Disclosure: Alison Goate, D.Phil.

Research Support / Grants	NIH-NIA, JPB Foundation, Rainwater Foundation, Anonymous Foundation, Neurodegeneration Consortium
Stock/Equity	None
Consulting	Cognition Therapeutics, Denali Therapeutics, AbbVie, Pfizer
Royalties	Taconic, Athena Diagnostics

A History of Alzheimer's disease Genetics

Friday Harbor, WA (September 6th, 2017)

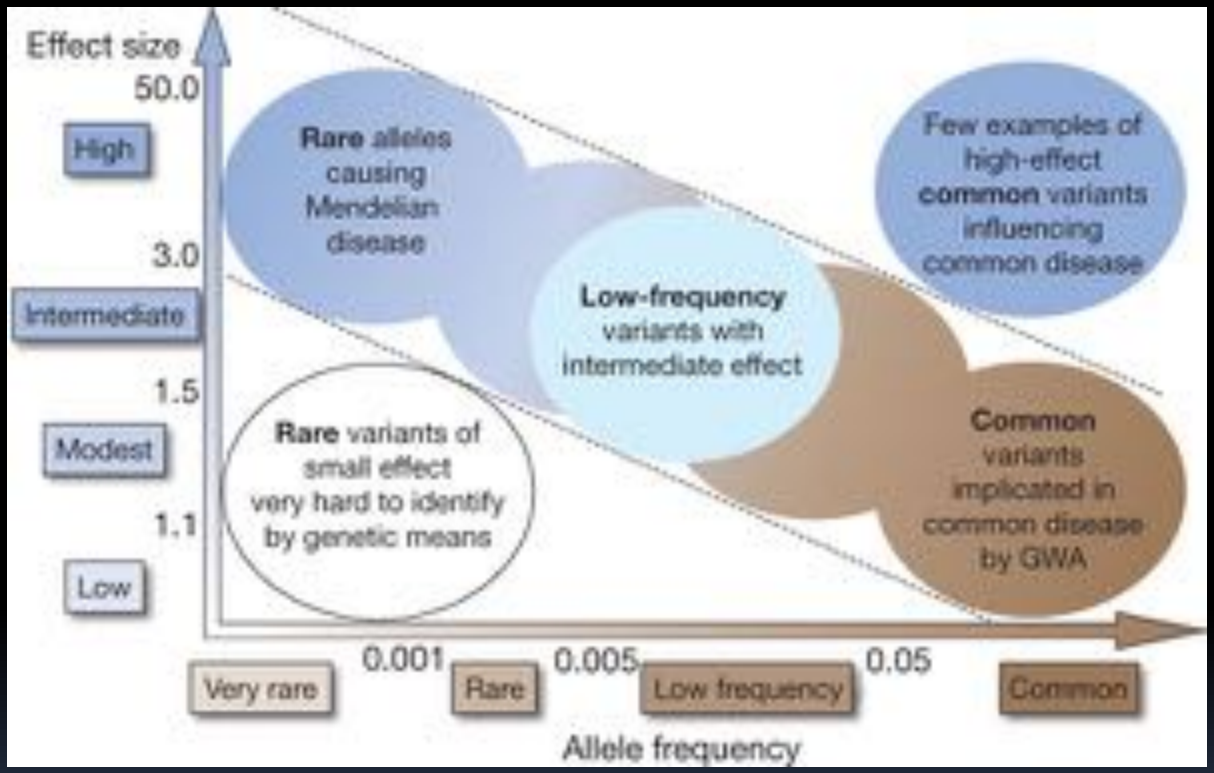
Alison Goate, D.Phil.
Alison.goate@mssm.edu

Icahn School of Medicine at Mount Sinai

Goals of this talk

- **Early AD Genetics I: Mendelian Causes of AD**
- Early AD Genetics II: Identification of common high effect variants influencing AD risk
- Genome-wide Association Studies (GWAS): Identification of common low-effect variants
  - Methods to fine map GWAS loci
- Whole Genome/Exome Sequencing: Identification of rare moderate-effect variants

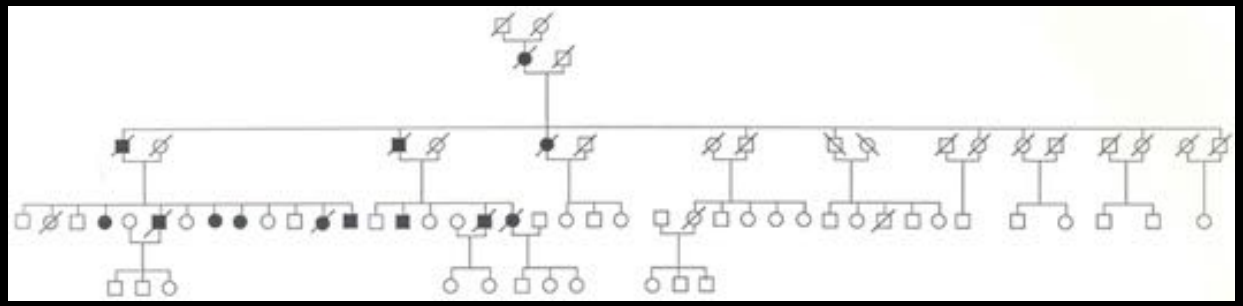
Genetic Architecture of human disease



TA Manolio *et al. Nature* 461, 747-753 (2009) doi:10.1038/nature08494

nature

Rare alleles causing Mendelian disease



- Autosomal Dominant Alzheimer's disease
  - *Amyloid Precursor Protein* (Goate et al., Nature, 1991)
  - *Presenilin 1* (Sherrington et al., Nature, 1995)
  - *Presenilin 2* (Rogaev et al., Nature, 1995; Levy-Lahad et al., Science, 1995)
- Autosomal recessive Alzheimer's disease
  - *Amyloid Precursor Protein* (Di Fede et al., Science 2009)

Most mutations causing Familial AD are in Presenilin 1

Gene	# Mutations		# Families	
APP	32	(14.3%) AAO: 39-59y	86	(17.2%)
PSEN1	177	(78.4%) AAO: 24-69y	392	(78.2%)
PSEN2	14	(6.3%) AAO: 45-73y	23	(4.6 %)
Total	223		501	

From AD mutations database:
http://molgen-www.uia.ac.be/ADMutations/

3.4% of clinically diagnosed NIALOAD families carry known functional variants

Gene	# of mutations	% of families (N=439)
APP	0	0
PSEN1	2	1.6%
PSEN2	0	0
MAPT	0	0
GRN	3	0.7%
C9orf72	1	1.1%

Cruchaga et al., PLoS One 2012; Harms et al., Jama Neurol. 2013

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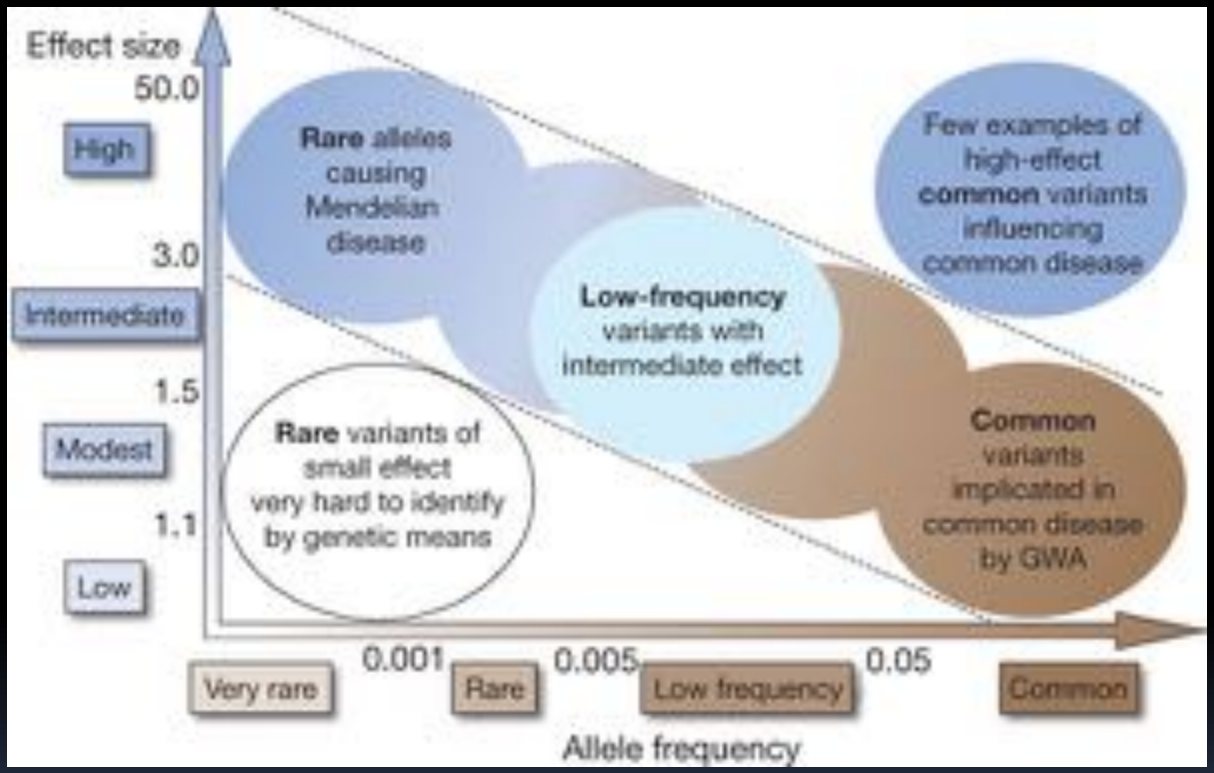

