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## **THE SORTILIN-RELATED RECEPTOR SORL1 IS FUNCTIONALLY AND GENETICALLY ASSOCIATED WITH ALZHEIMER'S DISEASE**

### **STATEMENT OF GOVERNMENTAL INTEREST**

[0001] This invention was made with Government support through grants from the US National Institute for Aging, National Institute of Health Grant no. R37 AG15473, and PO1-AG 07232. The US government has certain rights in this invention. US National Institute on Aging: R01-AG09029, R01-AG25259, R01-AG17173, and P30-AG13846.

### **BACKGROUND**

#### **1. Field of the Invention**

[0002] The present invention relates methods for determining if a patient is at risk of developing Alzheimer's Disease by analyzing DNA from the patient and determining whether there are any nucleotide variants in the region of the gene for SORL1 at SNP sites 4 or 8-12 and 19-26.

#### **2. Description of the Related Art**

[0003] The accumulation of amyloid beta peptide (A $\beta$  peptide), a neurotoxic proteolytic derivative of the amyloid precursor protein (APP) is a central event in the pathogenesis of Alzheimer's Disease (AD)<sup>1</sup>. Thus, inherited variants in the amyloid precursor protein (APP)<sup>2</sup>, presenilin 1 (PS1)<sup>3</sup> presenilin 2 (PS2)<sup>4</sup> and apolipoprotein E (APOE) all cause A $\beta$  accumulation in the brain<sup>5 6</sup>. The generation of A $\beta$  occurs in several subcellular compartments, but a principle location is during the re-entry and recycling of APP from the cell surface via the endocytic pathway (FIG. 1A)<sup>7-11</sup>. The recycling of the amyloid precursor protein (APP) from the cell surface via the endocytic pathways plays a key role in the generation of the neurotoxic amyloid  $\beta$ -peptide (A $\beta$ ), the accumulation of which is thought to be central to the pathogenesis of Alzheimer's Disease (AD). Changes in the expression of several proteins involved in these pathways have previously been observed in AD brain tissue, and have been correlated with increased A $\beta$  production. However, it is unclear whether these changes are causal or merely secondary reactions.

**[0004]** Moreover, early diagnosis of AD or identification of people at risk of developing AD would permit early intervention. Although clinicians can diagnose “probable” AD, by taking a complete medical history and conducting various neuropsychological tests to determine memory loss and mental acuity, a definitive diagnosis of AD can only be confirmed by autopsy. Proper diagnosis of AD is critical because there are dozens of other causes of dementia that share symptoms with AD.

**[0005]** Therefore there is still a great need to understand the cause of metabolic changes associated with Alzheimer’s disease, to accurately diagnose AD, and to identify individuals at risk of developing AD.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0006] The present invention is illustrated by way of example, and not by way of limitation, in the figures of the accompanying drawings and in which like reference numerals refer to similar elements and in which:

[0007] **FIG. 1 A: Top panel:** Domain diagram of the SORL1 protein. VPS10 = vacuolar protein sorting 10-like domain; YWTD = low density lipoprotein receptor YWTD domain; EGF = EGF-like domain; LDLA = low density lipoprotein receptor class A domain; FN3 = fibronectin type 3 domain; TM = transmembrane domain. **Bottom panel:** Genomic map of SORL1 gene showing the location of SNPs genotyped in this study. Orange bars represent the 5'UTR and 3'UTR, red bar represents intragenic regions, and vertical bars represent each of the 48 exons. SNPs 1, 28 and 29 are located in extragenic intervals.

[0008] **B:** Diagram of APP processing pathways. APP holoprotein is synthesized in the endoplasmic reticulum (ER) and Golgi. Proteolytic cleavage through the A $\beta$  peptide domain by ADAM17 and other  $\alpha$ -secretase enzymes generates N-terminal soluble APPs $\alpha$  and membrane-bound APP-CTF $\alpha$  fragments. Sequential cleavage by BACE1 ( $\beta$ -secretase) generates N-terminal APPs $\beta$  and membrane bound APP-CTF $\beta$  fragments. The latter undergoes presenilin-dependent  $\gamma$ -secretase cleavage to generate A $\beta$  and amyloid intracellular domain (AICD). SORL1 binds both APP holoprotein (see FIG. 3) and VPS35 (not shown) and acts as a sorting receptor for APP holoprotein. Absence of SORL1 switches the APP holoprotein away from the retromer recycling pathway. The APP holoprotein is instead directed into the  $\beta$ -secretase cleavage pathway, increasing APPs $\beta$  production (FIG 2c) and then into the  $\gamma$ -secretase cleavage pathway to generate A $\beta$  (see FIG. 2b). Blockade of the retromer complex (RC) by inhibiting retromer complex proteins such as VPS26 (FIG. 2d) or VPS35, or Golgi-localized gamma-ear-containing ARF-binding (GGA) adaptor proteins, has a similar effect, also increasing APPs $\beta$  and A $\beta$  production.

[0009] **FIG. 2 A:** Small quantities of endogenous APP holoprotein but not APP C-terminal fragments (APP-CTFs, generated by  $\alpha$ - or  $\beta$ -secretase) can be co-immunoprecipitated with endogenous SORL1 (*Top panel*). Conversely small quantities of endogenous SORL1 can be co-precipitated with endogenous APP holoprotein (*Bottom panel*).

[0010] **B:** SORL1 does not interact with BACE1 ( $\beta$ -secretase). Co-immunoprecipitations with antibodies to over-expressed BACE1-V5 fail to capture SORL1 (*Bottom panel*).

Conversely, SORL1-directed antibodies do not co-immunoprecipitate BACE1 (*Top panel*) even though BACE1 also traffics through the endosome to Golgi pathway.

**[0011]** FIG. 3 **A:** Over-expression of SORL1 reduces A $\beta$ 40 (and A $\beta$ 42 not shown) secretion ( $p < 0.05$ ). *Upper panel:* Representative data of Western blot for SORL1 and APP in HEK293 cells stably expressing APP<sub>Swe</sub>, and transiently transfected with empty vector (mock) or SORL1 ( $n = 2$  independent transfections). *Lower panel:* Bar charts of ELISA assays of secreted A $\beta$ 40 (and A $\beta$ 42 not shown) following SORL1 over-expression. Error bar: SD; \* $p < 0.05$  compared to Control (2-tailed t-test);  $n = 2$  replications.

**[0012]** **B:** *Left panel:* Suppression of SORL1 expression with three independent siRNA primers (LR1222, LR1318, and LR5806) did not alter the expression levels or maturation of APP, APP-C83 C-terminal fragments or PS1, but (*Right panel*) significantly increased A $\beta$ 40 and A $\beta$ 42 secretion and APPs secretion (\* $p < 0.005$ , \*\*  $p < 0.001$  2-tailed t-test compared to controls,  $n = 5$  replications, 3 siRNA oligomers).

**[0013]** **C:** anti-SORL1 siRNA treatment results in significant increases in APPs $\beta$  secreted into the media, but no significant change in APPs $\alpha$  levels. *Left panel:* Western blots of conditioned media from cells treated with nonsense siRNA oligo-nucleotides (Controls #1 and #2) or with anti-SORL1 siRNA oligonucleotides investigated with the 2H3 antibody to APPs $\alpha$  or with SW192 antibody to APPs $\beta$  ( $n = 5$  replications). *Right panel:* quantitation normalized to the control. \*\*  $p < 0.0001$  2-tailed t-test compared to controls,  $n = 5$  replications.

**[0014]** **D:** *Top panel:* suppression of VPS26, another member of the VPS10 family involved in the retromer pathways also did not alter APP or PS1 maturation, but (*Middle and Bottom panels*) did increase both A $\beta$ 40 and A $\beta$ 42 secretion (\* $p < 0.005$ , \*\*  $p < 0.001$  2-tailed t-test compared to controls,  $n = 5$  replications, 2 siRNA oligomers). The control primer had no such effect.

**[0015]** **FIG. 4** Linkage disequilibrium(LD) structure of SORL1. Relative locations of single nucleotide polymorphisms (SNPs) included in each dataset are shown on two parallel stick diagrams, with LD maps for the TGEN dataset located above and the north European familial AD dataset in the Primary Study below the gene structure. The measure of LD(D0) among all possible pairs of SNPs is shown graphically according to the shade of red where white represents very low D0 and dark red represents very high D0. High D0 estimates

associated with a large confidence interval (most likely owing to one of the alleles being rare) are denoted by blue squares. TGEN: Translational Genomics Research Institute.

## SUMMARY OF THE INVENTION

**[0016]** A method for determining if a patient is at risk of developing Alzheimer's Disease, by a. obtaining a DNA sample from the patient, b. determining if the DNA sample shows a high risk nucleotide variant in an SNP that is a member of the group comprising SNPs rs560573, rs985421, rs593769, rs12364988, rs668387 [SNPs 8-12], rs4935775, 17 rs12285364, rs2298813 [SNPs 16-18], rs11600231, rs2276346, rs10502262, SORL1-T833T, rs556349, rs7131432, rs11218340 and rs10892756.[SNPs 19-26].of the sortilin-related receptor low density lipoprotein receptor class A (SORL1) gene, and c. if a high risk nucleotide variant is detected concluding that the patient is at high risk of developing Alzheimer's Disease, or if a low risk variant is detected concluding that the patient is at low risk of developing Alzheimer's Disease.

**[0017]** In one aspect, of the invention high risk nucleotide variants include the high risk nucleotide variant is an allele variant that is a member selected from the group that includes C at SNP, G at SNP rs985421, C at SNP rs593769, G at SNP rs11600231, T at SNP rs556349, C at SNP rs668387, T at SNP rs668387, G at SNP rs2276346, and G at SNP rs10892756. In another aspect, high risk nucleotide variants include a haplotype that is a member selected from the group that includes CGC at SNP rs560573, rs985421, and rs593769, respectively, or at a site in linkage disequilibrium with it; CGC at SNP rs985421, rs593769, and rs12364988, respectively, or at a site in linkage disequilibrium with it, GCC at SNP rs985421, rs593769, and rs12364988, respectively, or at a site in linkage disequilibrium with it, ATA at SNP CTT at SNP rs560573, rs985421, and rs593769, respectively, or at a site in linkage disequilibrium with it, CTT SORL1-T833T, rs556349, rs7131432, respectively, or at a site in linkage disequilibrium with it, TTC at SNP rs556349, rs7131432, rs11218340, respectively, or at a site in linkage disequilibrium with it, ACT at SNP rs556349, rs7131432, rs11218340, respectively, or at a site in linkage disequilibrium with it, TGG at SNP rs556349, rs7131432, rs11218340, respectively, or at a site in linkage disequilibrium with it, CTG at SNP rs7131432, rs11218340 and rs10892756, respectively, or at a site in linkage disequilibrium with it, GGT at SNP rs7131432, rs11218340 and rs10892756, respectively, or at a site in linkage disequilibrium with it, ATA at SNP rs4935775, rs12285364 and 18 rs2298813, respectively, or at a site in linkage disequilibrium with it, GAC at SNP rs7131432, rs11218340 and rs10892756, respectively, or at a site in linkage disequilibrium with it, CCA

at SNP rs7131432, rs11218340 and rs10892756, respectively, or at a site in linkage disequilibrium with it, ATA at SNP rs4935775, rs12285364 and rs2298813 or at a site in linkage disequilibrium with it, TAG rs12285364, rs2298813 and rs11600231, respectively, or at a site in linkage disequilibrium with it, AGG at SNP rs2298813, rs11600231, and rs2276346, respectively, or at a site in linkage disequilibrium with it, GGC at SNP rs11600231, 20 rs2276346 and rs10502262, respectively, or at a site in linkage disequilibrium with it, GCC rs2276346, 21, respectively, rs10502262 and SORL1-T833T or at a site in linkage disequilibrium with it, CAT at SNP rs560573, rs985421, and rs593769, respectively, or at a site in linkage disequilibrium with it, CGT at SNP rs560573, rs985421, and rs593769, respectively, or at a site in linkage disequilibrium with it, and TTC at SNP, rs556349, rs7131432, rs11218340, respectively, or at a site in linkage disequilibrium with it.

**[0018]** In another aspect the low risk nucleotide variants include a haplotype variant that is a member selected from the group that includes GCG at SNP rs560573, rs985421, and rs593769, respectively; or in linkage disequilibrium with it, GCG at SNP rs985421, rs593769, and rs12364988, respectively, or in linkage disequilibrium with it, CGG at SNP rs985421, rs593769, and rs12364988, respectively, or in linkage disequilibrium with it, TAT at SNP CTT at SNP rs560573, rs985421, and rs593769, respectively, or in linkage disequilibrium with it, GAA SORL1-T833T, rs556349, rs7131432, respectively, or in linkage disequilibrium with it, AAG at SNP rs556349, rs7131432, rs11218340, respectively, or in linkage disequilibrium with it, TGA at SNP rs556349, rs7131432, rs11218340, respectively, or in linkage disequilibrium with it, ACC at SNP rs556349, rs7131432, rs11218340, respectively, or in linkage disequilibrium with it, GAC at SNP rs7131432, rs11218340 and rs10892756, respectively, or in linkage disequilibrium with it, CCA at SNP rs7131432, rs11218340 and rs10892756, respectively, or in linkage disequilibrium with it, TAT at SNP rs4935775, rs12285364 and rs2298813, respectively, or in linkage disequilibrium with it, CTG at SNP rs7131432, rs11218340 and rs10892756, respectively, or in linkage disequilibrium with it, GGT at SNP rs7131432, rs11218340 and rs10892756, respectively, or in linkage disequilibrium with it, TAT at SNP rs4935775, rs12285364 and rs2298813, respectively, or in linkage disequilibrium with it, ATC rs12285364, rs2298813 and rs11600231, respectively, or in linkage disequilibrium with it, TCC at SNP rs2298813, rs11600231, and rs2276346, respectively, or in linkage disequilibrium with it, CCG at SNP rs11600231, 20 rs2276346 and rs10502262, respectively, or in linkage disequilibrium with it,

CGGrS2276346, 21 rs10502262 and SORL1-T833T, respectively, or in linkage disequilibrium with it, GTA at SNP rs560573, rs985421, and rs593769, respectively, or in linkage disequilibrium with it, GCA at SNP rs560573, rs985421, and rs593769, respectively, or in linkage disequilibrium with it, and AAG at SNP, rs556349, rs7131432, rs11218340, respectively, or in linkage disequilibrium with it.

**[0019]** Another aspect is directed to a method for confirming a diagnosis of Alzheimer's Disease in a patient having a diagnosis of probable or possible Alzheimer's Disease by: a. obtaining a DNA sample from the patient, b. determining if the DNA sample shows a high risk nucleotide variant in an SNP that is a member of the group comprising SNPs rs560573, rs985421, rs593769, rs12364988, rs668387 [8-12], rs4935775, 17 rs12285364, rs2298813 [16-18], rs11600231, rs2276346, rs10502262, SORL1-T833T, rs556349, rs7131432, rs11218340 and rs10892756.[19-26].of the sortilin-related receptor low density lipoprotein receptor class A (SORL1) gene, and c. if a high risk nucleotide variant is detected, concluding that the patient has Alzheimer's Disease, or if a low risk nucleotide variant is detected concluding that the patient does not have Alzheimer's Disease. In another aspect the method further includes: d. obtaining a cell sample from the patient, e. determining the level of SORL1 expression in the patient cell sample, f. comparing the level of expression of SORL1 in the patient cell sample to the level of expression of SORL1 in a cell sample taken from a normal patient, and g. determining that the patient has Alzheimer's Disease if the level of SORL1 expression in patient sample is significantly lower than the level in the cell sample from the normal patient.

**[0020]** Another aspect of the invention is directed to a method for determining if a patient is at risk of developing AD, by a. obtaining a cell sample from the patient, b. determining the level of SORL1 expression in the patient cell sample, c. comparing the level of expression of SORL1 in the patient cell sample to the level of expression of SORL1 in a cell sample taken from a normal patient, and d. determining that the patient is at risk for Alzheimer's Disease if the level of SORL1 expression in patient sample is significantly lower than the level in the cell sample from the normal patient.

**[0021]** Another aspect of the invention is directed to a method for treating Alzheimer's Disease in a patient, by administering SORL1 (preferably human recombinant SORL1) in a therapeutically effective amount that reduces the level of amyloid beta in the serum, plasma or cerebrospinal fluid (csf) of the patient. In a preferred embodiment the SORL1 is

formulated in a pharmaceutical composition that crosses the blood brain barrier and is recombinant human SORL1.

**[0022]** Another aspect of the invention is directed to a pharmaceutical composition for treating or preventing Alzheimer's Disease that includes human recombinant SORL1 in a formulation that crosses the blood brain barrier.

## DEFINITIONS

[0023] By “haplotype” is meant a is a set of closely linked genetic markers present on one chromosome which tend to be inherited together (not easily separable by recombination). Some haplotypes may be in linkage disequilibrium. haplotype can be identified by patterns of SNPs. Maps of SNPs (Haplotype maps) can be used to identify complex genetic variations of inherited diseases. A haplotype is a contraction of the phrase "haploid genotype".

[0024] By “linkage disequilibrium” is meant the case where the observed frequencies of haplotypes in a population does not agree with haplotype frequencies predicted by multiplying together the frequency of individual genetic markers in each haplotype. This indicates that the two markers are physically close on the same DNA strand.

[0025] By “genotype” is meant the specific allelic composition of a gene.

[0026] Significantly lower means that the difference is statistically significant.

[0027] As used herein, by "protein" or "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation).

[0028] By “biological activity” is meant the functional activity of SORL1 in a standardized quantity of tissue or cells.

[0029] Peptide variants” means polypeptides that may contain one or more substitutions, additions, deletions and/or insertions such that the therapeutic, antigenic and/or immunogenic properties of the peptides encoded by the variants are not substantially diminished, relative to the corresponding peptide. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide directed site-specific mutagenesis as taught, for example, by Adelman et al. (DNA, 2:183, 1983). Preferably, the antigenicity or immunogenicity of a peptide variant is not substantially diminished. Variants also include what are sometimes referred to as “fragments.” Fragments also include peptides that may contain one or more amino acid substitutions, additions, deletions and/or insertions, such that the biologic activity, therapeutic, antigenic and/or immunogenic properties of the peptide variants are not substantially diminished, relative to the corresponding peptide. When SORL1 is discussed in the context of expression, activity or secretion, or therapeutic use the term includes biologically active fragments and variants thereof, and recombinant or synthesized proteins. The preferred therapeutic form of SORL1 is human recombinant SORL1.

**[0030]** The term "therapeutically effective" or "effective amount" is intended to mean an amount of a compound sufficient to substantially improve some symptom associated with a disease or a medical condition. For example, in the treatment of AD, an agent or compound which decreases, prevents, delays, suppresses, or arrests any symptom of the disease or condition would be therapeutically effective, or which depresses amyloid beta formation as can be measured in serum, plasma or csf or the level of APPsBeta. In this case, a therapeutic amount of SORL1 includes an amount that decreases amyloid beta and APPsBeta production. A therapeutically effective amount of an agent or compound is not required to cure a disease or condition but will provide a treatment for a disease or condition such that the onset of the disease or condition is delayed, hindered, or prevented, or the disease or condition symptoms are ameliorated, or the term of the disease or condition is changed or, for example, is less severe. or recovery is accelerated in an individual.

## DETAILED DESCRIPTION

**[0031]** We have discovered that nucleotide variants within two distinct regions of the sortilin-related receptor low density lipoprotein receptor class A gene (hereafter “the SORL1 gene” or “SORL1”) are associated with both familial and sporadic Alzheimer’s disease. Our results show that certain genetic variants in the genomic interval containing SNPs 4 or 8-12, and 16-26 (defined by the dbSNP rs numbers listed in Table 1C) were associated with Alzheimer’s Disease, particularly familial Alzheimer’s Disease. SNPs 4 or 8-12 are clustered near the 5’ end of the gene and SNPs 16-26 are clustered near the 3’ end. We have further discovered that genetic variants (nucleotide variants) at these SNP regions are further associated with a decreased expression of the SORL1 gene in patients having AD, which in turn causes an increase in amyloid beta and APPsBeta levels, as is described below. For convenience we have consecutively numbered the SNPs we analyzed in the three studies described below. By “associated with” is meant here that a particular SORL1 allele, genotype or haplotype is disproportionately represented in individuals with Alzheimer’s disease compared with control subjects or in individuals having a higher/larger outcome for a measureable trait compared with individuals having a lower/smaller outcome for that trait.

**[0032]** Certain aspects of the invention are described above. We have shown that there is a causal relationship between AD and certain nucleotide variants at SNPs 4, 8-11, 19, and 22-26. (Our results in the two studies below expand the SNP range further.) Tables 1A (allele variants) and 1B(haplotype variants) list high and low risk factors at specific SNPs in the SORL1 gene. The haplotypes listed in Table 1B also include haplotypes that are in linkage disequilibrium with them. Where there is a high risk for a given haplotype (e.g. CGC at SNPs 8-10), the reverse is also true: there is a low risk for the opposite haplotype (e.g. GCG at SNPs 8-10). Certain embodiments of the invention are directed to a method for determining if a patient is at risk of developing Alzheimer’s Disease, by a. obtaining a DNA sample from the patient, b. determining if the DNA sample shows a nucleotide variant in an SNP that is a member of the group that includes SNPs rs661057 (SNP 4), rs560573, rs985421, rs593769, rs12364988, rs668387 [8-12], rs4935775, 17 rs12285364, rs2298813 [16-18], rs11600231, rs2276346, rs10502262, SORL1-T833T, rs556349, rs7131432, rs11218340 and rs10892756.[19-26] of the sortilin-related receptor low density lipoprotein receptor class A (SORL1) gene, and c. if a high risk nucleotide variant is detected concluding that the patient

is at high risk of developing Alzheimer's Disease, or if a low risk variant is detected concluding that the patient is at low risk of developing Alzheimer's Disease. Patients at risk of developing AD can also be identified by testing to see if the patient's SORL1 levels are below normal, and concluding that the patient is at risk of developing AD if the SORL1 levels are below normal. Patient samples suitable for obtaining a DNA sample include any cell or tissue, preferably fibroblasts, lymphoblasts, or epidermal cells. If a patient has a high risk genetic variant in the SORL1 gene or low SORL1 levels or both, his or her risk of developing AD is heightened. Should there be a familial incidence of AD, such a patient should be carefully monitored for early intervention.

