Genetic variation in *PCDH11X* is associated with susceptibility to late onset Alzheimer's disease (LOAD)

Steven G Younkin, MD, Ph.D.
George M. Eisenberg Professor
Department of Neuroscience
Mayo Clinic
Jacksonville, Florida USA

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Younkin Laboratory:
Minerva Carrasquillo
Fanggeng Zou
Samantha Wilcox
Louise Walker
Ma Li
Gina Bisceglio
Linda Younkin
Samuel Younkin
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Nilufer Ertekin-Taner

Statistical Support:
Shane Pankratz
Julia Crook

Mayo Clinic Sample Collection:
Jacksonville
Neil Graff-Radford
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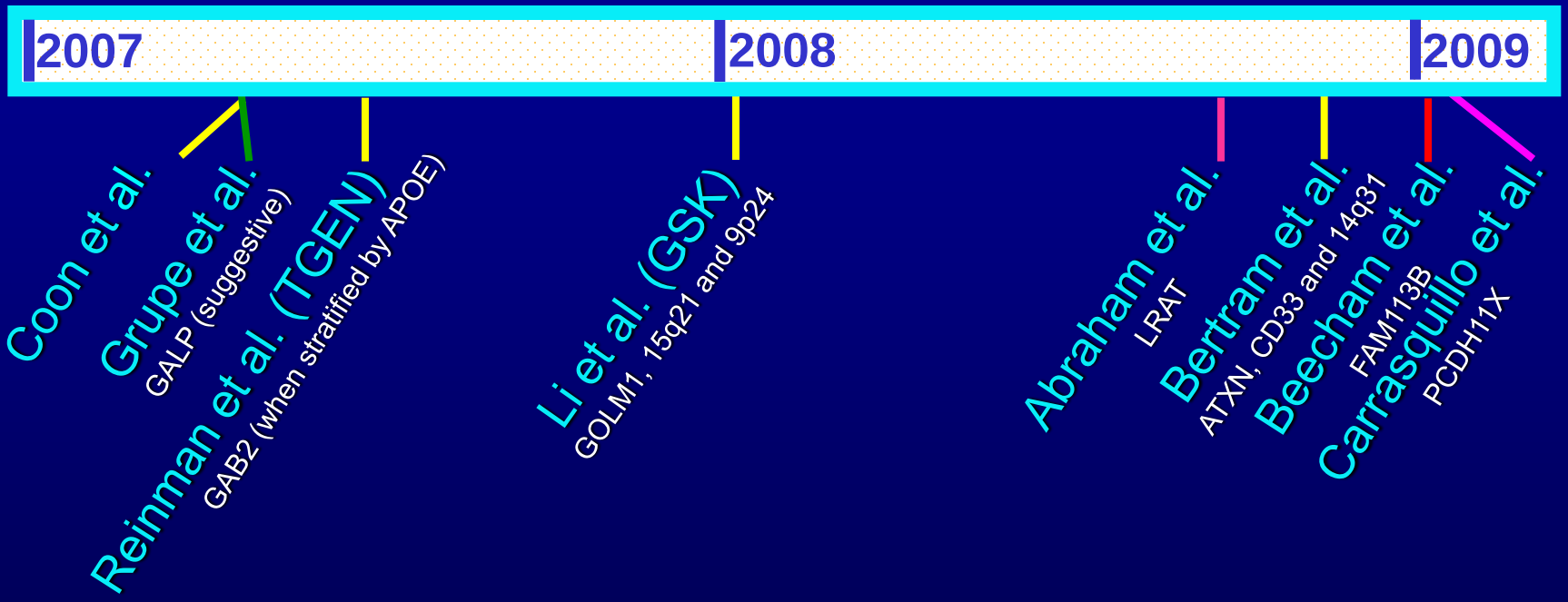
LOAD GWAS


2007
2008
2009
Coon et al.
Grupe et al.
GALP (suggestive)
Reinman et al. (TGEN)
GAB2 (when stratified by APOE)
Li et al. (GSK)
GOLM1, 15q21 and 9p24
Abraham et al.
LRAT
Bertram et al.
ATXN, CD33 and 14q31
Beecham et al.
FAM113B
Carrasquillo et al.
PCDH11X