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nature

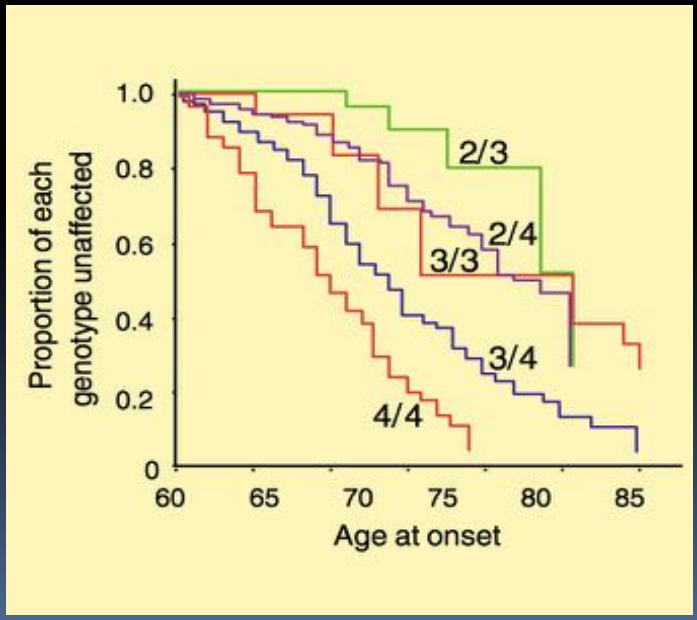
High effect-size common variants

- *Apolipoprotein E4* shows dose dependent increase in AD risk (OR=3 for 1 allele, OR=8 for 2 alleles) Corder et al., Science,1993; Strittmatter et al., PNAS, 1993

- *Apolipoprotein E2* decreases in AD risk (OR=0.5)

  Corder et al., Nat. Genet. 1994

- *APOE* genotype influences age at onset of AD

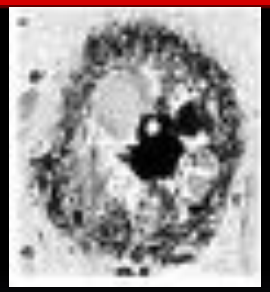


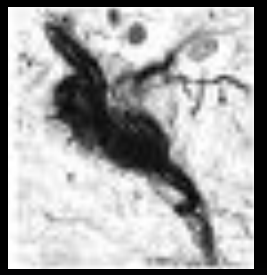
From: Roses et al., JNEN 1994

The Amyloid Hypothesis

Factors affecting clearance and conformation of Aß:
- APOE
- CLU
- Degrading proteases
- Anti-Aβ antibodies

Soluble Aβ → **Formation of β-sheets; fibrillogenesis** → **Synaptic and neuronal degeneration**





Factors increasing Aß production
- PS1 and PS2 mutations
- APP mutations
- Trisomy 21

Neurotoxic and inflammatory responses
- Cytokines
- Glial activation
- Free radical generation

Modified from David M. Holtzman, MD; photomicrographs courtesy of Daniel W. McKeel, MD.

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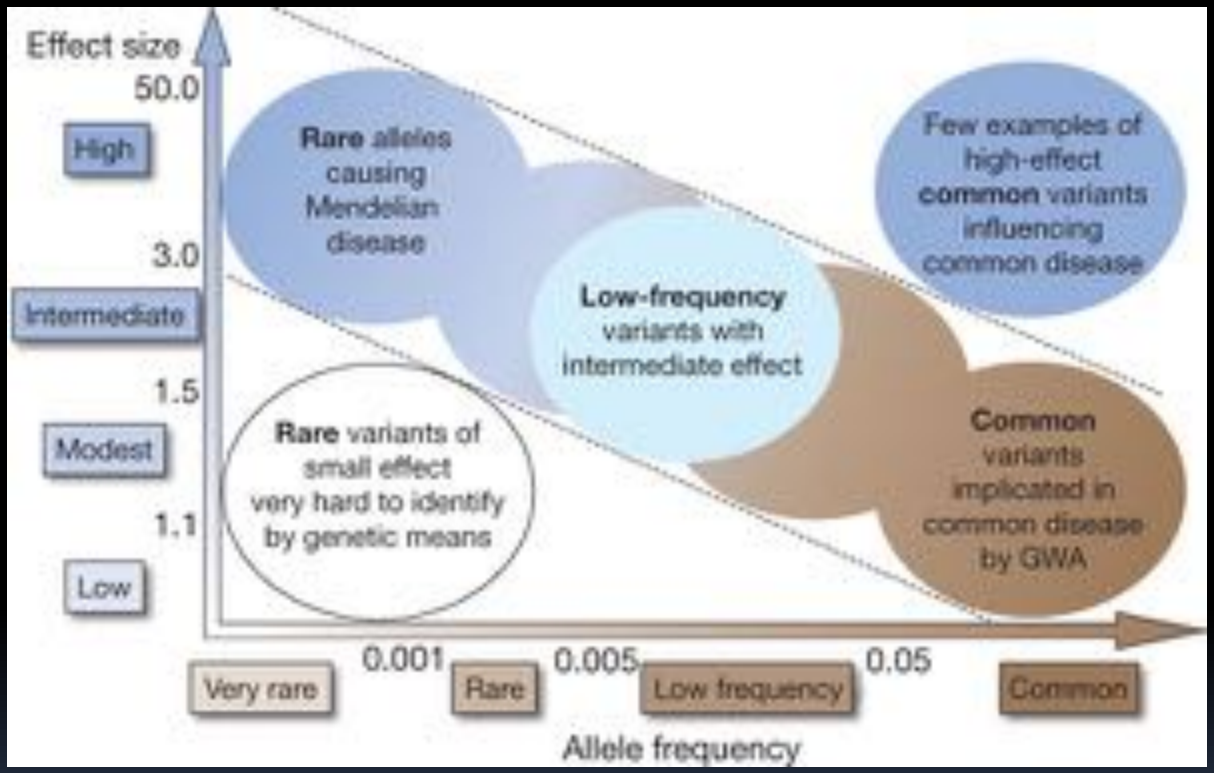

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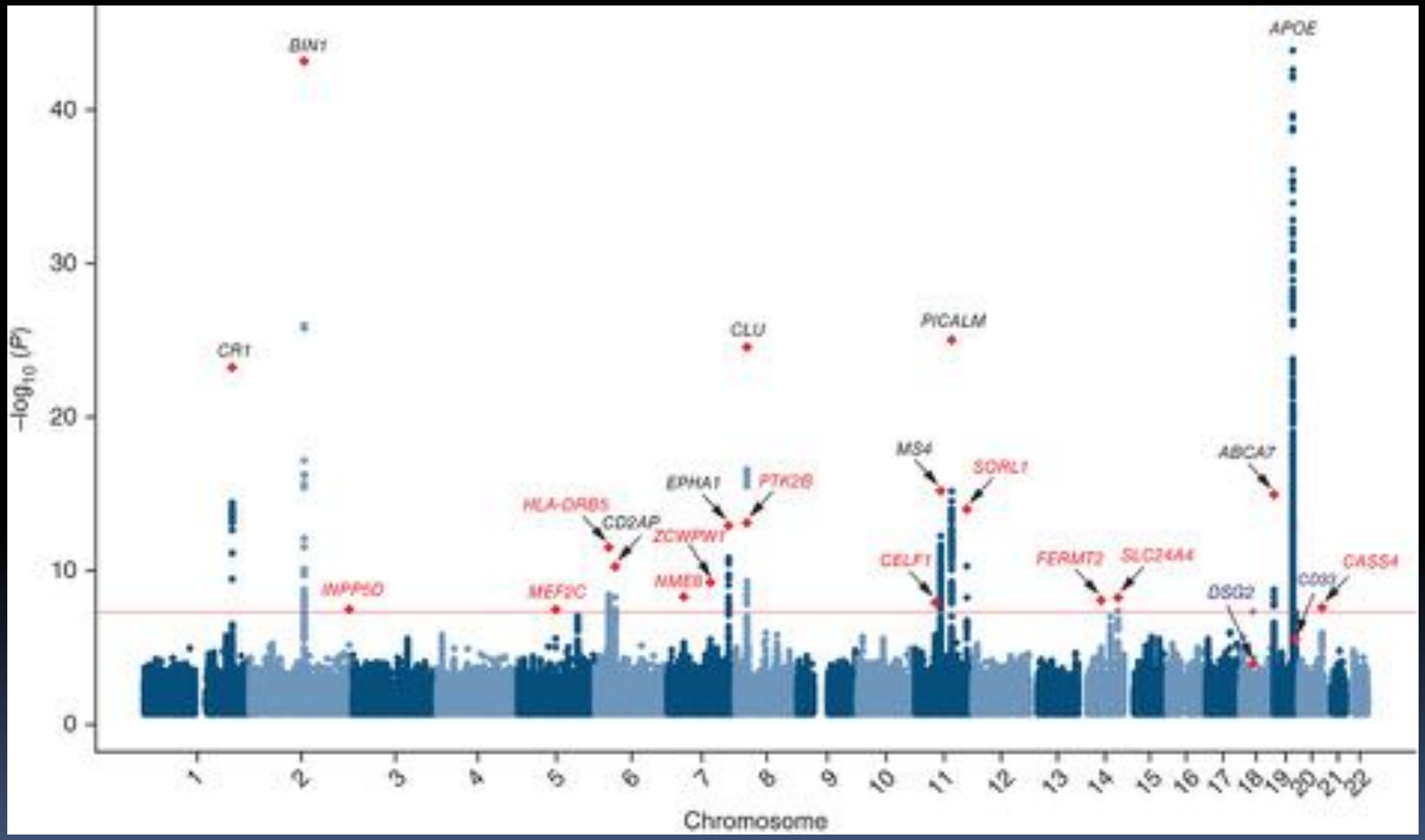
nature

