

# Genetics of Cerebrovascular Disease

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# Spectrum of Cerebrovascular Disease

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- Clinical Stroke: Ischemic, Hemorrhagic
- Carotid atherosclerosis
- Intracranial stenosis, arteriosclerosis, lipohyalinosis
- MRI: WMH, covert brain infarcts, total/lobar atrophy
- Spectrum of Vascular Cognitive Impairment

# Some Monogenic Causes of Stroke & VCI

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- Homocysteinuria
- CADASIL: Cerebral Autosomal Dominant Arteriopathy, subcortical infarcts and leukoencephalopathy
- Sickle Cell Disease
- Fabry's Disease
- Hereditary cerebral hemorrhage with amyloidosis (HCHWA types)

# Genes Underlying Stroke & VCI

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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)

# Putative Cerebrovascular Disease Genes

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- Surprisingly few genes/loci are known to alter risk in the community as a whole
  - *PDE4D, ALOX5AP, LTA4H*
  - *Specific pathway genes* →
    - renin-angiotensin
    - thrombosis and hemostasis
    - lipid metabolism
    - Inflammation
    - endothelial function
    - oxidative stress
  - *9p21, 4q25*
  - *APOE*
  - *APP*

*Debette S, Seshadri S. Genetics of Atherothrombotic and Lacunar Stroke. Circulation Cardiovascular Genetics, 2009 [in press].*

# Putative Brain Injury Modulators

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- Genes determining tissue response to
    - acute ischemia
    - Long term recovery
    - Non-ischemic injury
    - AD and aging processes
  - Genes determining brain & cognitive reserve
- Neurotrophic factors: BDNF, NGF  
Vascular growth factors  
*APOE*  
Matrix metalloproteinases  
Glutamate and GABA receptors  
Adhesion molecules  
Transcription factors  
Ion channels  
*NOS pathway genes*  
*Inflammation and Oxidative stress*

# Meta-analyses: Candidate Genes, Loci

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- *MTHFR*
- *F5, SERPINE1, F2, GPIBA*
- *ALOX5AP, PDE4D*
- *APOE*
- ACE insertion/deletion
- Intergenic SNPs at chromosome 4q25 and 9p21 related previously to Afib, CAD: rs2200733 and rs1537378

# Human genetic variation and its contribution to complex traits

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

*Nature Reviews Genetics, April 2009; 241-249.*

Single nucleotide variant

```
ATTGGCCTTAACCCCCGATTATCAGGAT  
ATTGGCCTTAACCTCCGATTATCAGGAT
```

Copy number variant

```
ATTGGCCTTAGGCCTTAACCCCGATTATCAGGAT  
ATTGGCCTTA-----ACCTCCGATTATCAGGAT
```

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

# What is a Genome-Wide Association Study (GWAS)?

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An agnostic study of genetic variation across the **entire human genome** designed to identify genetic associations with