Samples for 2-Stage LOAD GWAS

	n		Mean Age (SD)		% Females	
Series	Cases	Controls	Cases	Controls	Cases	Controls
Stage I						
JS 60-80	353	331	74.5 (4.4)	72.5 (4.8)	61.2	59.8
RS 60-80	245	701	73.8 (4.9)	74.0 (3.6)	58.4	51.4
AUT 60-80	246	223	73.5 (5.3)	71.8 (5.5)	50.0	37.2
Stage I combined	844	1255	74.0 (4.8)	73.2 (4.4)	57.1	51.1
Stage II						
JS 80+	237	260	83.8 (3.2)	85.1 (4.0)	62.0	60.8
RS 80+	276	624	86.0 (4.4)	84.0 (3.1)	66.7	57.1
AUT 80+	332	116	87.4 (4.8)	84.6 (6.0)	68.7	54.3
NCRAD 60+	702	209	75.2 (6.8)	78.3 (8.9)	64.8	61.7
Stage II combined	1547	1209	81.1 (7.8)	83.3 (5.6)	65.5	58.4
Stage I + II combined	2391	2464	78.6 (7.7)	78.2 (7.1)	62.6	54.7

- Diagnosis - NINCDS/ADRDA criteria
- Age at diagnosis/entry ≥ 60
- Series composition: JS - Mayo Clinic Jacksonville - N. Graff-Radford et al.
  RS - Mayo Clinic Rochester - R. Petersen et al.
  AUT - Mayo Clinic Autopsy Confirmed - D. Dickson
  NCRAD - National Cell Repository for Alzheimer's Disease (1 AD/family)

Stage I

- Genotyping:
  - Illumina's HumanHap300-Duo v2 arrays (318,237 SNPs)
- QC:
  - Sample call rate > 90% (90% passed QC) – 2099 (844 AD, 1244 CON)
  - SNP call rate > 90% & HW *P* value > 0.001 - 313,504 passed QC
  - Cryptic relatedness/duplicates - 17 samples eliminated
- Analysis:
  - PLINK (Purcell S. et al., *AJHG* 2007)
    - allelic association
    - logistic regression with an allelic dosage model
  - EIGENSTRAT (Price, A. et al., *Nat Genet* 2006)
    - evaluation of population substructure:

Stage II

- Follow up of top 25 allelic associations from Stage I
- Genotyping:
  - SEQUENOM's iPLEX multiplex assays
- QC:
  - SNP call rate > 95% & Hardy-Weinberg *p*-value > 0.10
  - All SNPs passed visual inspection of genotype clusters
  - Cryptic relatedness/duplicates – 61 samples eliminated
- Analysis
  - Allelic association using PLINK
  - Multivariable logistic regression

Top 25 PLINK Allelic Association Results in Stage I GWAS

- After Bonferroni correction, p-value cut-off for genome wide significance = 1.6×10^{-7} (0.05/313,330)
- Of the top 25 results, 10 map to the *APOE* linkage region, 6 *APOE* SNPs meet genome-wide significance
- No other SNPs meet genome wide significance
- 3 SNPs failed genotype cluster QC criteria upon visual inspection

