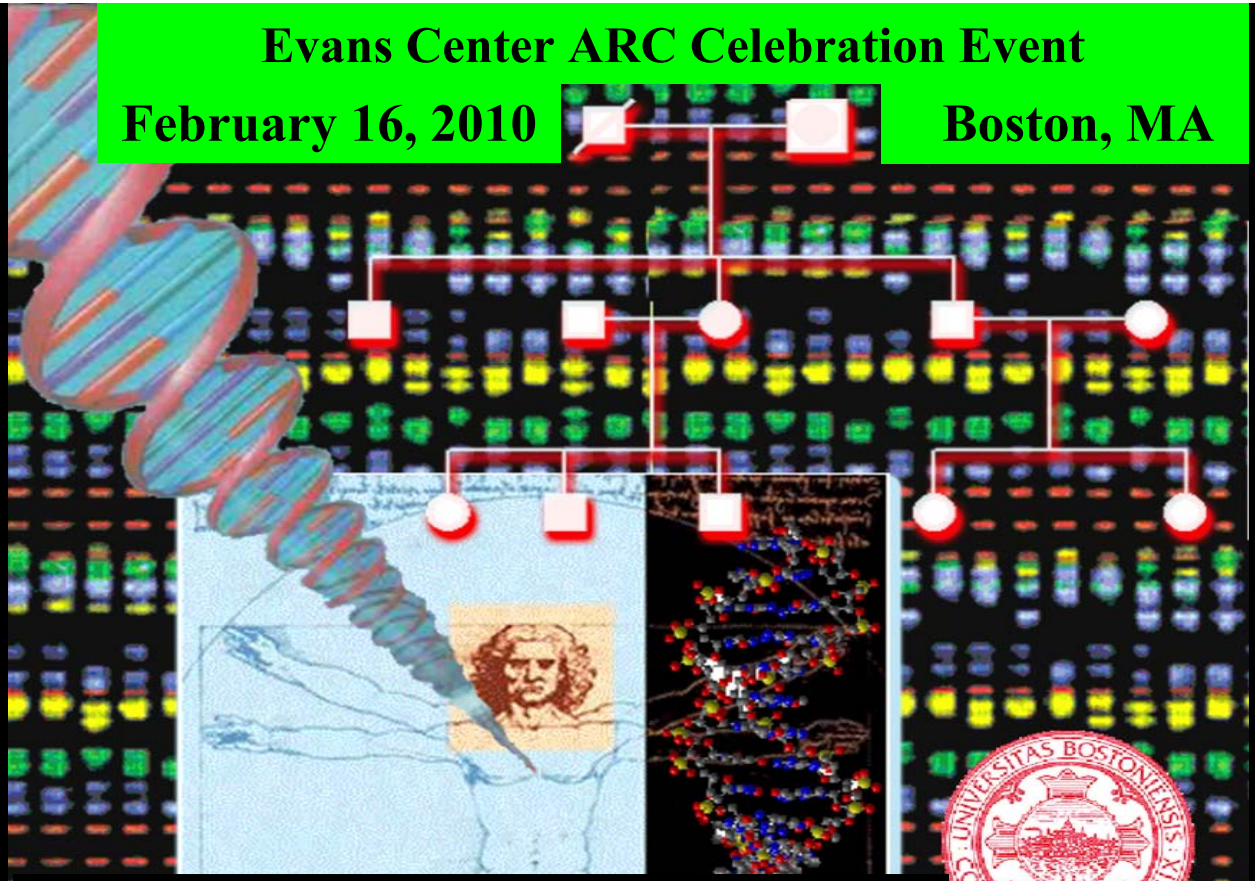
Evans Center ARC Celebration Event

February 16, 2010 Boston, MA

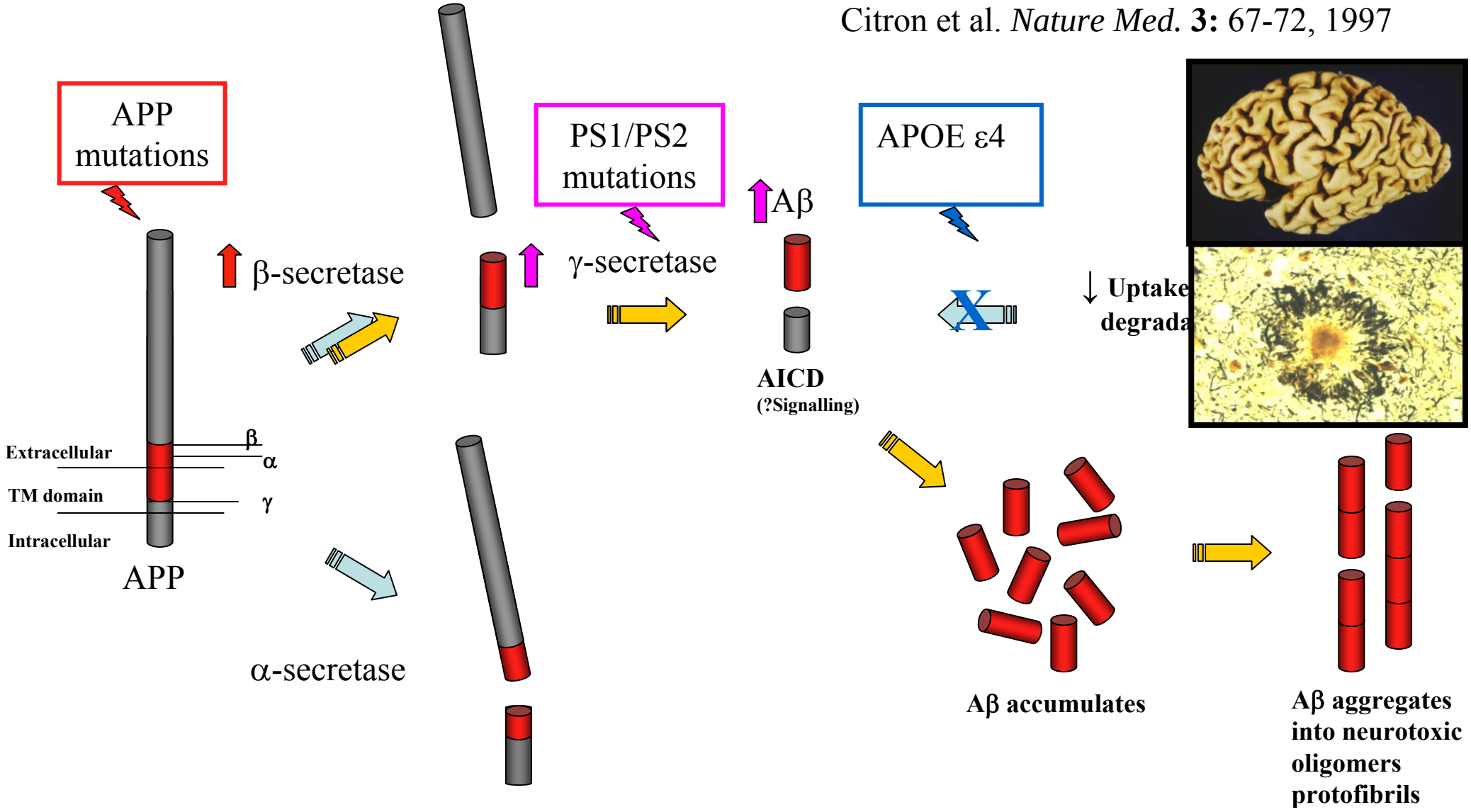


Protein Trafficking and Neurodegenerative Diseases: Alzheimer Disease as a First Model