**[0033]** Since there are many forms of dementia that mimic AD, another embodiment of the invention is directed to a method for confirming a diagnosis of Alzheimer's Disease in a patient having a diagnosis of probable or possible Alzheimer's Disease by determining if a DNA sample from the patient has a nucleotide variant in an SNPs 4, 8-12 or 16-26, and if a high risk nucleotide variant (Table 1A or B) is detected, concluding that the patient has Alzheimer's Disease, or if a low risk nucleotide variant is detected concluding that the patient does not have Alzheimer's Disease, but perhaps another form of dementia. A positive diagnosis of AD can also be confirmed by further testing to see if the patient's SORL1 levels are below normal, and concluding that the patient has AD if the patient has a diagnosis of probable AD and the SORL1 levels are below normal. Alternatively, both testing for genetic variants and below normal levels of SORL1 can be done to confirm a diagnosis of probable AD. These tests can also be used to identify individuals having a mild cognitive disorder who will progress to AD.

**[0034]** Other embodiments are directed to a method for treating or preventing AD by administering a therapeutically effective amount of SORL1, preferably human recombinant SORL1, preferably formulated to enhance its ability to cross the blood brain barrier. In one embodiment a therapeutically effective amount is an amount that decreases amyloid beta in plasma, serum or csf.

**Primary Study:**

**[0035]** The recycling of the amyloid precursor protein (APP) from the cell surface via the endocytic pathways plays a key role in the generation of the neurotoxic amyloid  $\beta$ -peptide ( $A\beta$ ), the accumulation of which is thought central to the pathogenesis of Alzheimer's Disease (AD). Prior data showed that: 1) the expression of several candidate proteins in the APP pathway (e.g. SORL1<sup>12</sup>, VPS35<sup>13</sup>) are reduced in AD brain tissue; and 2) reductions in the expression of some of these proteins are associated with increased  $A\beta$  production<sup>13-15</sup>. However, it was unclear whether these changes were causal, or were simply secondary and reactive to the AD process. We investigated several genes involved in these protein trafficking pathways. We discovered that inherited variants in two distinct regions of the neuronal SORL1 sorting receptor gene (at the 3' and 5' ends of the molecule) are reproducibly associated with late-onset AD in datasets from multiple different ethnic origins. We also discovered that SORL1 directs trafficking of APP into recycling pathways, and that when SORL1 is under-expressed, APP is sorted into the  $A\beta$ -generating late-endosomal compartments. (Data presented below.) These data lead to the conclusion that inherited changes in SORL1 gene expression or function are mechanistically involved in the AD-causal pathway. This means that genetic variants in these locations are consistently associated with AD. When combined with our biological studies (and those of others), variants that lower SORL1 are likely to be part of the cause of AD.

**[0036]** To address the question of causality, we investigated genetic associations between AD and single nucleotide polymorphisms (SNPs) in selected members of the vacuolar protein sorting (VPS) gene family including VPS35 (16q12); VPS26 (10q21); sortilin SORT1 (1p21-p13); sortilin-related VPS10 domain containing receptors 1-3 SORCS1 (10q23-q25), SORCS3 (10q23-q25), SORCS2 (4p16); and sortilin-related receptor, low density lipoprotein receptor class A repeats-containing; SORL1 (11q23-q24)]. The inheritance of SNPs in these genes was explored in six independent datasets of AD and unaffected subjects. These datasets have sufficient power to detect significant gene effects ( $\lambda_s = 1.5$ ). To minimize the confounding effects of allelic heterogeneity, which can be especially problematic with large cohorts<sup>16,17</sup>, the ethnic origins of each of these datasets

have been tightly controlled. Two of these six datasets (*Caribbean-Hispanic FAD* and *Israeli-Arab* datasets), have been drawn from population isolates with limited numbers of founders.<sup>18,19</sup>

**[0037]** We divided these six data sets into a ‘discovery cohort’ composed of families with late-onset familial Alzheimer disease (FAD) and a ‘replication cohort’ composed of discordant sibships and collections of individuals with Alzheimer disease and normal controls matched for age, gender and ethnic origin. We analyzed the FAD pedigrees in the discovery cohort (124 north European FAD families<sup>20,21</sup> and 228 Caribbean Hispanic FAD families<sup>22</sup>; Supplementary Table 1) with conservative family-based association (FBAT) methods, which are less sensitive to population stratification. We then reinvestigated positive results from the discovery cohort in the replication cohort (Supplementary Table 1). This replication cohort contained (i) northern European individuals from a case-control study (178 individuals with sporadic Alzheimer disease and 242 controls of self-identified Caucasian European ancestry)<sup>20</sup>, (ii) MIRAGE Caucasian sibships (276 Caucasian sibships from the MIRAGE Study)<sup>23,24</sup>, (iii) MIRAGE African American sibships (238 African American sibships from the MIRAGE Study)<sup>23,24</sup> and (iv) Israeli Arab affected individuals and controls (all 111 individuals with Alzheimer disease and 114 normal controls were from the Wadi Ara population study)<sup>19,25</sup>. Our results are reported in Rogaeva, E., et al., *Nature Genetics* 2007;39: 168-177, the contents of which are incorporated herein by reference.

**[0038]** We also obtained fully independent replication from a large data set composed of three cohorts of Americans of self-identified European Caucasian ancestries that were separately ascertained, genotyped and analyzed statistically at the Mayo Clinic (1,405 individuals with Alzheimer disease and 2,124 controls; Supplementary Table 1). The confirmation of the association among individuals from other ethnic groups increases the validity of the original observation. Differences in allele frequencies exist when comparing different racial and ethnic groups. However, when the same alleles are associated with a disease the likelihood that the association is real is increased, because it shows that the association is not affected by the genetic make-up of the population.

### **SNPs in SORL1 are associated with late-onset AD**

**[0039]** We initially screened at least two SNPs in the intragenic sequences of the SORL1, VPS26A, VPS35, SORCS1, SORCS3, SORCS2 and SORT1 genes for association with Alzheimer disease in the two independent FAD ‘discovery data sets’. We did not observe any allelic associations with VPS26A, VPS35, SORCS3 or SORT1 (Supplementary Tables 2 and 3). However, one SNP in SORCS1 (rs7082289:  $P \approx 0.013$ ), one SNP in SORCS2 (rs7694823:  $P \approx 0.015$ ) and two SNPs in SORL1 showed nominally significant association in at least one of the FAD data sets (rs2298813:  $P \approx 0.012$ ; rs2070045:  $P \approx 0.031$ ).

**[0040]** To validate these initial results, we investigated a second series of SNPs from the SORCS1, SORCS2 and SORL1 genes in the two FAD discovery data sets (Table 1C and FIG. 1b). We did not detect any association with the additional SNPs in SORCS1 (a total of nine SNPs) or in SORCS2 (a total of six SNPs) (Supplementary Tables 2 and 3). However, six SNPs clustered in two distinct regions of the SORL1 gene were significantly associated with Alzheimer disease in at least one discovery data set and also in at least one replication data set (Table 2 and Supplementary Table 4). Notably, at five of these SNPs, the alleles associated with Alzheimer disease were identical in both the discovery and replication data sets (Table 2 and Supplementary Table 4). Thus, at the 5’ end of SORL1, Alzheimer disease was associated with the C, G and C alleles at SNPs 8, 9 and 10, respectively, in the Caribbean Hispanic FAD ( $P \approx 0.013$ , 0.017 and 0.021, respectively), Israeli Arab case-control ( $P \approx 0.002$ , 0.007 and 0.005, respectively) and north European case-control data sets ( $P \approx 0.021$ , 0.040 and 0.067, respectively; Table 2 and Supplementary Table 4). Similarly, at the 3’ end of SORL1, Alzheimer disease was associated with the G and T alleles at SNPs 19 and 23, respectively, in the north European FAD ( $P \approx 0.031$  and 0.0031, respectively) and north European case-control data sets ( $P \approx 0.00082$  and 0.00073, respectively; Table 2 and Supplementary Table 4). Post hoc statistical adjustment for APOE genotype, age and gender did not alter the conclusions that (i) there were allelic associations between Alzheimer’s disease and two clusters of SNPs in distinct regions of SORL1 in different data sets and (ii) that these associations replicated in multiple independent data sets. We performed haplotypic analyses using a sliding window method<sup>26</sup> with a window size of three contiguous SNPs,

confirming the single-SNP analyses by demonstrating replicated haplotypic associations in two regions of SORL1 (Table 3 and Supplementary Table 5). At the 5' end of SORL1, the CGC haplotype at SNPs 8, 9 and 10 was associated with Alzheimer disease in the Caribbean Hispanic FAD (global  $P \leq 0.0098$ , haplotype  $P \leq 0.0053$ , haplotype Table 4). At the 5' end of SORL1, Alzheimer disease was associated with the C, G and C alleles at SNPs 8, 9 and 10, respectively, in the Caribbean Hispanic FAD ( $P \leq 0.013$ ,  $0.017$  and  $0.021$ , respectively), Israeli Arab case-control ( $P \leq 0.002$ ,  $0.007$  and  $0.005$ , respectively) and north European case-control data sets ( $P \leq 0.021$ ,  $0.040$  and  $0.067$ , respectively; Table 2 and Supplementary Table 4).

**[0041]** Similarly, at the 3' end of SORL1, Alzheimer disease was associated with the G and T alleles at SNPs 19 and 23, respectively, in the north European FAD ( $P \leq 0.031$  and  $0.0031$ , respectively) and north European case-control data sets ( $P \leq 0.00082$  and  $0.00073$ , respectively; Table 2 and Supplementary Table 4). Post hoc statistical adjustment for APOE genotype, age and gender did not alter the conclusions that (i) there were allelic associations between Alzheimer disease and two clusters of SNPs in distinct regions of SORL1 in different data sets and (ii) that these associations replicated in multiple independent data sets.

**[0042]** We performed haplotypic analyses using a sliding window method<sup>26</sup> with a window size of three contiguous SNPs (a person of skill in the art knows that the window size can be manipulated to capture larger haplotypes), confirming the single-SNP analyses by demonstrating replicated haplotypic associations in two regions of SORL1 (Table 3 and Supplementary Table 5). Thus, at the 5' end of SORL1, the CGC haplotype at SNPs 8, 9 and 10 was associated with Alzheimer disease in the Caribbean Hispanic FAD (global  $P \leq 0.0098$ , haplotype  $P \leq 0.0053$ , haplotype frequency estimated by FBAT  $\leq 0.638$  versus  $0.583$  in unrelated controls), the Israeli Arab case-control (global  $P \leq 0.023$ , haplotype  $P \leq 0.0085$ , frequency  $\leq 0.661$  in cases versus  $0.539$  in controls) and the north European case-control data set (haplotype  $P \leq 0.045$ , frequency  $\leq 0.638$  in cases versus  $0.566$  in controls; Table 3 and Supplementary Table 5). In the Israeli Arab data set, the overlapping GCC haplotype at SNPs 9, 10 and 11 showed even greater evidence for association (global  $P \leq 0.0080$ ; haplotype  $P \leq 0.0047$ ). As might be expected, SNPs 8, 9 and 10 also possessed a

protective haplotype. Thus, the TAT haplotype at SNPs 8, 9 and 10 was associated with decreased risk of Alzheimer's disease in these data sets (Hispanic FAD: haplotype P  $\frac{1}{4}$  0.0086; haplotype frequency estimated by FBAT  $\frac{1}{4}$  0.317 versus 0.394 in unrelated controls; Israeli Arab case-control: frequency  $\frac{1}{4}$  0.301 in affected individuals versus 0.434 in controls, and haplotype P  $\frac{1}{4}$  0.0037; north European Caucasian case control: frequency  $\frac{1}{4}$  0.351 in affected individuals versus 0.417 in controls, and haplotype P  $\frac{1}{4}$  0.068). We observed a second cluster of replicated haplotypic associations at the 3' end of SORL1 in the north European data sets. We found that the overlapping haplotypes of CTT at SNPs 22–24 and TTC at SNPs 23–25 were associated with Alzheimer disease in the north European FAD and north European case-control data sets (0.001 o haplotype P o 0.02; Table 3 and Supplementary Table 5). This region of SORL1 also showed significant haplotypic associations in the MIRAGE African American sibships. However, the haplotypic associations at SNPs 23–25 in the MIRAGE African American sibships were with different haplotypes (global P  $\frac{1}{4}$  0.0043; disease-associated 'ACT' haplotype-P  $\frac{1}{4}$  0.0025, frequency  $\frac{1}{4}$  0.513; protective 'ACC' haplotype P  $\frac{1}{4}$  0.0044, frequency  $\frac{1}{4}$  0.403; Table 3 and Supplementary Table 5). The conclusion that there are at least two distinct regions of SORL1 that are associated with Alzheimer's disease in different populations was supported when we examined shorter or longer haplotypes (Supplementary Tables 6–9).

**[0043]** To provide a completely independent confirmation of the association between Alzheimer disease and SORL1, we genotyped SNPs 4, 5, 8, 9, 12, 19 and 22–25 and analyzed them at an independent facility in three series of American affected individuals and controls of European ancestry ascertained at the Mayo Clinic (n  $\frac{1}{4}$  1,405 late-onset Alzheimer disease cases and 2,124 controls; Supplementary Table 1)<sup>27,28</sup>. The north European Caucasians and the Mayo data sets have slightly different allele frequencies and haplotype structures and may therefore have slightly different ancestral origins. Nevertheless, we observed significant associations at SNPs 4, 12, 19 and 23–25 in the overall Mayo data set (single- SNP:  $0.009 \leq P \leq 0.046$ ). Two of the three sub-data sets individually generated highly significant results ( $0.001 \leq P \leq 0.007$ ) for one or more of these SNPs (Table 4). Notably, the alleles and haplotypes at SNPs 19 and 22–25 that were associated with

increased risk for Alzheimer disease in the Mayo data sets (boldface in Tables 4 and 5) were the same as those associated with increased risk for Alzheimer disease in both the north European FAD data set and in the north European case-control data set (boldface in Tables 2 and 3). When we considered all of the Caucasian case control samples together (n = 1,583 Alzheimer disease cases and 2,366 controls), the associations remained robust (single-SNP:  $P = 0.002$  to  $P = 0.04$ , with three SNPs giving  $P = 0.008$ ). Notably, both the Mayo data set and the overall Caucasian case-control data set also detected association with SNP 4 ( $P = 0.009$  and  $P = 0.002$ , respectively), a result not evident in the individual data sets.

**[0044]** Risk is relative to the comparison group. In a case-control study or in families you are comparing the odds that an individual with a certain genotype is a case (affected with AD) compared to the odds that he is a control (unaffected). The ratio of the odds in large studies is roughly equivalent to risk ratio. Risk is then defined by the presence or absence of the risk factor - in this situation a particular genotype or allele or a particular haplotype. This is interpreted as a risk such that the risk of having AD is roughly X% higher among those with the allele than among those without. The reverse is also true that the risk is X% lower. Because we have identified SNPs that are useful for diagnosis, we have not yet found the true genetic effector, we can only assume that some defect in the gene will be identified that explains why certain genetic variations in the gene are associated with higher (or lower) risk.

**[0045]** We have shown that there is a causal relationship between AD if there is any nucleotide variant at SNPs 4, 8-11, 19, and 22-25. (Our results in the two studies below expand the SNP range further.) Tables 1A (allele variants) and Table 1B(haplotype variants) list high and low risk factors at specific SNPs in the SORL1 gene. The haplotypes listed in Table 1Balso include haplotypes that are in linkage disequilibrium with them. Where there is a high risk for a given haplotype (e.g. CGC at SNPs 8-10), the reverse is also true: there is a low risk for the opposite haplotype (e.g. GCG at SNPs 8-10). Certain embodiments are directed to a method for determining if a patient is at risk of developing Alzheimer's Disease, by a. obtaining a DNA sample from the patient, b. determining if the DNA sample shows a nucleotide variant in an SNP that is a member of the group that includes SNPs rs661057 (SNP 4), rs560573, rs985421, rs593769, rs12364988, rs668387 [8-12], rs4935775, 17

rs12285364, rs2298813 [16-18], rs11600231, rs2276346, rs10502262, SORL1-T833T, rs556349, rs7131432, rs11218340 and rs10892756.[19-26].of the sortilin-related receptor low density lipoprotein receptor class A (SORL1) gene, and c. if a high risk nucleotide variant is detected concluding that the patient is at high risk of developing Alzheimer's Disease, or if a low risk variant is detected concluding that the patient is at low risk of developing Alzheimer's Disease. Patients at risk of developing AD can also be identified by testing to see if the patient's SORL1 levels are below normal, and concluding that the patient is at risk of developing AD if the SORL1 levels are below normal. Patient samples suitable for obtaining a DNA sample include any cell or tissue, preferably fibroblasts, lymphoblasts, or epidermal cells. If a patient has both a high risk genetic variant in the SORL1 gene and low SORL1 levels, his or her risk of developing AD is heightened. Should there be a familial incidence of AD, such a patient should be carefully monitored for early intervention.

**[0046]** Since there are many forms of dementia that mimic AD, another embodiment of the invention is directed to a method for confirming a diagnosis of Alzheimer's Disease in a patient having a diagnosis of probable or possible Alzheimer's Disease by determining if a DNA sample from the patient has a nucleotide variant in an SNPs 4, 8-12 or 16-26, and if a high risk nucleotide variant (Table 1A or B) is detected, concluding that the patient has Alzheimer's Disease, or if a low risk nucleotide variant is detected concluding that the patient does not have Alzheimer's Disease, but perhaps another form of dementia. A positive diagnosis of AD can also be confirmed by further testing to see if the patient's SORL1 levels are below normal, and concluding that the patient has AD if the patient has a diagnosis of probable AD and the SORL1 levels are below normal. Alternatively, both testing for genetic variants and below normal levels of SORL1 can be done to determine if a patient with a probable diagnosis of AD actually has this disease.

### **Cell biology of SORL1**

**[0047]** To determine the role of SORL1 in AD, we conducted experiments to sequence the exons and immediate intron-exon boundaries in carriers of the disease-associated haplotypes at SNPs 8-10 or SNPs 22-24, and we investigated SORL1 splice forms recovered by RT-

PCR. We did not identify any pathogenic sequence variants enriched in individuals with Alzheimer disease (Supplementary Table 10). The possibility that the observed associations with SNPs inside SORL1 might reflect pathogenic variants outside SORL1 can be excluded because none of the SNPs flanking the 5' and 3' ends of SORL1 showed association with Alzheimer disease. It is therefore likely that the observed associations with SNPs reflect the presence of pathogenic variants within the intronic sequences of SORL1 near SNPs 8–10 and 22–24. We speculate that these putative intronic SORL1 variants modulate the cell type–specific transcription or translation of SORL1 in carriers of the Alzheimer disease–associated haplotypes. The individuals with high risk haplotypes would under-express SORL1. This hypothesis is supported by the recent observation of reduced expression of SORL1 in neurons but not glia of some individuals with sporadic Alzheimer disease<sup>12</sup>.

**[0048]** Direct exploration of this hypothesis is difficult. First, the variations in SORL1 expression in Alzheimer disease brain are cell-type specific, with SORL1 expression depressed in neurons but not glia<sup>12</sup>. Second, there are only limited brain tissue samples from individuals where SORL1 SNP marker phase (and thus haplotypes) are known. Nevertheless, we showed that Alzheimer disease–associated haplotypes in SORL1 are associated with reduced SORL1 transcription by quantitative real-time PCR studies of SORL1 expression in lymphoblasts from carriers of the CTT Alzheimer disease haplotype at SNPs 22–24. Sufficient numbers of samples were not available to test the effects of SNPs 8–10. These experiments demonstrated that SORL1 was expressed in Alzheimer disease haplotype carriers at less than half the levels observed in obligate carriers of non–Alzheimer disease haplotypes ( $10,324 \pm 8,215$  arbitrary units in carriers versus  $23,650 \pm 17,999$  in non-carriers (mean  $\pm$  s.d.), normalized to b-actin mRNA;  $P < 0.05$ , two-tailed Mann-Whitney U-test;  $n = 8$  independent samples;  $n = 3$  replications). However, it is also of note that univariate regression analyses showed that SORL1 haplotype status accounted for only 14% of this variance ( $P = 0.08$ ). This latter result implies, as expected, that other genetic and nongenetic factors can also modulate SORL1 expression and, perhaps, therefore, risk for Alzheimer disease.

**[0049]** The observation that specific nucleotide variants in SORL1 are associated with Alzheimer disease and that these same variants are accompanied by reduced SORL1 expression is significant because shows that the previously reported reductions in SORL1 expression in neurons in sporadic Alzheimer disease are causal rather than simply reactive. This conclusion is supported by that fact that SORL1 expression is not altered in other types of Alzheimer disease with known etiology (for example, FAD with mutant PSEN1)<sup>12,29</sup>. To determine how changes in SORL1 expression or function affect the risk for Alzheimer disease, we undertook cell biological experiments that demonstrated that SORL1 directly binds APP and differentially regulates its sorting into endocytic or recycling pathways (FIG. 1a). Co-immunoprecipitation experiments in native HEK cells demonstrated that endogenous SORL1 physically interacted with the endogenous APP holoprotein (FIG. 2) and with VPS35 (which drives cargo selection in the retromer via VPS10-containing proteins like SORL1 (ref. 30 and data not shown). SORL1, however, did not bind to APP C-terminal fragments produced by a-, b- or g-secretase cleavage (FIG. 2). These protein-protein interactions are specific because SORL1 did not bind to other type 1 membrane proteins (for example, BACE1 (ref. 31) and FIG. 3) or to VPS26 (which links VPS35 to the other structural elements of the retromer (ref. 30 and data not shown).

**[0050]** The interaction between SORL1, VPS35 and APP holoprotein provides a mechanism by which SORL1 regulates differential sorting of APP into the retromer recycling pathway or into the late endosomal pathway (where APP undergoes b- and g-secretase cleavage to generate Ab). Overexpression of SORL1, which would be predicted to divert APP holoprotein into the retromer recycling pathway, resulted decreased Ab production (82% of control,  $P < 0.05$ ,  $n = 5$  replications; FIG. 3a). Conversely, short interfering RNA (siRNA) suppression of SORL1 expression, which we speculate mimics the effects of Alzheimer disease-associated variants in SORL1, accordingly resulted in deflection of APP holoprotein away from the retromer recycling pathway and into the late endosome-lysosome pathway. As would be predicted, siRNA suppression of SORL1 led to (i) overproduction of the soluble N-terminal ectodomain of APP (APPsb) generated by BACE1 cleavage of APP holoprotein ( $149.45\% \pm 9.66$  of control (mean  $\pm$  s.e.m.),  $P < 0.0001$ ,  $n = 5$  replications; FIG.