Identification of common variants of low effect size

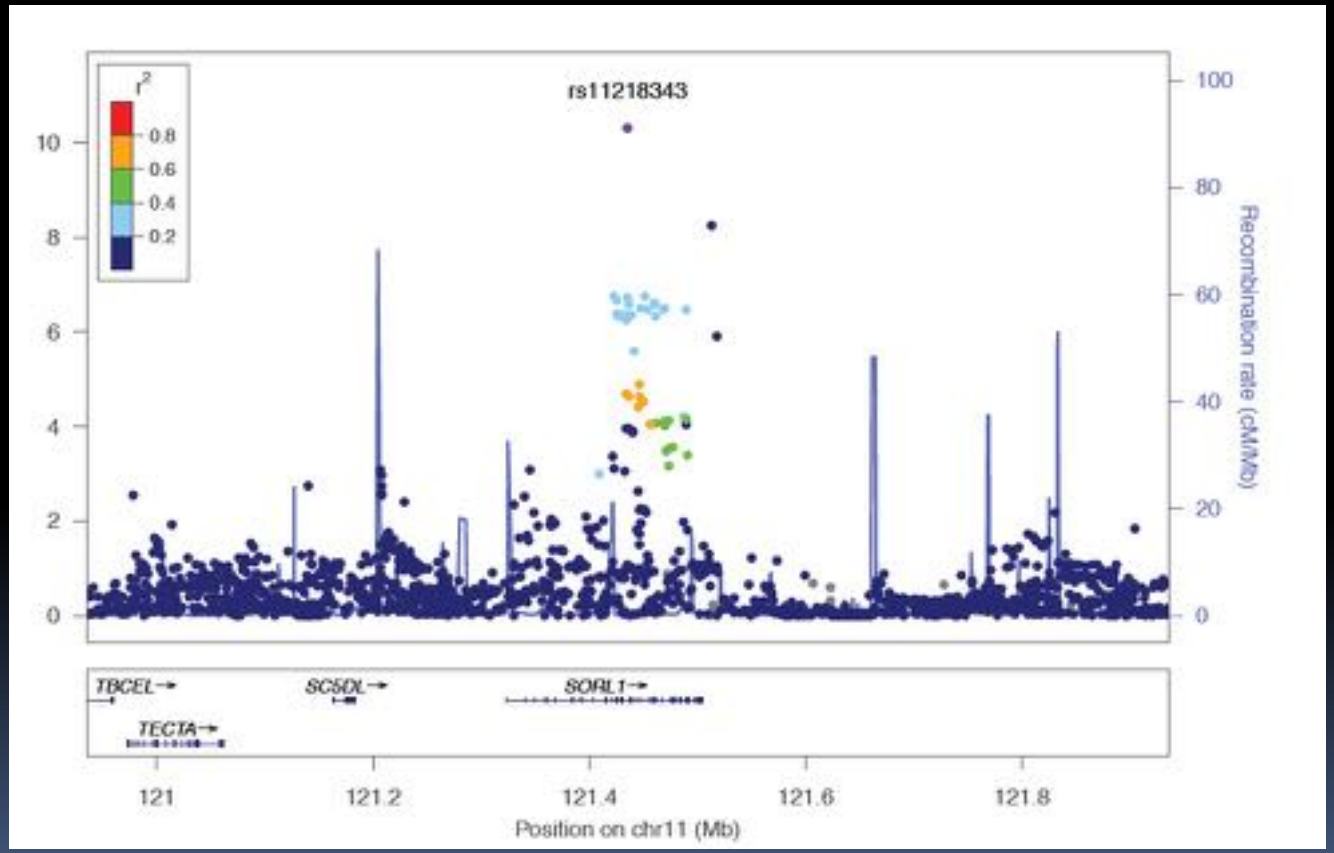
- Genome-Wide Association Studies (GWAS)
  - Allows evaluation of millions of DNA variants simultaneously
  - Most common design is a comparison of unrelated cases and controls
    - Families
    - Endophenotypes – quantitative traits e.g. imaging, fluid biomarkers
  - Very large sample sizes increase power

More than 20 loci associated with Alzheimer's disease risk



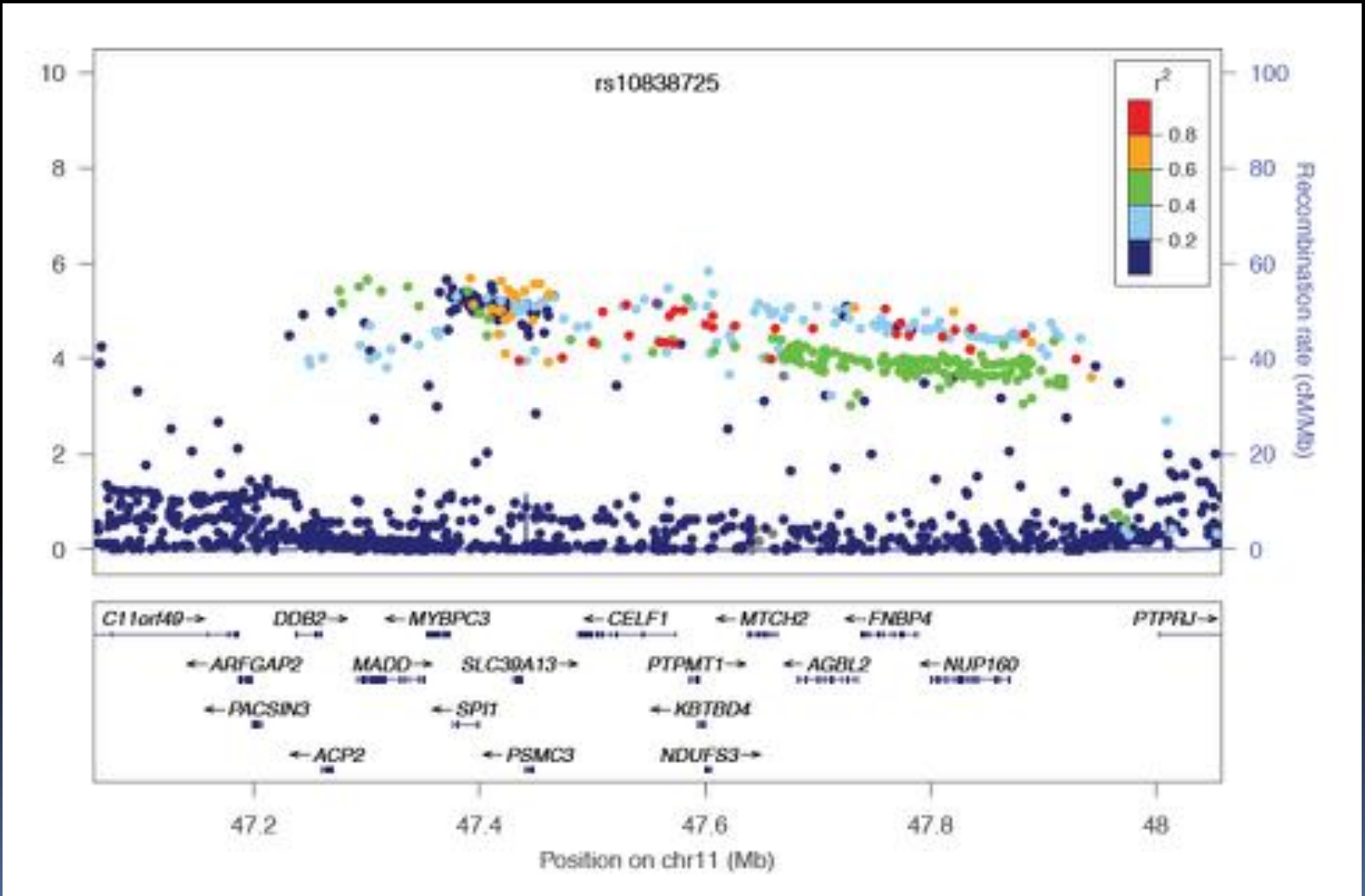
Lambert JC et al., Nature Genetics (2013) 45, 1452-1458

Some GWAS SNPs implicate a single gene



Lambert et al 2013

Some GWAS SNPs implicate many genes



Pathway analysis implicates efferocytosis in the etiology of AD

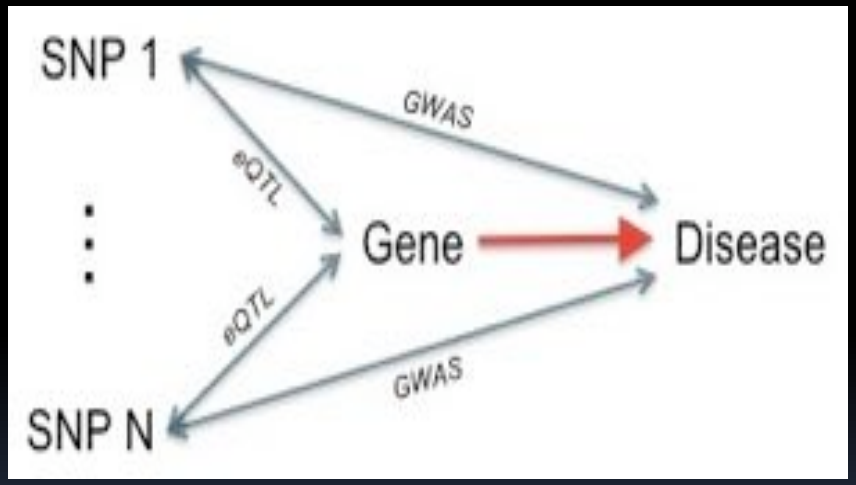
- Publicly available IGAP AD GWAS dataset
  - 26K cases/48K controls
- GATES SNP-to-gene algorithm to generate gene-level statistics
  - 298 candidate genes at 10% FDR
- Ingenuity Pathway Analysis