Chr	rs#	Position (bp)	Stage I Combined			
			MAF		*P* value	OR (95% CI)
			AD	CON		
19	rs2075650	50,087,459	0.34	0.15	4.8E-46	2.89 (2.49-3.36)
19	rs157580	50,087,106	0.25	0.40	3.0E-22	0.51 (0.45-0.59)
19	rs439401	50,106,291	0.26	0.39	2.4E-17	0.56 (0.48-0.64)
19	rs6859	50,073,874	0.54	0.41	1.7E-15	1.66 (1.47-1.89)
19	rs8106922	50,093,506	0.29	0.38	1.2E-09	0.66 (0.58-0.76)
19	rs405509	50,100,676	0.41	0.50	6.2E-09	0.69 (0.61-0.78)
19	rs10402271	50,021,054	0.39	0.32	8.4E-07	1.38 (1.22-1.57)
11	rs2746600	33,671,217	0.38	0.30	1.2E-06	1.39 (1.22-1.58)
12	rs11044668	19,593,783	0.24	0.31	1.5E-06	0.71 (0.62-0.82)
1	rs3007421	6,452,776	0.09	0.05	1.8E-06	1.83 (1.42-2.36)
13	rs7318037	81,367,146	0.40	0.33	2.9E-06	1.36 (1.20-1.55)
12	rs10841260	19,597,931	0.41	0.34	3.2E-06	1.35 (1.19-1.54)
9	rs3858095	93,944,438	0.30	0.23	3.3E-06	1.41 (1.22-1.63)
8	rs2318144	58,277,297	0.06	0.03	4.2E-06	1.96 (1.47-2.63)
10	rs701864	95,154,196	0.34	0.27	5.0E-06	1.37 (1.19-1.56)
14	rs856675	84,405,968	0.14	0.10	5.1E-06	1.56 (1.29-1.89)
1	rs639222	64,062,886	0.09	0.05	5.2E-06	1.77 (1.38-2.26)
19	rs377702	50,054,507	0.45	0.38	5.5E-06	1.34 (1.18-1.52)
19	rs1114832	50,328,041	0.13	0.08	5.9E-06	1.59 (1.30-1.94)
X	rs1279795	123,152,101	0.53	0.45	7.2E-06	1.38 (1.20-1.59)
1	rs649608	64,086,284	0.08	0.05	9.0E-06	1.77 (1.37-2.28)
X	rs5984894	91,280,393	0.53	0.45	1.2E-05	1.38 (1.19-1.59)
11	rs3740938	102,092,272	0.05	0.08	1.4E-05	0.56 (0.43-0.73)
15	rs8039031	34,954,382	0.17	0.23	1.5E-05	0.71 (0.6-0.83)
19	rs1048699	50,342,226	0.12	0.08	1.5E-05	1.57 (1.28-1.93)

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Stage II Allelic Association Results (Follow-up) of Top 25 SNPs from Stage I

- 7 APOE-linked SNPs show significance
- rs5984894 achieves significance, even after performing Bonferroni correction for the 25 variants tested on follow-up

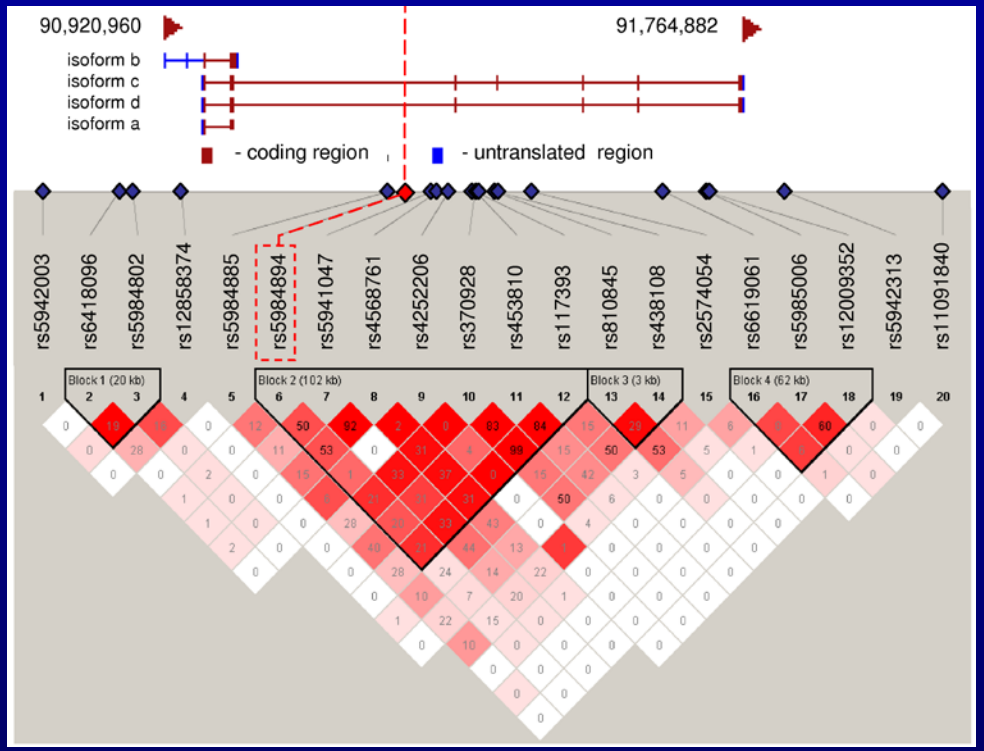
rs #	Chr	Position (bp)	Stage II Combined			
			MAF		*P* value	OR (95% CI)
			AD	CON		
rs2075650	19	50,087,459	0.32	0.11	9.5E-79	3.93 (3.39-4.57)
rs405509	19	50,100,676	0.42	0.55	1.1E-21	0.59 (0.53-0.66)
rs6859	19	50,073,874	0.52	0.40	1.1E-20	1.67 (1.50-1.86)
rs157580	19	50,087,106	0.27	0.39	1.4E-20	0.58 (0.52-0.65)
rs8106922	19	50,093,506	0.30	0.42	2.2E-20	0.59 (0.53-0.66)
rs439401	19	50,106,291	0.27	0.37	2.8E-15	0.63 (0.56-0.71)
rs10402271	19	50,021,054	0.39	0.30	3.5E-12	1.49 (1.33-1.67)
rs5984894	X	91,280,393	0.51	0.46	5.7E-04	1.23 (1.10-1.39)
rs1048699	19	50,342,226	0.11	0.09	2.2E-02	1.23 (1.03-1.47)
rs377702	19	50,054,507	0.42	0.39	3.1E-02	1.13 (1.01-1.26)
rs1114832	19	50,328,041	0.12	0.10	5.0E-02	1.19 (1.00-1.42)
rs701864	10	95,154,196	0.32	0.33	2.8E-01	0.94 (0.84-1.05)
rs1279795	X	123,152,101	0.46	0.47	4.3E-01	0.95 (0.84-1.08)
rs3858095	9	93,944,438	0.29	0.30	4.6E-01	0.96 (0.85-1.08)
rs7318037	13	81,367,146	0.34	0.35	4.6E-01	0.96 (0.86-1.07)
rs11044668	12	19,593,783	0.29	0.28	4.8E-01	1.04 (0.93-1.18)
rs10841260	12	19,597,931	0.38	0.38	5.0E-01	0.96 (0.86-1.08)
rs2318144	8	58,277,297	0.13	0.14	5.3E-01	0.95 (0.81-1.11)
rs649608	1	64,086,284	0.07	0.07	5.4E-01	0.93 (0.75-1.16)
rs8039031	15	34,954,382	0.21	0.21	5.6E-01	0.96 (0.84-1.10)
rs3007421	1	6,452,776	0.12	0.12	7.8E-01	1.02 (0.87-1.20)
rs2746600	11	33,671,217	0.34	0.34	8.5E-01	0.99 (0.88-1.11)
rs856675	14	84,405,968	0.12	0.12	8.7E-01	0.99 (0.84-1.16)
rs3740938	11	102,092,272	0.06	0.06	9.4E-01	1.01 (0.80-1.26)
rs639222	1	64,062,886	0.07	0.07	9.8E-01	1.00 (0.81-1.23)