3c) and (ii) overproduction of Ab by the subsequent g-secretase cleavage of the APP C-terminal stub generated by BACE1 (Ab40, 189% of control; Ab42, 202% of control,  $P < 0.001$ ; three independent siRNA oligonucleotides with five replications each; FIG. 3b). Our conclusion that SORL1 regulates sorting of APP into the retromer-recycling pathway is further supported by the observation of identical effects after suppression of the retromer proteins VPS26A (Ab40, 186% of control value; Ab42, 183% of control value,  $P < 0.001$ ,  $n = 5$  replications; FIG. 3d) or VPS35 (ref. 13). These results and conclusions are in very good agreement with independent reports that appeared during preparation of this manuscript<sup>14,15</sup>.

**[0051]** Taken together, our results show that genetic and possibly environmentally specified changes in SORL1 expression or function are causally linked to the pathogenesis of Alzheimer disease and have a significant effect on risk for this disease. The precise identity of the genetic effectors in SORL1 remains to be determined. However, the results described here show that (i) there are several different Alzheimer disease–associated allelic variants in distinct regions of the SORL1 gene in different populations; (ii) these variants are likely to be in intronic regulatory sequences that might govern cell type–specific or tissue-specific expression of SORL1 and (iii) these variants affect this risk by altering the physiological role of SORL1 in the processing of APP holoprotein.

**[0052]** In sharp contrast to APOE (where APOE e4 is associated with Alzheimer disease in most data sets<sup>32</sup>), no single SORL1 SNP or haplotype is associated with increased risk for Alzheimer disease in all six data sets. Although some data sets fail to show any association with SORL1, four points confirm that the association between SORL1 and Alzheimer disease is causal. First, the association was initially identified using conservative family-based association tests, which are less sensitive to confounding due to population stratification<sup>33</sup>. Second, at each set of SNP clusters, the same alleles and haplotypes were associated with increased risk for Alzheimer disease in at least three unrelated data sets, some of which were drawn from different ancestral origins. This shows that the haplotypes and allele variants cross ethnic lines and are true indicators of risk factors. Third, the discovery of association with different SNPs in different populations does not indicate a spurious result. The association of disease with a single allele in all data sets (that is, an APOE e4–like

association) is not a universal observation for either complex or monogenic diseases<sup>17</sup>. Thus, the occurrence of pathogenic mutations across multiple domains of disease genes (allelic heterogeneity) and the absence of these variants in some data sets (locus heterogeneity) are frequently observed in both monogenic and complex traits such as AD<sup>34,35</sup>. Fourth, the absence of significant associations in two data sets (MIRAGE Caucasian sibships and the Mayo Rochester data set) does not negate the findings from the other data sets. There are several potential explanations for the failure to detect a significant association in these two data sets. These potential explanations include (i) insufficient power to reliably detect the association in all series; (ii) locus heterogeneity (that is, non-SORL1 causes might have been over-represented and SORL1-associated causes underrepresented in some data sets) or (iii) allelic heterogeneity (that is, the association may have been obscured if the biologically active SORL1 alleles had occurred on multiple SNP backgrounds in some data sets).

**[0053]** Our results resolve the conundrum concerning the significance of reduced expression of SORL1 and several other genes potentially involved in APP trafficking in brain tissue from individuals with Alzheimer disease. Our results show that the reduction in SORL1 expression in affected brain tissue is likely to be a primary and pathogenic event, whereas the reduction in VPS35 expression is likely to be a secondary event. Finally, our data demonstrate that SORL1 has a key physiological role in the differential sorting of APP holoprotein. In the presence of SORL1, APP holoprotein is recovered via the retromer. In the absence of SORL1, APP is released into late endosomal pathways, where it is subjected to b-secretase cleavage, and subsequently g-secretase cleavage, which generates amyloid beta (FIG. 1a).

**[0054]** Now that the causal relationship between SORL1 and AD is known, another embodiment of the invention is directed to the therapeutic administration of SORL1 to treat or prevent AD; preferably human recombinant SORL1 is administered in a form that increases its ability of this large molecule (Molecular Weight of SORL1 is 248441 Daltons, and the Length of SORL1 is: 2214 amino acids) to cross the blood brain barrier. The effective amount of SORL1 can be determined by monitoring the amount of amyloid beta in serum, plasma or cerebrospinal fluid of an AD patient or a patient at risk of developing AD;

SORL1 should be administered in an amount that decreases amyloid beta. Sorl1 can be formulated and administered to treat or prevent AD by any means that produces contact of the active ingredient with the agent's site of action in the brain of a mammal. SORL1 can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic active ingredients or in a combination of therapeutic active ingredients. It can be administered alone, but is generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice for facilitating passage of a therapeutic agent through the blood brain barrier. Treatment of a subject having AD with a therapeutically effective amount of SORL1 can include a single treatment or, preferably, can include a series of treatments. The duration of administration and amount of drug can be influenced by the amelioration of the symptoms of the disease.

**[0055]** There is a great need for a means of identifying individuals having mild cognitive impairment who will progress to AD, as opposed to those who will not. One embodiment is directed to a method for identifying a patient having mild cognitive impairment who will progress to AD, by determining if the patient DNA has a genetic variant in the 3' or 5' end of the gene for SORL1, particularly at the SNPs we have identified. If the patient has a high risk variant, he or she will likely progress to AD and should be treated with early intervention accordingly.

| <b>TABLE 1A</b>      |                              |             |               |             |
|----------------------|------------------------------|-------------|---------------|-------------|
| <b>PRIMARY STUDY</b> |                              |             |               |             |
| <b>SNP LOCUS</b>     | <b>ALLELE</b>                | <b>RISK</b> | <b>ALLELE</b> | <b>RISK</b> |
|                      |                              |             |               |             |
| 8                    | C                            | HI          | G             | LOW         |
| 9                    | G                            | HI          | C             | LOW         |
| 10                   | C                            | HI          | G             | LOW         |
| 19                   | G                            | HI          | C             | LOW         |
| 23                   | T                            | HI          | A             | LOW         |
|                      |                              |             |               | LOW         |
|                      | <b>Autopsy<br/>Neurology</b> |             |               | LOW         |
| 8                    | C                            | HI          | G             | LOW         |
| 9                    | G                            | HI          | C             | LOW         |
|                      | <b>Archive Neuro</b>         |             |               | LOW         |
| 12                   | C                            | HI          | G             | LOW         |
| 12                   | T                            |             | A             | LOW         |
| 20                   | G                            | HI          | C             | LOW         |
| 26                   | G                            | HI          | C             | LOW         |

Table 1B

| <b>PRIMARY STUDY</b>         |                              |                               |
|------------------------------|------------------------------|-------------------------------|
| <b>SNP LOCUS</b>             | <b>HI RISK<br/>HAPLOTYPE</b> | <b>LOW RISK<br/>HAPLOTYPE</b> |
| 8, 9, 10                     | CGC                          | GCG                           |
| 9, 10, 11                    | CGC                          | GCG                           |
| 9, 10, 11                    | GCC                          | CGG                           |
| 8, 9, 10                     | ATA                          | TAT                           |
| 22, 23, 24                   | CTT                          | GAA                           |
| 23, 24, 25                   | TTC                          | AAG                           |
| 22-25                        | ACT                          | TGA                           |
| 22-25                        | TGG                          | ACC                           |
| 24, 25, 26                   | <i>CTG</i>                   | <i>GAC</i>                    |
| <b>24, 25, 26</b>            | <b><i>GGT</i></b>            | <b><i>CCA Protective</i></b>  |
| 16, 17, 18                   | ATA                          | TAT                           |
|                              |                              |                               |
| <b>AUTOPSY STUDY</b>         |                              |                               |
| 8, 9, 10                     | CGC                          | GCG                           |
| 23, 24, 25                   | ATC                          | TAG                           |
| <b>COMMUNITY BASED STUDY</b> |                              |                               |
| 24, 25, 26                   | <i>GAC</i>                   | <i>CTG</i>                    |
| <b>24, 25, 26</b>            | <b><i>CCA</i></b>            | <b><i>GGT</i></b>             |
| 16, 17, 18                   | ATA                          | TAT                           |
| 17, 18, 19                   | TAG                          | ATC                           |
| 18, 19, 20                   | AGG                          | TCC                           |
| 19, 20, 21                   | GGC                          | CCG                           |
| 20, 21, 22                   | GCC                          | CGG                           |
| 8, 9, 10                     | CAT                          | GTA                           |
| 8, 9, 10                     | CGT                          | GCA                           |
| 23, 24, 25                   | TTC                          | AAG                           |

**AUTOPSY**

**[0056]** To extend the results of the primary study, a series of autopsy confirmed cases with AD (n=103) of white, non-Hispanic origin were genotyped and compared to controls (n=93) from similar ethnic origins. The single SNP analyses revealed that the C and G alleles

in SNPs 8 and 9 were significantly associated with AD ( $0.015 \leq p \leq 0.017$ , Table 6a). Haplotype analysis confirmed this result, showing that the CGC haplotype in SNPs 8-9-10 was also significantly associated with AD (nominal  $p=0.0047$ , global  $p = 0.02$ , t 1b). Importantly, these were the same alleles within the same haplotype that was associated with AD in Caribbean Hispanic families, the Israeli Arabs and the northern European case-control series in the primary study. Haplotypes in the 3' region involving SNPs 23-24-25 previously found to be associated with AD were also significant (nominal  $p=0.015$ , global  $p =0.012$ , Table 6b), but the alleles differed; ATC in the autopsy series (Neurology 2007 in press) contrasted with TTC in the Primary Study.

#### **Community-based Study: Association of SORL1 Mutations with AD in Patients with Probable AD in an Urban, Multiethnic, Community-based Study**

**[0057]** To further explore the association between SORL1 and AD we studied an independent collection of patients with probably AD and healthy, elderly controls from a longitudinal prospective study of aging and dementia in a multiethnic community in northern Manhattan, NY, hereafter the “community-based study.” The investigation of a randomly sampled, prospectively studied, community-based cohort has advantages and disadvantages in replicating the earlier genetic association studies. The diagnoses for both affected and normal status are highly secure, and are relatively unaffected by referral bias inherent in clinic-based series (e.g. enrichment of patients from centers with known interests in genetics). However, sampling from community-based series ablates the ability to collate patients and controls according to their true genetic backgrounds. Nevertheless, we reasoned that, on balance, the investigation of samples from this study would serve as a credible source of independent replication of our earlier study, and provide insight as to the degree of allelic heterogeneity in the *SORL1* AD locus.

**[0058]     *Demographics.*** There were a total 724 participants in the study which included 296 (41%) individuals with probable AD. The mean age of the cohort was 81.1 years (s.d.=6.6) and the mean age of onset for the patients was 82.0 years (s.d.=7.2). As noted, Hispanics from the Dominican Republic were the most frequently represented ethnic group,

and there were more women than men in the analysis cohort. The other demographic characteristics are included in Table 7.

**[0059] *SORL1* Association.** The single SNP analyses revealed that three SNPs (SNPs 12, 20 and 26), were significantly associated with probable AD in at least one of the three case-control series (Table 8) ( $0.029 \leq p \leq 0.016$ ). SNP12, which is located 12.2 kb from the SNP 8-10 cluster associated with AD in multiple datasets in the primary study, was significantly associated with AD in the African-Americans and Caribbean Hispanics. The “T” allele at SNP 12 was significantly associated with AD in Caribbean Hispanics ( $p=0.029$ ). In the non-Hispanic Whites, the T allele was associated with AD, but was not significant ( $p=0.20$ ). The “T” allele was also associated with AD in both the Mayo Clinic autopsy cohort ( $p = 0.003$ ) and in the overall Mayo Clinic case-control cohort ( $p = 0.046$ ) in primary study showing an association between *SORL1* and AD (Table 4 in the Primary Study). In the African Americans, however, the “C” allele at SNP 12 was associated with AD ( $p=0.016$ ). SNP 20 was significantly associated with AD in the non-Hispanic white cases ( $p = 0.025$ , G allele). SNP 20 is closely flanked by both SNP 19 (-93 b.p.), which was associated with AD in several datasets in our report and by SNP 21 (+5966 b.p.), which was associated with AD in the North European case control dataset in the primary study. SNP 26 was associated with AD in the African American cohort ( $p = 0.019$ , G allele), and is located +6015 b.p. from the SNP23-25 cluster that had previously show a haplotypic association with AD in the MIRAGE African American sibships and in three different Caucasian datasets in the primary study.

#### ***SORL1* haplotype association.**

**[0060]** Haplotype analyses using a sliding window size of three contiguous SNPs demonstrated several haplotypic associations in all three ethnic group datasets (Table 9). Although these haplotypes were distributed across the *SORL1* gene and varied in frequency (0.01 to 0.037) several of them clustered. The common “TTC” haplotype at SNPs 23, 24, 25 was associated with AD in non-Hispanic Whites ( $p=0.035$ ). This same haplotype had previously been shown to be associated with AD in the North European FAD dataset and the

Israeli Arab, North European and Mayo Clinic - Jacksonville case-control datasets in the primary study. In this same region of the *SORL1* gene, the frequent “CCA” haplotype at SNPs 24, 25, 26 was robustly associated with AD in African-Americans (haplotype  $p=0.0006$ , empirical  $p=0.0005$  and global  $p$  value  $p=0.01$ ), while the common “CTG” haplotype at these same SNPs had a borderline protective effect ( $p = 0.06$ ). Intriguingly, these same haplotypes were also previously shown to be associated with AD in the MIRAGE African American cohort in the primary study. However, in the MIRAGE African Americans, “CCA” haplotype was protective while the “CTG” haplotype was deleterious. We interpret these opposite allelic associations to mean that one or more risk alleles nearby are in linkage disequilibrium with these haplotypes in this region in African Americans.

**[0061]** In the central region of *SORL1*, several frequent, overlapping haplotypes at SNPs 16-22 were also associated with AD. Thus the haplotypes “ATA” at SNPs 16-18, “TAG” at SNPs 17-19, “AGG” at SNPs 18-20, “GGC” at SNPs 19-21, and “GCC” at SNPs 20-22 were significantly associated with AD ( $0.0006 \leq \text{haplotype } p \leq 0.032$ ). The “ATA” haplotype at SNPs at 16-18 was also associated with risk for AD in the North European FAD dataset in the initial report (haplotype frequency 0.218,  $Z = 2.794$ , haplotype  $p = 0.005$ ; global  $p = 0.057$ ; Supplementary Table 5 herein).

**[0062]** At the 5' end of the gene, several low frequency haplotypes at SNPs 1-6 and SNPs 8-13 were also associated with AD in all three cohorts. The “CGC” haplotype at SNPs 8-10, which was previously associated with AD in the Caribbean Hispanic FAD, the Israeli Arab, and North European case-control datasets in the primary study, was not replicated in the sporadic Caribbean Hispanic case-control samples here. Nevertheless, at SNPs 8 -10, both the “CAT” haplotype in White non-Hispanics (haplotype  $p=0.014$ ) and the “CGT” haplotype in African-Americans (haplotype  $p=0.016$ ) were associated with AD.

**[0063]** These results independently confirm the previous conclusion that multiple nucleotide variants in *SORL1* are associated with AD. We directly replicated the previously reported association between AD and both the “TTC” haplotype at SNPs 23, 24, 25 and the “ATA” haplotype at SNPs 16-18 among Whites in the present and in the primary study. We have also shown that SNPs 26-28 display haplotypic association with AD in African

Americans in both the present dataset and in the previously studied MIIRAGE African Americans, although the some haplotypes had opposite effects on risk for AD in African Americans from these two datasets. A similar situation exists at SNPs 8-10 where different haplotypes were associated with AD in the community-based study and in the “initial” study (“CAT” in white non-Hispanics, and “CGT” in African Americans here, and “CGC” in datasets from our initial study<sup>1</sup>). The failure to detect an association at SNPs 8-10 in the sporadic Caribbean Hispanic AD cases from this population based cohort in Washington Heights does not contradict the findings from the earlier study of the Caribbean Hispanics FAD pedigrees. Indeed, weak allelic associations at SNP 12 (+11.7 kb from SNPs 8-10) and weak haplotypic associations at SNPs 4-6 (-7037 b.p. from SNPs 8-10) were observed in the 5’ region of the *SORL1* gene in this dataset. There are several possible explanations for the disparity in location of the association. First, there may be further allelic heterogeneity within the Caribbean Hispanic population. Second, it is conceivable that genetic factors such as *SORL1* play a relatively smaller role in community-based sporadic AD in Caribbean Hispanics, and are observable only with larger sample sizes or when investigated in sibships multiply affected with AD.

**[0064]** Taken together, this community-based study confirms that there is an association between AD and variants in the *SORL1* gene. The discovery of a significant association in multiple regions of the gene, and the discovery of different AD-associated haplotypes in different datasets supports the notion that there may be a high degree of allelic heterogeneity, with disease-associated variants occurring on multiple different haplotypic backgrounds. This situation differs markedly from the circumstances observed with *APOE*  $\epsilon 4$ . Two practical considerations arise from these conclusions. Further replication studies should include assess cohorts with as few founders as possible. Second attempts to identify the pathogenic variants in *SORL1* will likely have to investigate larger regions of the *SORL1* gene.

#### **Association between SORL1 and Alzheimer’s Disease in a Genome-Wide Study**

**[0065]** Recently, results of a genome-wide association study in AD were published [Reiman EM, et al. Neuron 2007; 54(5):713-720] with a focus on a novel association of

SNPs in the GAB2 gene with AD. This report however did not mention any results for SORL1. We were therefore interested to test whether the association of AD with SORL1 is replicated in this dataset (Translational Genomics Research Institute; TGEN), which is publicly available.

**[0066]** The TGEN database had 31 SORL1 SNPs, and eight of those overlapped the 29 SNPs in the primary study. These 31 SNPs are referred to by their sequential order on the physical map in TGEN database, such as T.1, T.2, T.n, T.31. Therefore, a total of 52 unique SNPs were analyzed in these two studies (Table 1). All SNPs are in Hardy–Weinberg equilibrium in control samples. The LD structures of SNPs in the 50 and 30 regions are similar in the north European family data in the primary study and TGEN data [Lee JH, et al., Arch Neurol 2007; 64:501–506.] (FIG. 4). Six SNPs (T.17, T.19, T.20, T.21, T.26, T.27) showed nominally significant association ( $0.01 < P < 0.05$ ) with AD under at least one model (Table 10). These six SNPs span a region of approximately 35 kb including SNPs 21–25 near the 30 end of SORL1 which were strongly associated with AD in the primary study. TGEN SNPs T.17 and T.19 are located between SNPs 20 and 21, TGEN SNPs T.20 and T.21 are between SNPs 22 and 23, and TGEN SNPs T.26 and T.27 are between SNPs 24 and 25 (Table 10). Haplotype analysis strengthened the association signal in the region including the two SNPs between SNPs 22 and 23 (global  $P < 0.005$ , data not shown).

**[0067]** Table 1A and Table 1B summarize the nucleotide variants at SNPs 8-12 and 19-26 from all 3 studies with their associated risk for developing AD.

**[0068]** The results from analysis of the TGEN data provide an independent replication of the association between AD and SORL1. We recognize that the magnitude of the significance of these results would not survive correction for multiple testing in a hypothesis-generating study (e.g. genome-wide association study); however, a P value of less than 0.05 is sufficient to confirm a previously reported association between variants within a specific gene and disease. Furthermore, although the particular SNP associations in this study are novel, a Bonferroni or similar correction is not appropriate or justified because all of the associated SNPs are within the region of and in linkage disequilibrium (LD) with SNPs implicated in Primary and the Autopsy studies, and therefore these are not truly independent

tests. Although the precise identity of genetic effectors in SORL1 remains unknown, the TGEN data support the hypothesis that at least one disease risk enhancing allele is located near the 3' end of SORL1.

### **Pharmaceutical Formulations**

**[0069]** Techniques for formulation and administration can be found in "Remington: The Science and Practice of Pharmacy" (20<sup>th</sup> edition, Gennaro (ed.) and Gennaro, Lippincott, Williams & Wilkins, 2000). Suitable routes of administration can include oral, intestinal, parenteral, transmucosal, transdermal, intramuscular, subcutaneous, transdermal, rectal, intramedullary, intrathecal, intravenous, intraventricular, intraatrial, intraaortal, intraarterial, or intraperitoneal administration. The pharmaceutical compositions of the present invention can be administered to the subject by a medical device, such as, but not limited to, catheters, balloons, implantable devices, biodegradable implants, prostheses, grafts, sutures, patches, shunts, or stents.

**[0070]** The dosage administered will be a therapeutically effective amount of the compound sufficient to result in amelioration of symptoms of the AD and will, of course, vary depending upon known factors such as the pharmacodynamic characteristics of the formulation of SORL1, its mode and route of administration; age, sex, health and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment, frequency of treatment and the effect desired.

Treatment of a subject having AD with a therapeutically effective amount of SORL1 can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with enough SORL1 to cause plasma or csf levels of amyloid beta to go down. This can be monitored using methods known in the art. The duration of administration and amount of drug can be influenced by the amelioration of the symptoms of the disease.

**[0071]** Dose determinations and formulations of the pharmaceutical compositions for use in accordance with the present invention are described in US Patent No. 20060165683, which

is incorporated herein by reference in its entirety, or may be formulated in any conventional manner known in the art.

**[0072]** Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). Since SORL1 is a naturally-occurring compound toxicity should be low. The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

**[0073]** For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

**[0074]** The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. In general, water, suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration contain preferably a water soluble salt of the active ingredient, suitable stabilizing agents and, if necessary, buffer substances. Antioxidizing agents such as sodium bisulfate, sodium sulfite or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium ethylenediaminetetraacetic acid (EDTA). In addition, parenteral solutions can contain preservatives such as benzalkonium chloride, methyl- or propyl-paraben and chlorobutanol. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, a standard reference text in this field.

**[0075]** In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long-acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

**[0076]** Additionally, standard pharmaceutical methods can be employed to control the duration of action. These are well known in the art and include controlled-release preparations and can include appropriate macromolecules, for example polymers, polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methyl cellulose, carboxymethyl cellulose or protamine sulfate. The concentration of macromolecules as well as the methods of incorporation can be adjusted in order to control release. Additionally, the

agent can be incorporated into particles of polymeric materials such as polyesters, polyamino acids, hydrogels, poly (lactic acid) or ethylenevinylacetate copolymers. In addition to being incorporated, these agents can also be used to trap the compound in microcapsules.

**[0077]**     Injectable: A parenteral composition suitable for administration by injection is prepared by stirring 1.5% by weight of active ingredients in 10% by volume propylene glycol and water. The solution is made isotonic with sodium chloride and sterilized.

**[0078]**     Suspension: An aqueous suspension is prepared for oral administration so that each 5 millimeters contain 100 milligrams of finely divided active ingredient, 200 milligrams of sodium carboxymethyl cellulose, 5 milligrams of sodium benzoate, 1.0 grams of sorbitol solution U.S.P. and 0.025 millimeters of vanillin.