Edoardo Marcora

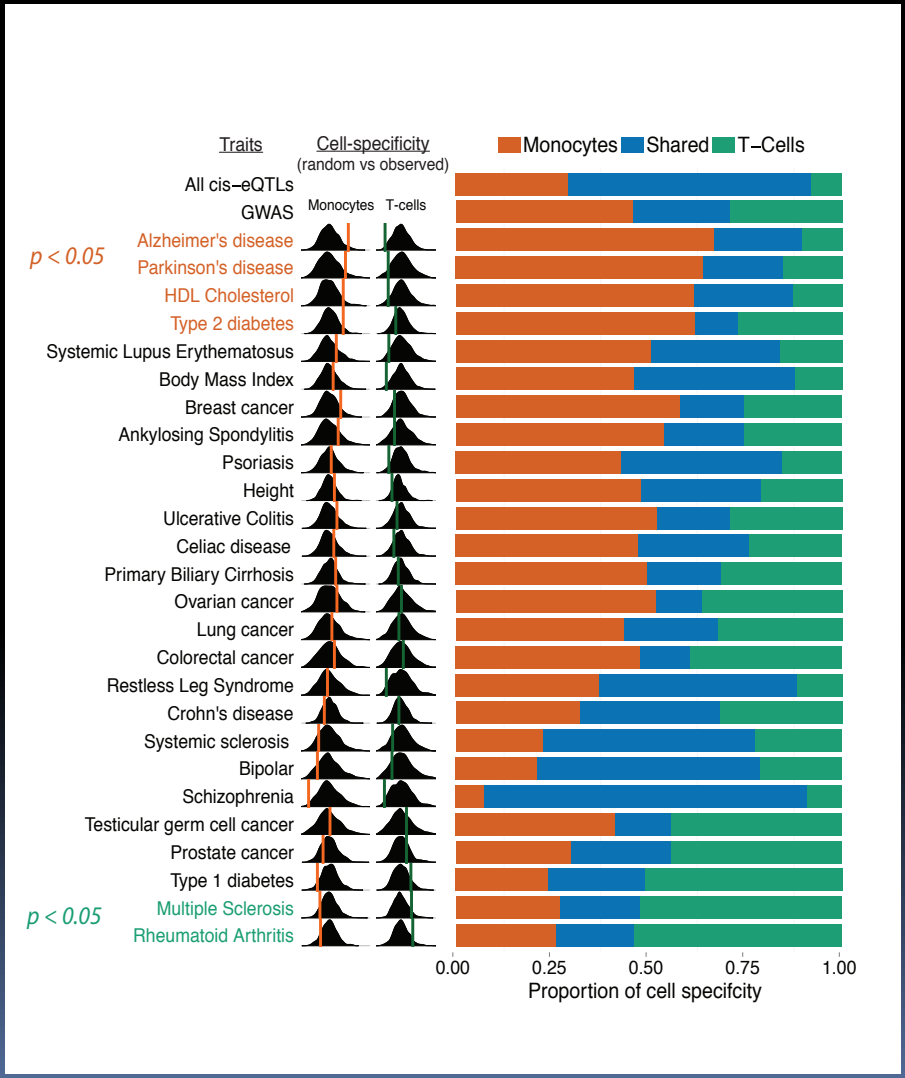
CANONICAL PATHWAYS	
LXR/RXR activation	9.61E-15
LPS/IL-1 mediated inhibition of RXR function	1.37E-11
Antigen presentation pathway	2.31E-10
IL-12 signaling and production in macrophages	4.87E-10

DISEASES & BIOLOGICAL FUNCTIONS	
Late-onset Alzheimer's disease	8.83E-14
Dementia	6.05E-08
Tauopathy	7.93E-08
Alzheimer's disease	1.02E-07
Multiple Sclerosis	4.24E-07
Engulfment of cells	2.16E-06
Phagocytosis of cells	4.74E-06

Integrative analysis of GWAS and expression eQTL data for the discovery of disease genes



Polarization of cis-regulatory effects of autoimmune and neurodegenerative risk alleles in monocytes and T-cells



Raj et al. Science 2014

AD risk alleles enriched in hematopoietic epigenomic annotations (LD score regression)

Annotation	Prop._SNPs	Prop._h2	Prop._h2_std_error	Enrichment	Enrichment_std_error	Enrichment_p	Coefficient	Coefficient_std_error	Coefficient_z-score
Hematopoietic	0.23	1.27	0.25	5.46	1.09	2.66E-07	4.48E-08	1.89E-08	2.37
Liver	0.07	0.73	0.19	10.09	2.62	5.84E-06	6.14E-08	2.14E-08	2.87
Other	0.20	1.00	0.24	4.95	1.18	1.07E-04	3.77E-08	1.74E-08	2.16
CNS	0.15	0.73	0.18	4.92	1.20	5.25E-04	9.74E-09	1.51E-08	0.65
Connective_Bone	0.11	0.65	0.21	5.62	1.83	2.69E-03	1.42E-08	2.08E-08	0.68
Adrenal_Pancreas	0.09	0.59	0.18	6.34	1.97	3.60E-03	1.75E-08	2.44E-08	0.72
GI	0.17	0.71	0.19	4.22	1.12	4.57E-03	2.41E-09	1.89E-08	0.13
Kidney	0.04	0.39	0.13	9.22	3.10	5.30E-03	1.11E-08	3.23E-08	0.34
SkeletalMuscle	0.10	0.54	0.17	5.23	1.66	1.01E-02	1.54E-09	2.38E-08	0.06
Cardiovascular	0.11	0.20	0.17	1.84	1.53	5.90E-01	-5.72E-08	2.49E-08	-2.30

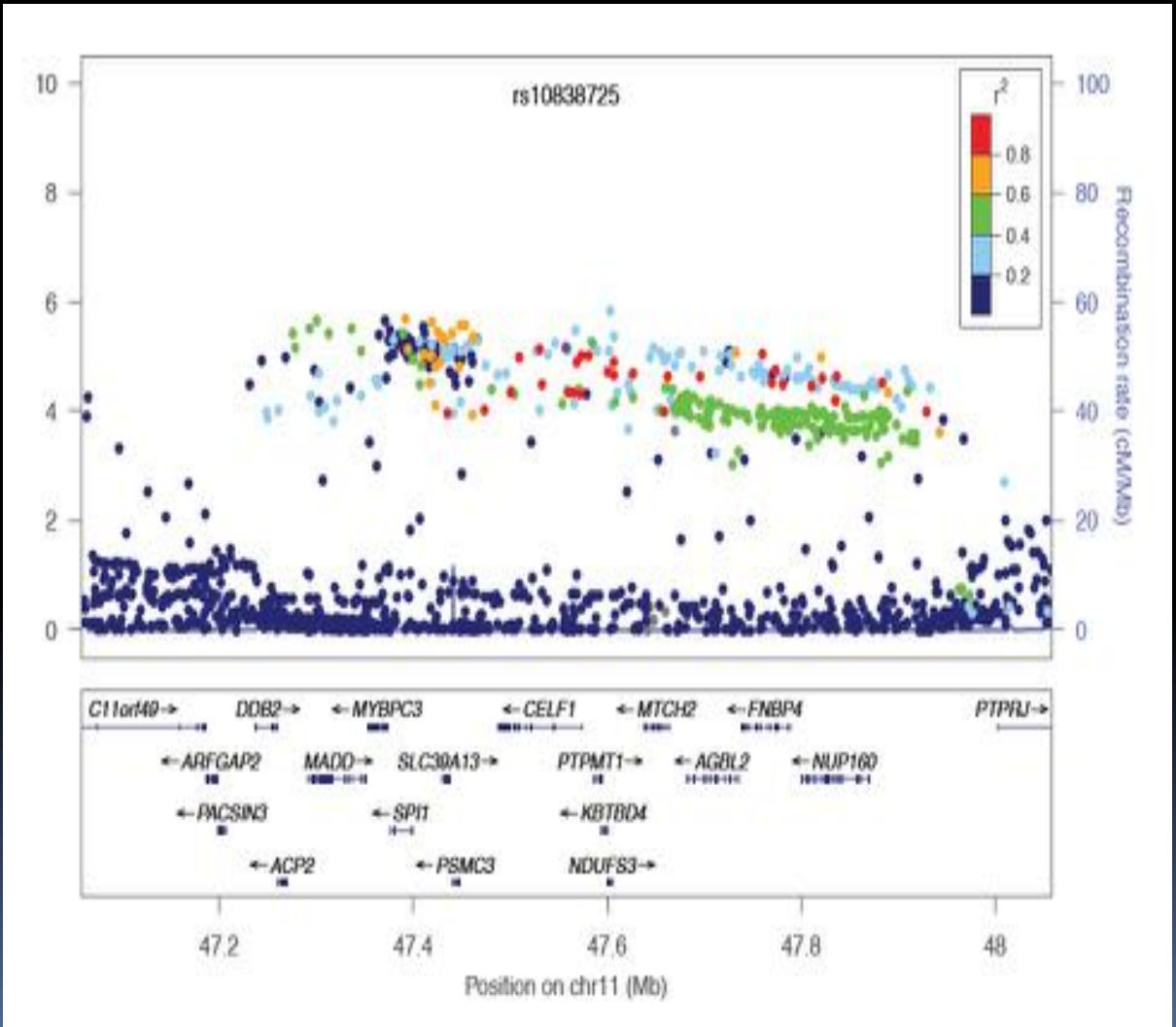
Shown are the 10 cell types that are enriched at P < 0.05 after Bonferroni correction