Stages I + II Combined Allelic Association Results of Top 25 SNPs from Stage I

- rs5984894 is the only non-APOE-linked SNP that meets genome-wide significance

rs #	Chr	Position (bp)	Stage I + II Combined			
			MAF		*P* value	OR (95% CI)
			AD	CON		
rs2075650	19	50,087,459	0.33	0.13	3.7E-120	3.29 (2.97-3.65)
rs157580	19	50,087,106	0.26	0.39	7.4E-42	0.55 (0.51-0.6)
rs6859	19	50,073,874	0.53	0.41	5.5E-34	1.65 (1.52-1.79)
rs439401	19	50,106,291	0.27	0.38	1.6E-31	0.60 (0.55-0.65)
rs405509	19	50,100,676	0.42	0.53	6.2E-27	0.64 (0.59-0.7)
rs8106922	19	50,093,506	0.3	0.4	7.6E-27	0.63 (0.58-0.69)
rs10402271	19	50,021,054	0.39	0.31	2.9E-17	1.44 (1.32-1.56)
rs5984894	X	91,280,393	0.52	0.46	3.8E-08	1.29 (1.18-1.41)
rs1048699	19	50,342,226	0.12	0.09	3.6E-06	1.37 (1.20-1.56)
rs377702	19	50,054,507	0.43	0.39	4.3E-06	1.21 (1.12-1.32)
rs1114832	19	50,328,041	0.12	0.09	5.9E-06	1.35 (1.19-1.54)
rs3007421	1	6,452,776	0.11	0.09	7.7E-05	1.32 (1.15-1.51)
rs2318144	8	58,277,297	0.11	0.08	1.4E-04	1.31 (1.14-1.50)
rs8039031	15	34,954,382	0.2	0.22	1.5E-03	0.85 (0.77-0.94)
rs3740938	11	102,092,272	0.06	0.07	1.6E-03	0.77 (0.65-0.91)
rs639222	1	64,062,886	0.08	0.06	2.2E-03	1.28 (1.09-1.50)
rs2746600	11	33,671,217	0.35	0.32	2.4E-03	1.14 (1.05-1.24)
rs856675	14	84,405,968	0.13	0.11	3.8E-03	1.20 (1.06-1.36)
rs3858095	9	93,944,438	0.29	0.27	4.0E-03	1.14 (1.04-1.25)
rs10841260	12	19,597,931	0.39	0.36	8.6E-03	1.12 (1.03-1.21)
rs649608	1	64,086,284	0.07	0.06	1.1E-02	1.24 (1.05-1.46)
rs11044668	12	19,593,783	0.27	0.29	1.3E-02	0.89 (0.82-0.98)
rs701864	10	95,154,196	0.32	0.3	1.3E-02	1.12 (1.02-1.22)
rs7318037	13	81,367,146	0.36	0.34	2.8E-02	1.10 (1.01-1.19)
rs1279795	X	123,152,101	0.48	0.46	3.0E-02	1.11 (1.01-1.21)

