Original Paper

Neurodegenerative Diseases

Neurodegenerative Dis 2008;5:60–64
DOI: 10.1159/000110789

Received: March 8, 2007
Accepted after revision: June 4, 2007
Published online: November 1, 2007

Sorl1 as an Alzheimer's Disease Predisposition Gene?

Jennifer A. Webster[a] Amanda J. Myers[b] John V. Pearson[a] David W. Craig[a] Diane Hu-Lince[a] Keith D. Coon[a] Victoria L. Zismann[a] Thomas Beach[c, d] Doris Leung[e] Leslie Bryden[e] Rebecca F. Halperin[a] Lauren Marlowe[e] Mona Kaleem[e] Matthew J. Huentelman[a] Keta Joshipura[a] Douglas Walker[c, d] Christopher B. Heward[f] Rivka Ravid[i] Joseph Rogers[c, d] Andreas Papassotiropoulos[a, j] John Hardy[b] Eric M. Reiman[a, d, g, h] Dietrich A. Stephan[a, d]

[a]Neurogenomics Division, Translational Genomics Research Institute, Phoenix, Ariz.; [b]Department of Psychiatry and Behavioral Sciences, University of Miami, Miller School of Medicine, Miami, Fla.; [c]Sun Health Research Institute, Sun City, Ariz.; [d]Arizona Alzheimer's Consortium, Phoenix, Ariz.; [e]Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Md.; [f]Kronos Science Laboratories, [g]Banner Alzheimer's Institute, Phoenix, Ariz., and [h]Department of Psychiatry, University of Arizona, Tucson, Ariz., USA; [i]Royal Dutch Academy of Sciences, Amsterdam, The Netherlands; [j]Division of Psychiatry Research, University of Zurich, Zurich, Switzerland

Key Words
Sorl1 · Alzheimer's disease · Predisposition gene · *APOE* gene · Sortilin-related receptor

Abstract
Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressively disabling impairments in memory, cognition, and non-cognitive behavioural symptoms. Sporadic AD is multifactorial and genetically complex. While several monogenic mutations cause early-onset AD and gene alleles have been suggested as AD susceptibility factors, the only extensively validated susceptibility gene for late-onset AD is the apolipoprotein E *(APOE)* ε4 allele. Alleles of the *APOE* gene do not account for all of the genetic load calculated to be responsible for AD predisposition. Recently, polymorphisms across the neuronal sortilin-related receptor *(SORL1)* gene were shown to be significantly associated with AD in several cohorts. Here we present the results of our large case-control whole-genome scan at over 500,000 polymorphisms which presents weak evidence for association and potentially narrows the association interval.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressively disabling impairments in memory and other cognitive domains, but also by non-cognitive behavioural symptoms. AD preferentially affects individuals over 60 years with prevalence rates as high as 40% in nonagenarians [1]. Sporadic AD is multifactorial and genetically complex. Twin studies suggest that genetic factors may account for as much as 80% of the disease risk [2]. While several monogenic mutations cause early-onset AD and gene alleles have been suggested as AD susceptibility factors, the only extensively validated susceptibility gene for AD is the apolipoprotein E *(APOE)* ε4 allele [3, 4]. Alleles of the *APOE* gene do not account for all of the genetic load calculated to be responsible for AD predisposition. Recently, Rogaeva et al. [5] reported polymorphisms across the neuronal sor-

J.A.W. and A.J.M. contributed equally.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/ndd

Dietrich A. Stephan
Neurogenomics Division, TGen, The Translational Genomics Research Institute
445 N. Fifth Street
Phoenix, AZ 85004 (USA)
Tel. +1 602 343 8727, Fax +1 602 343 8448, E-Mail dstephan@tgen.org

tilin-related receptor *(SORL1)* gene to be significantly associated with AD in several cohorts.