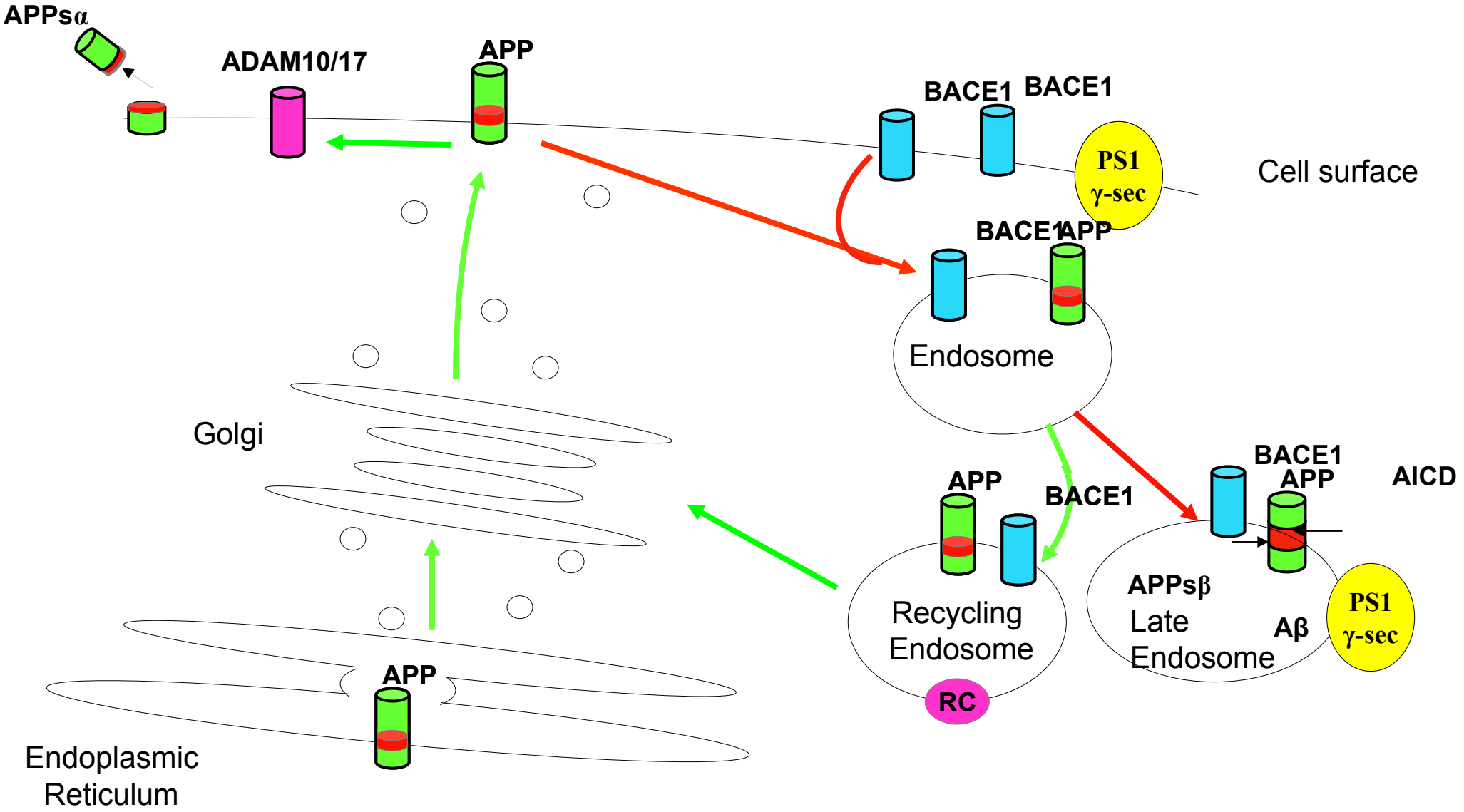
Lindsay A. Farrer
Genetics Program
Boston University School of Medicine