- complex diseases: stroke, dementia
- phenotypic traits such as MRI

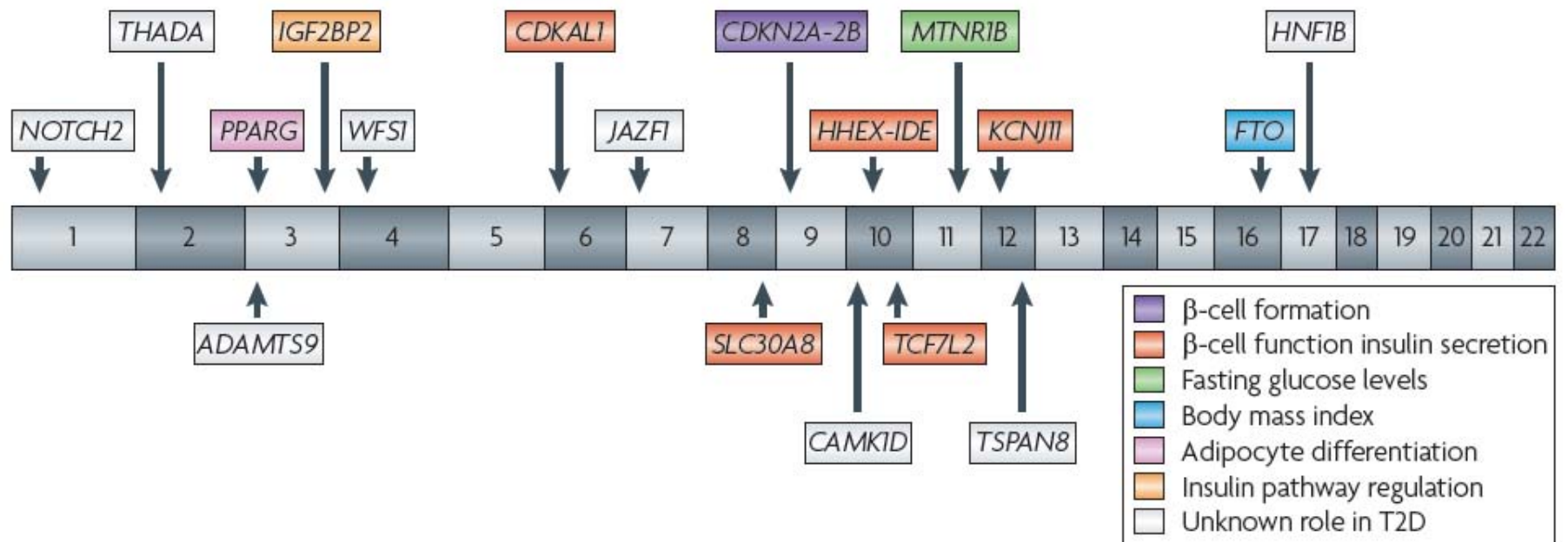


Figure 2 | **Insights into the genetic basis of type 2 diabetes (T2D).** Genome-wide association (GWA) studies have identified 18 genomic intervals that confer increased risk to T2D in Caucasians<sup>58,59,72-75,123-127</sup>. Four of these contain previously known candidate genes, based on the involvement 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

# GWAS of Stroke

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- 2 prior GWAS: 1 did not find any SNP reaching genome-wide significance
- Another related an Afib associated SNP to cardioembolic stroke



## A genome-wide genotyping study in patients with ischaemic stroke: initial analysis and data release

*\*Mar Matarín, \*W Mark Brown, \*Sonja Scholz, \*Javier Simón-Sánchez, \*Hon-Chung Fung, Dena Hernandez, J Raphael Gibbs, Fabienne Warrant De Vrieze, Cynthia Crews, Angela Britton, Carl D Langefeld, Thomas G Brott, Robert D Brown Jr, Bradford B Worrall, Michael Franke, Scott Silliman, L Douglas Case, Andrew Singleton, John A Hardy, Stephen S Rich, James F Meschia*

### Summary

*Lancet Neurol 2007; 6: 414-20*

**Background** Despite evidence of a genetic role in stroke, the identification of common genetic risk factors for this

# GWAS of Stroke

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## Risk Variants for Atrial Fibrillation on Chromosome 4q25 Associate with Ischemic Stroke

Solveig Gretarsdottir, PhD,<sup>1</sup> Gudmar Thorleifsson, PhD,<sup>1</sup> Andrei Manolescu, PhD,<sup>1</sup>  
Unnur Styrkarsdottir, PhD,<sup>1</sup> Anna Helgadóttir, MD,<sup>1</sup> Andreas Gschwendtner, MD,<sup>2</sup>  
Konstantinos Kostulas, MD, PhD,<sup>3</sup> Gregor Kuhlenbäumer, MD,<sup>4,5</sup> Steve Bevan, PhD,<sup>6</sup>  
Thorbjorg Jonsdottir, BSc,<sup>1</sup> Hjordis Bjarnason, BSc,<sup>1</sup> Jona Saemundsdottir, BSc,<sup>1</sup> Stefan Palsson, MSc,<sup>1</sup>  
David O. Arnar, MD, PhD,<sup>7</sup> Hilma Holm, MD,<sup>1</sup> Gudmundur Thorgeirsson, MD, PhD,<sup>7</sup>  
Einar Mar Valdimarsson, MD,<sup>7</sup> Sigurlaug Sveinbjörnsdottir, MD,<sup>7</sup> Christian Gieger, PhD,<sup>8,9</sup>  
Klaus Berger, MD,<sup>10</sup> H-Erich Wichmann, MD,<sup>8,9</sup> Jan Hillert, MD,<sup>3</sup> Hugh Markus, MD,<sup>6</sup>  
Jeffrey Robert Gulcher, MD, PhD,<sup>1</sup> E. Bernd Ringelstein, MD,<sup>4</sup> Augustine Kong, PhD,<sup>1</sup>  
Martin Dichgans, MD,<sup>2</sup> Daniel Fannar Gudbjartsson, PhD,<sup>1</sup> Unnur Thorsteinsdottir, PhD,<sup>1,11</sup> and  
Kari Stefansson, MD, PhD<sup>1,11</sup>

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**Objective:** To find sequence variants that associate with the risk for ischemic stroke (IS), we performed a genome-wide association study.

**Methods:** We genotyped 1,661 Icelandic IS patients and 10,815 control subjects using the Infinium HumanHap300 chip (Illumina, San Diego, CA). A total of 310,881 single nucleotide polymorphisms (SNPs) were tested for association with IS, and the most significant signals were replicated in two large European IS sample sets (2,224 cases/2,583 control subjects). Two SNPs, rs2200733 and rs10033464, were tested further in additional European IS samples (2,327 patients and 16,760 control subjects).