A genome-wide association study was performed using 502,627 SNPs on DNA samples extracted from brain tissue of donors who were at least 65 years of age at the time of their death. The donors included 664 patients who satisfied clinical and neuropathological criteria for the diagnosis of AD and 422 persons that did not meet clinical or neuropathological criteria for AD as controls. Their brain tissue and neuropathological diagnoses were supplied by investigators from 20 National Institute on Aging Alzheimer's Disease Centers (ADCs) in accordance with an agreement with these Centers, the National Institute on Aging, and the National Alzheimer's Coordinating Center (NACC). Additional postmortem samples were received from Sun Health Research Institute and The Netherlands Brain Bank. In addition to a neuropathologist's diagnostic decision, 18% of the patients and 16% of controls were assessed using CERAD criteria, which provide information about neuritic plaque density [6]. 59% of the patients and 75% of the controls were also assessed using Braak and Braak staging [7], which assesses the distribution of neurofibrillary tangles [6]. The patients included 362 females and 302 males, and averaged 82 $\pm$ 7.7 years of age at the time of death. The controls included 255 females and 167 males, and averaged 79 $\pm$ 11.0 years of age at the time of death. The software program STRUCTURE was used to test for underlying genetic stratification, using 5,000 randomly selected SNPs with at least 100 per chromosome. Initial analysis yielded empirical evidence of three populations. One, which contained 14 samples and was far removed from the rest of the study population, was removed from further analyses. STRUCTURE was re-run with K = 2, and each sample was assigned an admixture of the resulting populations. Comparison of the resulting case and control populations defined by these admixture vectors yielded a silhouette score of 0.06. This indicates that while the samples in this study are likely to be the result of admixture of two populations, the distribution of those populations is equivalent in cases and controls [8]. As previously reported [9], the most significant SNP identified through Fisher's exact tests through the scan, rs4420638 (uncorrected p value = 1.06×10^{-39}, Bonferonni corrected p value for multiple hypothesis testing = 5.30×10^{-34} based on 500,000 comparisons), which was located on chromosome 19, 14 kb from the *APOE* ε4 variant, speaking to the technical robustness of the whole-genome association strategy at this SNP density.

The hypothesis that a specific allele of *SORL1* is associated with increased AD risk was tested. The 46 SNPs located in the *SORL1* genomic locus on the Affymetrix 500K Mapping Array were directly targeted for significance analysis in our whole-genome data set, and thus no multiple-testing correction was applied to the significance values. Calculation of a SNP's allelic frequency differences between cases and controls was based on the Fisher's exact test using each contiguous SNP within the gene sequentially. Table 1 identifies four SNPs with $p < 0.05$ between chromosome 11 SNPs rs668387 (120,873,131 bp) and rs1790208 (120,932,469 bp). This region overlaps the Rogaeva et al. significant region from rs668387 (120,873,131 bp) to rs12285364 (120,898,436 bp). The risk-associated alleles are presented in table 1, and articulate a 59-kb interval, which encompasses 12 exons (exons 7 through 18) of the *SORL1* gene. Of the 9 significant SNPs in Rogaeva et al., 3 were directly measured in our study and each of the remaining 6 are in LD with at least 1 SNP on the Affymetrix Array (fig. 1) [5]. Thus we have captured all of the significant SNPs from the Rogaeva et al. study, including those we have not directly measured. Potentially problematic is that SNPs rs7131432 and rs2101756 have very rare minor allele frequencies (1.6 and 0.5% respectively) and thus the p values are very sensitive to subtle distribution differences, residual genotype error, and subtle stratification between cases and controls and should be interpreted with caution. We do not see significant association with the SNPs that were reported in Rogaeva et al. that were present on the Affymetrix 500k SNP array (in italics in table 1, significant SNPs from Rogaeva et al. underlined), nor do we see significance at the 3′ region of the locus noted in the original report [5]. The 5′ region of the locus shows nominally significant replication with low odds ratio (OR) of 1.551 at rs11218313 (maximum value of the four associated SNPs with MAF >2%, and in contrast to an OR of 4 for *APOE*ε*4* heterozygotes, and 15–20 for homozygotes). Of note, we would not see these effects if we corrected for multiple testing by Bonferoni. Replication of only one region of the *SORL1* locus is consistent with the findings of Rogaeva et al. Significance of this locus in our population using a different series of SNPs than the original report potentially gives insight into SORL1-mediated biology, and refining the association signal to 59 kb could help to narrow the search for disease causing alleles. Whole-genome association studies using well-phenotyped and well-powered study designs may provide disparate results for subtle allelic association findings, such as SORL1. The importance of the *SORL1* finding is also that it articulates a common prob-