Annotation	Prop._SNPs	Prop._h2	Prop._h2_std_error	Enrichment	Enrichment_std_error	Enrichment_p	Coefficient	Coefficient_std_error	Coefficient_z-score
Hematopoietic:CD34_primary:H3K4me1	0.03	0.70	0.19	21.47	5.84	1.61E-06	2.09E-07	5.58E-08	3.74
Hematopoietic:Mobilized_CD34_primary:H3K4me1	0.07	0.85	0.21	12.26	3.03	2.21E-06	1.12E-07	3.15E-08	3.56
Hematopoietic:CD15_primary:H3K4me1	0.04	0.53	0.12	14.44	3.30	9.16E-07	1.11E-07	3.45E-08	3.23
Hematopoietic:Mobilized_CD34:H3K27ac	0.03	0.53	0.16	16.57	4.94	3.73E-05	1.32E-07	4.20E-08	3.14
Hematopoietic:CD14_primary:H3K4me1	0.04	0.64	0.15	16.06	3.80	1.93E-05	1.54E-07	4.92E-08	3.13
Hematopoietic:Spleen:H3K4me1	0.05	0.59	0.16	12.80	3.39	4.08E-05	1.23E-07	3.96E-08	3.11
Hematopoietic:Mobilized_CD34_primary:H3K4me3	0.02	0.55	0.15	24.43	6.61	1.52E-05	2.28E-07	7.57E-08	3.01
Hematopoietic:CD19_primary_(BI):H3K4me1	0.04	0.57	0.15	13.91	3.63	1.90E-06	1.02E-07	3.45E-08	2.96
Hematopoietic:CD15_primary:H3K4me3	0.01	0.33	0.09	23.24	6.70	1.43E-04	1.67E-07	7.39E-08	2.26
Hematopoietic:CD19_primary_(UW):H3K4me1	0.04	0.46	0.14	11.13	3.39	1.73E-04	6.45E-08	3.46E-08	1.86

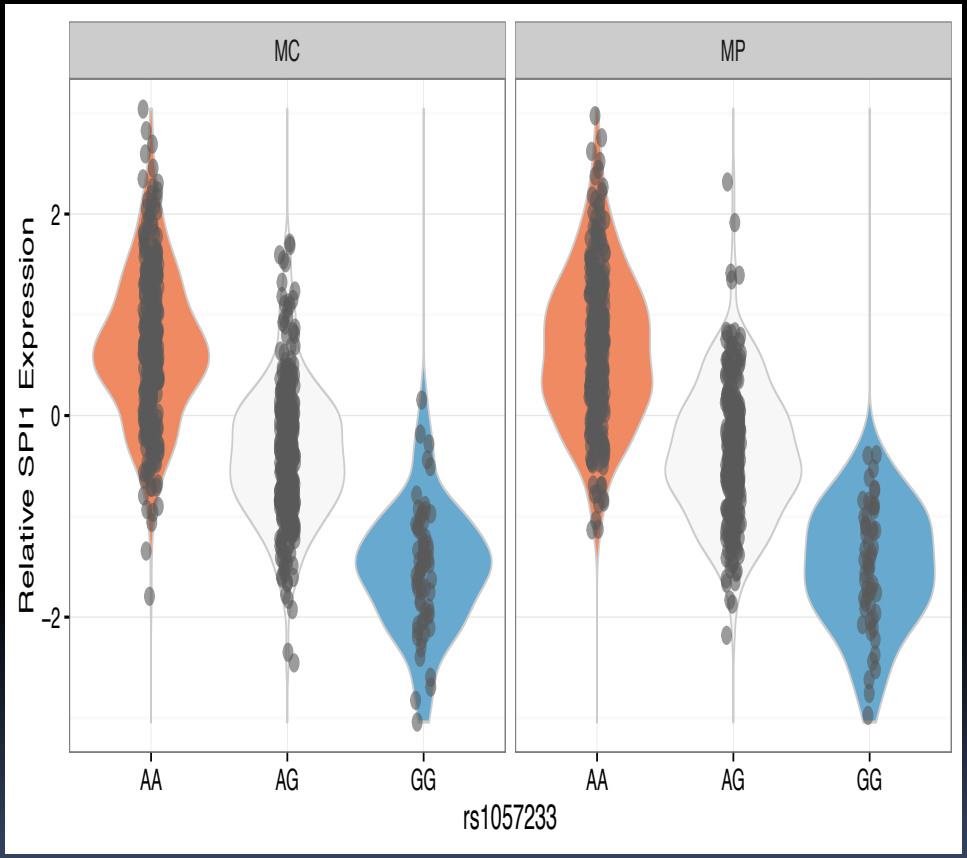
IGAP phase 1 SNP summary stats used as input

Huang et al., Nat. Neurosci. 20: 1052-1061, 2017

Some GWAS SNPs implicate many genes



Protective allele associated with lower *SPI1* expression in monocytes and macrophages



Huang et al., Nat. Neurosci. 20: 1052-1061, 2017

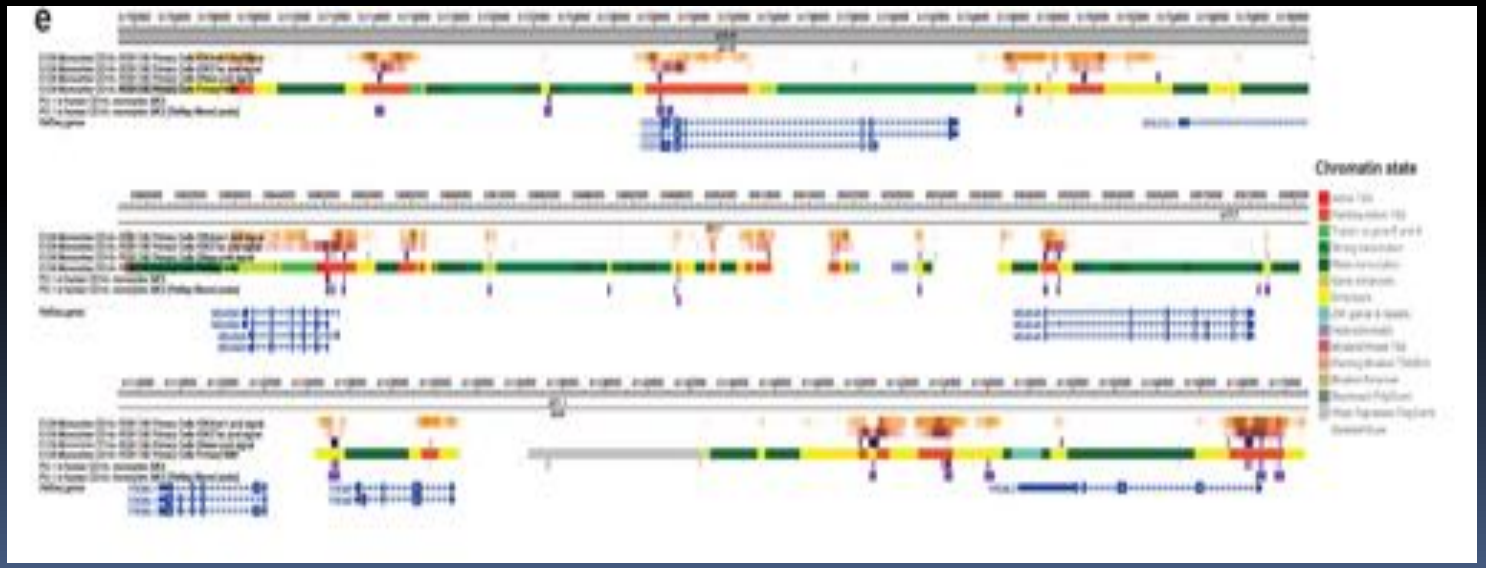
PU.1 cistrome enriched in AD GWAS loci

- LD score regression used to estimate enrichment of AD heritability across the entire PU.1 cistrome, using ChIP-Seq data in human peripheral blood monocytes and macrophages derived from them
  - Enrichment of AD heritability in both monocytes (56 fold enrichment, P = 0.003) and macrophages (60 fold enrichment, P = 0.001) but not schizophrenia heritability

Huang et al., Nat. Neurosci. 20: 1052-1061, 2017

PU.1 binds to the promoter and regulatory regions of 10/22 AD GWAS risk loci in CD14+ monocytes

Functional PU.1 binding motifs in multiple AD loci including INPP5D, MS4A4A, MS4A6A, TREML2, TREM2, PILRB, PICALM, CD33, TRIP4 and TYROBP



Huang et al., Nat. Neurosci. 20: 1052-1061, 2017

Variants that reduce risk for AD

- Common Variants
  - *APOE2* decreases risk for AD by 50%
  - Some GWAS loci reduce risk by 10-15%
    - *CLU, PICALM, MS4A6A, CD33, EPHA1, SORL1, SLC24A/RIN3, DSG2, MEF2C, NME8, ZCWPW1, CASS4, TREML2 S144G*

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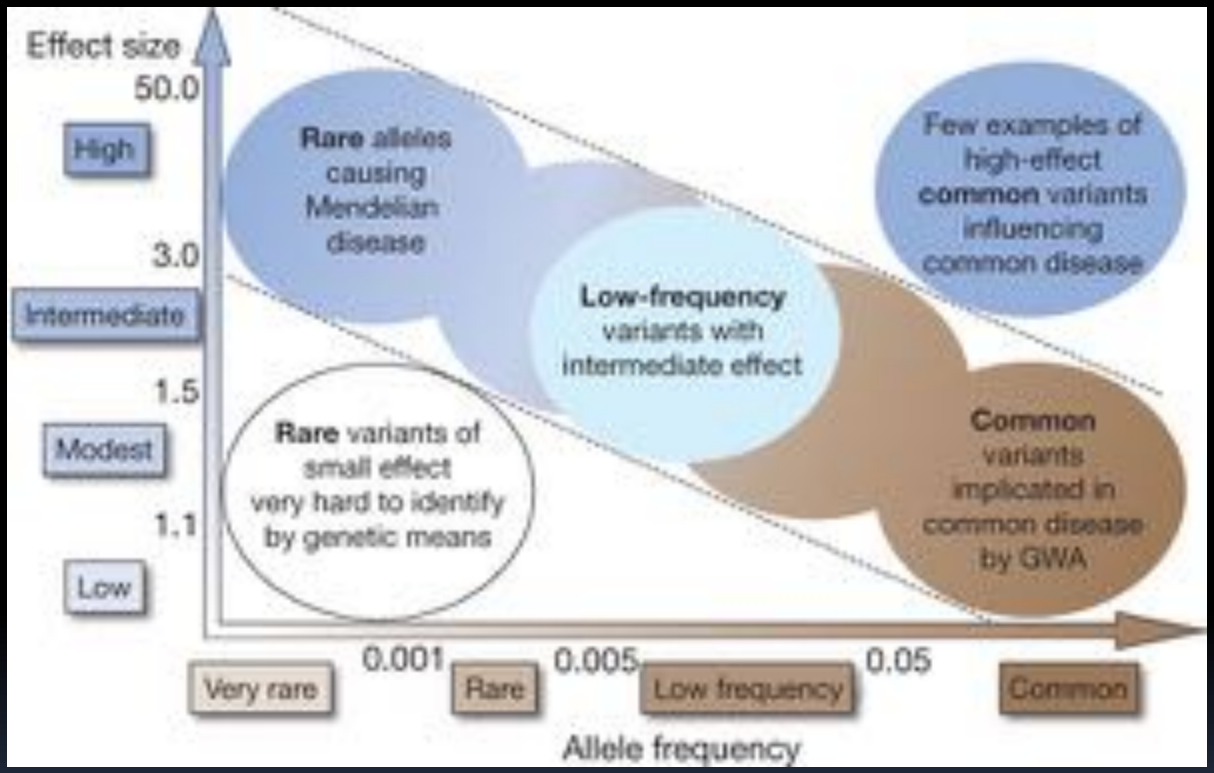