**Results:** In the Icelandic samples and the two replication sets combined, rs2200733 associated significantly with cardioembolic stroke (CES) (odds ratio [OR], 1.54;  $p = 8.05 \times 10^{-9}$ ). No other variants associated with IS or any of its subtypes. rs2200733

# Problems

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- Genetic heterogeneity
- Multiple genes with modest effects
- Gene-gene and gene-environment interactions
- Selection and survival biases
  
- Relatively late manifestation of clinical disease
  - Competing mortality

# Solutions

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- Careful phenotype definition
- Collaborative efforts to increase numbers
- Gene-gene and GEI
  
- Study incident disease
  
- Study endophenotypes

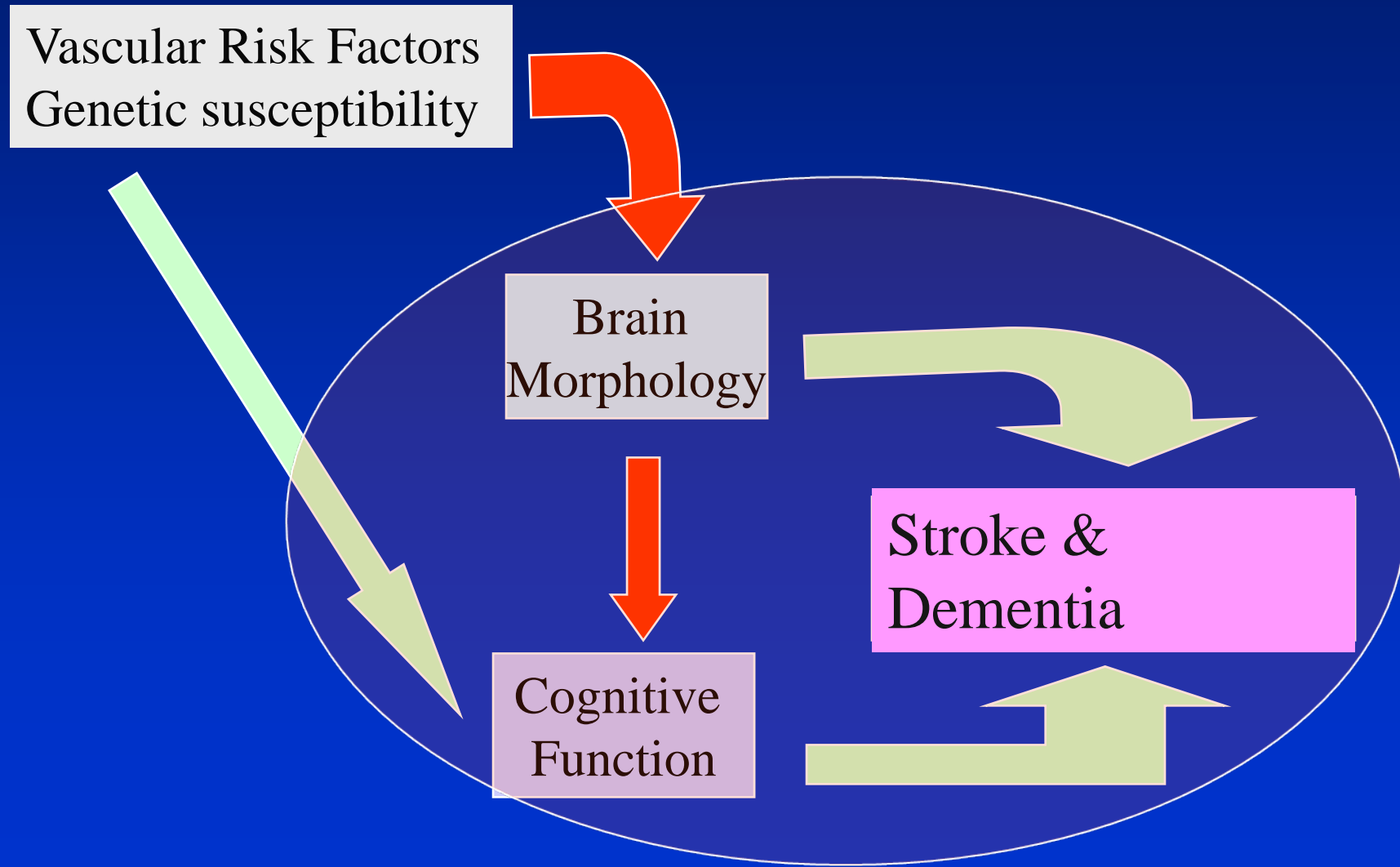
# What is an Endophenotype?