Table 1. Fisher's exact p values across the *SORL1* locus from a whole-genome association scan using 1,086 AD cases and controls

dbSNP RS ID	Position	p value	Risk allele	MAF	OR (95% CI)
rs4935774	*120826964*	*0.8094*		*0.209*	
rs17125349	120829417	0.2355		0.077	
rs610188	120834606	0.415		0.038	
rs1784934	120843203	0.5133		0.331	
rs676160	120845443	0.3994		0.094	
rs676759	120864475	0.6479		0.383	
rs560573	*120866094*	*0.119*		*0.38*	
rs2298525	120866225	0.8829		0.098	
rs985421	120867526	0.343		0.012	
rs9665981	120869213	0.6092		0.382	
rs12364988	*120872836*	*0.3524*		*0.486*	
rs668387	*120873131*	*0.5226*		*0.422*	
rs2101756	**120874460**	**0.01923**	**C**	**0.005**	**1.19 (0.866–1.74)**
rs11218313	**120888081**	**0.02026**	**C**	**0.118**	**1.55 (0.756–2.12)**
rs17125423	120909761	0.3375		0.003	
rs626885	**120914166**	**0.03616**	**T**	**0.479**	**1.19 (0.968–1.42)**
rs2276346	*120919686*	*0.09332*		*0.355*	
rs10502262	120920522	0.106		0.283	
rs7131432	**120932080**	**0.03869**	**A**	**0.016**	**1.73 (0.998–3.67)**
rs1790208	120932469	0.3479		0.001	
rs11218340	120936564	0.6076		0.143	
rs10892756	120939766	0.7295		0.075	
rs11218343	120940797	0.6357		0.082	
rs11218346	120944391	0.4664		0.081	
rs1792124	120946730	0.6149		0.017	
rs1790213	120947399	0.2949		0.385	
rs11218347	120951758	0.7815		0.077	
rs1699103	120957136	0.8596		0.42	
rs7116734	120957150	0.1016		0.408	
rs1792127	120957244	0.1566		0.001	
rs11218350	120957861	0.7873		0.227	
rs10892759	120969298	0.1122		0.341	
rs7127359	120970156	0.6789		0.358	
rs11218360	120978601	0.9359		0.031	
rs7128608	120978808	0.946		0.034	
rs1629493	120982306	0.2664		0.381	
rs2282648	120983705	0.2016		0.339	
rs2282649	*120984168*	*0.97*		*0.285*	
rs726601	120986617	0.19		0.308	
rs1784931	120988148	0.1377		0.397	
rs1010159	*120988611*	*0.2545*		*0.355*	
rs1503413	120992024	0.5687		0.057	
rs1614735	*120998211*	*0.4347*		*0.471*	
rs17125558	120999237	0.7932		0.025	
rs17125561	120999299	0.4623		0.004	
rs10892761	121003094	0.4663		0.412	

SNPs highlighted in bold are significant in our population, SNPs italicized are those reported in the Rogaeva et al. study populations, and those that are underlined are reported as significant by Rogaeva et al.