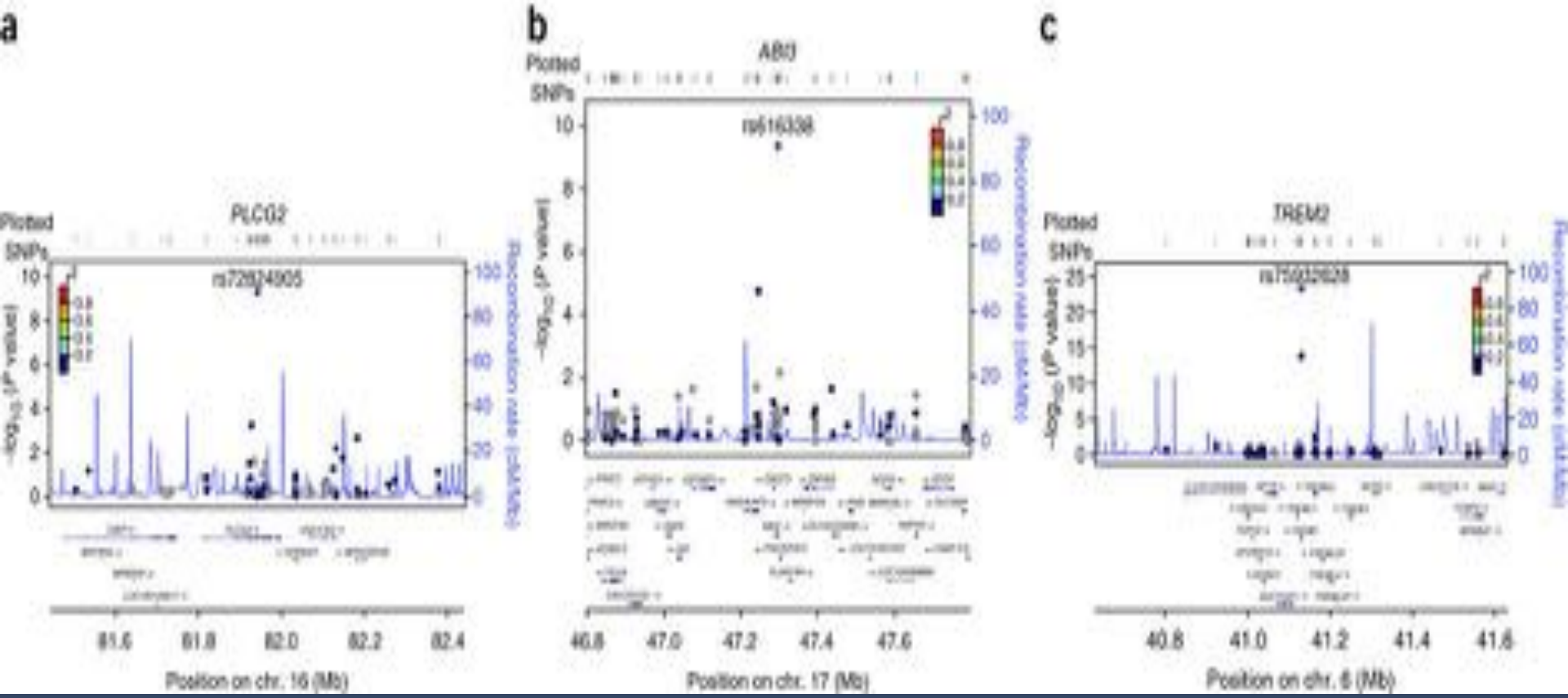
TA Manolio *et al. Nature* 461, 747-753 (2009) doi:10.1038/nature08494

nature

Identification of low frequency and rare variants of moderate effect size

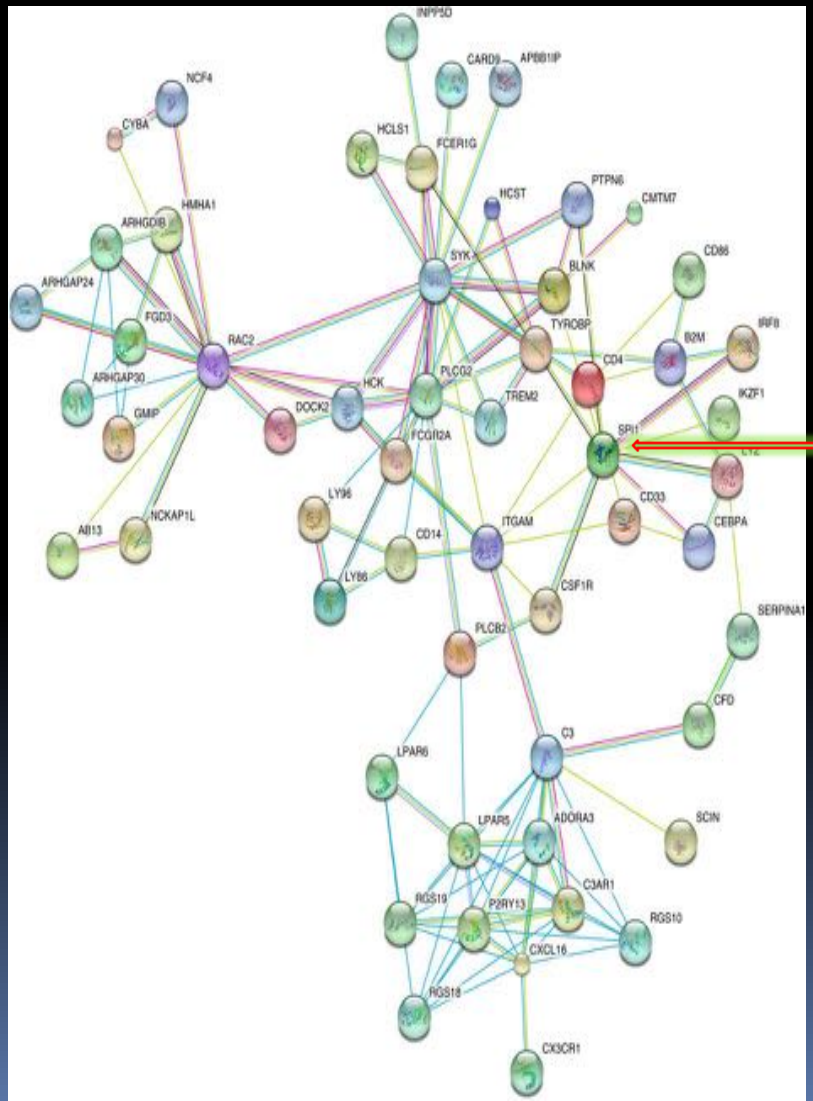
- Whole Genome Sequencing (WGS)
- Whole Exome Sequencing (WES)
- Targeted Sequencing
- RNA Sequencing (RNAseq)
  - Performed in unrelated individuals or families
  - Likely have bigger impact on risk

TREM2, ABI3 and *PLCG2* associated with AD risk



Sims et al., Nature Genetics 2017

Microglial Protein Interaction Network Contains AD risk genes

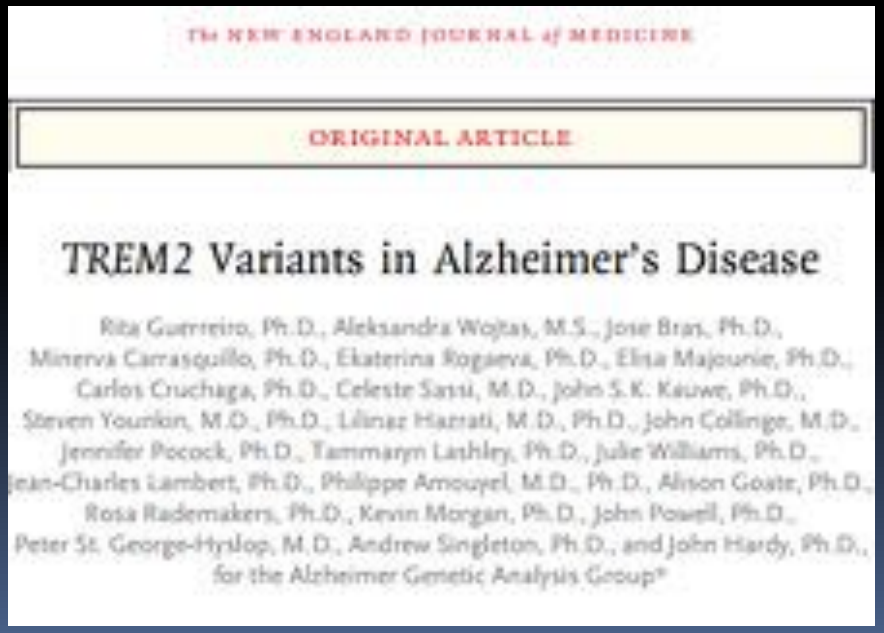


Sims et al., Nat. Genet. 2017

Many genes likely to carry rare variants associated with increased AD risk

- ADAM10 (Kim et al., 2009)
- TREM2 (Jonsson et al., 2013; Guerreiro et al., 2013)
- PLD3 (Cruchaga et al., 2014)
- AKAP9 (Logue et al., 2014)
- UN5C (Wetzel-Smith et al., 2014)
- SORL1 (Pottier et al. Vardarajan et al., 2015; Sleegers et al., AAIC2015)
- ABCA7 (Steinberg et al., 2015; Cuyvers et al. 2015)