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- 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

# Conceptual Model for Pathways from Genes to Stroke/Dementia

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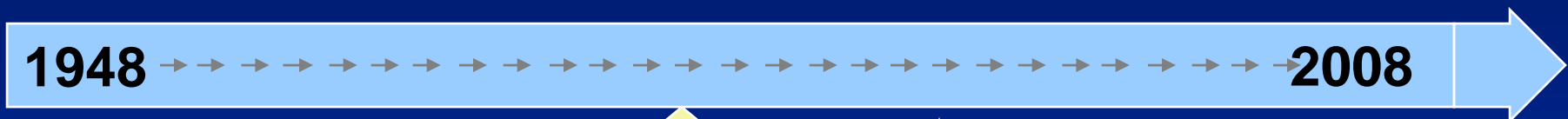


# Cerebrovascular Endophenotypes

- MRI and cognitive tests define **endophenotypes** (**WMH, SCI, MCI-EF**) that are
- Quantitative traits
- With moderate to high heritability

# Framingham Heart Study

Stroke, Dementia and MRI/NP Studies



1948

2008

**Original cohort (Gen 1)**

**Exam 14 (1975)** (N=2,842; mean age 67)

**Exam 26: 313 Gen 1 survivors MR and NP**



1971

2008

**Offspring cohort (Gen 2)**

**Exam 7 (1999-02) MRI & NP**  
(N=2442; mean age 42)

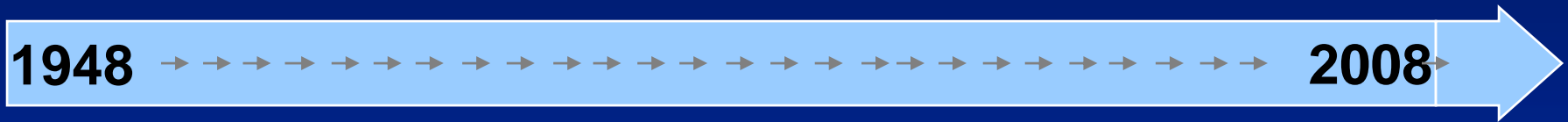
Brain Bank: 1995

**Exam 8 (2005-08)**  
**Repeat: MRI & NP**  
(N=1800; mean age 67)

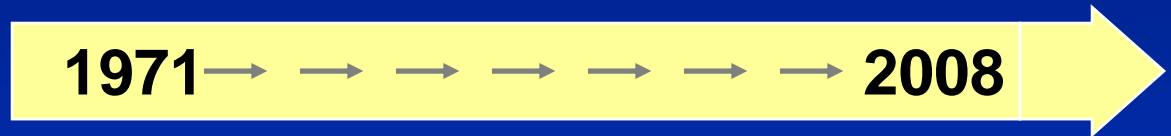
# Framingham Heart Study

Longitudinal Community-Based Family Study

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**Gen 1 Original cohort**



**Gen 2 Offspring cohort**



**Gen 3 cohort**

Exam 2 (2009-11) MRI  
NP & Biomarkers



# *SORL1: New kid on the AD Block*

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nature  
genetics

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## The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease

Ekaterina Rogaeva<sup>1,15</sup>, Yan Meng<sup>2,15</sup>, Joseph H Lee<sup>3,15</sup>, Yongjun Gu<sup>1,15</sup>, Toshitaka Kawarai<sup>1,15</sup>, Fanggeng Zou<sup>4,15</sup>, Taiichi Katayama<sup>1</sup>, Clinton T Baldwin<sup>2</sup>, Rong Cheng<sup>3</sup>, Hiroshi Hasegawa<sup>1</sup>, Fusheng Chen<sup>1</sup>, Nobuto Shibata<sup>1</sup>, Kathryn L Lunetta<sup>2</sup>, Raphaele Pardossi-Piquard<sup>1</sup>, Christopher Bohm<sup>1</sup>, Yosuke Wakutani<sup>1</sup>, L Adrienne Cupples<sup>2</sup>, Karen T Cuenco<sup>2</sup>, Robert C Green<sup>2</sup>, Lorenzo Pinessi<sup>5</sup>, Innocenzo Rainero<sup>5</sup>, Sandro Sorbi<sup>6</sup>, Amalia Bruni<sup>7</sup>, Ranjan Duara<sup>8</sup>, Robert P Friedland<sup>9</sup>, Rivka Inzelberg<sup>10</sup>, Wolfgang Hampe<sup>11</sup>, Hideaki Bujo<sup>12</sup>, You-Qiang Song<sup>13</sup>, Olav M Andersen<sup>14</sup>, Thomas E Willnow<sup>14</sup>, Neill Graff-Radford<sup>4</sup>, Ronald C Petersen<sup>4</sup>, Dennis Dickson<sup>4</sup>, Sandy D Der<sup>1</sup>, Paul E Fraser<sup>1</sup>, Gerold Schmitt-Ulms<sup>1</sup>, Steven Younkin<sup>4</sup>, Richard Mayeux<sup>3</sup>, Lindsay A Farrer<sup>2</sup> & Peter St George-Hyslop<sup>1</sup>

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

Research

Open Access

## Genetic correlates of brain aging on MRI and cognitive test measures: a genome-wide association and linkage analysis in the Framingham study

Sudha Seshadri<sup>\*1,2</sup>, Anita L DeStefano<sup>1,3</sup>, Rhoda Au<sup>1,2</sup>, Joseph M Massaro<sup>1,3,4</sup>, Alexa S Beiser<sup>1,2,3</sup>, Margaret Kelly-Hayes<sup>1,2</sup>, Carlos S Kase<sup>1,2</sup>, Ralph B D'Agostino Sr<sup>1,4</sup>, Charles DeCarli<sup>5</sup>, Larry D Atwood<sup>1,2</sup> and Philip A Wolf<sup>1,2</sup>