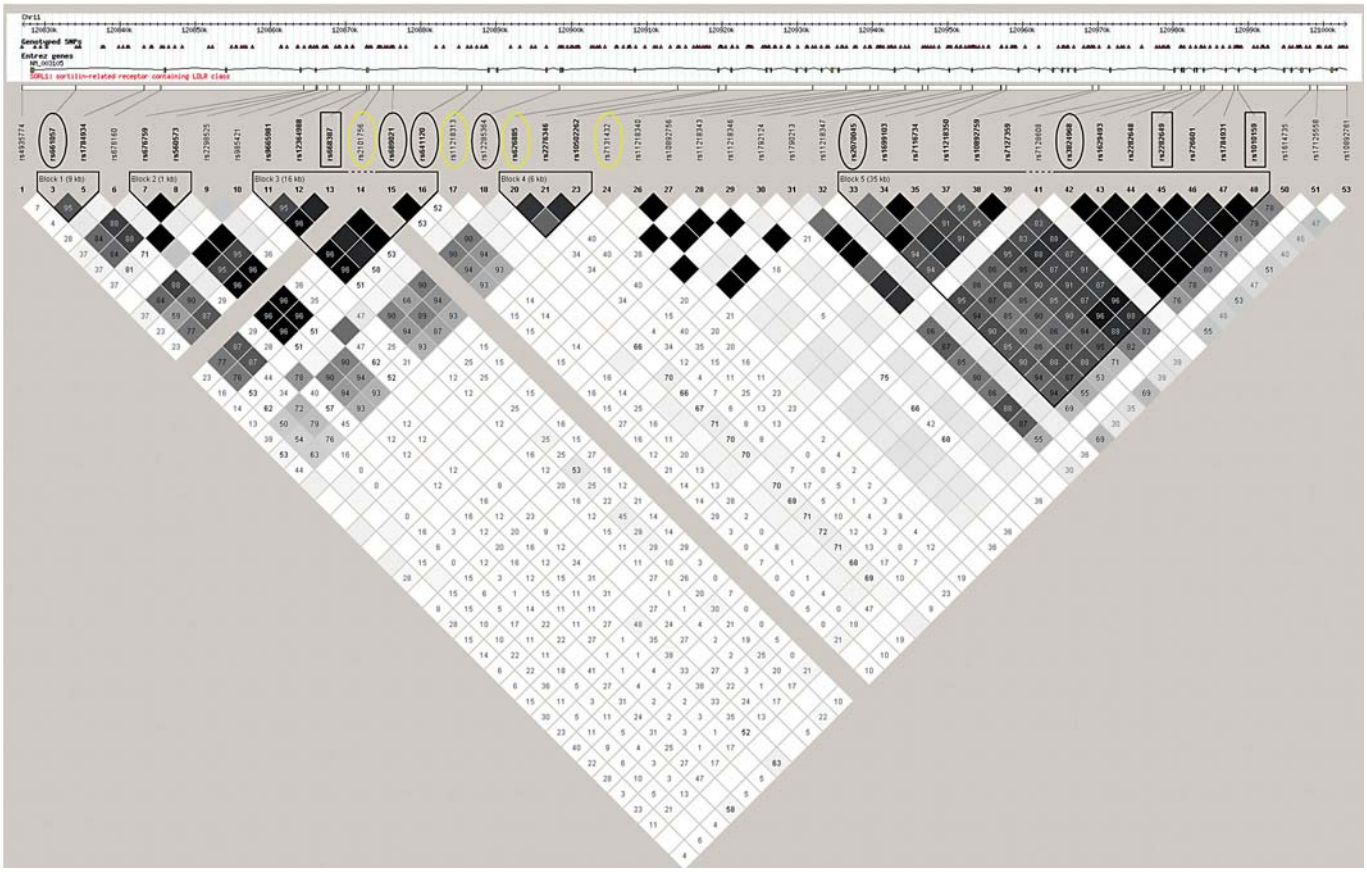

Fig. 1. Out of the nine markers in SORL1 found to be significantly associated with disease risk in Rogaeva et al. [5], we have typed three of those markers (black rectangles). To examine whether we were able to capture the remaining 6 significant SNPs typed from the Rogaeva screen (black circles), we mapped the LD relationships between our SNPs and the Rogaeva SNPs using the HapMap database and the program Haploview. For each of those significant SNPs, there is at least one marker within our screen which is in complete or almost complete linkage disequilibrium (as measured by r^2) with the Rogaeva et al. markers. Thus, we have captured if not typed all of the significant Rogaeva markers. Yellow circles indicate significant SNPs from our screen demonstrating that we have potentially replicated one block of the association (rs668387 to rs7131432 from our data set) but not the other (rs2070045 to rs1010159).

lem with low OR associations in that power, population structure and history, allelic heterogeneity and SNP coverage across the locus may confound the ability to tease out subtle allelic associations in complex traits. If *SORL1* does demonstrate allelic heterogeneity, the presence of the admixture of two populations that STRUCTURE revealed for our study population could dilute any association signals that were present in the two original populations. Clearly our results can be interpreted either as a failure to replicate or as a partial replication depending on one's point of view and this illustrates a current major problem in association studies. Even analyses in large, well-characterized studies such as ours fail to give signals clear enough to distinguish between no signal and variable signals in different cohorts.