Sequencing identifies rare variant in triggering receptor expressed on myeloid cells 2 (*TREM2*) as risk factor for AD



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

TREM2 Variants in Alzheimer's Disease

Rita Guerreiro, Ph.D., Aleksandra Wojtas, M.S., Jose Bras, Ph.D., Minerva Carrasquillo, Ph.D., Ekaterina Rogaeva, Ph.D., Elisa Majounie, Ph.D., Carlos Cruchaga, Ph.D., Celeste Sassi, M.D., John S.K. Kauwe, Ph.D., Steven Younkin, M.D., Ph.D., Lilinaz Hazrati, M.D., Ph.D., John Collinge, M.D., Jennifer Pocock, Ph.D., Tammaryn Lashley, Ph.D., Julie Williams, Ph.D., Jean-Charles Lambert, Ph.D., Philippe Amouyel, M.D., Ph.D., Alison Goate, Ph.D., Rosa Rademakers, Ph.D., Kevin Morgan, Ph.D., John Powell, Ph.D., Peter St. George-Hyslop, M.D., Andrew Singleton, Ph.D., and John Hardy, Ph.D., for the Alzheimer Genetic Analysis Group*

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Variant of *TREM2* Associated with the Risk of Alzheimer's Disease

Thorlakur Jonsson, Ph.D., Hreinn Stefansson, Ph.D., Stacy Steinberg Ph.D., Ingileif Jonsdottir, Ph.D., Palmi V. Jonsson, M.D., Jon Snaedal, M.D., Sigurbjorn Bjornsson, M.D., Johanna Huttenlocher, B.S., Allan I. Levey, M.D., Ph.D., James J. Lah, M.D., Ph.D., Dan Rujescu, M.D., Harald Hampel, M.D., Ina Giegling, Ph.D., Ole A. Andreassen, M.D., Ph.D., Knut Engedal, M.D., Ph.D., Ingun Ulstein, M.D., Ph.D., Srdjan Djurovic, Ph.D., Carla Ibrahim-Verbaas, M.D., Albert Hofman, M.D., Ph.D., M. Arfan Ikram, M.D., Ph.D., Cornelia M van Duijn, Ph.D., Unnur Thorsteinsdottir, Ph.D., Augustine Kong, Ph.D., and Kari Stefansson, M.D., Ph.D.

TREM2 variants associated with AD risk

TREM2-gene-based analysis

Jin et al., 2014; Jin et al., 2015

Resequencing 2,082 cases and 1,648 controls (European Americans)

All variants	→	**P = 5.37×10^{-7} OR = 2.55 (1.80-3.67)**
Excluding R47H	→	**P = 7.72×10^{-5} OR = 2.47 (1.62-3.87)**
Excluding R47H and R62H	→	**P = 0.09 OR = 2.95 (1.23-8.09)**

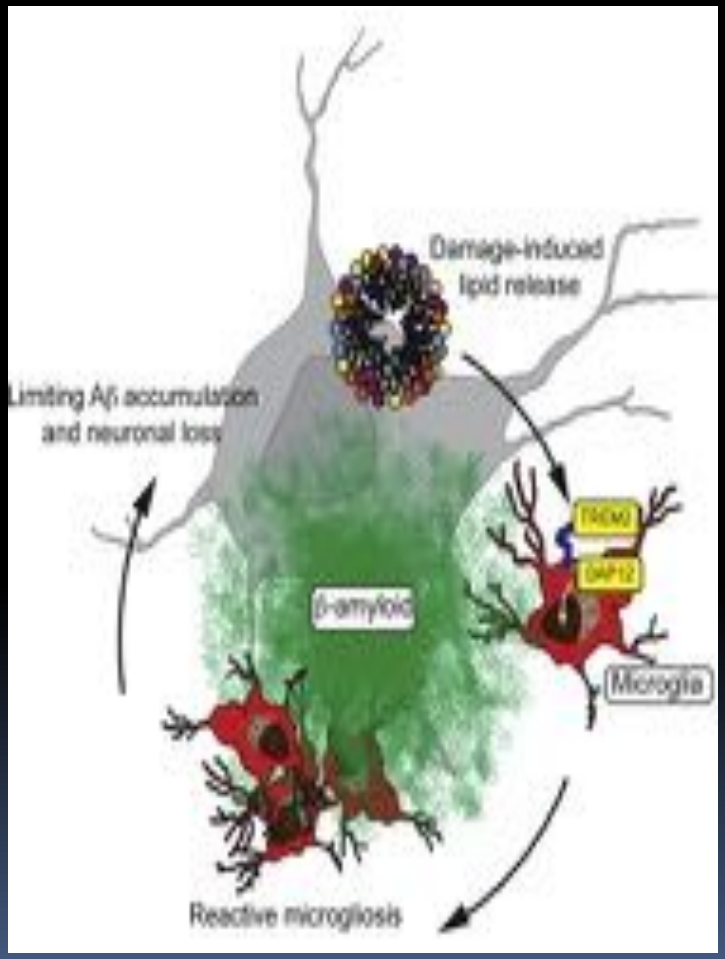
Resequencing 412 cases and 139 controls followed by direct genotyping six variants in 1,058 cases and 2,738 controls (African Americans)

R47H, R62H, D87N, E151K, W191X, L211P	→	**P = 0.02 OR = 1.22 (1.01-1.47)**

TREM2 and disease

- Recessive loss of function mutations in *TREM2* cause Nasu-Hakola disease
  - Bone cysts and fractures
  - Personality changes and dementia
  - White matter disease, no plaques and tangles
  - Death in forties
- Rare heterozygous mutations (R47H, R62H) associated with 2-3 fold increase in risk for late onset AD

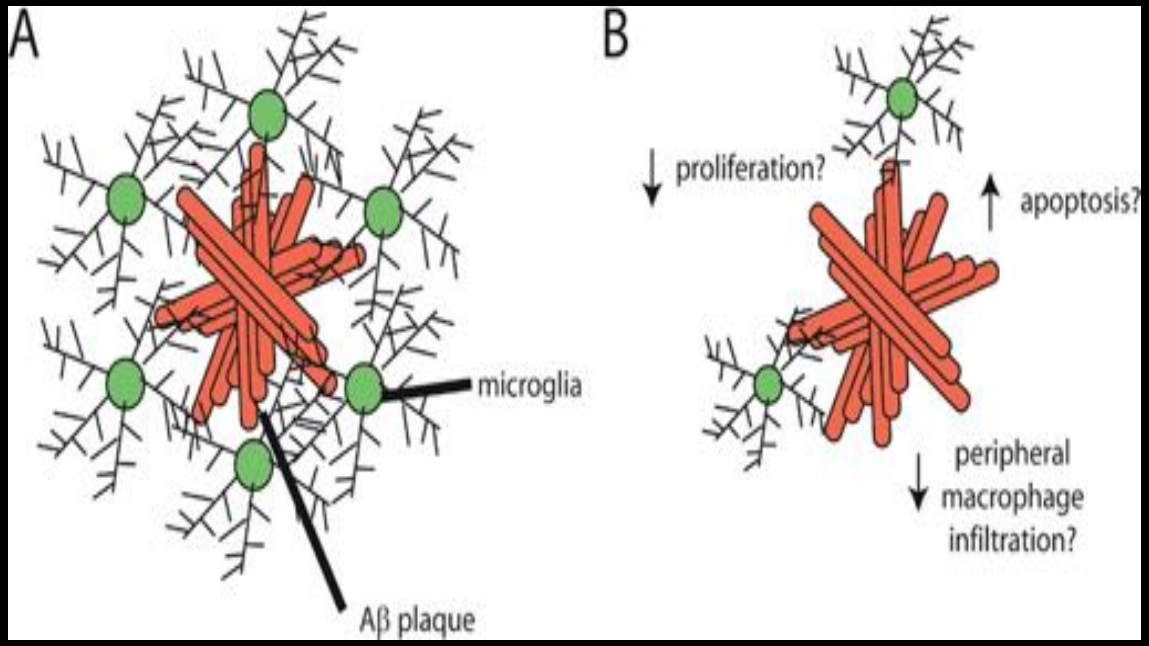
Role of *TREM2* in Neurodegeneration



Wang et al., Cell 160: 1061-71 (2015)

- TREM2 senses lipids exposed after membrane damage
- TREM2 is a receptor for APOE containing lipoproteins
- Activation of TREM2 triggers protein tyrosine phosphorylation through SYK
- AD risk variants thought to be partial loss of function

TREM2 is critical for Aβ-associated microgliosis



(A) Microglia cluster around Aβ plaque deposits in humans and mouse models of Aβ deposition.
(B) TREM2 deficiency markedly impairs microgliosis around Aβ plaques.