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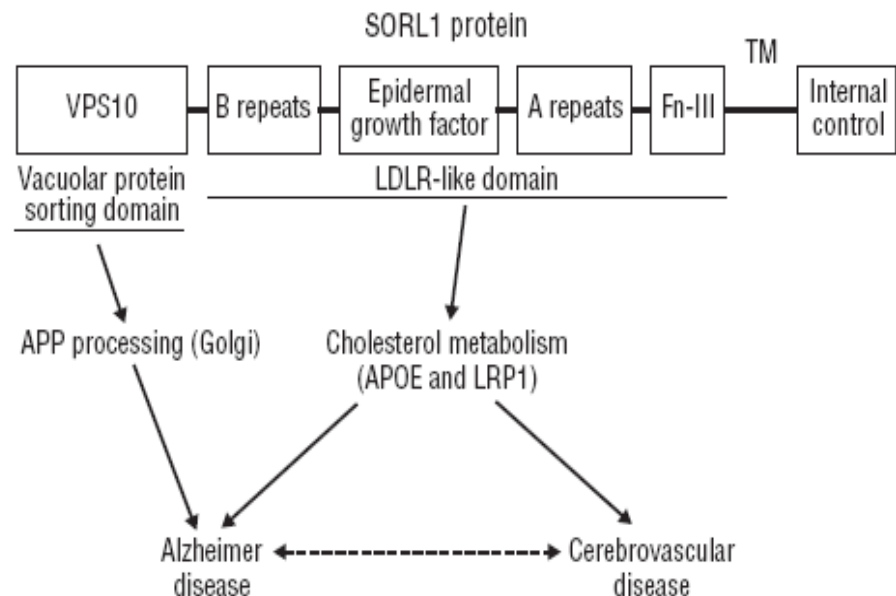
# Association of Distinct Variants in *SORL1* With Cerebrovascular and Neurodegenerative Changes Related to Alzheimer Disease

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**

SNP	P Value (No. of Informative Families) <sup>a</sup>			
	WMH	CVR	CA	MTA
1	.053 (73)	<b>.046</b> (73)	.43 (73)	.21 (T <sup>b</sup> /73)
6	<b>.03</b> (66)	.16 (66)	.65 (66)	.18 (T <sup>b</sup> /66)
8	<b>.001</b> (81)	<b>.006</b> (81)	.35 (81)	.34 (C <sup>b</sup> /81)
9	<b>&lt;.001</b> (76)	<b>.002</b> (76)	.44 (76)	.29 (G <sup>b</sup> /76)
10	<b>.006</b> (78)	<b>.02</b> (78)	.94 (78)	.16 (C <sup>b</sup> /78)
11	.08 (76)	.42 (76)	.57 (T <sup>b</sup> /76)	<b>.050</b> (T <sup>b</sup> /76)
15	<b>.04</b> (G <sup>b</sup> /80)	.47 (80)	.42 (G <sup>b</sup> /80)	.12 (80)
16	.33 (A <sup>b</sup> /31)	.21 (A <sup>b</sup> /31)	<b>.004</b> (31)	.36 (A <sup>b</sup> /31)
18	.15 (29)	<b>.03</b> (29)	.45 (29)	.98 (29)
21	.38 (G <sup>b</sup> /38)	.35 (G <sup>b</sup> /38)	<b>.02</b> (38)	.24 (38)



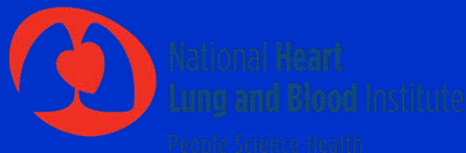
# SNP Health Association

## 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

# Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium

## Design of Prospective Meta-Analyses of Genome-Wide Association Studies From 5 Cohorts

Bruce M. Psaty, MD, PhD; Christopher J. O'Donnell, MD, MPH; Vilmundur Gudnason, MD, PhD; Kathryn L. Lunetta, PhD; Aaron R. Folsom, MD; Jerome I. Rotter, MD; André G. Uitterlinden, PhD; Tamara B. Harris, MD; Jacqueline C.M. Witteman, PhD; Eric Boerwinkle, PhD;  
on Behalf of the CHARGE Consortium

**Background**—The primary aim of genome-wide association studies is to identify novel genetic loci associated with interindividual variation in the levels of risk factors, the degree of subclinical disease, or the risk of clinical disease. The requirement for large sample sizes and the importance of replication have served as powerful incentives for scientific collaboration.

