^b/38)</td>
<td>.02 (38)</td>
<td>.24 (38)</td>
</tr>
</tbody>
</table>
SNP Health Association Resource (SHARe): A Genome-Wide Association Study in the NHLBI’s Framingham Heart Study

Collaboration Between National Heart, Lung, and Blood Institute And Boston University School of Medicine

550,000 SNPs, 9934 persons across 3 generations Became available October 2007
Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium

http://depts.washington.edu/chargeco/wiki/Main_Page
Overview of CHARGE

- CVD/Aging cohorts with GWAS data
  - ARIC, CHS, AGES, ASPS, FHS and Rotterdam
  - Sharing of within-study analyses for cross-study meta-analysis
  - Imputation to HapMap permitted meta-analyses despite use of different platforms in each study
Genes Underlying Stroke & VCI

Expected to belong to 1 or both of 2 classes:

• Genes that predispose individuals to cerebrovascular disease, and

• Genes that determine tissue responses to cerebrovascular disease (e.g. ischemic tolerance)
Genomewide Association Studies of Stroke

M. Arfan Ikram, M.D., Sudha Seshadri, M.D., Joshua C. Bis, Ph.D., Myriam Fornage, Ph.D., Anita L. DeStefano, Ph.D., Yurii S. Aulchenko, Ph.D., Stephanie Debette, M.D., Ph.D., Thomas Lumley, Ph.D., Aaron R. Folsom, M.D., M.P.H., Evita G. van den Herik, M.D., Michiel J. Bos, M.D., Ph.D., Alexa Beiser, Ph.D., Mary Cushman, M.D., M.Sc., Lenore J. Launer, Ph.D., Eyal Shahar, M.D., M.P.H., Maksim Struchalin, M.Sc., Yangchun Du, B.A., Nicole L. Glazer, Ph.D., Wayne D. Rosamond, Ph.D., Fernando Rivadeneira, M.D., Ph.D., Margaret Kelly-Hayes, R.N., D.Ed., Oscar L. Lopez, M.D., Josef Coresh, M.D., Ph.D., Albert Hofman, M.D., Ph.D., Charles DeCarli, M.D., Susan R. Heckbert, M.D., Ph.D., Peter J. Koudstaal, M.D., Ph.D., Qiong Yang, Ph.D., Nicholas L. Smith, Ph.D., Carlos S. Kase, M.D., Kenneth Rice, Ph.D., Talin Haritunians, Ph.D., Gerwin Roks, M.D., Ph.D., Paul L.M. de Kort, M.D., Ph.D., Kent D. Taylor, Ph.D., Lonneke M. de Lau, M.D., Ph.D., Ben A. Oostra, Ph.D., Andre G. Uitterlinden, Ph.D., Jerome I. Rotter, M.D., Eric Boerwinkle, Ph.D., Bruce M. Psaty, M.D., Ph.D., Thomas H. Mosley, Ph.D., Cornelia M. van Duijn, Ph.D., Monique M.B. Breteler, M.D., Ph.D., W.T. Longstreth, Jr., M.D., and Philip A. Wolf, M.D.

Results of Tests for the Association between Stroke and Each SNP Measured in the Genomewide Association Study

Associations in the Region Centered on rs11833579 and Containing NINJ2
• Genome-wide significant association of stroke with 2 SNPs located in the regulatory region of *Ninjurin-2* (rs11833579 and rs12425791).

• *Ninjurin-2*: Transmembrane protein in the “nerve-injury-induced protein” family  
  • cell-cell adhesion molecule, expressed in glia-  
  • shown to promote neurite extension after nerve injury  
  • may also modify brain response to ischemic injury
Brain Aging, AD & Cerebrovascular Disease Phenotypes in CHARGE

- Total and ischemic stroke

- Total dementia, AD, Pure AD, VaD, MCI

- Cerebral MRI measures
  - White matter disease
  - Covert brain infarcts
  - Total cranial & brain volumes, hippocampal, lobar

- Cognitive Function
  - Tests of verbal and visual memory; processing speed, executive function; other domains
Univariate vs. Cross-Phenotype Analyses

Information on multiple correlated phenotypes is not utilized

• Cross Phenotype Analyses
  – Pros:
    • increase power to detect associations of genetic variants with disease by making younger subjects informative
    • Increase statistical power to detect associations with each phenotype
    • identify genetic variants with pleiotropic effects ➔ common biological pathways

Klei, Genet Epidemiol 2008
Need Sequencing & Functional Studies to Find Causal Variant(s).

- An allele is associated with a phenotype when its frequency differs between cases and controls more than would be predicted by chance.
  
  ➔ But this does NOT necessarily imply causality
Use Genetic, Risk Factor, Biomarker & Phenotype Data

Replication and finding causal variant

Look at gene-environment and gene-gene interactions

Explore links genes → gene expression → endophenotype → disease

Develop Predictive Models
Mendelian Randomization

• Association of cholesterol/statin use with decreased AD risk could be causal or an effect of disease-

Genetic variation is determined at birth, if we stratify...


CHOLESTEROL-RELATED GENETIC RISK SCORES ARE ASSOCIATED WITH HYPOMETABOLISM IN ALZHEIMER’S-AFFECTED BRAIN REGIONS

Eric M. Reiman, M.D.¹,²,⁹,¹², Kewei Chen, Ph.D.¹,³,⁴,¹², Richard J. Caselli, M.D.⁶,¹², Gene E. Alexander, Ph.D.⁵,¹², Daniel Bandy, M.S.¹,¹², Jennifer L. Adamson, M.B.A.⁸, Wendy Lee, M.S.¹,¹², Ashley Cannon, B.S.⁸, Elizabeth A. Stephan, Ph.D.⁹,¹², Dietrich A. Stephan, Ph.D.⁹,¹², and Andreas Papassotiropoulos, M.D.⁹,¹⁰,¹¹,¹²
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