Published in: Jason D. Ulrich; David M. Holtzman; *ACS Chem. Neurosci.* **2016,** 7, 420-427.
DOI: 10.1021/acschemneuro.5b00313

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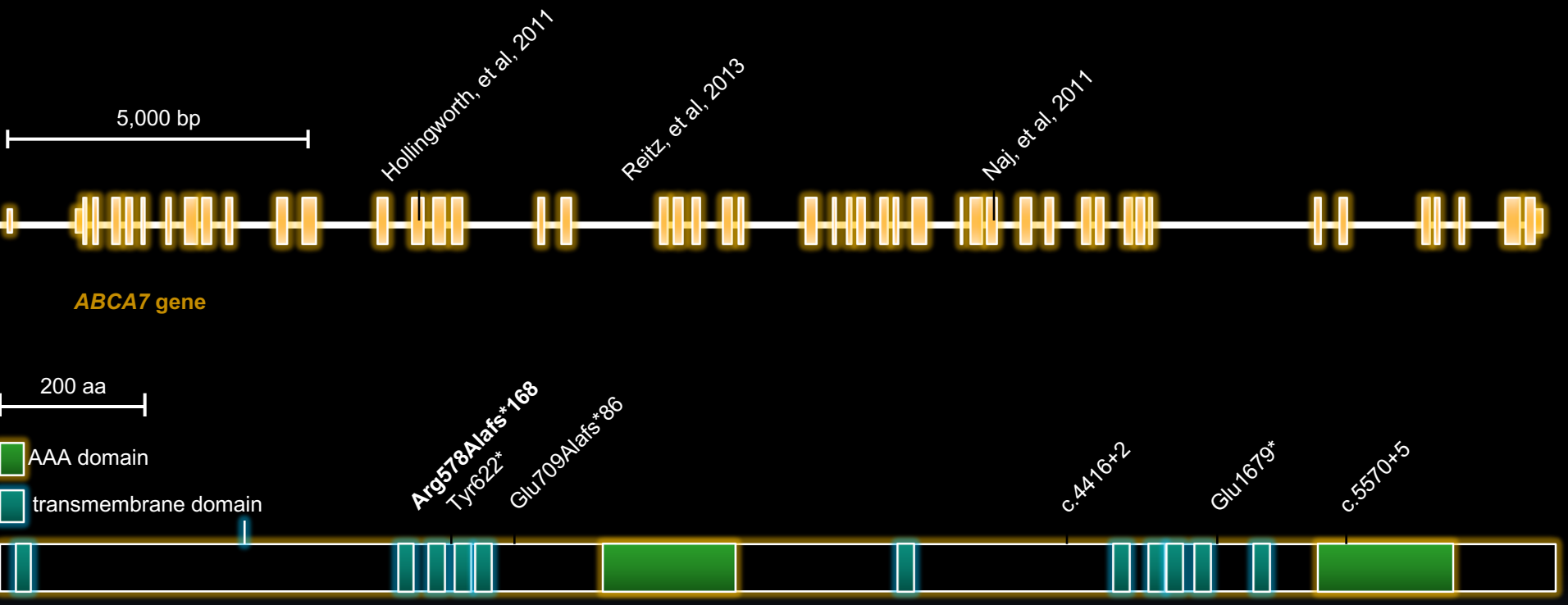
- SORL1 (Pottier et al. 2012; Vardarajan et al., 2015; Sleegers et al., AAIC2015)
- ABCA7 (Steinberg et al., 2015; Cuyvers et al. 2015)

ABCA7 Implicated as an AD risk factor by GWAS

- European populations
  - Hollingworth et al 2011, Naj et al 2011, Lambert et al 2013
- African American population
  - Reitz et al. 2013
- Odds ratio for risk higher in African Americans than Europeans

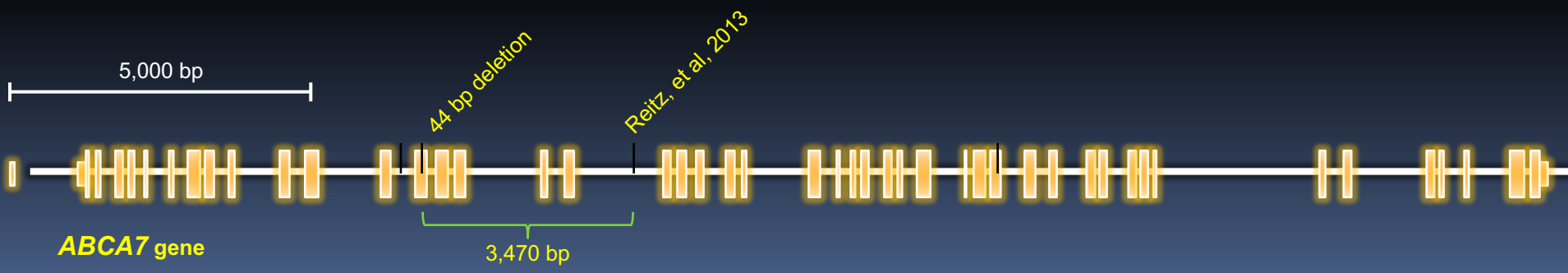
Loss of function variants in *ABCA7* increase risk for AD



Steinberg, et al, 2015, Vardarajan, et al, 2015, Cuyvers, et al, 2015, Cukier et al., submitted, Del Aguila et al., submitted

ABCA7 deletion increases AD risk in African American cohorts

HIHG	samples	deletion frequency	odds ratio	95% CI	Pr > |Z|
cases	531	16.20%	2.13	1.42-3.20	0.0002
controls (>65)	527	9.30%			
ADGC					
cases	447	14.90%	1.65	1.12-2.44	0.0117
controls (>65)	880	10.00%			
Joint Analysis					
cases	978	15.20%	1.81	1.38-2.37	1.41×10^{-5}
controls (>65)	1407	9.74%			



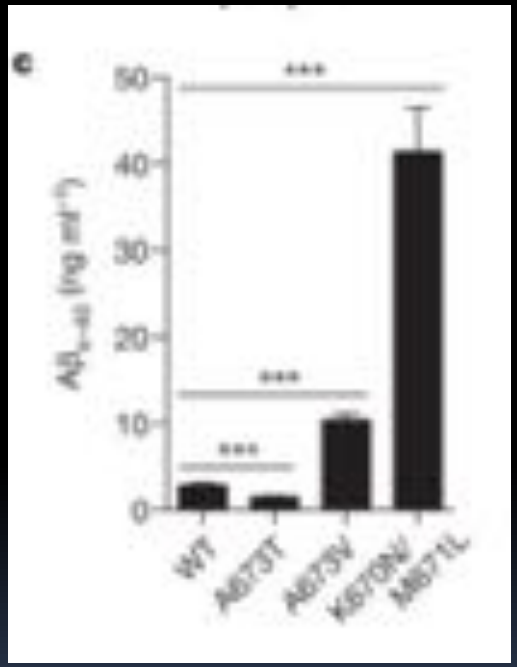
Cukier, Kunkle, Vardarajan, et al. (submitted) presented at AAIC2015

Whole Exome and Whole Genome Sequencing in AD

- The NIA/NHGRI AD sequencing project (ADSP)
  - Whole Exome Sequencing
    - 5000 AD cases
    - 5000 nondemented elderly controls
    - 685 unrelated cases from LOAD families
  - Whole Genome Sequencing
    - 584 individuals from 100 LOAD families
- Other cohorts with WES or WGS
  - ADNI
  - NIMH LOAD families
  - NIA LOAD families
  - Various European cohorts

APPA673T protects against AD by reducing BACE cleavage of APP

Analysis	Odds Ratio	P value	Freq (%)
AD			0.13
AD vs population controls	0.24	4.2x10-5	0.45
AD vs controls >85yrs	0.19	4.8x10-7	0.62
AD vs cognitively intact >85yrs	0.13	6.9x10-6	0.79



From Jonsson et al., Nature (2012) 488:96-99

Summary

- More than 20 risk loci identified by GWAS
- Several pathways are enriched for GWAS loci including immune response, lipid metabolism and endocytosis
- Expression of 1/3 of GWAS genes enriched in monocytes and microglia
- Many risk/protective genes have already been identified by WES and WGS
- Different variants found in different populations making replication tougher
- A more complete atlas of these risk alleles will identify the key dysregulated networks leading to AD providing a new generation of drug targets for treatment

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NIALOAD investigators

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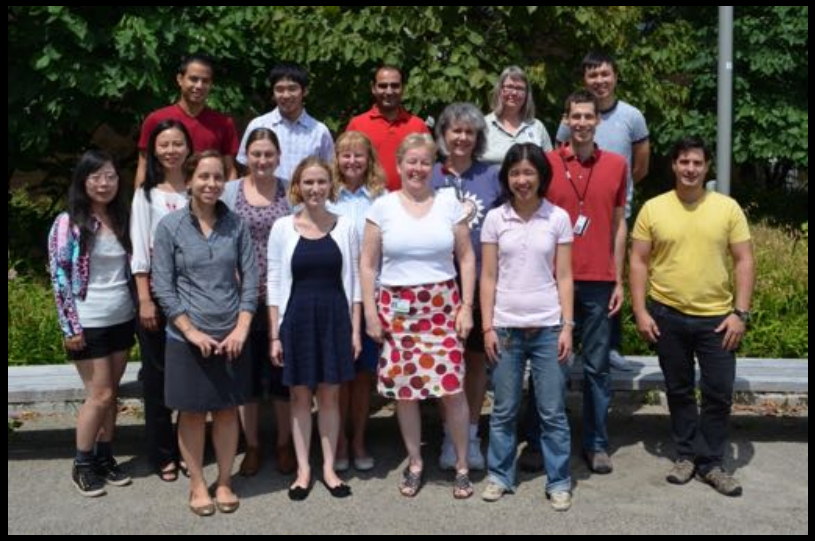

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IGAP
INTERNATIONAL
GENOMICS OF ALZHEIMER'S PROJECT

Thank you to the many families who have made this genetics research possible over the last 4 decades


STEVE ETHERINGTON

Carol Jennings and her children Emily and John who have been involved in research into Alzheimer's disease

"It's marvellous. In future it may be possible to prevent the disorder happening. While it will not help me. I hope it will help my children" Carol Jennings, The Times, February 16th, 1991