**Methods**—The Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium was formed to facilitate genome-wide association studies meta-analyses and replication opportunities among multiple large population-based cohort studies, which collect data in a standardized fashion and represent the preferred method for estimating disease incidence. The design of the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium includes 5 prospective cohort studies from the United States and Europe: the Age, Gene/Environment Susceptibility—Reykjavik Study, the Atherosclerosis Risk in Communities Study, the Cardiovascular Health Study, the Framingham Heart Study, and the Rotterdam Study. With genome-wide data on a total of about 38 000 individuals, these cohort studies have a large number of health-related phenotypes measured in similar ways. For each harmonized trait, within-cohort genome-wide association study analyses are combined by meta-analysis. A prospective meta-analysis of data from all 5 cohorts, with a properly selected level of genome-wide statistical significance, is a powerful approach to finding genuine phenotypic associations with novel genetic loci.

**Conclusions**—The Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium and collaborating non-member studies or consortia provide an excellent framework for the identification of the genetic determinants of risk factors, subclinical-disease measures, and clinical events. (*Circ Cardiovasc Genet.* 2009;2:73-80.)

Key Words: epidemiology ■ meta-analysis ■ genetics ■ genomics

# Overview of CHARGE

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- 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

# Brain Aging, AD & Cerebrovascular Disease Phenotypes in CHARGE

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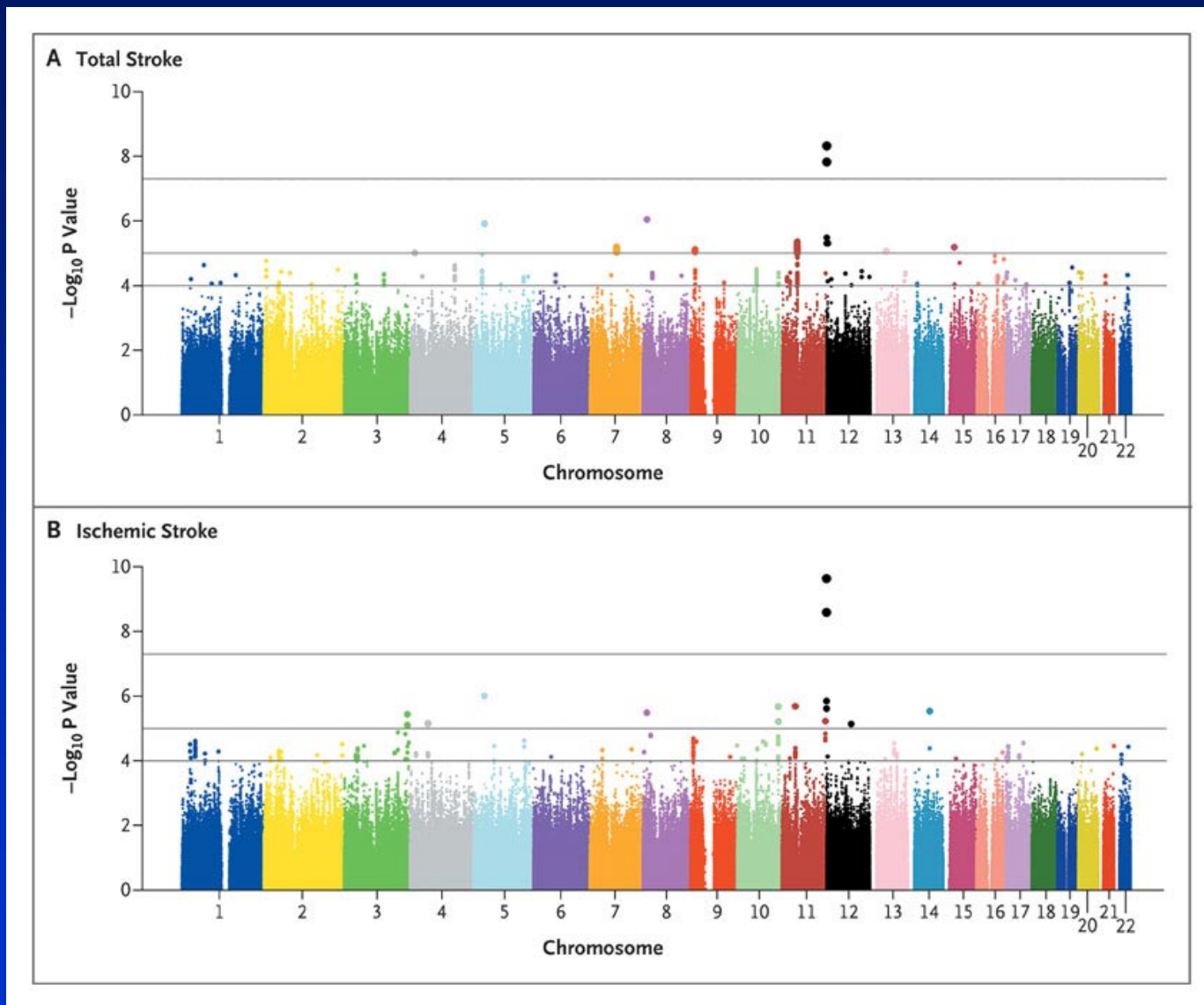
- Total and ischemic stroke
- Total dementia, AD, Pure AD, VaD, MCI
- Cerebral MRI measures
  - White matter disease
  - Covert brain infarcts
  - Total cranial & brain volumes, hippocampal, lobar
- Cognitive Function
  - Tests of verbal and visual memory; processing speed, executive function; other domains