**[0079]**     Accordingly, the pharmaceutical composition of the present invention may be delivered via various routes and to various sites in an animal body to achieve a particular effect (see, e.g., Rosenfeld et al., 1991, *supra*; Rosenfeld et al., 1991, Clin. Res., 39(2), 311A; Jaffe et al., *supra*; and Berkner, *supra*). One skilled in the art will recognize that although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route. Local or systemic delivery can be accomplished by administration including application or instillation of the formulation into body cavities, inhalation or insufflation of an aerosol, or by parenteral or intracranial introduction, and intramuscular, intravenous, peritoneal, subcutaneous, intradermal, as well as topical administration.

**[0080]**     A composition of the present invention can also be formulated as a sustained and/or timed release formulation. Such sustained and/or timed release formulations may be made by sustained release means or delivery devices that are well known to those of ordinary skill in the art, such as those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 4,710,384; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; and 5,733,566, the disclosures of which are each incorporated herein by reference. "Sustained release" refers to release of a therapeutic or prophylactic amount of a drug or an active metabolite thereof over a period of time that is longer than a conventional formulation of the drug. For oral formulations, the term "sustained release" typically means

release of the drug within the gastrointestinal tract lumen over a period of from about 2 to about 30 hours, more typically over a period of about 4 to about 24 hours. Sustained release formulations achieve therapeutically effective concentrations of the drug in the systemic blood circulation over a prolonged period of time relative to that achieved by oral administration of a conventional formulation of the drug. "Delayed release" refers to release of the drug or an active metabolite thereof into the gastrointestinal lumen after a delay time period, typically a delay of about 1 to about 12 hours, relative to that achieved by oral administration of a conventional formulation of the drug.

**[0081]** The pharmaceutical compositions of the present invention can be used to provide slow or sustained release of one or more of the active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or the like, or a combination thereof to provide the desired release profile in varying proportions. Suitable sustained release formulations known to those of ordinary skill in the art, including those described herein, may be readily selected for use with the pharmaceutical compositions of the invention. Thus, single unit dosage forms suitable for oral administration, such as, but not limited to, tablets, capsules, gelcaps, caplets, powders, and the like, that are adapted for sustained release are encompassed by the present invention.

**[0082]** The methods described herein are by no means all-inclusive, and further methods to suit the specific application will be apparent to the ordinary skilled artisan. Moreover, the effective amount of the compositions can be further approximated through analogy to compounds known to exert the desired effect.

#### **Methods to Enhance Penetration of the Blood Brain Barrier**

**[0083]** Because SORL1 is a very large molecule, preferred embodiments are directed to pharmaceutical formulations that optimize delivery of the drug to the brain. There are several ways to optimize SORL1 delivery to the brain. In one method SORL1 is derivatized to enhance BBB penetration by formation of a reversible linkage with one or more suitable groups so as to yield "pro-drugs", i.e. chemical derivatives that, after having passed through

the blood-brain barrier, are converted (back) to the original compound itself inside the patient's brain. Liberation of the parent compound may be by chemical hydrolysis or enzymatic attack. A derivative or pro-drug has an "enhanced blood-brain barrier permeability" according to the present invention or an "enhanced blood-brain barrier penetration" if, after administration of a pro-drug or derivative thereof to a living organism, a higher amount of the compound penetrates through the BBB, resulting in a higher level of effective agent in the brain, as compared to administration of the base compound without derivatization.

Known derivatives that facilitate penetration across the BBB include quaternary ammonium salts with a labile nitrogen-carbon bond at R5; mono- or diacyl derivatives (esters) of the hydroxyl groups of the base compounds (R1, R2); sugar derivatives, preferably glucuronides (R1, R2); derivatives coupled with nicotinic acid (R1, R2); and selected halogenides (R3).

**[0084]** Another derivative that increases BBB penetration is a lipophilic dihydropyridinium carrier. This Redox Chemical Delivery System (RCDS; Misra A. et al. 2003 J Pharm Pharmaceut Sci 6:252-273) is known to significantly enhance drug delivery through the BBB into the brain parenchyma. Once inside the brain, the dihydropyridinium moiety is enzymatically oxidized to the corresponding ionic pyridinium salt. Subsequent cleavage of the original compound from the carrier leads to liberation of the original compound and to sustained levels of it in the brain tissue. The derivatives obtained by chemical modification do not need to work as medicaments but rather may initially be pro-drugs that, after penetration through the blood-brain barrier, are converted (e.g., by brain enzymes) to the parent compound or a metabolite thereof and work as such as a medicament.

**[0085]** The BBB has specific receptors that allow the transport from the blood to the brain of several macromolecules. These transporters include those that transport insulin, transferrin, insulin-like growth factors 1 and 2 (IGF1 and IGF2), leptin, and lipoproteins. One noninvasive approach for the delivery of drugs to the CNS is to attach the agent (SORL1 or a fragment, variant or derivative of it) of interest to a molecule that binds with receptors on the BBB. The molecule then serves as a vector for transporting the agent across the BBB. Such structures are often referred to as "molecular Trojan horses (MTH)." Typically, though not necessarily, a MTH is an exogenous peptide or peptidomimetic moiety (e.g., a MAb to a

transport receptor) capable of binding to an endogenous BBB receptor mediated transport system that traverses the BBB on the endogenous BBB receptor-mediated transport system. In international publication no. WO2004/060403, the inventors have disclosed that AngioPep-1 (SEQ ID NO.:67) and aprotinin (SEQ ID NO. 98) are effective vectors for transporting desirable molecules across the blood brain barrier. Other peptides having similar domains as aprotinine and Angiopep-1 and a modified form of Angiopep-1 (amidated, peptide no. 67) are also used as potential carrier vectors. These derived peptides resemble aprotinine and Angiopep-1 but comprise different amino acid insertions and bear different charges. US application 20060189515.

**[0086]** Pharmaceutical compositions comprising micro particles having an average diameter ranging from 40 to 150 nm, consisting of one or more lipids, a drug and, optionally, a steric stabilizer have also been used successfully to transport large molecules across the blood-brain barrier. US Patent No. 6,419,949.

### **Protein Modifications**

**[0087]** Therapeutic SORL1 includes any biologically active fragment, epitope, modifications, derivatives or variants thereof. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of SORL1. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Variants of SORL1 include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or other molecule that facilitates transport through the BBB . (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein. For example, polypeptide variants containing amino acid substitutions of

charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity; see, e.g. Pinckard, Clin. Exp. Immunol. 2 (1967), 331-340; Bobbins, Diabetes 36 (1987), 838-845; Cleland. Crit. Rev. Therapeutic Drug Carrier Systems 10 (1993), 307-377.

**[0088]** "Amino acid residue" (including those making up SORL1p) refers to an amino acid which is part of a polypeptide. The amino acid residues described herein are preferably in the L" isomeric form. However, residues in the D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH<sub>2</sub> refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxyl group present at the carboxyl terminus of a polypeptide. "Amino acid residue" is broadly defined to include the 20 amino acids commonly found in natural proteins, as well as modified and unusual amino acids, such as those referred to in 37 C.F.R. Sections 1.821-1.822, and incorporated herein by reference. In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and can be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al. Molecular Biology of the Gene, 4th Edition, 1987, The Benjamin/Cummings Pub. Co., p.224).

**[0089]** Such substitutions are preferably made as follows: Original residue Conservative substitution Ala (A) Gly; Ser Arg (R) Lys Asn (N) Gln; His Cys (C) Ser Gln (Q) Asn Glu (E) Asp Gly (G) Ala; Pro His (H) Asn; Gln Ile (I) Leu; Val Leu (L) Ile; Val Lys (K) Arg; Gln; Glu Met (M) Leu; Tyr; Ile Phe (F) Met; Leu; Tyr Ser (S) Thr Thr (T) Ser Trp (W) Tyr Tyr (Y) Trp; Phe Val (V) Ile; Leu. Other substitutions are also permissible and can be determined empirically or in accord with known conservative substitutions.

**[0090]** Acylation of the N-terminal amino group can be accomplished using a hydrophilic compound, such as hydroorotic acid or the like, or by reaction with a suitable

isocyanate, such as methylisocyanate or isopropylisocyanate, to create a urea moiety at the N-terminus. Other agents can also be N-terminally linked that will increase the duration of action of the SRIF analog as known in this art.

**[0091]** Reductive amination is the process by which ammonia is condensed with aldehydes or ketones to form imines which are subsequently reduced to amines. For drugs bearing one or more amino groups, reductive amination is a potentially useful method for conjugation to PEG. Covalent linkage of poly(ethylene glycol) (PEG) to drug molecules results in water-soluble conjugates with altered bioavailability, pharmacokinetics, immunogenic properties, and biological activities. For drugs bearing one or more amino groups, reductive amination is a potentially useful method for conjugation to PEG. Bentley et al., J Pharm Sci. 1998 Nov;87(11):1446-9.

**[0092]** As is also well known, polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of post-translation events, including natural processing events and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translational natural processes and by synthetic methods.

**[0093]** Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. Blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally-occurring and synthetic polypeptides. For instance, the amino-terminal residue of polypeptides made in E. coli, prior to proteolytic processing, almost invariably will be N-formylmethionine.

**[0094]** The modifications can be a function of how the protein is made. For recombinant polypeptides, for example, the modifications will be determined by the host cell posttranslational modification capacity and the modification signals in the polypeptide amino acid sequence. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic cell. Insect cells often carry out the same posttranslational glycosylations as mammalian cells, and, for this reason, insect cell

expression systems have been developed to efficiently express mammalian proteins having native patterns of glycosylation. Similar considerations apply to other modifications. The same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain more than one type of modification.

| Protein Modification | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Acetylation          | <p>Acetylation of N-terminus or e-lysines. Introducing an acetyl group into a protein, specifically, the substitution of an acetyl group for an active hydrogen atom.</p> <p>A reaction involving the replacement of the hydrogen atom of a hydroxyl group with an acetyl group (<math>\text{CH}_3\text{CO}</math>) yields a specific ester, the acetate. Acetic anhydride is commonly used as an acetylating agent, which reacts with free hydroxyl groups.</p> <p>Acylation may facilitate addition of other functional groups. A common reaction is acylation of e.g., conserved lysine residues with a biotin appendage.</p> |
| ADP-ribosylation     | Covalently linking proteins or other compounds via an arginine-specific reaction.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Alkylation           | Alkylation is the transfer of an alkyl group from one                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

| Protein Modification | Description                                                                                                                                                                                                                                                                                                                                                                       |
|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                      | molecule to another. The alkyl group may be transferred as an alkyl carbocation, a free radical or a carbanion (or their equivalents). Alkylation is accomplished by using certain functional groups such as alkyl electrophiles, alkyl nucleophiles or sometimes alkyl radicals or carbene acceptors. A common example is methylation (usually at a lysine or arginine residue). |
| Amidation            | Reductive animation of the N-terminus. Methods for amidation of insulin are described in U.S. 4,489,159.                                                                                                                                                                                                                                                                          |
| Carbamylation        | Nigen et al. describes a method of carbamylating hemoglobin.                                                                                                                                                                                                                                                                                                                      |
| Carboxylation        | Carboxylation typically occurs at the glutamate residues of a protein, which may be catalyzed by a carboxylase enzyme (in the presence of Vitamin K – a cofactor).                                                                                                                                                                                                                |
| Citrullination       | Citrullination involves the addition of citrulline amino acids to the arginine residues of a protein, which is catalyzed by peptidylarginine deaminase enzymes (PADs). This generally converts a positively charged arginine into a neutral citrulline residue, which may affect the                                                                                              |

| Protein Modification                                | Description                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|-----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                     | hydrophobicity of the protein (and can lead to unfolding).                                                                                                                                                                                                                                                                                                                                                                                            |
| Condensation of amines with aspartate or glutamate  | Such reactions, may be used, e.g., to attach a peptide to other proteins labels.                                                                                                                                                                                                                                                                                                                                                                      |
| Covalent attachment of flavin                       | Flavin mononucleotide (FAD) may be covalently attached to serine and/or threonine residues. May be used, e.g., as a light-activated tag.                                                                                                                                                                                                                                                                                                              |
| Covalent attachment of heme moiety                  | A heme moiety is generally a prosthetic group that consists of an iron atom contained in the center of a large heterocyclic organic ring, which is referred to as a porphyrin. The heme moiety may be used, e.g., as a tag for the peptide.                                                                                                                                                                                                           |
| Attachment of a nucleotide or nucleotide derivative | May be used as a tag or as a basis for further derivatising a peptide.                                                                                                                                                                                                                                                                                                                                                                                |
| Cross-linking                                       | Cross-linking is a method of covalently joining two proteins. Cross-linkers contain reactive ends to specific functional groups (primary amines, sulfhydryls, etc.) on proteins or other molecules. Several chemical groups may be targets for reactions in proteins and peptides. For example, Ethylene glycol bis[succinimidylsuccinate, Bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone, and Bis[sulfosuccinimidyl] suberate link amines to amines. |

| Protein Modification     | Description                                                                                                                                                                                                                                                                                                                                                                                      |
|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cyclization              | For example, cyclization of amino acids to create optimized delivery forms that are resistant to, e.g., aminopeptidases (e.g., formation of pyroglutamate, a cyclized form of glutamic acid).                                                                                                                                                                                                    |
| Disulfide bond formation | Disulfide bonds in proteins are formed by thiol-disulfide exchange reactions, particularly between cysteine residues (e.g., formation of cystine).                                                                                                                                                                                                                                               |
| Demethylation            | See, e.g., U.S. 4,250,088 (Process for demethylating lignin).                                                                                                                                                                                                                                                                                                                                    |
| Formylation              | The addition of a formyl group to, e.g., the N-terminus of a protein. See, e.g., U.S. Patent Nos. 4,059,589, 4,801,742, and 6,350,902.                                                                                                                                                                                                                                                           |
| Glycylation              | The covalent linkage of one to more than 40 glycine residues to the tubulin C-terminal tail.                                                                                                                                                                                                                                                                                                     |
| Glycosylation            | Glycosylation may be used to add saccharides (or polysaccharides) to the hydroxy oxygen atoms of serine and threonine side chains (which is also known as O-linked Glycosylation). Glycosylation may also be used to add saccharides (or polysaccharides) to the amide nitrogen of asparagine side chains (which is also known as N-linked Glycosylation), e.g., via oligosaccharyl transferase. |

| Protein Modification | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| GPI anchor formation | The addition of glycosylphosphatidylinositol to the C-terminus of a protein. GPI anchor formation involves the addition of a hydrophobic phosphatidylinositol group - linked through a carbohydrate containing linker (e.g., glucosamine and mannose linked to phosphoryl ethanolamine residue) - to the C-terminal amino acid of a protein.                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Hydroxylation        | Chemical process that introduces one or more hydroxyl groups (-OH) into a protein (or radical). Hydroxylation reactions are typically catalyzed by hydroxylases. Proline is the principal residue to be hydroxylated in proteins, which occurs at the C <sup>γ</sup> atom, forming hydroxyproline (Hyp). In some cases, proline may be hydroxylated at its C <sup>β</sup> atom. Lysine may also be hydroxylated on its C <sup>δ</sup> atom, forming hydroxylysine (Hyl). These three reactions are catalyzed by large, multi-subunit enzymes known as prolyl 4-hydroxylase, prolyl 3-hydroxylase and lysyl 5-hydroxylase, respectively. These reactions require iron (as well as molecular oxygen and α-ketoglutarate) to carry out the oxidation, and use ascorbic acid to return the iron to its reduced state. |
| Iodination           | See, e.g., U.S. 6,303,326 for a disclosure of an enzyme that                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

| Protein Modification  | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                       | is capable of iodinating proteins. U.S. 4,448,764 discloses, e.g., a reagent that may be used to iodinate proteins.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| ISGylation            | Covalently linking a peptide to the ISG15 (Interferon-Stimulated Gene 15) protein, for, e.g., modulating immune response.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Methylation           | <p>Reductive methylation of protein amino acids with formaldehyde and sodium cyanoborohydride has been shown to provide up to 25% yield of N-cyanomethyl (-CH<sub>2</sub>CN) product. The addition of metal ions, such as Ni<sup>2+</sup>, which complex with free cyanide ions, improves reductive methylation yields by suppressing by-product formation. The N-cyanomethyl group itself, produced in good yield when cyanide ion replaces cyanoborohydride, may have some value as a reversible modifier of amino groups in proteins. (Gidley et al.) Methylation may occur at the arginine and lysine residues of a protein, as well as the N- and C-terminus thereof.</p> |
| <b>Myristoylation</b> | <p>Myristoylation involves the covalent attachment of a myristoyl group (a derivative of myristic acid), via an amide bond, to the alpha-amino group of an N-terminal glycine residue. This addition is catalyzed by the N-myristoyltransferase enzyme.</p>                                                                                                                                                                                                                                                                                                                                                                                                                    |

| Protein Modification            | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Oxidation</b>                | <p>-Oxidation of cysteines.</p> <p>-Oxidation of N-terminal Serine or Threonine residues (followed by hydrazine or aminooxy condensations).</p> <p>-Oxidation of glycosylations (followed by hydrazine or aminooxy condensations).</p>                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Palmitoylation</b>           | <p>Palmitoylation is the attachment of fatty acids, such as palmitic acid, to cysteine residues of proteins.</p> <p>Palmitoylation increases the hydrophobicity of a protein.</p>                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>(Poly)glutamylation</b>      | <p>Polyglutamylation occurs at the glutamate residues of a protein. Specifically, the gamma-carboxy group of a glutamate will form a peptide-like bond with the amino group of a free glutamate whose alpha-carboxy group may be extended into a polyglutamate chain. The glutamylation reaction is catalyzed by a glutamylase enzyme (or removed by a deglutamylase enzyme). Polyglutamylation has been carried out at the C-terminus of proteins to add up to about six glutamate residues. Using such a reaction, Tubulin and other proteins can be covalently linked to glutamic acid residues.</p> |
| <b>Phosphopantetheinylation</b> | The addition of a 4'-phosphopantetheinyl group.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Phosphorylation</b>          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

| Protein Modification          | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                               | A process for phosphorylation of a protein or peptide by contacting a protein or peptide with phosphoric acid in the presence of a non-aqueous apolar organic solvent and contacting the resultant solution with a dehydrating agent is disclosed e.g., in U.S. 4,534,894. Insulin products are described to be amenable to this process. See, e.g., U.S. 4,534,894. Typically, phosphorylation occurs at the serine, threonine, and tyrosine residues of a protein. |
| <b>Prenylation</b>            | Prenylation (or isoprenylation or lipidation) is the addition of hydrophobic molecules to a protein. Protein prenylation involves the transfer of either a farnesyl (linear grouping of three isoprene units) or a geranyl-geranyl moiety to C-terminal cysteine(s) of the target protein.                                                                                                                                                                           |
| <b>Proteolytic Processing</b> | Processing, e.g., cleavage of a protein at a peptide bond.                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Selenoylation</b>          | The exchange of, e.g., a sulfur atom in the peptide for selenium, using a selenium donor, such as selenophosphate.                                                                                                                                                                                                                                                                                                                                                   |
| <b>Sulfation</b>              | Processes for sulfating hydroxyl moieties, particularly tertiary amines, are described in, e.g., U.S. 6,452,035. A process for sulphation of a protein or peptide by contacting the protein or peptide with sulphuric acid in the presence of a non-aqueous apolar organic solvent and contacting the resultant solution with a dehydrating agent is disclosed.                                                                                                      |

| Protein Modification                                              | Description                                                                                                                                                                                   |
|-------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                   | Insulin products are described to be amenable to this process. See, e.g., U.S. 4,534,894.                                                                                                     |
| <b>SUMOylation</b>                                                | Covalently linking a peptide a SUMO (small ubiquitin-related Modifier) protein, for, e.g., stabilizing the peptide.                                                                           |
| <b>Transglutamination</b>                                         | Covalently linking other protein(s) or chemical groups (e.g., PEG) via a bridge at glutamine residues                                                                                         |
| <b>tRNA-mediated addition of amino acids (e.g., arginylation)</b> | For example, the site-specific modification (insertion) of an amino acid analog into a peptide.                                                                                               |
| <b>Ubiquitination</b>                                             | The small peptide ubiquitin is covalently linked to, e.g., lysine residues of a protein. The ubiquitin-proteasome system can be used to carryout such reaction. See, e.g., U.S. 2007-0059731. |

## **EXAMPLES**

### **Example 1: Primary Study**

**[0095] Subjects.** Informed consent was obtained from all participants using procedures approved by institutional review boards at each of the clinical research centers collecting human subjects. The clinical diagnosis of ‘probable’ or ‘possible’ Alzheimer disease was defined according to the National Institute of Neurological and Communication Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) diagnosis criteria at clinics specializing in memory disorders. Clinical characteristics of the north European, MIRAGE, Caribbean Hispanic FAD, Israeli Arab and Mayo Caucasian American data sets are summarized in Supplementary Table 1<sup>19,20,22–25,27,2</sup>). The north European case-control set is drawn from the same populations as the north European FAD data set<sup>20,22</sup>. The three Mayo data sets were drawn from Caucasian affected individuals and controls assessed in clinical series at the Rochester and Jacksonville Mayo Clinics or from Caucasian brains in which the presence or absence of Alzheimer disease was determined neuropathologically by autopsy (‘AUT’ in Supplementary Table 1).

**[0096] Genetic analyses.** Genotyping was performed using the GenomeLab SNPstream System®, and primer sets were as in Supplementary Table 3 (Beckman Coulter). We genotyped 100 DNA samples twice for every SNP marker (the concordance rate was 499%). APOE was genotyped as described<sup>5</sup>. Genotyping of the Mayo samples was performed on an ABI 7900 instrument using TaqMan chemistry with primers and probes designed by Applied Biosystems. The entire ORF of the SORL1 gene was sequenced in 12 individuals with sporadic Alzheimer disease, 12 individuals with familial Alzheimer disease and two normal controls selected from the north European and Caribbean Hispanic data sets (Supplementary Tables 3 and 10). Alternatively spliced transcripts were sought by conventional RT-PCR in eight overlapping fragments using total RNA isolated from frontal cortex (16 normal controls and 17 individuals with sporadic Alzheimer disease from the Canadian Brain Tissue Bank and the New York Brain Bank; Supplementary Table 3).

**[0097] Statistical analyses.** SNP marker data were assessed for deviations from Hardy-Weinberg equilibrium (using Pedstats software) and for Mendelian inheritance errors (using Pedcheck software). Single-point family-based association was assessed with FBAT v1.5.5 (ref. 36), using an additive genetic model with the null hypothesis of no linkage and no association. Allele frequencies were estimated by FBAT using the EM algorithm. APOE e4 carrier status was included in the analyses using PBAT v2.6 (refs. 37–40). The w2 test (or the Fisher's exact test) was used to assess genotypic and allelic associations between Alzheimer disease. Multivariate logistic regression analysis was performed to adjust for APOE e4, sex and age-at-onset or age-at-examination.