Mutations Causing Alzheimer Disease cause mis-processing of APP

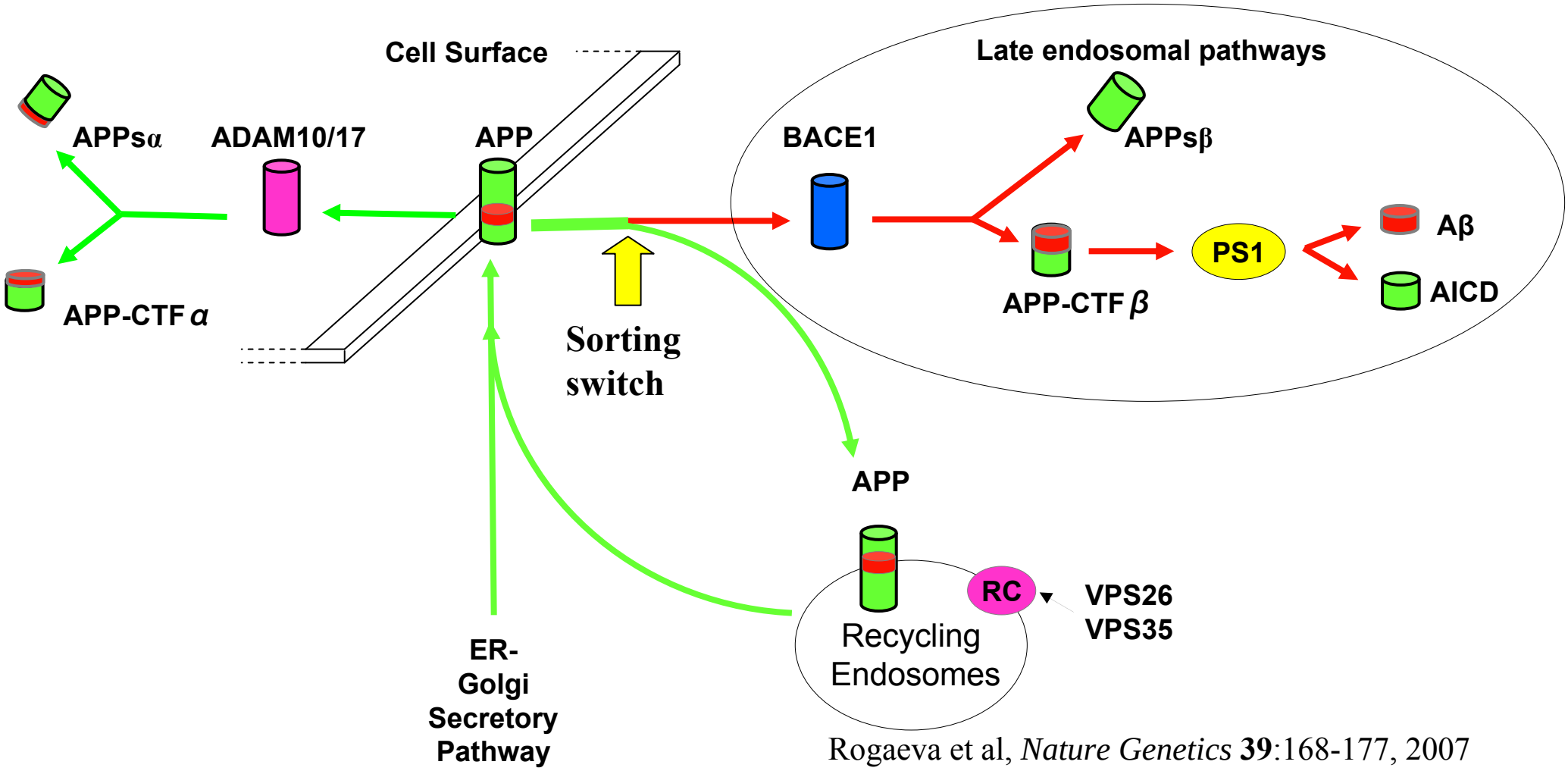
Citron et al. *Nature Med.* **3:** 67-72, 1997



APP is cleaved at sites which require subcellular trafficking of APP



Generation of Aβ requires trafficking into selected subcellular compartments



Rogaeva et al, *Nature Genetics* **39**:168-177, 2007

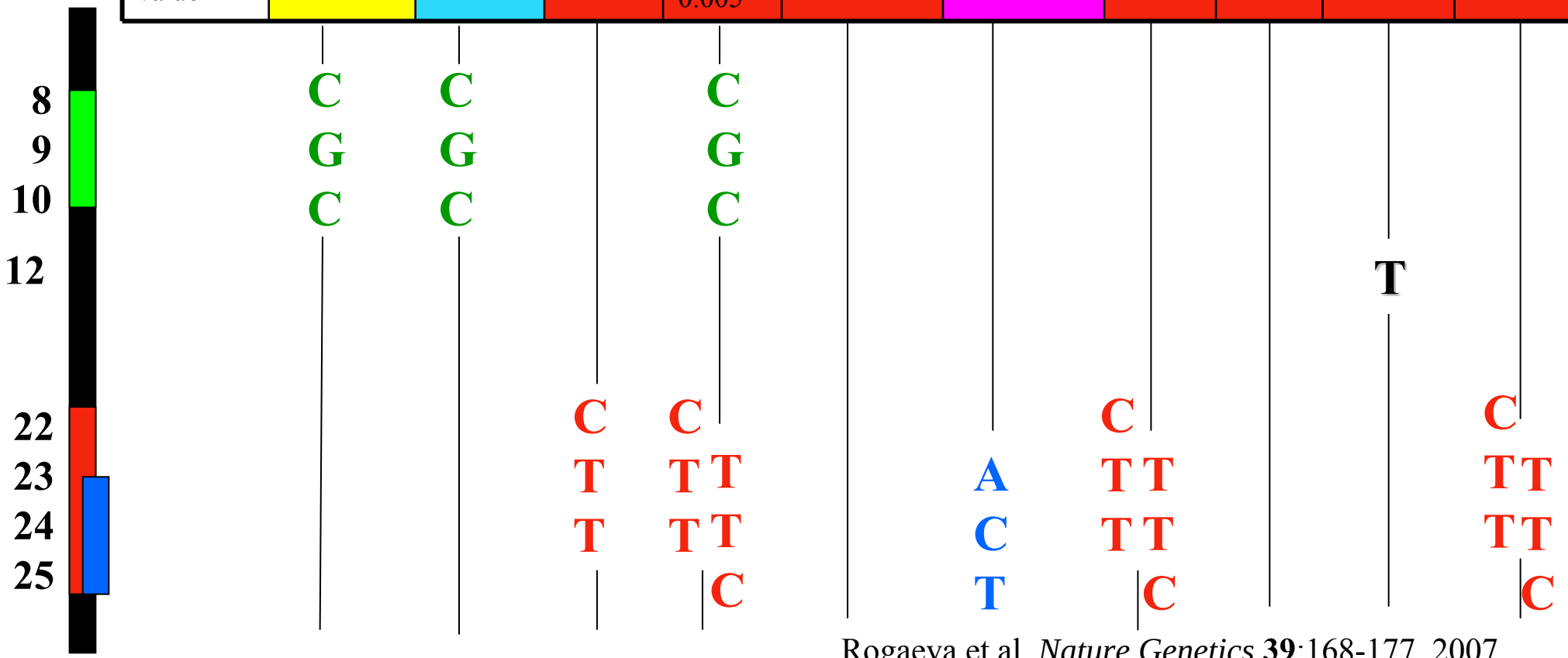
nature genetics 2007; 39:168-177

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

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

Ethnic origin → = Hispanic = Arab = Caucasian = Afro-American

	Carib Hisp FAD **	Israeli Arab	NE FAD **	NE spAD	MIRAGE Caucasian	MIRAGE African American	Mayo Jack	Mayo Roch	Mayo Aut	All Cauc
# AD samples	605	111	321	178	279	244	549	433	423	1583
Haplo p-value	0.005	0.0085	0.005	0.045/ 0.005	-	0.0025	<0.003	-	0.003	<0.02