Where is rs5984894?



- On Xq21.31, in a haplotype block that lies entirely within the protocadherin 11X gene (*PCDH11X*)
  - Deep within intron 2
- Region does not undergo X inactivation
- The Y chromosome has a similar functional gene PCDH11Y, that does not have the rs5984894 variant

Why is *PCDH11X* an interesting LOAD gene?

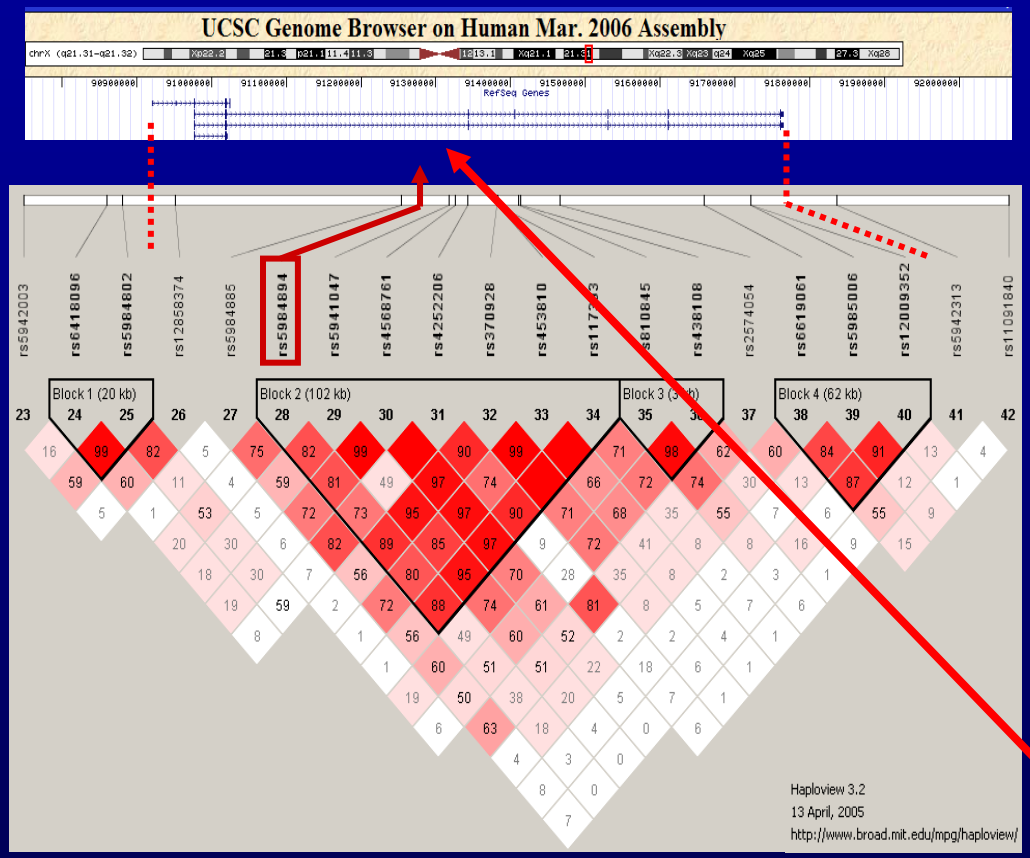
- Xq21.31 not previously implicated in AD
- Contributes substantially to increased risk in females
- The *PCDH11X* gene is a member of the protocadherin subfamily of calcium-dependent cell adhesion and recognition proteins, which are particularly prevalent in the central nervous system.
- Expression is particularly strong in the cortex and hippocampus and weaker in the cerebellum.
- Some protocadherins are known to undergo presenilin-dependent processing.