**[0098] Statistical significance and multiple testing corrections.** The Benjamini corrected false discovery rate (FDR)<sup>41</sup> was used with a cutoff level of 0.1 to correct for multiple testing. The P values presented are nominal P values. The cutoff P values for significance in each data set are shown in the table legends.

**[0099] Linkage disequilibrium.** LD structure was examined using Haploview. Haplotype blocks were defined using the confidence intervals algorithm. The default settings were used in these analyses, which create 95% confidence bounds on D' to define SNP pairs in strong LD.

**[0100] Haplotype analyses.** Haplotype analyses were carried out with a sliding window of three contiguous SNPs using FBAT for family data and Haplo.stats v1.1.1 for case-control data<sup>16,26,42–44</sup>. The analyses were repeated using sliding windows of two, four, five and six SNPs. Expression plasmids and cDNA constructs for human SORL1. The cDNA clones encoding APP K670N/M671L Swedish mutation (APPSwe) and BACE1 (V5-tagged at the C terminus) were as described previously<sup>45,46</sup>.

**[0101] Cell culture and transfection.** The HEK293 cell line stably expressing APPS was as described<sup>47</sup>. Transient transfection of BACE1 cDNA was performed using LipofectAMINE 2000® (Invitrogen).

**[0102] RNA interference.** siRNA oligonucleotides were designed using the online siRNA Design Tool ® (Dharmacon Research). The siRNAs for SORL1 are in

Supplementary Table 3. The siCONTROL Non-Targeting siRNAs #1 and #2 (Dharmacon Research) were used as a negative control.

**[0103]** Transfections were performed using LipofectAMINE 2000® according to the manufacturer's recommendations. In case of consecutive transfections, cells were split after 24 h and then retransfected 24 h later. After culturing for an additional 24 h, the conditioned medium was collected for the Ab assay, and the cells were harvested for protein blotting.

**[0104]** Antibodies, immunoprecipitation and protein blotting. Antibodies were as follows: mouse monoclonal anti-human LR11/SORL1gp250 (BD Transduction Laboratories) and 5-4-30-19-2 (from H.B.); rabbit antibody to the C terminus of SORL1 (from W.H.); rabbit polyclonal antibody to PS1-NTF (Ab14, from S. Gandy, Temple University); mouse monoclonal anti-myc (Invitrogen); rabbit polyclonal antibody to the C terminus of APP (Sigma) and anti-BACE1 (EE-17, Sigma). Proteins were immunoprecipitated in 1% digitonin<sup>48</sup>, subjected to protein blot and visualized by ECL (Amersham). Ab, APPsa and APPsb assays. Ab40 and Ab42 peptide levels were measured by sandwich ELISA<sup>49</sup>. APPs, APPsa and APPsb were measured by protein blotting using antibodies 22C11 (Chemicon), 2H3 and SW192 (Elan Pharmaceuticals), respectively. Differences were assessed by two-tailed Student's t-test.

**[0105]** **Quantitative RT-PCR.** PCR primer pairs targeting SORL1 exon 23 were as in Supplementary Table 3. Total RNA (5 mg) was reverse transcribed using a random hexamer. Real-time PCR was performed in a 384-well format using an ABI Prism 7900HT instrument and the Sybr Green detection method. Samples were analyzed in triplicate, and mean expression levels corresponding to SORL1 mRNA expression were normalized to b-actin mRNA levels.

## **EXAMPLE 2**

### **AUTOPSY STUDY**

**[0106]** Frozen brain tissue was obtained from 103 autopsy confirmed cases of AD, and from 17 elderly controls with a normal postmortem examination, and without a history of dementia or another neurological disorder. . To augment the number of controls from the same ethnic background, we included 76 non-demented elderly participants who have been

followed prospectively at approximately 18-month intervals as part of a study of aging and dementia among Medicare recipients residing in northern Manhattan since 1999. The average age of onset for the patients was 80.5 and 52.4% were women. The mean age of the combined group of controls was 79.7 years and 48.4% were women. The Institutional Review Boards of Columbia University Medical Center and the New York Psychiatric Institute approved recruitment, informed consent and study procedures.

**[0107]** Genotyping was performed using matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (Sequenom). Detailed information on genotyping was previously described (1), and is available upon request. The numbering system for the SNPs 1 to 29 reflects their relative order on the physical map of *SORLI*, and was the same system used in the primary study. We restricted the work to include only 12 of the previously genotyped SNPs to focus on 5' (SNPs 1, 2 and 7-10) and 3' (SNPs 13, 17 and 22-25) regions highlighted in the previous report. SNP marker data were assessed for deviations from Hardy-Weinberg equilibrium using the HAPLOVIEW program (Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263-265.), and none deviated. The  $\chi^2$  test (or the Fisher's exact test) was used to for analysis of genotypic and allelic associations between AD and each of the SNP markers. The HAPLOVIEW program was used to perform single point analysis, estimates of linkage disequilibrium (LD) structure and haplotype blocks. Haplotype analyses were performed with HAPLO.STATS v1.1.1 for case-control data using the same sliding window of three contiguous SNPs as described in the primary study. The objective of this study was to confirm associations in the primary study and in the community-based study (below), therefore a nominal p-value of 0.05 was considered sufficient evidence of confirmation (Ott J. *Analysis of human genetic linkage*, 3rd ed. Baltimore: Johns Hopkins University Press, 1999.). Consequently, nominal p-values are presented in the table for single point and haplotype analyses.

### EXAMPLE 3

### **Association Between Genetic Variants in SORLI and AD in an Urban, Multiethnic, Community-Based Cohort**

**[0108]     *Subjects and Setting.*** The patients with AD and non-demented elderly assessed here were participants in a prospective study of aging and dementia in Medicare recipients, 65 years and older, residing in northern Manhattan (Washington Heights, Hamilton Heights and Inwood). This epidemiological study originally consisted of a stratified random sample of 50% of all persons older than 65 years that was obtained from the Health Care Finance Administration (HCFA). Individuals were sent a letter from HCFA explaining that they had been selected to participate in a study of aging by investigators at Columbia University. The sampling procedures have been described in detail elsewhere<sup>2,3</sup>. Each participant underwent an in-person interview of general health and functional ability at the time of entry into the study followed by a standardized assessment, including medical history, physical and neurological examination and a neuropsychological battery especially developed for this community. Pittman J, Andrews H, Tatemichi T, et al. Diagnosis of dementia in a heterogeneous population. A comparison of paradigm-based diagnosis and physician's diagnosis. *Arch Neurol.* 1992;49(5):461-467; Stern Y, Andrews H, Pittman J, et al. Diagnosis of dementia in a heterogeneous population. Development of a neuropsychological paradigm-based diagnosis of dementia and quantified correction for the effects of education. *Arch Neurol.* May 1992;49(5):453-460; and Stricks L, Pittman J, Jacobs DM, Sano M, Stern Y. Normative data for a brief neuropsychological battery administered to English- and Spanish-speaking community-dwelling elders. *J Int Neuropsychol Soc.* Jul 1998;4(4):311-318. Ethnic group was classified by participant's self-report using the format of the 1990 US Census. Census of Population and Housing Summary Tape File1, Technical Documentation. Washington, DC: Bureau of the Census; 1991. Participants were asked if they considered themselves white, black or other, and then asked if they were Hispanic. Participants were recruited at two time points (1992-1994 and 1999-2002). They have been followed at approximately 18-month intervals with similar assessments at each interval. The Institutional Review Boards of Columbia University Medical Center and the New York Psychiatric Institute approved recruitment, informed consent and study procedures.

[0109] In the present study, in order to maximize diagnostic accuracy, we included all patients with probable AD who had a Clinical Dementia Rating Scale score of 1 or higher, and who had been followed-up, using the criteria described below, on at least two occasions. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. *Br J Psychiatry*. 1982;140:566-572. (Table 1). Similarly, the healthy elderly controls included subjects who were also followed-up on at least two occasions separated by approximately 18 months, and who had had no evidence of AD or mild cognitive impairment on either assessment (Table 1). Because our prior study revealed evidence for allelic heterogeneity, with different SNPs and haplotypes showing association with AD in datasets with different ancestries, we collated cases and controls in the present study into three nested subsets: Caribbean Hispanic (178 cases, 194 controls); African American (88 cases, 158 controls) and White, non-Hispanic Europeans (30 case, 76 controls). While these cohorts are small, statistical power estimates, assuming the parameters from our initial study (e.g., SNP 8, allele frequency of 0.39 in cases; OR of 1.5), reveal that the current study had 98% power to detect significant allelic association between genetic variants in SORL1 and AD at  $\alpha$  of 0.05 for Caribbean Hispanics; 90% power for African Americans; and 53% power for Caucasians based on the model by Gordon and colleagues<sup>9</sup>. For rarer SNPs (e.g., SNP 23; allele frequency of 0.125 and OR of 2), the current study has 84% power for Caribbean Hispanics, 66% for African Americans, and 32% for Caucasians.

#### **Assessment and Neurological Diagnosis.**

[0110] All participants received structured neurological and functional assessments by physicians, and underwent a standardized neuropsychological battery that included measures of memory, orientation, language, abstract reasoning, and visuospatial ability. Stern Y, Andrews H, Pittman J, et al. Diagnosis of dementia in a heterogeneous population. Development of a neuropsychological paradigm-based diagnosis of dementia and quantified correction for the effects of education. *Arch Neurol*. May 1992;49(5):453-460; Stricks L, Pittman J, Jacobs DM, Sano M, Stern Y. Normative data for a brief neuropsychological battery administered to English- and Spanish-speaking community-dwelling elders. *J Int*

*Neuropsychol Soc.* Jul 1998;4(4):311-318. The diagnosis of dementia was established at a consensus conference that included neurologists, neuropsychologists and psychiatrists and based on all available information gathered from the initial and follow-up assessments and medical records. The diagnosis was based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease Related Disorders Association (NINCDS-ADRDA) criteria for probable AD. *DSM-III and IV American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders.* Third Edition, revised ed: Washington, DC: American Psychiatric Association; 1987; McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology.* Jul 1984;34(7):939-944. The diagnosis of dementia required evidence of cognitive decline, including memory impairment, the neuropsychological test battery as well as evidence of impairment in social or occupational function (clinical dementia rating, CDR >1.0). Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. *Br J Psychiatry.* 1982;140:566-572

**[0111]**     *Genotyping.* Genotyping was performed using the GenomeLab SNPstream System and primer sets were as described in Example 1. 100 DNA samples were genotyped twice for every SNP marker (concordance rate >99%). APOE was genotyped as previously described<sup>5</sup>. We numbered the SNPs 1 to 29 reflecting their relative order on the physical map of *SORL1*, and is the same nomenclature used in (Table 2).

**[0112]**     *Statistical Analyses.* SNP marker data were assessed for deviations from Hardy-Weinberg equilibrium using the HAPLOVIEW program. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* Jan 15 2005;21(2):263-265. The  $\chi^2$  test (or the Fisher's exact test) was used to assess genotypic and allelic associations between AD and each of the SNP markers. The HAPLOVIEW program was used to perform single point analysis as well as estimation of linkage disequilibrium (LD) structure and haplotype blocks. For LD structure estimation, the default settings were used, which created 95% confidence bounds on  $D'$  to define SNP pairs in strong LD.

Haplotype analyses were performed with HAPLO.STATS v1.1.1 for case-control data using the same sliding window of three contiguous SNPs as described in our previous publication. We designed this study to confirm the primary study. Under these circumstances, a nominal p-value of 0.05 is widely considered to be sufficient for confirmation. Ott J. *Analysis of human genetic linkage*. Baltimore, Johns Hopkins University Press; 1999. Consequently, nominal p-values are presented in Table 2 for single point analysis. However, to minimize the risk of a false positive finding from rare haplotypes, we computed empirical p-values by generating the null distribution based on 10,000 replicates of the haplotype analyses.

#### **EXAMPLE 4**

##### **Association between SIRT1 and AD in a Genome-Wide Study**

###### **Subjects**

**[0113]** The TGEN dataset was obtained from the website <http://www.tgen.org/neurogenomics/data>. Although both the primary study and the study by Reiman et al. *Neuron* 2007; 54:713–720 describing the TGEN dataset included individuals ascertained at the Mayo Clinic in Rochester, Minnesota, the Mayo individuals in the TGEN data set and the Mayo individuals included in Rogaeva et al. study are independent. Consequently, we analyzed SIRT1 data for 1408 individuals in the TGEN database which included 1044 autopsied individuals (641 cases, 403 controls) and 364 clinically examined individuals from the Mayo Clinic (218 cases and 146 controls).

###### **Statistical analyses**

**[0114]** SNP marker data were assessed for deviations from Hardy–Weinberg equilibrium using Haploview [Barrett JC, et al., *Bioinformatics* 2005; 21:263–2655] software (<http://www.broad.mit.edu/mpg/haploview/index.php>). Single point allelic and genotypic tests were performed using PLINK (<http://pngu.mgh.harvard.edu/Bpurcell/plink/>) [Purcell S, et al., *Am J Hum Genet* 2007; 81:559–5756]. Marker genotype distributions in cases and controls were compared in several ways: (i) a genotypic test with two degrees of freedom, models

assuming (ii) dominant and (iii) recessive inheritance, and (iv) the Cochran–Armitage trend test.

**[0115] Linkage disequilibrium** The linkage disequilibrium (LD) structure among the SORL1 SNPs was examined using Haploview. Haplo- type blocks were defined using the confidence intervals algorithm. The default settings were used in these analyses, which create 95% confidence bounds on D0 to delineate SNP pairs in strong LD.

**[0116]** In the foregoing specification, the invention has been described with reference to specific embodiments thereof. It will, however, be evident that various modifications and changes may be made thereto without departing from the broader spirit and scope of the invention. The specification and drawings are, accordingly, to be regarded in an illustrative rather than a restrictive sense.

All of the references cited above are incorporated herein by reference in their entirety.

## REFERENCES

**Applicants hereby incorporate by reference all of the citations listed below in their entirety:**

1. Mattson, M.P. Pathways towards and away from Alzheimer's disease. *Nature* 430, 631–639 (2004).
2. Goate, A. et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer disease. *Nature* 349, 704–706 (1991).
3. Sherrington, R. et al. Cloning of a gene bearing missense mutations in early onset familial Alzheimer's disease. *Nature* 375, 754–760 (1995).
4. Rogaev, E.I. et al. Familial Alzheimer's disease in kindreds with missense mutations in a novel gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* 376, 775–778 (1995).
5. Saunders, A.M. et al. Association of apolipoprotein E allele  $\epsilon 4$  with the late-onset familial and sporadic Alzheimer disease. *Neurology* 43, 1467–1472 (1993).
6. Bales, K.R. et al. Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat. Genet.* 17, 263–264 (1997).
7. Golde, T.E., Estus, S., Younkin, L.H., Selkoe, D.J. & Younkin, S.G. Processing of the amyloid protein precursor to potentially amyloidogenic derivatives. *Science* 255, 728–730 (1992).
8. Haass, C. & Selkoe, D.J. Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide. *Cell* 75, 1039–1042 (1993).
9. Bayer, T.A. et al. Key factors in Alzheimer's disease: beta-amyloid precursor protein processing, metabolism and intraneuronal transport. *Brain Pathol.* 11, 1–11 (2001).
10. Kinoshita, A. et al. Demonstration by FRET of BACE interaction with the amyloid precursor protein at the cell surface and in early endosomes. *J. Cell Sci.* 116, 3339–3346 (2003).
11. Vetrivel, K.S. et al. Spatial segregation of gamma -secretase and substrates in distinct membrane domains. *J. Biol. Chem.* 280, 25892–25900 (2005).

12. Scherzer, C.R. et al. Loss of apolipoprotein E receptor LR11 in Alzheimer disease. *Arch. Neurol.* 61, 1200–1205 (2004).
13. Small, S.A. et al. Model-guided microarray implicates the retromer complex in Alzheimer's disease. *Ann. Neurol.* 58, 909–919 (2005).
14. Andersen, O.M. et al. Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* 102, 13461–13466 (2005).
15. Offe, K. et al. The lipoprotein receptor LR11 regulates amyloid beta production and amyloid precursor protein traffic in endosomal compartments. *J. Neurosci.* 26, 1596–1603 (2006).
16. Pritchard, J.K. Are rare variants responsible for susceptibility to complex diseases? *Am. J. Hum. Genet.* 69, 124–137 (2001).
17. Pritchard, J.K. & Cox, N.J. The allelic architecture of human disease genes: common disease-common variant or not? *Hum. Mol. Genet.* 11, 2417–2423 (2002).
18. Athan, E.S. et al. A founder mutation in presenilin 1 causing early-onset Alzheimer disease in unrelated Caribbean Hispanic families. *J. Am. Med. Assoc.* 286, 2257–2263 (2001).
19. Bowirrat, A., Treves, T.A., Friedland, R.P. & Korczyn, A.D. Prevalence of Alzheimer's type dementia in an elderly Arab population. *Eur. J. Neurol.* 8, 119–123 (2001).
20. Rogaeva, E.A. et al. An alpha-2-macroglobulin insertion-deletion polymorphism in Alzheimer's disease. *Nat. Genet.* 22, 19–22 (1999).
21. Rogaeva, E. et al. Evidence for an Alzheimer disease susceptibility locus on chr 12, and for further locus heterogeneity. *JAMA* 280, 614–618 (1998).
22. Lee, J.H. et al. Fine mapping of 10q and 18q for familial Alzheimer's disease in Caribbean Hispanics. *Mol. Psychiatry* 9, 1042–1051 (2004).
23. Graff-Radford, N.R. et al. Association between apolipoprotein E genotype and Alzheimer disease in African American subjects. *Arch. Neurol.* 59, 594–600 (2002).
24. Green, R.C. et al. Risk of dementia among white and African American relatives of patients with Alzheimer disease. *JAMA* 287, 329–336 (2002).

25. Farrer, L.A. et al. Identification of multiple loci for Alzheimer disease in a consanguineous Israeli-Arab community. *Hum. Mol. Genet.* 12, 415–422 (2003).
26. Lin, S., Chakravarti, A. & Cutler, D.J. Exhaustive allelic transmission disequilibrium tests as a new approach to genome-wide association studies. *Nat. Genet.* 36, 1181–1188 (2004).
27. Ertekin-Taner, N. et al. Elevated amyloid beta protein (Abeta42) and late onset Alzheimer's disease are associated with single nucleotide polymorphisms in the urokinase-type plasminogen activator gene. *Hum. Mol. Genet.* 14, 447–460 (2005).
28. Ertekin-Taner, N. et al. Genetic variants in a haplotype block spanning IDE are significantly associated with plasma Abeta42 levels and risk for Alzheimer disease. *Hum. Mutat.* 23, 334–342 (2004).
29. Dodson, S.E. et al. LR11/SorLA expression is reduced in sporadic Alzheimer disease but not in familial Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 65, 866–872 (2006).
30. Seaman, M.N. Recycle your receptors with retromer. *Trends Cell Biol.* 15, 68–75 (2005).
31. He, X., Li, F., Chang, W.P. & Tang, J. GGA proteins mediate the recycling pathway of memapsin 2 (BACE). *J. Biol. Chem.* 280, 11696–11703 (2005).
32. Farrer, L.A. et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer's Disease. A meta-analysis. APOE and Alzheimer's Disease Meta Analysis Consortium. *JAMA* 278, 1349–1356 (1997).
33. Rabinowitz, D. & Laird, N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum. Hered.* 50, 211–223 (2000).
34. Vermeire, S. & Rutgeerts, P. Current status of genetics research in inflammatory bowel disease. *Genes Immun.* 6, 637–645 (2005).
35. Owen, M.J., Craddock, N. & O'Donovan, M.C. Schizophrenia: genes at last? *Trends*

Genet. 21, 518–525 (2005).

36. Horvath, S. et al. Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. *Genet. Epidemiol.* 26, 61–69 (2004).

37. Lange, C., DeMeo, D., Silverman, E.K., Weiss, S.T. & Laird, N.M. PBAT: tools for family-based association studies. *Am. J. Hum. Genet.* 74, 367–369 (2004).

38. Lange, C., Silverman, E.K., Xu, X., Weiss, S.T. & Laird, N.M. A multivariate familybased association test using generalized estimating equations: FBAT-GEE. *Biostatistics* 4, 195–206 (2003).

39. Lange, C. & Laird, N.M. Power calculations for a general class of family-based association tests: dichotomous traits. *Am. J. Hum. Genet.* 71, 575–584 (2002).

40. Van Steen, K. et al. Genomic screening and replication using the same data set in family-based association testing. *Nat. Genet.* 37, 683–691 (2005).

41. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* 57, 289–300 (1995).

42. Jannot, A.S., Essioux, L., Reese, M.G. & Clerget-Darpoux, F. Improved use of SNP information to detect the role of genes. *Genet. Epidemiol.* 25, 158–167 (2003).

43. Terwilliger, J.D. & Weiss, K.M. Linkage disequilibrium mapping of complex disease: fantasy or reality? *Curr. Opin. Biotechnol.* 9, 578–594 (1998).

44. Schaid, D.J., Rowland, C.M., Tines, D.E., Jacobson, R.M. & Poland, G.A. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am. J. Hum. Genet.* 70, 425–434 (2002).

45. Chen, F. et al. Nicastrin binds to membrane-tethered Notch. *Nat. Cell Biol.* 3, 751–754 (2001).

46. Benjannet, S. et al. Post-translational processing of beta-secretase (beta-amyloidconverting enzyme) and its ectodomain shedding. The pro- and transmembrane/ cytosolic domains affect its cellular activity and amyloid-beta production. *J. Biol. Chem.* 276, 10879–10887 (2001).

47. Yu, G. et al. A novel protein (nicastrin) modulates presenilin-mediated Notch/Glp1 and betaAPP processing. *Nature* 407, 48–54 (2000).
48. Gu, Y. et al. The presenilin proteins are components of multiple membrane-bound complexes which have different biological activities. *J. Biol. Chem.* 279, 31329–31336 (2004).
49. Hasegawa, H. et al. Both the sequence and length of the C terminus of PEN-2 are critical for intermolecular interactions and function of presenilin complexes. *J. Biol. Chem.* 279, 46455–46463 (2004).