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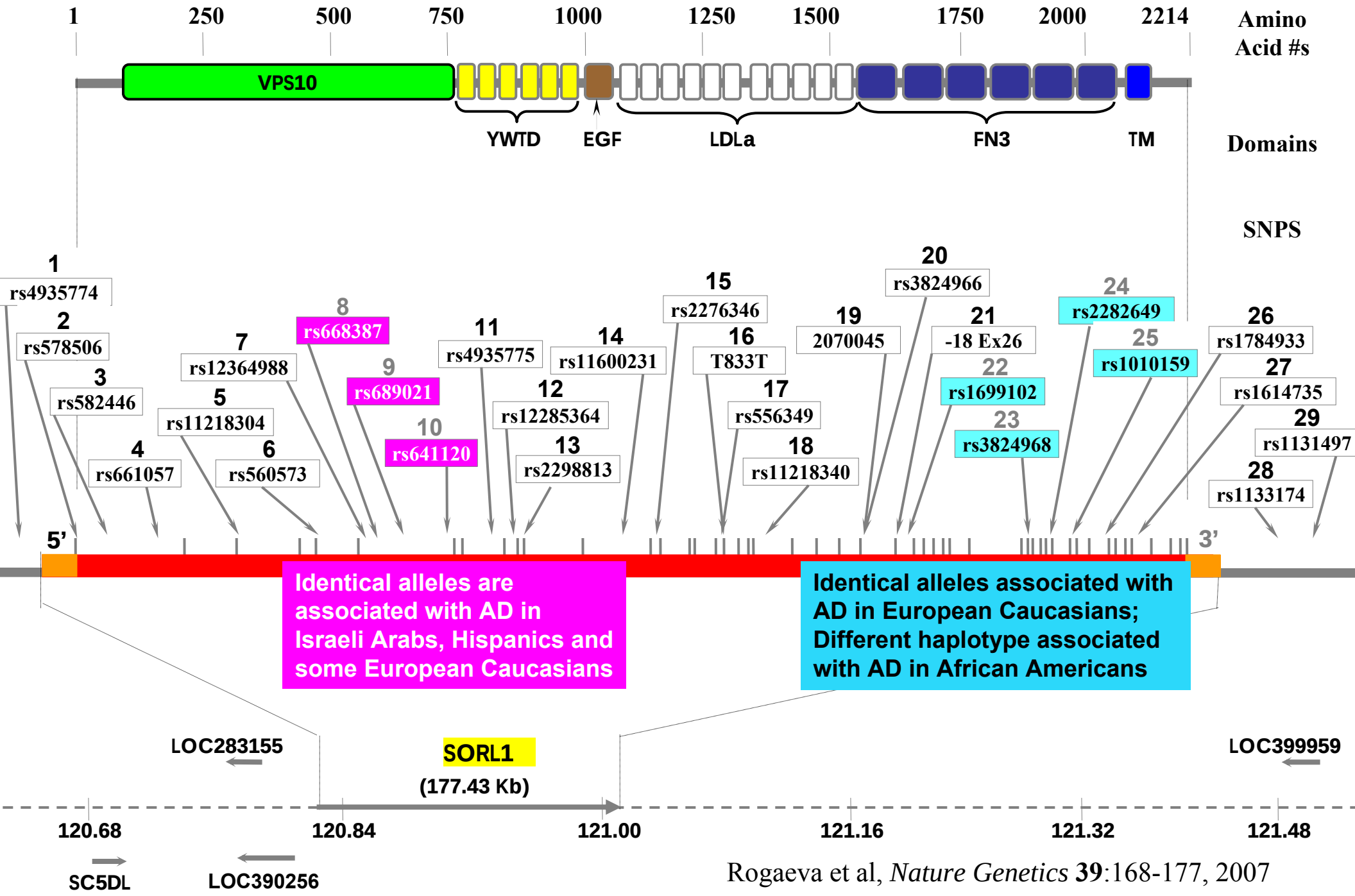
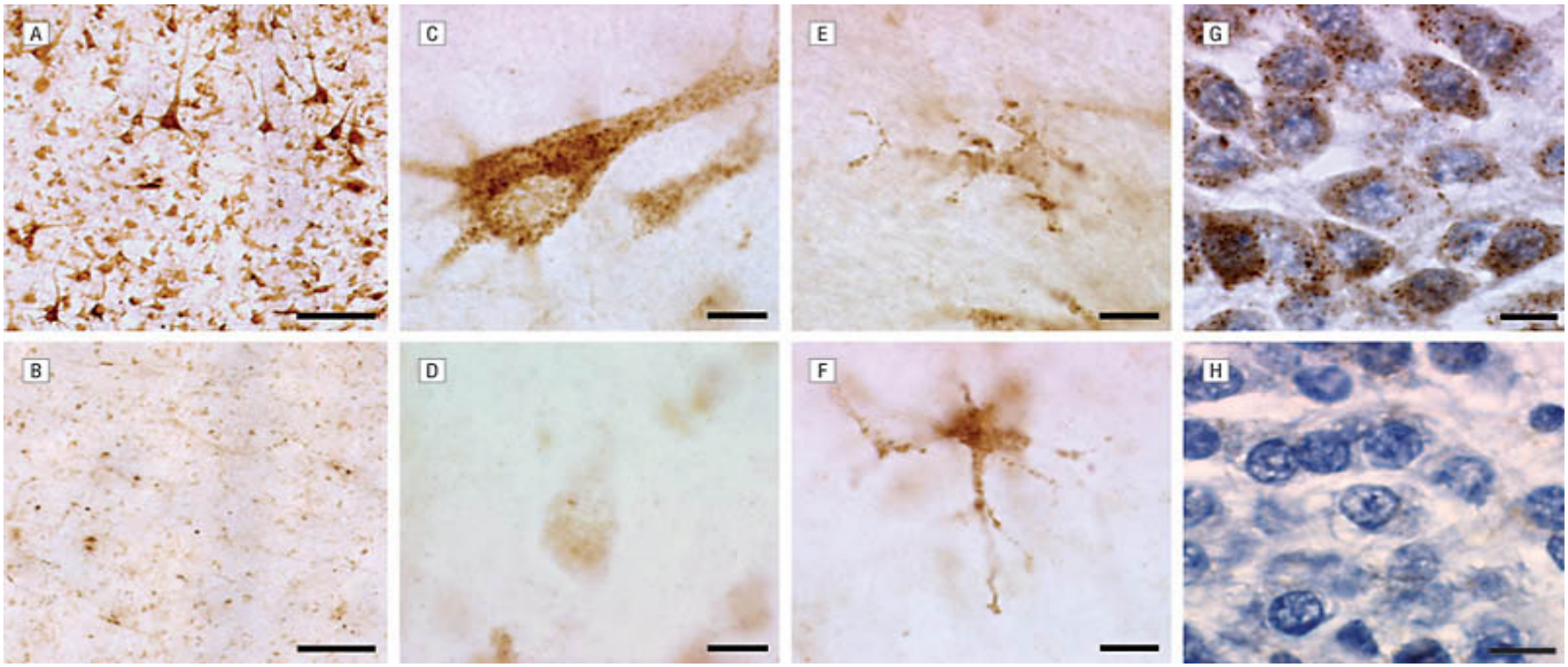