# Discovery Sample

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19,602 white persons (age  $63 \pm 8$ ) who developed 1,544 incident strokes (1,164 ischemic) over an average follow-up of 11 years

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

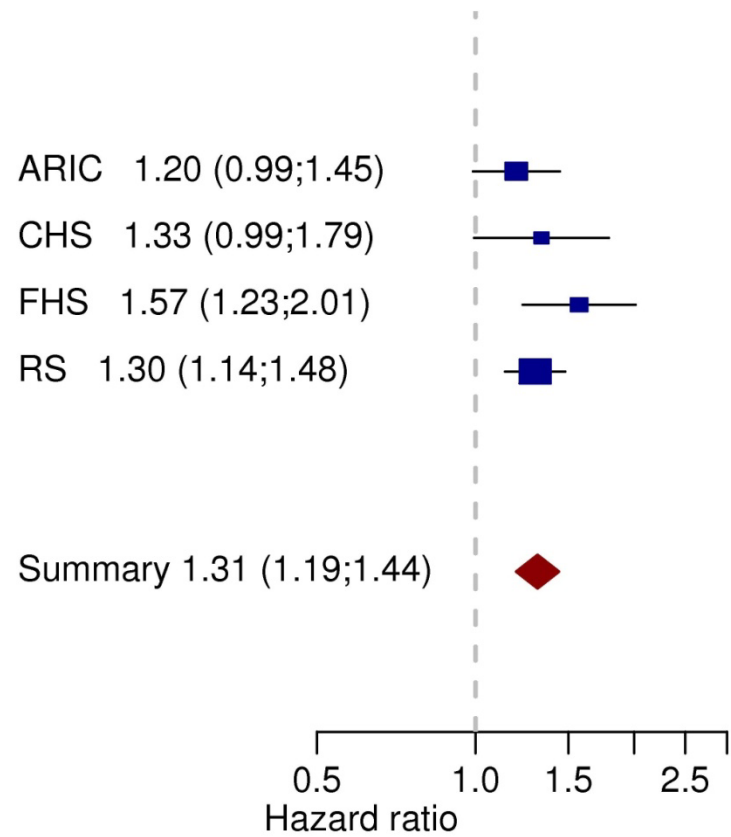
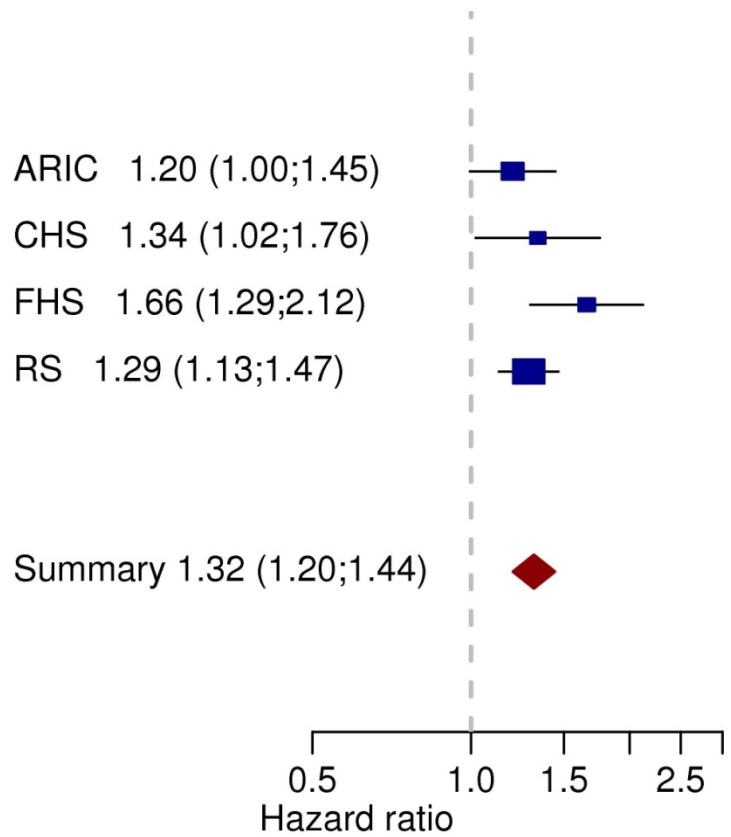