Table 1. SORL1 SNP INFORMATION

| SNP order | dbSNP rs Number   | Physical Map Location (bp) | Distance (bp) from Previous Marker | SNP Type            | Orig order | NE FAD       | HIS FAD       | NE csct       | Israeli Arab   | TGEN         | TGEN order |
|-----------|-------------------|----------------------------|------------------------------------|---------------------|------------|--------------|---------------|---------------|----------------|--------------|------------|
| 1         | <b>rs4935774</b>  | 120,826,964                | —                                  | Upstream of 5' UTR  | 1          | 0.204        | 0.777         | 0.111         | 0.182          | 0.388        | T.1        |
| 2         | rs578506          | 120,828,687                | 1,723                              | intron              | 2          | 0.072        | 0.331         | 0.105         | ND             |              |            |
| 3         | rs582446          | 120,833,069                | 4,382                              | intron              | 3          | 0.324        | 0.830         | 0.794         | 0.082          |              |            |
| 4         | rs661057          | 120,834,164                | 1,095                              | intron              | 4          | 0.119        | 0.407         | <b>0.0026</b> | 0.070          |              |            |
| 5         | rs676160          | 120,845,443                | 11,279                             |                     |            |              |               |               |                | 0.488        | T.2        |
| 6         | rs11218304        | 120,854,321                | 8,878                              | intron              | 5          | 0.272        | 0.964         | <b>0.0012</b> | <b>0.046</b>   | 0.569        | T.3        |
| 7         | rs676759          | 120,864,475                | 10,154                             |                     |            |              |               |               |                | 0.547        | T.4        |
| 8         | <b>rs560573</b>   | 120,866,094                | 1,619                              | intron              | 6          | 0.643        | 0.167         | 0.050         | 0.287          | 0.276        | T.5        |
| 9         | rs985421          | 120,867,526                | 1,432                              |                     |            |              |               |               |                | 0.572        | T.6        |
| 10        | rs593769          | 120,869,213                | 1,687                              |                     |            |              |               |               |                | 0.334        | T.7        |
| 11        | <b>rs12364988</b> | 120,872,836                | 3,623                              | H269H               | 7          | 0.149        | 0.174         | <b>0.0078</b> | <b>0.041</b>   | 0.324        | T.8        |
| 12        | <b>rs668387</b>   | 120,873,131                | 295                                | intron              | 8          | 0.515        | <b>0.013</b>  | <b>0.002</b>  | <b>0.021</b>   |              |            |
| 13        | rs689021          | 120,876,330                | 3,199                              | intron              | 9          | 0.708        | <b>0.017</b>  | <b>0.007</b>  | <b>0.040</b>   |              |            |
| 14        | rs641120          | 120,886,175                | 9,845                              | intron              | 10         | 0.565        | <b>0.021</b>  | <b>0.0051</b> | 0.067          |              |            |
| 15        | rs11218313        | 120,888,081                | 1,906                              |                     |            |              |               |               |                | 0.831        | T.9        |
| 16        | rs4935775         | 120,894,712                | 6,631                              | intron              | 11         | 0.225        | 0.780         | <b>0.0006</b> | <b>0.030</b>   |              |            |
| 17        | rs12285364        | 120,898,436                | 3,724                              | intron              | 12         | <b>0.014</b> | 0.800         | 1.000         | 0.557          |              |            |
| 18        | rs2298813         | 120,898,894                | 458                                | T528A               | 13         | <b>0.012</b> | 0.952         | 1.000         | 0.431          |              |            |
| 19        | rs11600231        | 120,911,918                | 13,024                             | intron              | 14         | 0.687        | <b>0.0062</b> | 0.462         | 0.124          | 0.249        | T.10       |
| 20        | <b>rs2276346</b>  | 120,919,686                | 7,768                              | intron              | 15         | 0.614        | 0.821         | <b>0.049</b>  | 0.363          | 0.168        | T.11       |
| 21        | rs10502262        | 120,920,522                | 836                                |                     |            |              |               |               |                |              |            |
| 22        | SORL1-T833T       | 120,931,165                | 10,643                             | T833T               | 16         | 0.406        | 0.910         | 0.600         | <b>0.0026</b>  |              |            |
| 23        | rs556349          | 120,931,417                | 252                                | intron              | 17         | <b>0.006</b> | 0.170         | <b>0.042</b>  | 0.205          |              |            |
| 24        | rs7131432         | 120,932,080                | 663                                |                     |            |              |               |               |                | 0.143        | T.12       |
| 25        | rs11218340        | 120,936,564                | 4,484                              | intron              | 18         | 0.075        | 0.817         | 0.137         | 0.141          |              |            |
| 26        | rs10892756        | 120,939,766                | 3,202                              |                     |            |              |               |               |                | 0.493        | T.13       |
| 27        | rs11218346        | 120,944,391                | 4,625                              |                     |            |              |               |               |                | 0.479        | T.14       |
| 28        | rs1790213         | 120,947,399                | 3,008                              |                     |            |              |               |               |                | 0.332        | T.15       |
| 29        | rs11218347        | 120,951,758                | 4,359                              |                     |            |              |               |               |                | 0.782        | T.16       |
| 30        | rs2070045         | 120,953,300                | 1,542                              | S1187S              | 19         | <b>0.031</b> | 0.617         | 0.499         | <b>0.00082</b> |              |            |
| 31        | rs3824966         | 120,953,393                | 93                                 | intron              | 20         | 0.154        | 0.883         | 0.494         | 0.148          |              |            |
| 32        | rs1699103         | 120,957,136                | 3,743                              |                     |            |              |               |               |                | <b>0.045</b> | T.17       |
| 33        | rs7116734         | 120,957,150                | 14                                 |                     |            |              |               |               |                | 0.297        | T.18       |
| 34        | rs11218350        | 120,957,861                | 711                                |                     |            |              |               |               |                | <b>0.040</b> | T.19       |
| 35        | SORL1-18ex26      | 120,959,359                | 1,498                              | (-18) 5' of exon 26 | 21         | 0.412        | 0.467         | 0.239         | 0.0091         |              |            |
| 36        | rs1699102         | 120,962,172                | 2,813                              | N1246N              | 22         | 0.056        | 0.386         | 0.051         | 0.907          |              |            |
| 37        | rs10892759        | 120,969,298                | 7,126                              |                     |            |              |               |               |                | <b>0.021</b> | T.20       |
| 38        | rs1792113         | 120,970,156                | 858                                |                     |            |              |               |               |                | 0.201        | T.21       |
| 39        | rs11218360        | 120,978,601                | 8,445                              |                     |            |              |               |               |                | 0.420        | T.22       |
| 40        | rs7128608         | 120,978,808                | 207                                |                     |            |              |               |               |                | 0.443        | T.23       |
| 41        | rs3824968         | 120,981,132                | 2,324                              | A1584A              | 23         | <b>0.003</b> | 0.513         | 0.672         | <b>0.00073</b> |              |            |
| 42        | rs1629493         | 120,982,306                | 1,174                              |                     |            |              |               |               |                | 0.085        | T.24       |
| 43        | <b>rs2282649</b>  | 120,984,168                | 1,862                              | intron              | 24         | <b>0.020</b> | 0.266         | 0.902         | 0.637          | 0.261        | T.25       |
| 44        | rs726601          | 120,986,617                | 2,449                              |                     |            |              |               |               |                | 0.065        | T.26       |
| 45        | rs1784931         | 120,988,148                | 1,531                              |                     |            |              |               |               |                | <b>0.049</b> | T.27       |
| 46        | <b>rs1010159</b>  | 120,988,611                | 463                                | intron              | 25         | 0.086        | 0.190         | 0.365         | 0.915          | 0.139        | T.28       |
| 47        | rs1784933         | 120,994,626                | 6,015                              | intron              | 26         | 0.069        | 0.291         | 0.728         | 0.796          |              |            |
| 48        | <b>rs1614735</b>  | 120,998,211                | 3,585                              | intron              | 27         | <b>0.010</b> | 0.600         | 0.139         | 0.964          | 0.188        | T.29       |
| 49        | rs17125558        | 120,999,237                | 1,026                              |                     |            |              |               |               |                | 0.695        | T.30       |
| 50        | rs10892761        | 121,003,094                | 3,857                              |                     |            |              |               |               |                | 0.565        | T.31       |
| 51        | rs1133174         | 121,006,965                | 3,871                              | downstream of 3'UTR | 28         | 0.068        | 0.903         | 0.004         | 0.087          |              |            |
| 52        | rs1131497         | 121,007,955                | 990                                | downstream of 3'UTR | 29         | 0.201        | 0.564         | 0.017         | 0.065          |              |            |

Note: The nominal *P* values of allelic association with AD are shown for each dataset. NE FAD -- north European familial AD dataset; HIS FAD: Caribbean Hispanic familial AD dataset; NE csct -- north European case-control dataset; Israeli-Arab -- Israeli-Arab case-control data sets, and TGEN -- TGEN dataset. Corrections for multiple testing were not applied in these replication analyses. Boldface rs number indicates identical SNPs in the Primary Study and Reiman et al. Neuron 2007;54:713-720. Bold face p-values indicates that the SNP is nominally associated with Alzheimer Disease. Bold face p-values with white font, black background refer to results from TGEN data.

Table 2 Single-SNP association results

| SNP | Minor allele | Discovery data sets     |               |                     |                             |       | Replication data sets     |       |             |                             |        |
|-----|--------------|-------------------------|---------------|---------------------|-----------------------------|-------|---------------------------|-------|-------------|-----------------------------|--------|
|     |              | North European families |               |                     | Caribbean Hispanic families |       | Israeli Arab case-control |       |             | North European case-control |        |
|     |              | MAF                     | Info families | P                   | Risk allele                 | MAF   | Info families             | P     | Risk allele | MAF                         | P      |
|     |              |                         |               |                     |                             |       |                           |       |             |                             |        |
| 8   | T            | 0.429                   | 51            | 0.515               | C                           | 0.388 | 80                        | 0.013 | C           | 0.399                       | 0.002  |
| 9   | A            | 0.429                   | 54            | 0.708               | G                           | 0.379 | 78                        | 0.017 | G           | 0.397                       | 0.0071 |
| 10  | T            | 0.429                   | 55            | 0.565               | C                           | 0.374 | 79                        | 0.021 | C           | 0.385                       | 0.0051 |
| 17  | T            | 0.277                   | 51            | 0.0057 <sup>a</sup> | T                           | 0.488 | 91                        | 0.170 | G           | 0.384                       | 0.042  |
| 19  | G            | 0.207                   | 42            | 0.031               | G                           | 0.245 | 84                        | 0.617 | G           | 0.236                       | 0.499  |
| 23  | T            | 0.284                   | 53            | 0.0031 <sup>a</sup> | T                           | 0.288 | 78                        | 0.513 | G           | 0.345                       | 0.672  |

After adjustment for multiple testing with an FDR level of 0.1, the cutoff *P* values for significant association are 0.024 in the north European family data set and 0.003 in the Hispanic family data set. MAF, minor allele frequency; OR, odds ratio; *P*, single-SNP  $\chi^2$  *P* value; Info families, number of informative families. Supplementary Table 3 also shows allele and genotype frequencies. The alleles putatively associated with Alzheimer disease are depicted only for SNPs generating nominal *P* values  $\leq 0.10$ . Separate confirmatory analyses were performed in four independent 'replication data sets': North European and Israeli Arab case-control data sets, and MAFGE Caucasian Americans and African-American (db pats). Corrections for multiple testing were not applied in these replication analyses. Boldface indicates identical alleles that are nominally associated with disease in at least two independent data sets. The *P* values in the case-control cohort are for allelic association. Alleles putatively associated with Alzheimer disease are depicted only for those SNPs generating nominal *P* values  $< 0.10$ . Complete data are contained in Supplementary Table 4. <sup>a</sup>Association was significant after correction for multiple testing.

Table 3 Haplotypes for all three-SNP windows that have a global  $P$  value for association with Alzheimer disease of  $P \leq 0.05$  in at least one data set

| Discovery data sets     |       |               |               |         |               |                |                             |               |         |               |                |  |
|-------------------------|-------|---------------|---------------|---------|---------------|----------------|-----------------------------|---------------|---------|---------------|----------------|--|
| North European families |       |               |               |         |               |                | Caribbean Hispanic families |               |         |               |                |  |
| SNP#                    | HAP   | HAP frequency | Info families | Z score | Hap $P$ value | Global sim $P$ | Hap frequency               | Info families | Z score | Hap $P$ value | Global sim $P$ |  |
| 8 9 10                  | C G C | 0.579         | 52            | -0.392  | 0.695         | 0.152          | 0.628                       | 75            | 2.785   | 0.0053        | 0.0098         |  |
| 8 9 10                  | T A T | 0.395         | 52            | 0.893   | 0.372         | 0.317          | 0.317                       | 72            | -2.628  | 0.0086        |                |  |
| 9 10 11                 | G C C | 0.404         | 57            | -1.259  | 0.208         | 0.553          | 0.217                       | 62            | -1.202  | 0.229         | 0.022          |  |
| 9 10 11                 | A T A | 0.380         | 57            | 0.860   | 0.390         | 0.319          | 0.319                       | 83            | -2.005  | 0.045         |                |  |
| 9 10 11                 | G C A | 0.178         | 42            | 0.465   | 0.642         | 0.428          | 0.428                       | 79            | 2.557   | 0.011         |                |  |
| 22 23 24                | C T T | 0.186         | 45            | 2.779   | 0.0054        | 0.018          | 0.205                       | 66            | -0.103  | 0.918         | 0.554          |  |
| 22 23 24                | G A T | 0.003         | 2             | *       | *             |                | 0.008                       | 3             | *       | *             |                |  |
| 22 23 24                | T T T | 0.014         | 4             | *       | *             |                | 0.019                       | 7             | *       | *             |                |  |
| 23 24 25                | A C T | 0.735         | 58            | -1.742  | 0.082         | 0.041          | 0.551                       | 80            | 1.351   | 0.177         | 0.824          |  |
| 23 24 25                | T T C | 0.196         | 47            | 1.878   | 0.060         | 0.220          | 0.220                       | 70            | -0.811  | 0.417         |                |  |
| 23 24 25                | A C C | 0.087         | 13            | 0.475   | 0.635         | 0.183          | 0.183                       | 51            | -0.608  | 0.543         |                |  |
| 23 24 25                | A T C | 0.004         | 2             | *       | *             |                | 0.008                       | 3             | *       | *             |                |  |

| Replication data sets     |       |                   |                |         |               |                |                             |                |         |               |                |        |
|---------------------------|-------|-------------------|----------------|---------|---------------|----------------|-----------------------------|----------------|---------|---------------|----------------|--------|
| Israeli Arab case-control |       |                   |                |         |               |                | North European case-control |                |         |               |                |        |
| SNP#                      | HAP   | Control frequency | Case frequency | Z score | Hap $P$ value | Global sim $P$ | Control frequency           | Case frequency | Z score | Hap $P$ value | Global sim $P$ |        |
| 8 9 10                    | C G C | 0.539             | 0.661          | 2.633   | 0.0085        | 0.023          | 0.566                       | 0.638          | 2.001   | 0.045         | 0.154          |        |
| 8 9 10                    | T A T | 0.434             | 0.301          | -2.901  | 0.0037        | 0.0037         | 0.417                       | 0.351          | -1.836  | 0.068         | 0.510          | 0.707  |
| 9 10 11                   | G C C | 0.282             | 0.407          | 2.828   | 0.0047        | 0.0080         | 0.385                       | 0.455          | 1.972   | 0.049         | 0.223          | 0.582  |
| 9 10 11                   | A T A | 0.410             | 0.247          | -3.390  | 0.0007        | 0.0007         | 0.393                       | 0.323          | -1.904  | 0.057         | 0.071          | 0.280  |
| 9 10 11                   | G C A | 0.256             | 0.251          | -0.258  | 0.796         | 0.796          | 0.184                       | 0.191          | 0.337   | 0.736         | 0.399          | 0.893  |
| 22 23 24                  | C T T | 0.250             | 0.227          | -0.579  | 0.563         | 0.286          | 0.069                       | 0.134          | 2.795   | 0.0052        | 0.00055        | 0.314  |
| 22 23 24                  | C A T | *                 | *              | *       | *             |                | 0.170                       | 0.092          | -3.096  | 0.0020        | 0.001          | 1      |
| 22 23 24                  | T T T | 0.015             | 0.041          | 1.387   | 0.165         | 0.165          | 0.011                       | 0.033          | 2.272   | 0.023         | 0.007          | 3      |
| 23 24 25                  | A C T | 0.535             | 0.563          | 0.668   | 0.504         | 0.547          | 0.667                       | 0.674          | 0.129   | 0.897         | 0.00035        | 0.0043 |
| 23 24 25                  | T T C | 0.265             | 0.259          | -0.135  | 0.892         | 0.892          | 0.048                       | 0.167          | 3.468   | 0.0011        | 0.047          | 18     |
| 23 24 25                  | A C C | 0.103             | 0.103          | -0.057  | 0.955         | 0.955          | 0.066                       | 0.046          | -1.080  | 0.280         | 0.403          | 53     |
| 23 24 25                  | A T C | *                 | *              | *       | *             |                | 0.168                       | 0.097          | -2.508  | 0.012         | 0.003          | 1      |

All  $P$  values of  $P \leq 0.05$  are in boldface. Haplotypes that show increased risk for Alzheimer disease in at least two independent data sets are shown in boldface. Haplotypes that show reduced risk in at least two independent data sets are underlined. Complete data for all SNPs is contained in Supplementary Table 5. Hap  $P$  value, haplotypes  $P$  value (HaploStats v1.1.1); Global sim  $P$  (permutation-based global haplotype  $P$  value reported by FBAT (fast-p option) or HaploStats); \*, sample size too small to generate meaningful result; info families, number of informative families.

Table 4 Single-SNP association results for independent replication dataset from Mayo Clinic

| SNP# | Minor allele | Mayo Jacksonville series |             |                  |       | Mayo Rochester series |             |                  |       | AUS series |             |                  |       |
|------|--------------|--------------------------|-------------|------------------|-------|-----------------------|-------------|------------------|-------|------------|-------------|------------------|-------|
|      |              | MAF                      | Risk allele | OR (95% c.i.)    | P     | MAF                   | Risk allele | OR (95% c.i.)    | P     | MAF        | Risk allele | OR (95% c.i.)    | P     |
| 4    | C            | 0.418                    |             | 1.10 (0.92-1.31) | 0.305 | 0.455                 |             | 1.05 (0.90-1.23) | 0.504 | 0.424      |             | 1.19 (0.98-1.45) | 0.076 |
| 5    | C            | 0.413                    |             | 0.99 (0.83-1.18) | 0.876 | 0.397                 |             | 1.03 (0.92-1.26) | 0.373 | 0.397      |             | 1.11 (0.91-1.35) | 0.301 |
| 8    | T            | 0.431                    |             | 1.13 (0.94-1.34) | 0.187 | 0.440                 |             | 0.97 (0.83-1.13) | 0.692 | 0.431      |             | 1.20 (0.98-1.45) | 0.071 |
| 9    | A            | 0.435                    |             | 1.12 (0.94-1.34) | 0.202 | 0.443                 |             | 0.95 (0.82-1.13) | 0.640 | 0.448      |             | 1.10 (0.97-1.34) | 0.313 |
| 12   | T            | 0.044                    |             | 1.14 (0.74-1.75) | 0.548 | 0.050                 |             | 1.10 (0.77-1.55) | 0.606 | 0.052      | T           | 1.98 (1.26-3.12) | 0.003 |
| 19   | G            | 0.224                    |             | 1.14 (0.93-1.41) | 0.210 | 0.242                 |             | 1.19 (1.00-1.42) | 0.055 | 0.230      |             | 1.15 (0.92-1.44) | 0.225 |
| 22   | C            | 0.321                    |             | 1.20 (1.00-1.45) | 0.052 | 0.349                 |             | 1.07 (0.91-1.26) | 0.413 | 0.331      |             | 1.13 (0.92-1.39) | 0.227 |
| 23   | T            | 0.296                    | T           | 1.31 (1.08-1.59) | 0.006 | 0.321                 |             | 1.09 (0.93-1.29) | 0.287 | 0.302      |             | 1.12 (0.91-1.37) | 0.294 |
| 24   | T            | 0.278                    | T           | 1.31 (1.08-1.59) | 0.007 | 0.299                 |             | 1.06 (0.89-1.25) | 0.513 | 0.281      |             | 1.15 (0.93-1.42) | 0.199 |
| 25   | C            | 0.333                    | C           | 1.35 (1.12-1.63) | 0.001 | 0.355                 |             | 1.03 (0.88-1.21) | 0.712 | 0.340      |             | 1.15 (0.94-1.41) | 0.163 |

| SNP# | Minor allele | Combined Mayo series |             |                  |       | Combined Caucasian data sets |             |                  |        |
|------|--------------|----------------------|-------------|------------------|-------|------------------------------|-------------|------------------|--------|
|      |              | MAF                  | Risk allele | OR (95% c.i.)    | P     | MAF                          | Risk allele | OR (95% c.i.)    | P      |
| 4    | C            | 0.437                | T           | 1.14 (1.03-1.26) | 0.009 | 0.431                        | T           | 1.16 (1.06-1.27) | 0.002  |
| 5    | C            | 0.402                |             | 1.06 (0.96-1.17) | 0.227 | 0.403                        |             | 1.09 (0.99-1.19) | 0.081  |
| 8    | T            | 0.435                |             | 1.08 (0.98-1.19) | 0.113 | 0.432                        | C           | 1.11 (1.01-1.22) | 0.027  |
| 9    | A            | 0.442                |             | 1.05 (0.95-1.16) | 0.322 | 0.438                        |             | 1.08 (0.98-1.19) | 0.109  |
| 12   | T            | 0.049                | T           | 1.25 (1.00-1.56) | 0.046 | 0.049                        |             | 1.20 (0.97-1.48) | 0.087  |
| 19   | G            | 0.234                | G           | 1.13 (1.01-1.26) | 0.038 | 0.238                        | G           | 1.18 (1.06-1.31) | 0.0023 |
| 22   | C            | 0.335                |             | 1.09 (0.98-1.20) | 0.108 | 0.334                        |             | 1.08 (0.98-1.19) | 0.119  |
| 23   | T            | 0.309                | T           | 1.12 (1.01-1.24) | 0.031 | 0.292                        | T           | 1.15 (1.04-1.27) | 0.0075 |
| 24   | T            | 0.289                | T           | 1.12 (1.00-1.24) | 0.042 | 0.286                        | T           | 1.11 (1.00-1.23) | 0.040  |
| 25   | C            | 0.345                | C           | 1.12 (1.01-1.24) | 0.025 | 0.343                        | C           | 1.11 (1.01-1.22) | 0.033  |