Rogaeva et al, *Nature Genetics* **39**:168-177, 2007

SORL1 is reduced specifically in cortical neurons in late onset AD



neocortex (bar =100µm)

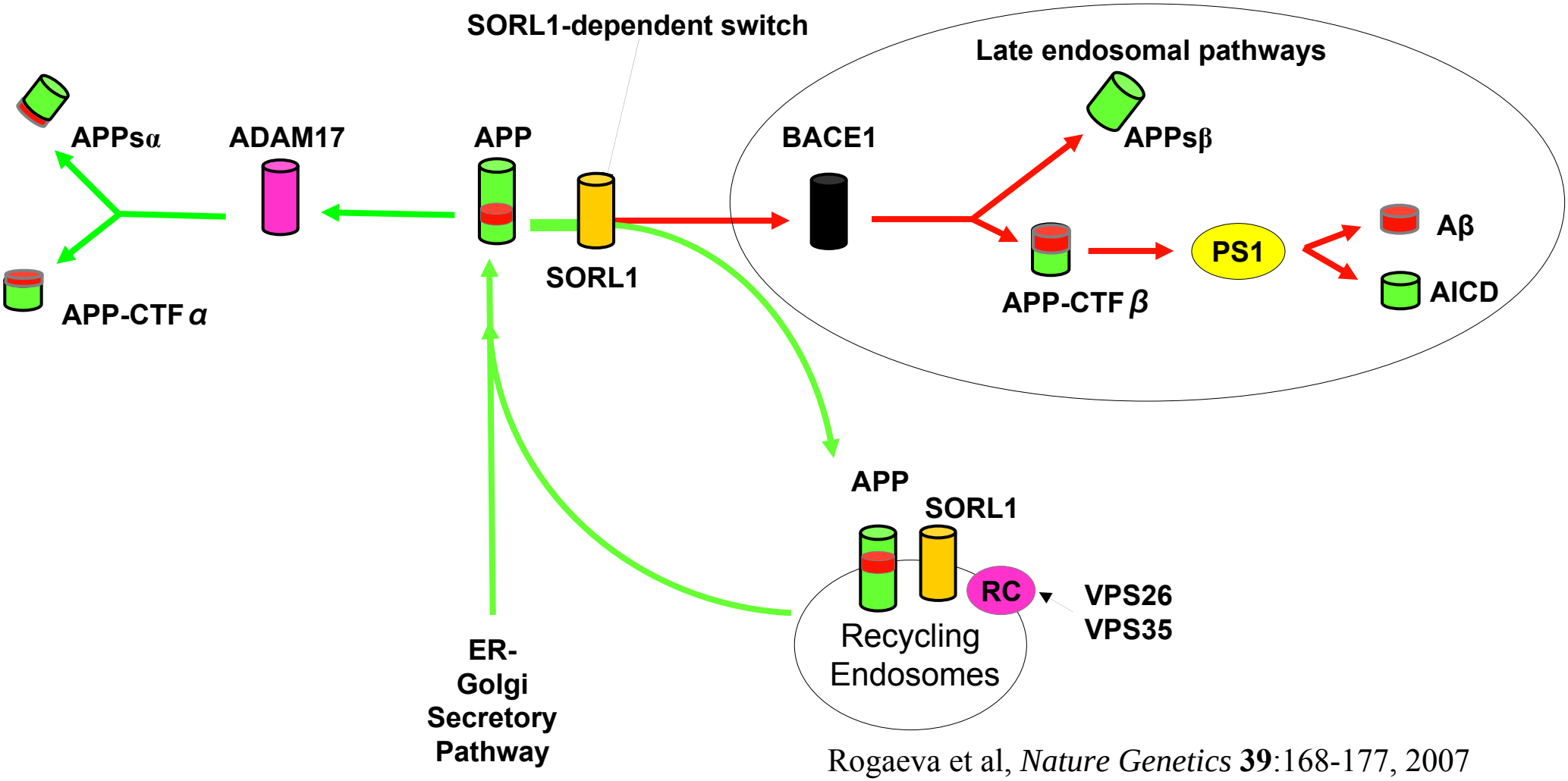
neocortical pyramidal neuron (bar =10µm)

neocortical astrocyte (bar =10µm)

dendate granule neuron (bar =10µm)

Scherzer, C. R. et al. *Arch Neurol* 61, 1200-1205, 2004.

SORL1 is a sorting receptor for APP



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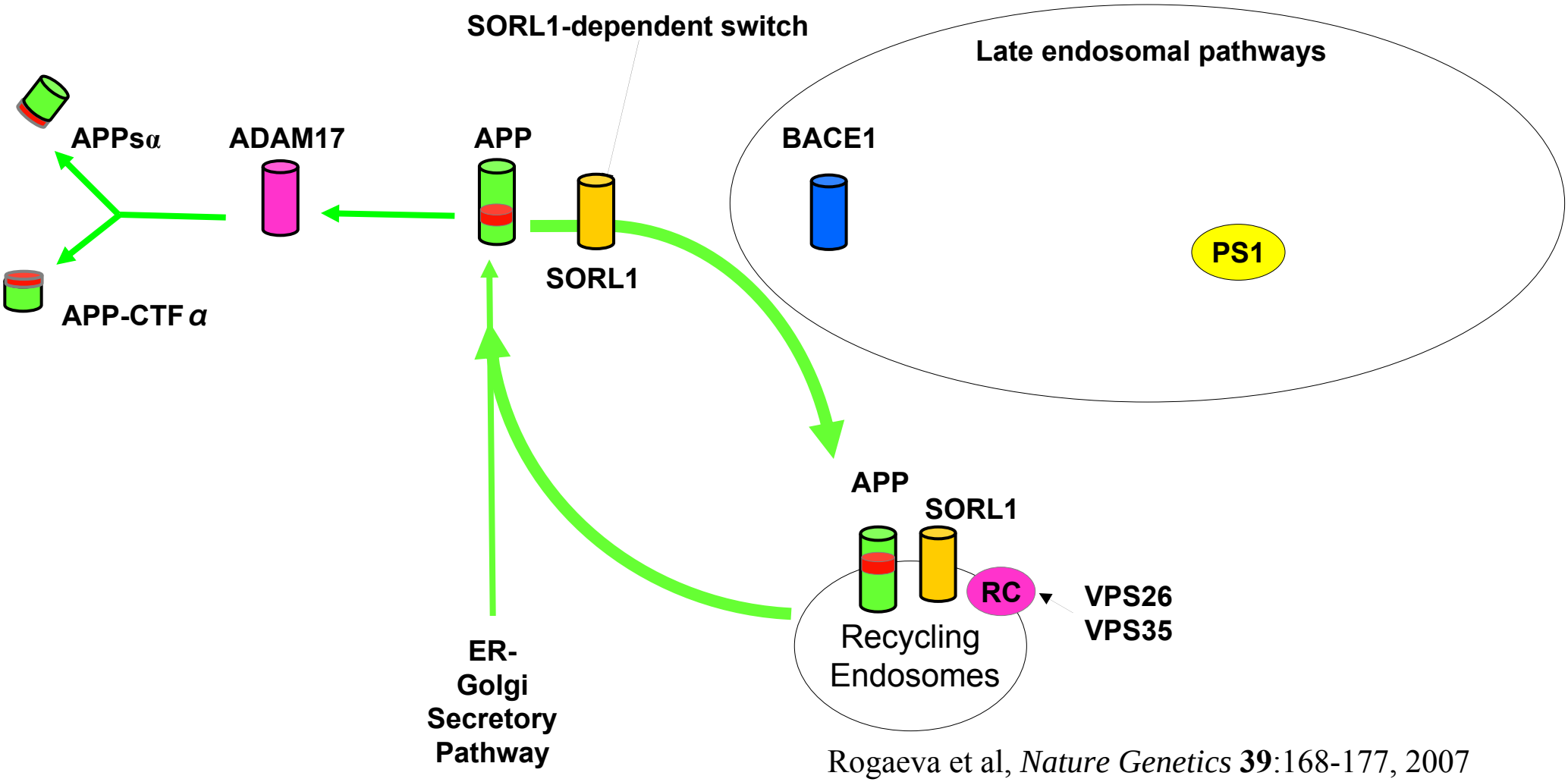