Ikram M et al. N Engl J Med 2009;10.1056/NEJMoa0900094

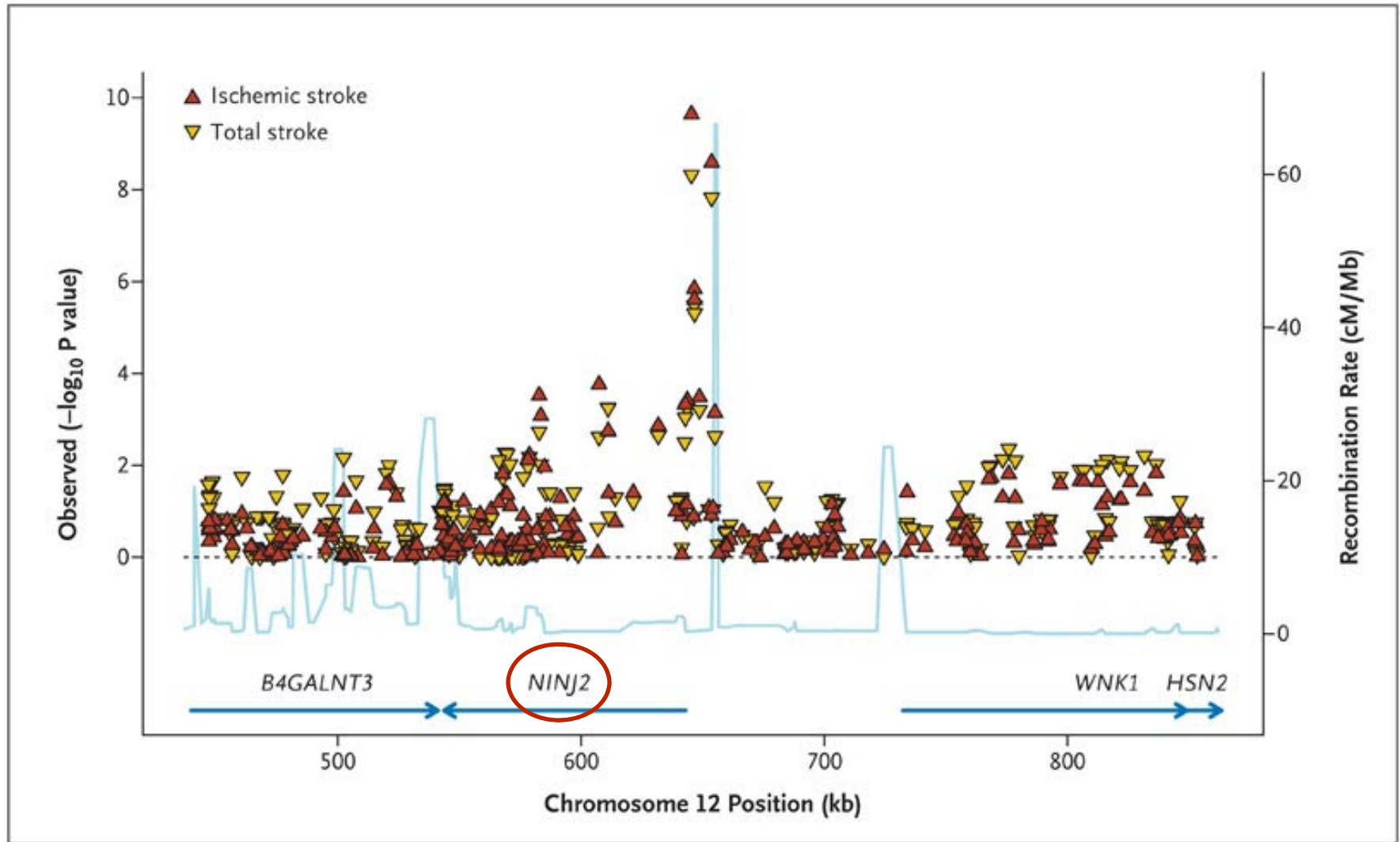


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# Forest plots: Total Stroke



# Associations in the Region Centered on rs11833579 and Containing NINJ2



Ikram M et al. N Engl J Med 2009;10.1056/NEJMoa0900094



The NEW ENGLAND  
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- 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

# Next Steps

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- Larger Meta-analyses to find more genes
- Cross-Phenotype analyses
- Functional Studies: expression, animal models



CARE: The NHLBI's Candidate Gene Association Resource

[http://www.broad.mit.edu/gen\\_analysis/care/index.php/Main\\_Page](http://www.broad.mit.edu/gen_analysis/care/index.php/Main_Page)

# CARe Genotyping Plan

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## Phase II (Illumina iSelect---IBC Chip)

-~49,000 SNPs covering ~2100 candidate genes  
typed on ~50,000 persons from all CARe Cohorts

# Use Genetic, Risk Factor, Biomarker & Phenotype Data

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**Replication** and finding **causal variant**

Look at gene-environment and gene-gene interactions

Explore links genes → gene expression  
endophenotype → disease

**Develop Predictive Models**

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- Framingham and other CHARGE study participants
- Talented and generous colleagues

*Thank you, Oscar!*

# Framingham Neurology Research Team

---

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- **Yangchun Du**
- Linda Farese
- Linda Clark
- Deb Foulkes
- Lois Abel
- Barbara Inglese
- Coreyann Poly

Justin Nyborn, Jackie Harvey, Sarah J Greene & others

*Neurogenetic group in yellow*