Table 5 Haplotype results for three-SNP windows for SNPs 22–25 in Mayo Clinic replication data sets

| Mayo Jacksonville series |    |     |   |                 |              |         |         |              |                 |                 | Mayo Rochester series |         |         |              |                 | AUT series      |              |         |         |              |                 |                              |  |  |  |  |
|--------------------------|----|-----|---|-----------------|--------------|---------|---------|--------------|-----------------|-----------------|-----------------------|---------|---------|--------------|-----------------|-----------------|--------------|---------|---------|--------------|-----------------|------------------------------|--|--|--|--|
| SNP#                     |    | HAP |   | Control<br>freq | Case<br>freq | Z score | P value | Hap<br>sim P | Global<br>sim P | Control<br>freq | Case<br>freq          | Z score | P value | Hap<br>sim P | Global<br>sim P | Control<br>freq | Case<br>freq | Z score | P value | Hap<br>sim P | Global<br>sim P |                              |  |  |  |  |
| 22                       | 23 | 24  | C | T               | T            | 0.235   | 0.285   | 2.641        | 0.0083          | 0.015           | 0.279                 | 0.296   | 0.926   | 0.354        | 0.220           | 0.248           | 0.271        | 1.051   | 0.293   | 0.435        |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | T | A               | C            | 0.690   | 0.644   | -2.248       | 0.025           |                 | 0.641                 | 0.624   | -0.920  | 0.358        |                 | 0.666           | 0.638        | -1.566  | 0.117   |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | C | A               | C            | 0.039   | 0.034   | -0.732       | 0.464           |                 | 0.041                 | 0.036   | -0.644  | 0.520        |                 | 0.038           | 0.050        | 1.146   | 0.252   |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | C | T               | C            | 0.021   | 0.019   | -0.390       | 0.697           |                 | 0.022                 | 0.026   | 0.745   | 0.456        |                 | 0.028           | 0.022        | -0.731  | 0.465   |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | T | T               | T            | 0.008   | 0.017   | 1.708        | 0.088           |                 | 0.014                 | 0.010   | -0.926  | 0.354        |                 | 0.015           | 0.018        | 0.627   | 0.530   |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | C | A               | T            | ^       | ^       | ^            | ^               |                 | ^                     | ^       | ^       | ^            |                 | ^               | ^            | ^       | ^       |              |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | T | T               | C            | 0.242   | 0.309   | 2.959        | 0.0031          | 0.0076          | 0.293                 | 0.305   | 0.681   | 0.496        | 0.386           | 0.263           | 0.288        | 1.115   | 0.265   | 0.432        |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | A | C               | T            | 0.790   | 0.631   | -3.261       | 0.0011          |                 | 0.646                 | 0.629   | -0.913  | 0.362        |                 | 0.668           | 0.633        | -1.445  | 0.148   |              |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | A | C               | C            | 0.029   | 0.046   | 1.911        | 0.056           |                 | 0.036                 | 0.031   | -0.752  | 0.452        |                 | 0.036           | 0.046        | 0.936   | 0.349   |              |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | T | C               | C            | 0.022   | 0.017   | -0.767       | 0.443           |                 | 0.019                 | 0.026   | 1.121   | 0.262        |                 | 0.022           | 0.021        | -0.208  | 0.835   |              |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | A | T               | C            | ^       | ^       | ^            | ^               |                 | ^                     | ^       | ^       | ^            |                 | ^               | ^            | ^       | ^       |              |                 |                              |  |  |  |  |
| Combined Mayo series     |    |     |   |                 |              |         |         |              |                 |                 |                       |         |         |              |                 |                 |              |         |         |              |                 | Combined Caucasian data sets |  |  |  |  |
| SNP#                     |    | HAP |   | Control<br>freq | Case<br>freq | Z score | P value | Hap<br>sim P | Global<br>sim P | Control<br>freq | Case<br>freq          | Z score | P value | Hap<br>sim P | Global<br>sim P |                 |              |         |         |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | C | T               | T            | 0.263   | 0.284   | 1.964        | 0.050           | 0.332           | 0.247                 | 0.279   | 2.258   | 0.034        | 0.0051          |                 |              |         |         |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | T | A               | C            | 0.657   | 0.633   | -2.063       | 0.039           |                 | 0.658                 | 0.635   | -2.101  | 0.036        |                 |                 |              |         |         |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | C | A               | C            | 0.040   | 0.039   | -0.178       | 0.859           |                 | 0.042                 | 0.043   | 0.005   | 0.996        |                 |                 |              |         |         |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | C | T               | C            | 0.023   | 0.022   | -0.171       | 0.864           |                 | 0.022                 | 0.022   | 0.004   | 0.997        |                 |                 |              |         |         |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | T | T               | T            | 0.013   | 0.015   | 0.846        | 0.397           |                 | 0.013                 | 0.017   | 1.538   | 0.124        |                 |                 |              |         |         |              |                 |                              |  |  |  |  |
| 22                       | 23 | 24  | C | A               | T            | ^       | ^       | ^            | ^               |                 | 0.017                 | 0.016   | -2.315  | 0.021        |                 |                 |              |         |         |              |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | T | T               | C            | 0.276   | 0.298   | 2.039        | 0.041           | 0.099           | 0.259                 | 0.286   | 2.504   | 0.012        | 0.017           |                 |              |         |         |              |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | A | C               | T            | 0.662   | 0.631   | -2.670       | 0.0076          |                 | 0.663                 | 0.636   | -2.443  | 0.016        |                 |                 |              |         |         |              |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | A | C               | C            | 0.035   | 0.041   | 1.361        | 0.174           |                 | 0.037                 | 0.042   | 0.873   | 0.383        |                 |                 |              |         |         |              |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | T | C               | C            | 0.020   | 0.021   | 0.118        | 0.906           |                 | 0.019                 | 0.020   | 0.341   | 0.732        |                 |                 |              |         |         |              |                 |                              |  |  |  |  |
| 23                       | 24 | 25  | A | T               | C            | ^       | ^       | ^            | ^               |                 | 0.017                 | 0.011   | -2.208  | 0.027        |                 |                 |              |         |         |              |                 |                              |  |  |  |  |

Although the Mayo Clinic data sets and north European data sets have slightly different haplotype structures, the GTT haplotype at SNP 22–24 and the overlapping TTC haplotype at SNPs 23–25 (in boldface) show increased risk for Alzheimer disease in the Mayo Clinic and north European Caucasians (in boldface in Table 3). Haplotypes with reduced risk of Alzheimer disease are underlined but are different from those in the north European data sets, suggesting the potential existence of several protective alleles in this region. MAF, minor allele frequency; OR, odds ratio;  $P$ , single-SNP  $\chi^2$   $P$  value; Hap  $P$  value, haplotype  $P$  value (Haplo.stats v1.1.1); Global sim  $P$ , permutation-based global haplotypic  $P$  value reported by FBAT (hbat -p option) or Haplo.stats; ^, sample size too small to generate meaningful result.

Tables 6A and 6B. Results of genotyping of the 12 SNPs in *SORL1* by single point and haplotype analysis. The SNP order refers to original order of the SNPs in our previous publication (1). The single SNPs and haplotypes in boldface were significant. The associated alleles for SNPs 8 and 9 were identical to those in the original manuscript as was the haplotype CGC from SNPs 8-9-10 (1).

| Table 6A SORL1        |  |            |   | SNP    | Associated                 | Frequency        |          |                   |                |
|-----------------------|--|------------|---|--------|----------------------------|------------------|----------|-------------------|----------------|
| SNP Order (1)         |  | Name       |   | Allele |                            | Cases : Controls |          | $X^2$             | P-value        |
| 1                     |  | rs4935774  |   | A      |                            | 0.789 : 0.703    |          | 2.793             | 0.0947         |
| 2                     |  | rs578506   |   | C      |                            | 0.531 : 0.451    |          | 1.843             | 0.1746         |
| 7                     |  | rs12364988 |   | G      |                            | 0.588 : 0.506    |          | 2.106             | 0.1467         |
| 8                     |  | rs668387   |   | C      |                            | 0.665 : 0.534    |          | 5.885             | 0.0153         |
| 9                     |  | rs689021   |   | G      |                            | 0.654 : 0.518    |          | 5.655             | 0.0174         |
| 10                    |  | rs641120   |   | C      |                            | 0.654 : 0.588    |          | 1.401             | 0.2365         |
| 13                    |  | rs2298813  |   | A      |                            | 0.047 : 0.034    |          | 0.319             | 0.572          |
| 17                    |  | rs556349   |   | T      |                            | 0.338 : 0.330    |          | 0.025             | 0.8733         |
| 22                    |  | rs1699102  |   | C      |                            | 0.354 : 0.335    |          | 0.136             | 0.7123         |
| 23                    |  | rs3824968  |   | A      |                            | 0.316 : 0.259    |          | 1.198             | 0.2736         |
| 24                    |  | rs2282649  |   | T      |                            | 0.301 : 0.212    |          | 3.229             | 0.0724         |
| 25                    |  | rs1010159  |   | T      |                            | 0.662 : 0.625    |          | 0.451             | 0.502          |
| 6B Haplotype Location |  | Haplotype  |   |        | Frequency Cases : Controls |                  | Z score  | Haplotype P-value | Global P-value |
| 8-9-10                |  | T          | A | T      | 0.334 : 0.432              |                  | -1.8875  | 0.0591            | 0.02863        |
|                       |  | C          | A | C      | 0.001 : 0.024              |                  | -1.79354 | 0.0729            |                |
|                       |  | T          | G | C      | 0.001 : 0.012              |                  | -1.31871 | 0.1873            |                |
|                       |  | T          | A | C      | 0.002 : 0.023              |                  | -1.28693 | 0.1981            |                |
|                       |  | C          | G | C      | 0.662 : 0.509              |                  | 2.82608  | 0.0047            |                |
| 23-24-25              |  | T          | C | C      | 0.029 : 0.103              |                  | -2.42463 | 0.0153            | 0.01255        |
|                       |  | A          | C | C      | 0.007 : 0.052              |                  | -2.11649 | 0.0343            |                |
|                       |  | T          | T | C      | 0.009 : 0.013              |                  | -1.37823 | 0.1681            |                |
|                       |  | T          | C | T      | 0.654 : 0.628              |                  | 0.49746  | 0.6189            |                |
|                       |  | A          | T | C      | 0.301 : 0.204              |                  | 2.00448  | 0.0450            |                |

**Table 7**  
**Characteristics of genotyped cases vs. controls in three ethnic groups residing in northern Manhattan.**

|                                                 | Caribbean Hispanic* |             | African-American |             | White, non-Hispanic European |             |
|-------------------------------------------------|---------------------|-------------|------------------|-------------|------------------------------|-------------|
|                                                 | Case                | Control     | Case             | Control     | Case                         | Control     |
| <b>N (724 total)</b>                            | 178                 | 194         | 88               | 158         | 30                           | 76          |
| <b>Age at onset<sup>†</sup></b><br><b>(±SD)</b> | 80.9 (±6.8)         | 79.1 (±5.1) | 83.3 (±7.3)      | 81.1 (±6.1) | 84.4 (±8.0)                  | 82.7 (±7.2) |
| <b>Education</b><br><b>(in years)</b>           | 4.8 (±4.0)          | 7.6 (±4.5)  | 8.5 (±3.7)       | 11.7 (±3.7) | 10.0 (±3.7)                  | 13.0 (±3.0) |
| <b>Women (%)</b>                                | 73.0                | 73.7        | 78.4             | 73.4        | 80.0                         | 75.0        |
| <b>APOE ε4 (%)</b>                              | 21.1                | 11.3        | 18.8             | 17.1        | 13.3                         | 11.8        |

Demographic characteristics of the cases and controls from the prospective, longitudinal, multi-ethnic community-based study of aging and dementia in northern Manhattan, who met diagnostic criteria for either probable AD or for normal aging on at least two occasions separated by 18 months in time. \* Ethnicity based on self-report. † For prevalent cases, we used the age at which the first complaint was reported, if available; otherwise, age at first evaluation was used to conservatively approximate age at onset.

Table 8 Allelic association between SNPs in *SORL1* and Alzheimer disease.

| SNP | Name          | Caribbean Hispanic   |       |          |        | African-American |       |          |        | Non-Hispanic Whites |       |          |        |
|-----|---------------|----------------------|-------|----------|--------|------------------|-------|----------|--------|---------------------|-------|----------|--------|
|     |               | Minor Allele         | Cases | Controls | Chi Sq | Minor Allele     | Cases | Controls | Chi Sq | Minor Allele        | Cases | Controls | Chi Sq |
| 1   | rs4935774     | G                    | 0.353 | 0.380    | 0.55   | A                | 0.422 | 0.484    | 1.67   | G                   | 0.315 | 0.288    | 0.14   |
| 2   | rs578506      | C                    | 0.334 | 0.317    | 0.24   | C                | 0.107 | 0.068    | 2.14   | C                   | 0.431 | 0.457    | 0.11   |
| 3   | rs582446      | G                    | 0.279 | 0.285    | 0.03   | G(A)             | 0.413 | 0.402    | 0.05   | G                   | 0.125 | 0.118    | 0.02   |
| 4   | rs661057      | C                    | 0.353 | 0.394    | 1.27   | C                | 0.274 | 0.291    | 0.15   | C                   | 0.276 | 0.382    | 2.04   |
| 5   | rs11218304    | C                    | 0.272 | 0.251    | 0.40   | C                | 0.187 | 0.226    | 0.98   | C                   | 0.431 | 0.393    | 0.25   |
| 6   | rs560573      | A                    | 0.334 | 0.351    | 0.22   | A                | 0.341 | 0.291    | 1.30   | A                   | 0.310 | 0.396    | 1.29   |
| 7   | rs12364988    | A <sub>1</sub> G(A)* | 0.488 | 0.492    | 0.28   | A(G)             | 0.494 | 0.471    | 0.24   | G(A)                | 0.500 | 0.493    | 0.01   |
| 8   | rs668387      | T                    | 0.384 | 0.395    | 0.09   | T                | 0.366 | 0.314    | 1.37   | T                   | 0.362 | 0.459    | 1.59   |
| 9   | rs689021      | A                    | 0.397 | 0.430    | 0.72   | A                | 0.391 | 0.323    | 1.98   | A                   | 0.420 | 0.493    | 0.78   |
| 10  | rs641120      | T                    | 0.348 | 0.379    | 0.74   | T                | 0.311 | 0.257    | 1.53   | T                   | 0.407 | 0.410    | 0.00   |
| 11  | rs4935775     | C                    | 0.249 | 0.254    | 0.03   | C                | 0.178 | 0.173    | 0.02   | C                   | 0.379 | 0.382    | 0.00   |
| 12  | rs12285364    | T                    | 0.215 | 0.146    | 4.74   | T                | 0.083 | 0.173    | 5.77   | T                   | 0.120 | 0.062    | 1.64   |
| 13  | rs2298813     | A                    | 0.101 | 0.071    | 2.03   | A                | 0.057 | 0.084    | 1.24   | A                   | 0.056 | 0.033    | 0.52   |
| 14  | rs11600231    | C                    | 0.104 | 0.104    | 0.00   | C                | 0.100 | 0.096    | 0.02   | C                   | 0.077 | 0.096    | 0.17   |
| 15  | rs2276346     | T                    | 0.202 | 0.171    | 1.12   | T                | 0.145 | 0.102    | 2.03   | T                   | 0.350 | 0.289    | 0.74   |
| 16  | SORL1-T833T   | T                    | 0.062 | 0.057    | 0.07   | T                | 0.041 | 0.042    | 0.01   | T                   | 0.037 | 0.116    | 2.89   |
| 17  | rs556349      | G(T)                 | 0.474 | 0.484    | 0.07   | G                | 0.343 | 0.382    | 0.70   | T                   | 0.433 | 0.313    | 2.72   |
| 18  | rs11218340    | T                    | 0.074 | 0.072    | 0.01   | T                | 0.066 | 0.045    | 1.00   | T                   | 0.067 | 0.140    | 2.14   |
| 19  | rs2070045     | G                    | 0.231 | 0.237    | 0.04   | G                | 0.098 | 0.132    | 1.17   | G                   | 0.300 | 0.194    | 2.40   |
| 20  | rs3824966     | G                    | 0.232 | 0.261    | 0.77   | G                | 0.116 | 0.127    | 0.13   | G                   | 0.345 | 0.197    | 5.03   |
| 21  | SORL1-ex26c-g | G                    | 0.170 | 0.155    | 0.26   | G                | 0.282 | 0.274    | 0.03   | G                   | 0.083 | 0.123    | 0.68   |
| 22  | rs1699102     | C                    | 0.471 | 0.476    | 0.01   | C(T)             | 0.466 | 0.487    | 0.20   | C                   | 0.446 | 0.329    | 2.43   |
| 23  | rs3824968     | T                    | 0.283 | 0.303    | 0.34   | T                | 0.140 | 0.155    | 0.22   | T                   | 0.385 | 0.264    | 2.63   |
| 24  | rs2282649     | T                    | 0.281 | 0.276    | 0.02   | T                | 0.110 | 0.166    | 2.68   | T                   | 0.321 | 0.208    | 2.83   |
| 25  | rs1010159     | C                    | 0.483 | 0.460    | 0.38   | C                | 0.405 | 0.444    | 0.70   | C                   | 0.426 | 0.380    | 0.35   |
| 26  | rs1784933     | G                    | 0.178 | 0.164    | 0.23   | G                | 0.262 | 0.371    | 5.53   | G                   | 0.069 | 0.088    | 0.20   |
| 27  | rs1614735     | C                    | 0.299 | 0.322    | 0.42   | C                | 0.157 | 0.117    | 1.50   | C <sub>1</sub> A(A) | 0.431 | 0.486    | 1.14   |
| 28  | rs1133174     | G                    | 0.438 | 0.500    | 2.78   | G                | 0.287 | 0.332    | 1.04   | G <sub>1</sub> A(A) | 0.450 | 0.411    | 3.32   |
| 29  | rs1131497     | G                    | 0.287 | 0.289    | 0.00   | G                | 0.151 | 0.190    | 1.12   | G                   | 0.431 | 0.414    | 0.05   |

Allelic association between individual *SORL1* SNPs and AD was evident in at least one of the nested cohorts at SNPs 12, 20 and 26. Nominal p-values are reported because this is a replication study. The minor allele is depicted using the same nomenclature as in reference<sup>1</sup>. The minor allele for the African-Americans and for the non-Hispanic Whites was obtained from previously published data from these ethnic groups<sup>1</sup>.

Table 9. Haplotype frequencies among patients and controls by ethnic group.

| SNPs | Haplotype   | Caribbean Hispanics |       |               |                            | African American |       |               |                            | European White, Non-Hispanic |       |               |                            |
|------|-------------|---------------------|-------|---------------|----------------------------|------------------|-------|---------------|----------------------------|------------------------------|-------|---------------|----------------------------|
|      |             | Hap Freq*           | Z     | P             | Permutation<br>Empirical P | Hap Freq*        | Z     | P             | Permutation<br>Empirical P | Hap Freq*                    | Z     | P             | Permutation<br>Empirical P |
| 1    | 2 3 C C G   | .                   | .     | .             | .                          | 0.01             | 2.35  | <b>0.0186</b> | <b>0.0140</b>              | 0.183                        | .     | .             | .                          |
| 2    | 3 4 C G C   | 0.04                | -1.46 | 0.1455        | 0.1520                     | 0.01             | 2.08  | <b>0.0380</b> | <b>0.0280</b>              | 0.530                        | .     | .             | .                          |
| 4    | 5 6 C C A   | 0.01                | 2.09  | <b>0.0367</b> | <b>0.0380</b>              | 0.416            | .     | .             | .                          | .                            | .     | .             | .                          |
| 8    | 9 10 C G T  | 0.01                | -0.09 | 0.9264        | 0.8950                     | 0.01             | 2.40  | <b>0.0164</b> | <b>0.0250</b>              | 0.01                         | 0.32  | 0.7463        | 0.7807                     |
| 8    | 9 10 C A T  | 0.01                | 0.74  | 0.4596        | 0.4630                     | 0.01             | -0.49 | 0.6243        | 0.6310                     | 0.01                         | 2.46  | <b>0.0138</b> | <b>0.0304</b>              |
| 10   | 11 12 C C T | 0.03                | 0.34  | 0.7344        | 0.7550                     | 0.01             | -1.11 | 0.2653        | <b>0.2520</b>              | 0.03                         | 2.15  | <b>0.0315</b> | <b>0.0300</b>              |
| 10   | 11 12 C A T | 0.11                | 1.54  | 0.1227        | 0.1330                     | 0.10             | -2.01 | <b>0.0447</b> | <b>0.0470</b>              | 0.04                         | 0.64  | 0.5205        | 0.5550                     |
| 11   | 12 13 C T G | 0.03                | 0.40  | 0.6927        | 0.7110                     | 0.01             | -1.16 | 0.2464        | 0.2420                     | 0.02                         | 2.16  | <b>0.0305</b> | <b>0.0250</b>              |
| 16   | 17 18 A T A | 0.44                | 0.09  | 0.9318        | 0.9370                     | 0.58             | 0.39  | 0.6935        | 0.6940                     | 0.23                         | 2.75  | <b>0.0060</b> | <b>0.0050</b>              |
| 16   | 17 18 T G A | 0.06                | 0.15  | 0.8821        | 0.8710                     | 0.04             | -0.10 | 0.9186        | 0.8640                     | 0.09                         | -1.54 | 0.1231        | 0.1340                     |
| 17   | 18 19 T A G | 0.22                | -0.57 | 0.5716        | 0.5670                     | 0.11             | -0.83 | 0.4040        | 0.4210                     | 0.20                         | 2.30  | <b>0.0214</b> | <b>0.0200</b>              |
| 18   | 19 20 A G G | 0.23                | -0.62 | 0.5375        | 0.5500                     | 0.12             | -0.58 | 0.5650        | 0.5890                     | 0.23                         | 2.13  | <b>0.0328</b> | <b>0.0250</b>              |
| 19   | 20 21 G G C | 0.23                | -0.66 | 0.5072        | 0.5150                     | 0.12             | -0.50 | 0.6192        | 0.6340                     | 0.22                         | 2.25  | <b>0.0242</b> | <b>0.0180</b>              |
| 20   | 21 22 G C C | 0.24                | -0.87 | 0.3827        | 0.3710                     | 0.12             | -0.56 | 0.5748        | 0.5840                     | 0.19                         | 2.28  | <b>0.0225</b> | <b>0.0230</b>              |
| 20   | 21 22 G G C | .                   | .     | .             | .                          | 0.01             | 2.19  | <b>0.0287</b> | <b>0.0230</b>              | 0.01                         | -0.42 | 0.6771        | 0.7350                     |