Rogaeva et al, *Nature Genetics* **39**:168-177, 2007

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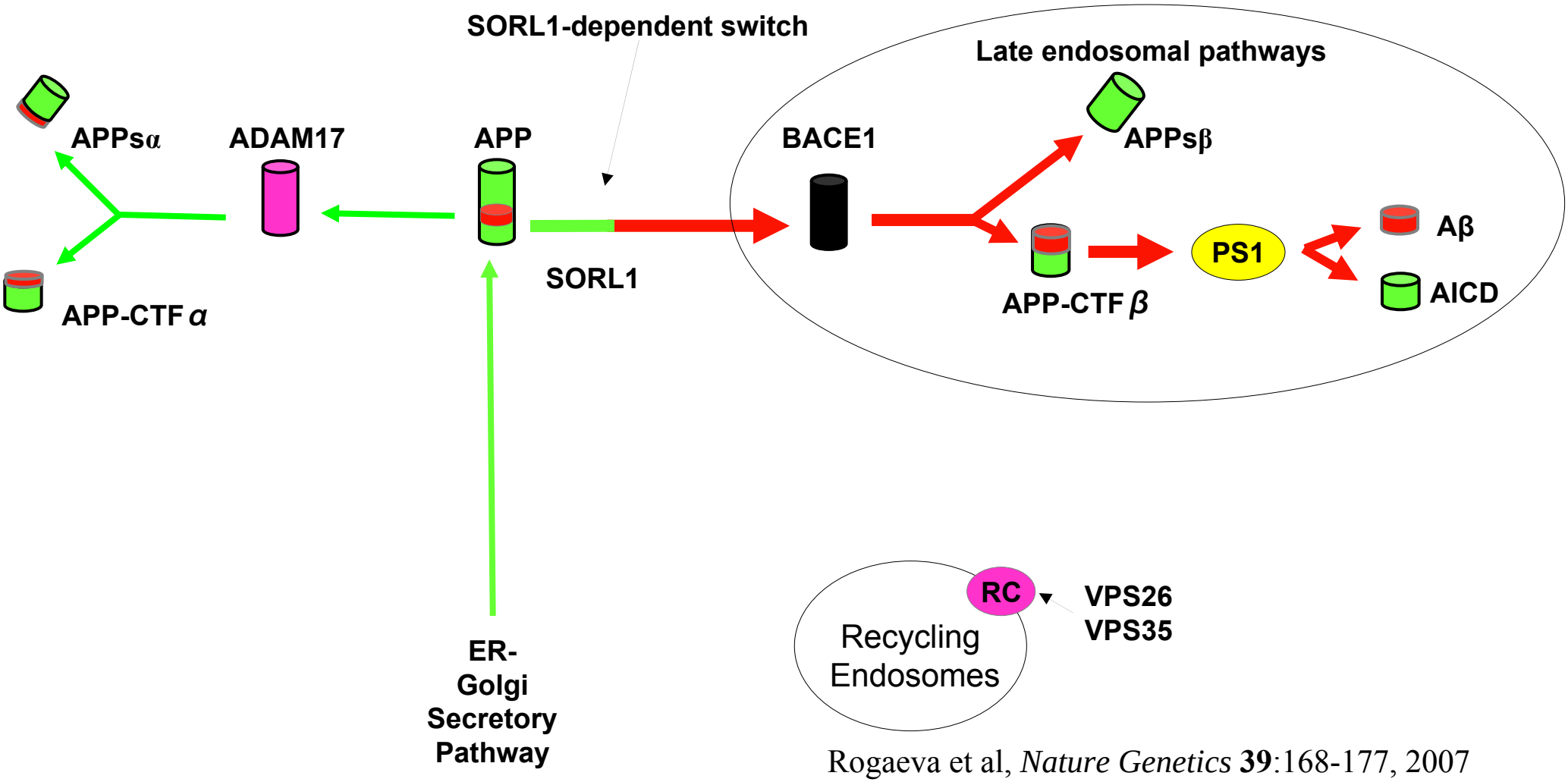
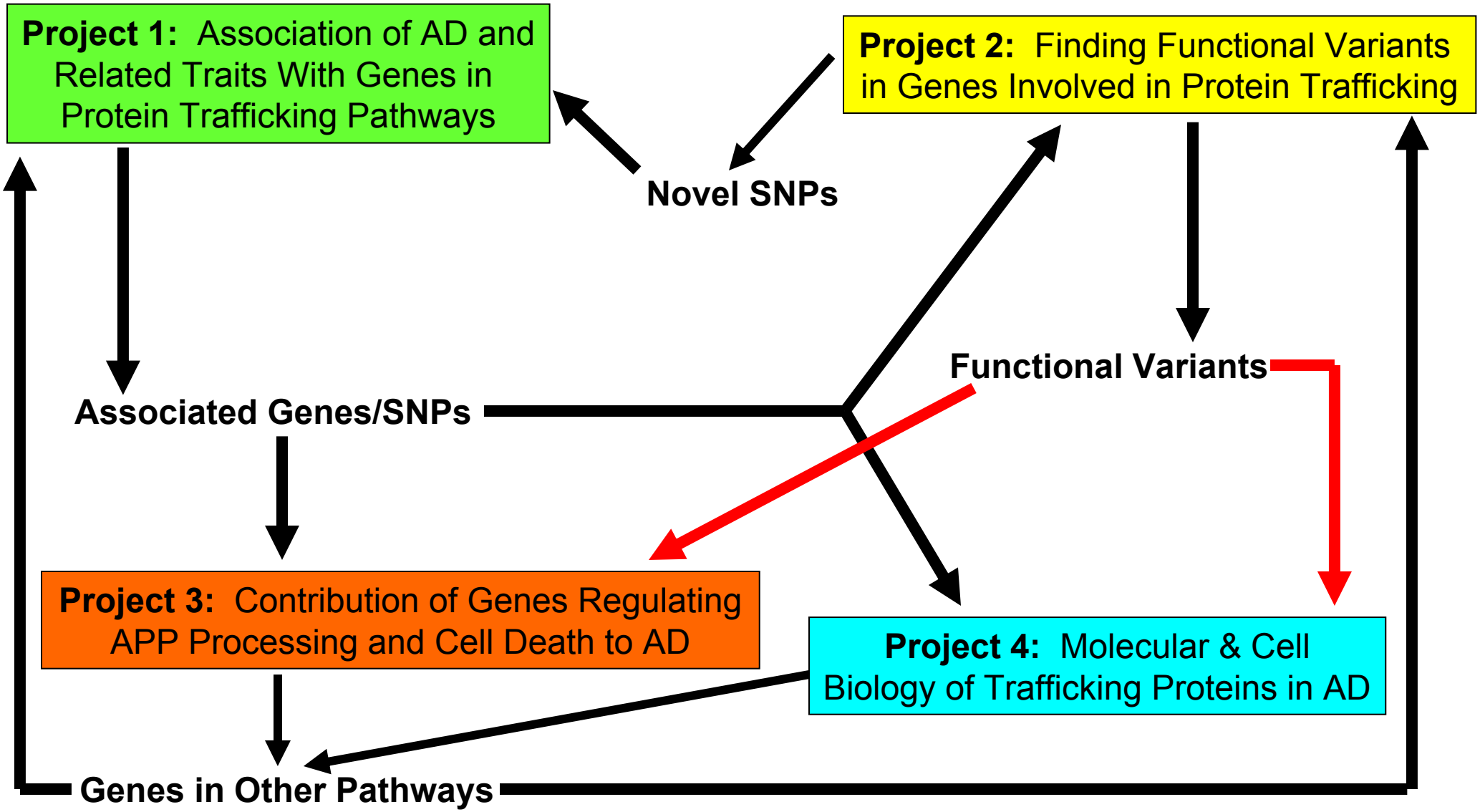