Multivariable Logistic Regression rs5984894

Series	Sex OR (95% CI)	*P*	Male Hemizygotes OR (95% CI)	*P*	Female Heterozygotes OR (95% CI)	*P*	Female Homozygotes OR (95% CI)	*P*	Global *P*
Stage I									
JS 60-80	1.28 (0.78-2.11)	0.33	1.28 (0.79-2.09)	0.31	1.66 (1.04-2.63)	0.03	1.96 (1.14-3.36)	0.01	0.09
RS 60-80	1.00 (0.58-1.72)	0.99	1.20 (0.76-1.90)	0.43	1.46 (0.87-2.44)	0.16	2.02 (1.12-3.64)	0.02	0.04
AUT 60-80	0.79 (0.41-1.53)	0.48	1.40 (0.85-2.32)	0.19	1.55 (0.77-3.12)	0.22	2.00 (0.91-4.40)	0.03	0.03
All Stage I	**0.98 (0.72-1.33)**	**0.90**	**1.33 (1.02-1.74)**	**0.04**	**1.43 (1.06-1.92)**	**0.02**	**1.92 (1.36-2.70)**	**0.0002**	**5.70E-05**
Stage II									
JS 80+	1.33 (0.73-2.44)	0.35	0.96 (0.54-1.71)	0.89	1.58 (0.91-2.72)	0.10	1.33 (0.70-2.55)	0.38	0.6
RS 80+	0.75 (0.46-1.22)	0.25	1.19 (0.74-1.91)	0.48	1.04 (0.67-1.62)	0.86	2.28 (1.39-3.73)	0.001	0.0001
AUT 80+	0.44 (0.19-1.01)	0.05	0.97 (0.49-1.92)	0.94	0.76 (0.36-1.61)	0.47	0.97 (0.39-2.39)	0.95	0.05
NCRAD 60+	1.11 (0.67-1.84)	0.67	0.95 (0.58-1.58)	0.86	1.19 (0.75-1.88)	0.47	1.71 (0.98-2.97)	0.06	0.35
All Stage II	**0.89 (0.69-1.15)**	**0.37**	**1.04 (0.82-1.33)**	**0.74**	**1.19 (0.94-1.50)**	**0.15**	**1.70 (1.29-2.24)**	**0.0002**	**4.80E-06**
All Stage I + II	**0.86 (0.71-1.05)**	**0.14**	**1.18 (0.99-1.41)**	**0.07**	**1.26 (1.05-1.51)**	**0.01**	**1.75 (1.42-2.16)**	**2.0×10^{-7}**	**3.90E-12**

- Global P = 5.7×10^{-5} in Stage I, 4.8×10^{-6} in Stage II, and 3.9×10^{-12} in Stages I + II
- Female homozygotes show significantly increased risk not only when compared to female non-carriers, (P=2×10^{-7}) but also when compared to female heterozygotes (*P*=0.0005) or male hemizygotes (*P*=1.4×10^{-7}) (data not shown).

PCDH11X Fine Mapping



- Identified additional SNPs which were evolutionarily conserved between human and mouse (identity ≥ 70% over 100 bp windows)
- 1 conserved SNP, rs2573905, is in strong LD with rs5984894 ($r2$=0.98, D'=0.99) and also showed highly significant association (global $P = 5.4 \times 10^{-13}$) when analyzed by multivariable logistic regression adjusted for sex.
- rs2573905 is also located deep in intron 2, 62 kb upstream from exon 3.

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Summary

- The results from our 2-stage GWAS provide the first evidence that genetic variation in *PCDH11X* is significantly ($P = 3.9 \times 10^{-12}$) associated with LOAD.
- *PCDH11X* alleles appear to have a dose-dependent effect. Females homozygote for the minor allele were at significantly greater risk than female heterozygotes and male hemizygotes.
- We are currently testing rs2573905 for association with the level of PCDH11X transcript in human brain to determine if rs2573905 is an eSNP that alters PCDH11X expression

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