Acknowledgements

Sponsors: Kronos Life Sciences Laboratories, The Arizona Alzheimer's Disease Core Center (P30 AG19610), the National Alzheimer's Coordinating Center (U01 AG016976), and the State of Arizona. We thank Drs. Creighton Phelps, Marcelle Morrison-Bogorad, Marilyn Miller, and Walter Kukull for their assistance in the acquisition of tissue samples and data. None of this work

would be possible without the generous participation of the patients and controls and their families. This work was partially supported by the NIA/NIH Intramural Research Program, USA. Many data and biomaterials were collected from several NIA-NACC funded sites. The directors, pathologist and technicians involved include: National Institute on Aging: Ruth Seemann, Dan Brady; John Hopkins Alzheimer's Disease Research Center (NIA grant # AG 05146): Juan C. Troncoso, Olga Pletnikova; University of California, Los Angeles (NIA grant # P50 AG16570): Harry Vinters, Justine Pomakian; The Kathleen Price Bryan Brain Bank, Duke University Medical Center (NIA grant # AG05128, NINDS grant # NS39764, NIMH MH60451 also funded by GlaxoSmithKline): Christine Hulette; Stanford University: Dikran Horoupian, Ahmad Salehi; New York Brain Bank, Taub Institute, Columbia University (NYBB): Jean Paul Vonsattel; Massachusetts General Hospital: E. Tessa Hedley-Whyte, Karlotta Fitch; University of Michigan (NIH grant P50-AG08671): Roger Albin, Lisa Bain, Eszter Gombosi; University of Kentucky: William Markesbery, Sonya Anderson; Mayo Clinic, Jacksonville: Dennis W. Dickson, Natalie Thomas; University Southern California: Caroll A. Miller, Jenny Tang, Dimitri Diaz; Washington University, St Louis Alzheimer's Disease Research Center: Dan McKeel, John C. Morris, Eugene Johnson, Jr., Virginia Buckles, Deborah Carter; University of Washington, Seattle: Thomas Montine, Aimee Schantz; University of Pennsylvania School of Medicine, Alzheimer's Disease Research Center (NIH grant): John Q Trojanowski, Virginia M Lee, Vivianna Van Deerlin, Terry Schuck; Boston University Alzheimer's Disease Research Center (NIH grant P30-AG13846): Ann C. McKee, Carol Kubilus; Sun Health Research Institute, Arizona: Lucia I. Sue; Emory University: Bruce H. Wainer, Marla Gearing; University of Texas, Southwestern Medical School: Charles L. White, III, Roger Rosenberg, Marilyn Howell, Joan Reisch, University of California, Davis: William Ellis, Mary Ann Jarvis; Rush University Medical Center, Rush Alzheimer's Disease Center: David A. Bennett, Julie A. Schneider, Karen Skish, Wayne T Longman; University of Miami/NPF Brain Endowment Bank: Deborah C. Mash, Margaret J Basile, Mitsuko Tanaka. A.J.M. is supported in part by the Johnnie B. Byrd Sr. Alzheimer's Disease and Research Institute. A.P. is supported by the Swiss National Science Foundation (PP00B-68859). Both A.J.M. and J.H. would like to thank the Verum Foundation. D.W.C., J.V.P., and D.A.S. are supported by the Bisgrove Charitable Donation, the NIH Neuroscience Blueprint (U24NS051872), the ENDGAME Consortium (UO1HL084744), and the State of Arizona.

References

1 Evans DA, Funkenstein HH, Albert MS, Scherr PA, Cook NR, Chown MJ, Hebert LE, et al: Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. JAMA 1989;262:2551–2556.
2 Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, Fiske A, et al: Role of genes and environments for explaining Alzheimer disease. Arch Gen Psychiatry 2006;63:168–174.
3 Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, et al: Association of apolipoprotein E allele ε4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43:1467–1472.
4 Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, et al: Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 1997;278:1349–1356.
5 Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, Katayama T, et al: The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet 2007;39:168–177.
6 Murayama S, Saito Y: Neuropathological diagnostic criteria for Alzheimer's disease. Neuropathology 2004;24:254–260.
7 Braak H, Braak E, Bohl J: Staging of Alzheimer-related cortical destruction. Eur Neurol 1993;33:403–408.
8 Pritchard JK, Stephens M, Donnelly P: Inference of population structure using multilocus genotype data. Genetics 2000;155:945–959.
9 Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Hu-Lince D, Zismann VL, et al: A high density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. J Clin Psychiatry 2007;68:613–618.