Rogaeva et al, *Nature Genetics* **39**:168-177, 2007

Genetics of Protein Trafficking in Alzheimer Disease



Sponsored ARC Projects

Project 1 (Lindsay Farrer and Clinton Baldwin)

Deep sequencing of SORL1 and 1-2 other protein trafficking genes for further genetic association, expression and functional studies

Project 2 (Peter Morin and Angela Ho)

Examine the effects of retromer malfunction in a double transgenic $APP_{SWE}/PS1_{\Delta E9}$ mouse line which exhibits amyloid β pathology as early as 4-6 months of age.

Project 3 (Carmela Abraham and Ci-Di Chen)

Multiple experiments with Klotho (KL), a protein associated with increased lifespan:

- Examine effects of KL polymorphisms on trafficking and shedding
- Examine effects of SORL1 overexpression or knockdown on KL isoforms and α secretases
- Determine mechanism by which insulin promotes secretion of KL and APP

Current ARC Members

Name	Department	Area of Expertise
Lindsay Farrer, Ph.D. (Director)	**Medicine (Genetics)**	**Genetic Epidemiology**
Carmela Abraham, Ph.D.	**Biochemistry**	**Neurobiology of AD**
Clinton Baldwin, Ph.D.	**Center for Human Genetics**	**Human Molecular Genetics**
Ci-Di Chen, Ph.D.	**Biochemistry**	**Molecular & Cell Biology**
James Collins, Ph.D.	Biomedical Engineering	Systems Biology
Adrienne Cupples, Ph.D.	Biostatistics	Statistical Genetics
Susan Doctrow, Ph.D.	Medicine (Pulmonary)	Oxidative Stress
Robert Green, M.D., MPH	Neurology	Behavioral Neurology
Angela Ho, Ph.D.	**Biology**	**AD transgenic models**
Ron Killiany, Ph.D.	Anatomy & Neurobiology	Brain MRI analysis
Kostya Kondror, Ph.D.	Biochemistry	Cell Vesicular Trafficking
Jeanne Latourelle, Ph.D.	Neurology	Genetics of Parkinson Disease
Marc Lenburg, Ph.D.	Pathology	Bioinformatics
Kathy Lunetta, Ph.D.	Biostatistics	Statistical Genetics
Ann McKee, M.D.	Neurology	Neuropathology
Peter Morin, M.D., Ph.D.	**Neurology/Biochemistry**	**Neurochemistry**
Orian Shirihai, Ph.D.	Medicine (Obesity)	Mitochondria Biology
Faina Schwartz, Ph.D.	Medicine (Genetics)	Mitochondrial Genetics
Martin Steffen	Pathology	Proteomics
Benjamin Wolozin, M.D., Ph.D.	Pharmacology	Neurobiology
Vassilis Zannis	Medicine (Molecular Medicine)	Lipoprotein molecular biology

Project 1: Association of AD and Related Traits With Genes in Protein Trafficking Pathways
Leader: Farrer

AIM 1. Identify associations of AD with genes in the protein trafficking pathway as follows:

a. Conduct a meta analysis of genes in this pathway using genome wide association study (GWAS) datasets assembled by our group, available in the public domain, and through collaboration.

b. Investigate statistical interaction among genes in the protein trafficking pathway and other leading candidate genes on AD risk using pathway analysis and data mining methods.

AIM 2. Replicate the findings in Aim 1 by genotyping and analyzing those SNPs in several other large family and case-control datasets.

AIM 3. In the basis of findings from Aims 1 and 2, deep sequencing performed in Project 2, systems biological analyses in Project 3, and siRNA screens in Project 4, analyze association with additional SNPs in all of our sample collections.

AIM 4. Evaluate the association of genes in the protein trafficking pathway with brain changes associated with AD (endophenotypes) measured by MRI scan in the MIRAGE and Alzheimer Disease Neuroimaging Initiative (ADNI) cohorts and neuropathologically in an autopsy sample from the BU ADC Brain Bank.

AIM 5. Assess the association of trafficking genes with quantitative in vivo measures of amyloid deposition obtained from 750 individuals using a PET tracer called Pittsburgh Compound-B (PiB).

Project 2: Finding Functional Variants in Genes Involved in Protein Trafficking
Leader: Baldwin

AIM 1. Identification of common gene variants in the APP-Protein Sorting Pathway contributing to AD: Use state-of-the-art sequencing technology to discover the complete polymorphism content of genes that are members of the APP-Protein Sorting Pathway. Genes to study will be prioritized based on evidence of genetic association (project 1), from the model system studies (project 3) or the siRNA screens (project 4).

AIM 2. Examine the functional impact of polymorphisms identified in Aim 1 on gene expression/protein function.. We will utilize *in vitro* approaches (ie protein-DNA binding assays) to identify the functional variants responsible for AD and demonstrate more conclusively the relationship between the genetic variation and altered function.

AIM 3. Compare gene expression patterns in AD brain tissue and lymphocytes from AD subjects with variants identified in Aim 1 and 2.

A. Examination correlation between SNP and gene expression levels. Using a collection of AD/control brain tissue samples with detailed neuropathology, compare the genotype for the polymorphisms identified in aim 1, confirmed in aim 2, with changes in gene expression patterns for that protein and its downstream targets.

B. Proteomic Analysis of Brain Tissue. Confirm that the changes in gene expression/ mRNA levels translate to a change in amount or structure of the protein produced in the AD brain.

Project 3: Contribution of Genes Regulating APP Processing and Cell Death to AD
Leader: Wolozin

Aim 1: Systems biology analysis for proteins regulate processing of APP and neuronal vulnerability to Aβ or aging.

Aim 2: Determine whether identified proteins modify the processing of APP in cell culture, and whether SORL1 modifies processing of identified proteins.

Aim 3: Determine whether identified proteins modify the vulnerability to Aβ, oxidative stress or aging.

A. Use a simple animal model, *C. elegans* (non-TG or expressing Aβ) to screen 500 genes identified in Aim 1, to determine whether they modify susceptibility to Aβ, oxidative stress or aging.

B. Generate expression arrays from nematode lines under conditions designed to optimize genetic effects, and feed the data back into Aim 1 to optimize the interactome map.

C. Test the most significant genes in primary neuronal cultures to determine whether they increase vulnerability to Aβ in mammalian cells and whether the genes modify mitochondrial function

Project 4: Molecular & Cell Biology of Trafficking Proteins in AD

Leader: Seaman (Univ. Cambridge, UK)

Aim 1: Compare the list of genes which affect cell surface to endosome, and endosome-to-Golgi retrieval with lists of gene candidates produced from GWAS (Project 1). Genes that appear in both lists will then be directly tested to validate whether siRNA knockdown of their expression alters APP trafficking and Aβ production.

Aim 2: Genes that alter Aβ production in Aim 1 will be resequenced in Project 2 to identify polymorphisms for genetic association studies (Project 1). The AD-associated variants will be investigated by siRNA-mediated knockdown of expression and also by transient overexpression to confirm that they do in fact alter APP processing and Aβ production.

Aim 3: Adapt the antibody-uptake assay used to identify genes required for cell surface to endosome and endosome-to-Golgi retrieval of the CIMPR by changing the reporter gene to APP in order to search for genes which specifically modulate APP localization within the post-Golgi endo-membrane system.

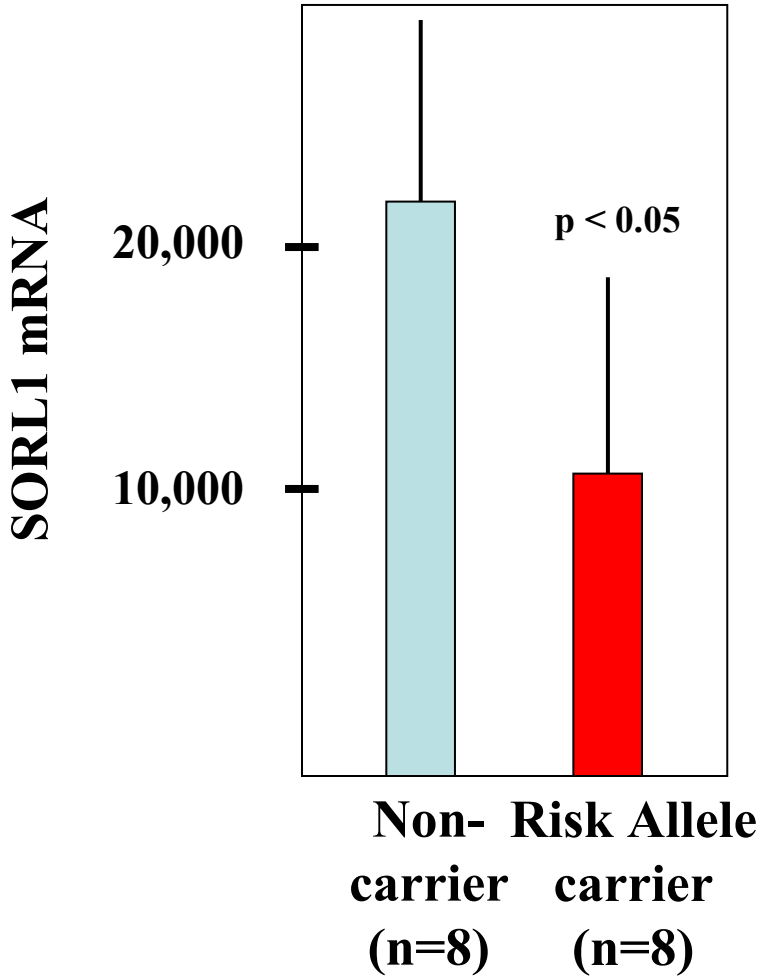
Aim 4: Re-screen the siRNA library for genes that specifically alter APP trafficking using the assay developed in Aim 3.

Risk Factors for Alzheimer Disease

- Advanced age
- Positive family history
- Low education
- Head trauma

How are sequence variants in SORL1 functionally associated with AD?

- Do not affect coding sequence or splicing;
- Intronic variants may affect tissue-specific regulation of transcription
- $CTT_{22\text{-}24}$ haplotype associated with reduced transcription in lymphoblasts (***not*** *very robust*);
- Genotype accounts for 14% of variance in expression level;
- ***Corollary:*** modifiers of SORL1 expression could be other causes of AD or potential therapies.



Rogaeva et al, *Nature Genetics* **39**:168-177, 2007

Suppressing SORL1 gene does not alter expression of APP or PS1 but increases BACE and γ-secretase cleavage of APP (more Aβ peptide and APPsβ)