Table 9  
continued

|    |    |    |   |   |   |      |       |        |        |       |      |       |               |               |              |      |       |               |               |              |
|----|----|----|---|---|---|------|-------|--------|--------|-------|------|-------|---------------|---------------|--------------|------|-------|---------------|---------------|--------------|
| 22 | 23 | 24 | C | T | T | 0.24 | -0.18 | 0.8596 | 0.8580 | 0.630 | 0.09 | -1.22 | 0.2227        | 0.2130        | 0.242        | 0.20 | 2.00  | <b>0.0451</b> | <b>0.0420</b> | 0.430        |
| 23 | 24 | 25 | T | T | C | 0.25 | 0.02  | 0.9857 | 0.9860 | 0.571 | 0.12 | -1.54 | 0.1246        | 0.1240        | 0.143        | 0.23 | 2.11  | <b>0.0348</b> | <b>0.0350</b> | 0.113        |
| 24 | 25 | 26 | C | T | A | 0.43 | -0.87 | 0.3821 | 0.3870 |       | 0.26 | 0.64  | 0.5195        | 0.5274        |              | 0.61 | -0.91 | 0.3614        | 0.3220        | 0.310        |
| 24 | 25 | 26 | C | T | G | 0.09 | 0.71  | 0.4792 | 0.4690 |       | 0.17 | -1.82 | 0.0683        | 0.0626        |              |      |       |               |               |              |
| 24 | 25 | 26 | C | C | A | 0.13 | 0.62  | 0.5357 | 0.5590 | 0.862 | 0.27 | 3.43  | <b>0.0006</b> | <b>0.0005</b> | <b>0.010</b> | 0.09 | -0.78 | 0.4370        | 0.4680        | 0.420        |
| 26 | 27 | 28 | A | A | A | 0.35 | 1.68  | 0.0922 | 0.0830 | 0.247 | 0.38 | 2.09  | <b>0.0362</b> | <b>0.0330</b> | 0.081        | 0.31 | 1.81  | 0.0700        | 0.0700        | 0.310        |
| 27 | 28 | 29 | C | A | C | 0.01 | 0.32  | 0.7495 | 0.8040 |       | 0.02 | 1.60  | 0.1104        | 0.1350        |              | 0.02 | 2.31  | <b>0.0207</b> | <b>0.0230</b> |              |
| 27 | 28 | 29 | A | A | C | 0.26 | 1.75  | 0.0802 | 0.1010 |       | 0.53 | 0.99  | 0.3205        | 0.3180        |              | 0.03 | 2.10  | <b>0.0356</b> | <b>0.0300</b> |              |
| 27 | 28 | 29 | A | G | G | 0.02 | -0.67 | 0.5034 | 0.5270 | 0.203 | 0.03 | -1.91 | 0.0566        | <b>0.0500</b> | 0.428        |      |       |               |               | <b>0.048</b> |

Table 10. Single-SNP association results of SORL1 SNPs in TGEN data.

| order | SNP        | Interval of SNPs in the original paper | A1 | F_A     | F_U     | A2 | CHISQ | OR    | Allele.P     | Geno.P       | DOM.P        | REC.P | TREND.P      |
|-------|------------|----------------------------------------|----|---------|---------|----|-------|-------|--------------|--------------|--------------|-------|--------------|
| T.1   | rs4935774  | 1                                      | 1  | 0.2145  | 0.2009  | 2  | 0.746 | 1.086 | 0.388        | 0.540        | 0.542        | 0.293 | 0.379        |
| T.2   | rs676160   | 4~5                                    | 2  | 0.1     | 0.09201 | 1  | 0.480 | 1.097 | 0.488        | 0.776        | 0.502        | 0.731 | 0.481        |
| T.3   | rs676759   | 5~6                                    | 2  | 0.3888  | 0.378   | 1  | 0.325 | 1.047 | 0.569        | 0.352        | 0.885        | 0.188 | 0.565        |
| T.4   | rs560573   | 6                                      | 2  | 0.3856  | 0.3743  | 1  | 0.363 | 1.049 | 0.547        | 0.405        | 0.953        | 0.211 | 0.548        |
| T.5   | rs985421   | 6~7                                    | 2  | 0.01641 | 0.02214 | 1  | 1.189 | 0.737 | 0.276        | NA           | NA           | NA    | 0.271        |
| T.6   | rs593769   | 6~7                                    | 1  | 0.3849  | 0.3743  | 2  | 0.320 | 1.046 | 0.572        | 0.480        | 0.980        | 0.255 | 0.572        |
| T.7   | rs12364988 | 7                                      | 2  | 0.4959  | 0.4772  | 1  | 0.935 | 1.078 | 0.334        | 0.558        | 0.611        | 0.285 | 0.338        |
| T.8   | rs668387   | 8                                      | 2  | 0.4296  | 0.4107  | 1  | 0.973 | 1.080 | 0.324        | 0.566        | 0.522        | 0.310 | 0.323        |
| T.9   | rs11218313 | 10~11                                  | 2  | 0.08795 | 0.08558 | 1  | 0.045 | 1.030 | 0.831        | 0.920        | 0.775        | 0.822 | 0.833        |
| T.10  | rs2276346  | 15                                     | 1  | 0.3506  | 0.372   | 2  | 1.327 | 0.912 | 0.249        | 0.218        | 0.102        | 0.947 | 0.243        |
| T.11  | rs10502262 | 15~16                                  | 1  | 0.2875  | 0.312   | 2  | 1.904 | 0.890 | 0.168        | 0.329        | 0.136        | 0.600 | 0.165        |
| T.12  | rs7131432  | 17~18                                  | 2  | 0.01615 | 0.02399 | 1  | 2.141 | 0.668 | 0.143        | NA           | NA           | NA    | 0.139        |
| T.13  | rs10892756 | 18~19                                  | 1  | 0.07477 | 0.06789 | 2  | 0.471 | 1.109 | 0.493        | 0.398        | 0.651        | 0.181 | 0.492        |
| T.14  | rs11218346 | 18~19                                  | 1  | 0.08523 | 0.07763 | 2  | 0.502 | 1.107 | 0.479        | 0.599        | 0.546        | 0.374 | 0.469        |
| T.15  | rs1790213  | 18~19                                  | 2  | 0.375   | 0.3932  | 1  | 0.942 | 0.926 | 0.332        | 0.344        | 0.167        | 0.992 | 0.328        |
| T.16  | rs11218347 | 18~19                                  | 1  | 0.07567 | 0.07286 | 2  | 0.077 | 1.042 | 0.782        | 0.942        | 0.811        | 0.776 | 0.780        |
| T.17  | rs1699103  | 20~21                                  | 1  | 0.4035  | 0.4419  | 2  | 4.001 | 0.855 | <b>0.045</b> | 0.129        | 0.075        | 0.139 | <b>0.043</b> |
| T.18  | rs7116734  | 20~21                                  | 1  | 0.3993  | 0.4193  | 2  | 1.089 | 0.921 | 0.297        | 0.420        | 0.613        | 0.189 | 0.293        |
| T.19  | rs11218350 | 20~21                                  | 2  | 0.2145  | 0.2477  | 1  | 4.218 | 0.829 | <b>0.040</b> | 0.108        | 0.106        | 0.072 | <b>0.045</b> |
| T.20  | rs10892759 | 22~23                                  | 1  | 0.3251  | 0.3675  | 2  | 5.310 | 0.829 | <b>0.021</b> | <b>0.040</b> | <b>0.011</b> | 0.354 | <b>0.020</b> |
| T.21  | rs1792113  | 22~23                                  | 1  | 0.3514  | 0.3752  | 2  | 1.632 | 0.902 | 0.201        | 0.063        | <b>0.041</b> | 0.682 | 0.206        |
| T.22  | rs11218360 | 22~23                                  | 1  | 0.02687 | 0.03211 | 2  | 0.652 | 0.832 | 0.420        | NA           | NA           | NA    | 0.413        |
| T.23  | rs7128608  | 22~23                                  | 1  | 0.04382 | 0.03781 | 2  | 0.588 | 1.166 | 0.443        | 0.747        | 0.447        | 0.845 | 0.446        |
| T.24  | rs1629493  | 23~24                                  | 2  | 0.3556  | 0.388   | 1  | 2.963 | 0.871 | 0.085        | 0.200        | 0.154        | 0.143 | 0.077        |
| T.25  | rs2282649  | 24                                     | 2  | 0.2887  | 0.3087  | 1  | 1.262 | 0.909 | 0.261        | 0.524        | 0.310        | 0.431 | 0.256        |
| T.26  | rs726601   | 24~25                                  | 2  | 0.2964  | 0.3294  | 1  | 3.404 | 0.858 | 0.065        | 0.116        | <b>0.038</b> | 0.557 | 0.063        |
| T.27  | rs1784931  | 24~25                                  | 1  | 0.3772  | 0.4147  | 2  | 3.885 | 0.855 | <b>0.049</b> | 0.122        | <b>0.049</b> | 0.244 | <b>0.046</b> |
| T.28  | rs1010159  | 25                                     | 1  | 0.3357  | 0.3631  | 2  | 2.192 | 0.886 | 0.139        | 0.324        | 0.207        | 0.245 | 0.135        |
| T.29  | rs1614735  | 27                                     | 2  | 0.4788  | 0.5047  | 1  | 1.737 | 0.902 | 0.188        | 0.269        | 0.105        | 0.579 | 0.176        |
| T.30  | rs17125558 | 27~28                                  | 2  | 0.01993 | 0.0221  | 1  | 0.154 | 0.900 | 0.695        | NA           | NA           | NA    | 0.702        |
| T.31  | rs10892761 | 27~28                                  | 2  | 0.4113  | 0.4223  | 1  | 0.332 | 0.956 | 0.565        | 0.069        | 0.100        | 0.317 | 0.564        |

Note: A1: Minor allele name (based on whole sample); F\_A: Frequency of this allele in cases; F\_U: Frequency of this allele in controls; A2: Major allele name. CHISQ: Chi-square test of allele; OR: estimated Odds ratio of Allele A1; Allele.P: p-value for allelic association; Geno.P: p-value for genotypic (2 df) test; DOM.P: p-value for genotypic (1 df) test with dominant model; REC.P: p-value for genotypic (1 df) test with recessive model; TREND.P: Cochran-Armitage trend test. "NA": The allelic, genotypic and dominant/recessive tests will only be conducted if at least one of the cells has a frequency less than 1. Boldface rs number indicates identical SNPs in Rogaeva et al. and Reiman et al. Bold face p-values with white font, black background refer to results from TGEN data

Supplementary Table 1 Characteristics of genotyped subjects

|                                                 | Number of genotyped subjects in familial data sets |                    |                  |                         | Number of genotyped subjects in case-control data sets |              |                   |                |            |  |
|-------------------------------------------------|----------------------------------------------------|--------------------|------------------|-------------------------|--------------------------------------------------------|--------------|-------------------|----------------|------------|--|
|                                                 | North European                                     | Caribbean-Hispanic | MIRAGE Caucasian | MIRAGE African-American | North European                                         | Israeli Arab | Mayo Jacksonville | Mayo Rochester | Mayo AUT   |  |
| Total number of Alzheimer disease families      | 124                                                | 228                | 276              | 238                     |                                                        |              |                   |                |            |  |
| Total number of subjects                        | 685                                                | 1180               | 531              | 371                     |                                                        |              |                   |                |            |  |
| Individuals with Alzheimer disease              | 321                                                | 605                | 279              | 244                     | 178                                                    | 111          | 549               | 433            | 423        |  |
| Controls                                        |                                                    |                    |                  |                         | 242                                                    | 114          | 477               | 1217           | 430        |  |
| At-risk relatives                               | 342                                                | 517                | 252              | 127                     |                                                        |              |                   |                |            |  |
| Nuclear sibships ( $\geq 2$ siblings genotyped) | 163                                                | 303                | 186              | 122                     |                                                        |              |                   |                |            |  |
| Patients per family, mean (range)               | 2.6 (1–6)                                          | 2.7 (1–12)         | 1.0 (1–2)        | 1.0 (1–2)               |                                                        |              |                   |                |            |  |
| At-risk persons per family, mean (range)        | 2.8 (0–18)                                         | 2.3 (0–15)         | 0.9 (0–4)        | 0.5 (0–3)               |                                                        |              |                   |                |            |  |
| Mean age at onset (years)                       | 70 $\pm$ 9                                         | 73 $\pm$ 11        | 74 $\pm$ 8       | 77 $\pm$ 8              | 76 $\pm$ 7                                             | 83 $\pm$ 8   | 77 $\pm$ 5        | 76 $\pm$ 6     | 76 $\pm$ 6 |  |
| Mean age of controls                            |                                                    |                    |                  |                         | 73 $\pm$ 8                                             | 76 $\pm$ 6   | 75 $\pm$ 6        | 77 $\pm$ 5     | 74 $\pm$ 6 |  |
| Autopsy confirmation (cases/controls)           | 58                                                 | 5                  | 0                | 0                       | 0/0                                                    | 0/0          | 11/0              | 91/42          | 423/430    |  |

Shown are characteristics for the six initial independent data sets composed four data sets of families multiply affected by late-onset familial Alzheimer disease and two sporadic case-control data sets. The three data sets from the Mayo Clinic were used for independent confirmation of the results in Caucasians. Age at death is shown for the subjects in which the presence or absence of Alzheimer disease was determined neuropathologically by autopsy (AUT)

Supplementary Table 2

| Gene Name | Chromosome | dbSNP rs Number | North European Families | Caribbean Hispanic Families |
|-----------|------------|-----------------|-------------------------|-----------------------------|
| SORT1     | 1          | rs370088        | 0.197                   | 0.181                       |
|           |            | rs2228604       | 0.090                   | 0.357                       |
|           |            | rs11142         | 0.415                   | 0.173                       |
|           |            | rs2140924       | 0.175                   | 0.283                       |
|           |            | rs1880670       | 0.585                   | 0.235                       |
| SORCS2    | 4          | rs4689630       | 0.396                   | 0.628                       |
|           |            | rs7676923       | 0.757                   | 0.956                       |
|           |            | rs7694823       | <b>0.015</b>            | <b>0.049</b>                |
|           |            | rs2285789       | 0.634                   | 0.206                       |
|           |            | rs4689833       | 0.583                   | 0.653                       |
|           |            | rs2285781       | 0.134                   | 0.848                       |
| VPS35     | 16         | rs794279        | 0.557                   | 0.213                       |
|           |            | rs700582        | 0.714                   | 0.141                       |
| VPS26     | 10         | rs4572029       | 0.550                   | 0.666                       |
|           |            | rs4746819       | 0.498                   | 0.599                       |
| SORCS3    | 10         | rs7084834       | 0.548                   | 0.680                       |
|           |            | rs4532962       | 0.657                   | 0.529                       |
|           |            | rs10884054      | 0.191                   | 0.267                       |
|           |            | rs10884064      | 0.110                   | 0.194                       |
|           |            | rs791125        | 0.315                   | 0.071                       |
|           |            | rs4259767       | 0.799                   | 0.871                       |
| SORCS1    | 10         | rs1269918       | 0.498                   | 0.784                       |
|           |            | rs7082289       | 0.842                   | <b>0.013</b>                |
|           |            | rs4917481       | 0.426                   | 0.915                       |
|           |            | rs3802553       | 0.236                   | 0.878                       |
|           |            | rs11192993      | 0.158                   | 0.983                       |
|           |            | rs7893643       | 0.589                   | 0.483                       |
|           |            | rs10736189      | 0.685                   | 0.772                       |
|           |            | rs10884373      | 0.747                   | 0.141                       |
|           |            | rs2993097       | 0.807                   | 0.659                       |

Supplementary Table 2: Single SNP results for VPS10 genes other than SORL1  
 FBAT analyses for the VPS-containing retromer complex genes and VPS  
 containing genes involved in regulating trafficking in the endosomal and endosomal-  
 Golgi-cell surface recycling pathways. Single SNP association results (p-values)  
 generated in two independent datasets of families multiply affected by late-onset AD  
 (North European and Caribbean Hispanic datasets). NE - North European; HIS -  
 Hispanic.

**Supplementary Table 3: Characteristics of SNPs in SORL1**

**A:** Genotype and allele frequencies (or raw genotype counts) of SORL1 single nucleotide polymorphisms (SNPs) for all nine datasets analyzed. Sibs – siblings.

**B:** SNPs and genotyping assays used in genetic association studies, primer sequence (5'-3');

**C:** Primers used for SORL1 genomic Sequencing Analysis;

**D:** Primer sequences and assay conditions used for analysis of the SORL1 gene by RT-PCR.

**E:** Primer sequences used for siRNA studies.

**F:** Linkage disequilibrium (LD) block structure around SORL1 estimated using the Haploview software. The five-color scheme (white to red) represents the increasing strength of LD. Boxes with a  $D'$  of 1 are shaded in bright red. Cells with  $D' < 1$  are shades of pink or red. Blue represents  $D' = 1$ , but with a low confidence estimate for  $D'$ . The markers generally show weak evidence of inter-marker LD. However, there are several haplotype blocks. In the North European FAD dataset (A), Block 1 contains SNPs 1 and 2 (1 kb); Block 2 contains SNPs 6 to 10 (20 kb); Block 3 contains SNPs 19 and 20 (0 kb); Block 4 contains SNPs 23 to 25 (7 kb); and Block 5 contains SNPs 28 and 29 (0 kb). Similar results were observed in all other datasets (A, B, C) and non-AD subjects used in the HapMap project.

Supplementary Table 3A

| Gene | RefSeq ID Number | Gene Description | Normal Exon/Intron | Pathologic Exon/Intron | BRCA1 Exon/Intron | BRCA2 Exon/Intron | Normal Exon/Intron |        | Pathologic Exon/Intron |        | BRCA1 Exon/Intron |        | BRCA2 Exon/Intron |        | Normal Exon/Intron |        | Pathologic Exon/Intron |        | BRCA1 Exon/Intron |        | BRCA2 Exon/Intron |        |
|------|------------------|------------------|--------------------|------------------------|-------------------|-------------------|--------------------|--------|------------------------|--------|-------------------|--------|-------------------|--------|--------------------|--------|------------------------|--------|-------------------|--------|-------------------|--------|
|      |                  |                  |                    |                        |                   |                   | Exon               | Intron | Exon                   | Intron | Exon              | Intron | Exon              | Intron | Exon               | Intron | Exon                   | Intron | Exon              | Intron | Exon              | Intron |
| 1    | 4935774          | A                | 0.750              | 0.808                  | 0.744             | 0.483             | 258                | 321    | 180                    | 185    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | G                | 0.250              | 0.394                  | 0.256             | 0.537             | 88                 | 107    | 38                     | 51     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AA               |                    |                        |                   |                   | 107                | 123    | 78                     | 84     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GA               |                    |                        |                   |                   | 44                 | 75     | 28                     | 37     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | C                | 0.497              | 0.338                  | 0.480             | 0.158             | 140                | 180    | 77                     | 98     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 2    | 578508           | G                | 0.503              | 0.662                  | 0.520             | 0.842             | 162                | 200    | 141                    | 128    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CC               |                    |                        |                   |                   | 28                 | 18     | 12                     | 29     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CG               |                    |                        |                   |                   | 84                 | 128    | 53                     | 38     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GG               |                    |                        |                   |                   | 38                 | 38     | 44                     | 45     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | A                | 0.862              | 0.781                  | 0.827             | 0.498             | 278                | 388    | 174                    | 181    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 3    | 582448           | G                | 0.138              | 0.239                  | 0.173             | 0.504             | 30                 | 80     | 44                     | 43     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AA               |                    |                        |                   |                   | 124                | 158    | 70                     | 74     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AG               |                    |                        |                   |                   | 28                 | 50     | 34                     | 33     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GG               |                    |                        |                   |                   | 1                  | 5      | 5                      | 5      |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | C                | 0.381              | 0.408                  | 0.455             | 0.311             | 110                | 180    | 58                     | 81     | 441               | 408    | 381               | 1100   | 329                | 381    |                        |        |                   |        |                   |        |
| 4    | 681067           | T                | 0.619              | 0.592                  | 0.545             | 0.689             | 214                | 288    | 159                    | 133    | 841               | 538    | 475               | 1300   | 489                | 475    |                        |        |                   |        |                   |        |
|      |                  | CC               |                    |                        |                   |                   | 24                 | 31     | 7                      | 17     | 83                | 98     | 81                | 237    | 88                 | 90     |                        |        |                   |        |                   |        |
|      |                  | CT               |                    |                        |                   |                   | 62                 | 118    | 45                     | 57     | 275               | 210    | 219               | 628    | 193                | 201    |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 78                 | 74     | 57                     | 38     | 183               | 184    | 128               | 337    | 148                | 137    |                        |        |                   |        |                   |        |
|      |                  | C                | 0.377              | 0.278                  | 0.398             | 0.202             | 121                | 145    | 88                     | 83     | 450               | 384    | 349               | 943    | 337                | 329    |                        |        |                   |        |                   |        |
| 5    | 11218304         | T                | 0.623              | 0.722                  | 0.604             | 0.798             | 143                | 237    | 118                    | 185    | 844               | 558    | 503               | 1481   | 485                | 525    |                        |        |                   |        |                   |        |
|      |                  | CC               |                    |                        |                   |                   | 31                 | 28     | 18                     | 10     | 87                | 78     | 73                | 178    | 71                 | 55     |                        |        |                   |        |                   |        |
|      |                  | TC               |                    |                        |                   |                   | 59                 | 89     | 50                     | 43     | 278               | 238    | 203               | 581    | 195                | 219    |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 42                 | 74     | 33                     | 81     | 184               | 158    | 150               | 435    | 145                | 153    |                        |        |                   |        |                   |        |
|      |                  | A                | 0.379              | 0.349                  | 0.422             | 0.293             | 105                | 141    | 82                     | 84     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 6    | 560573           | T                | 0.621              | 0.651                  | 0.578             | 0.707             | 223                | 253    | 158                    | 142    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AA               |                    |                        |                   |                   | 19                 | 17     | 7                      | 14     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AT               |                    |                        |                   |                   | 87                 | 107    | 48                     | 58     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 78                 | 73     | 54                     | 43     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | A                | 0.474              | 0.499                  | 0.511             | 0.505             | 122                | 201    | 80                     | 111    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 7    | 12384988         | G                | 0.526              | 0.501                  | 0.489             | 0.495             | 186                | 225    | 132                    | 109    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AA               |                    |                        |                   |                   | 30                 | 44     | 18                     | 31     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AG               |                    |                        |                   |                   | 82                 | 113    | 44                     | 49     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GG               |                    |                        |                   |                   | 62                 | 56     | 44                     | 30     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | C                | 0.571              | 0.812                  | 0.551             | 0.895             | 182                | 219    | 148                    | 114    | 838               | 528    | 474               | 1350   | 481                | 485    |                        |        |                   |        |                   |        |
| 8    | 688387           | T                | 0.429              | 0.388                  | 0.449             | 0.305             | 94                 | 166    | 72                     | 102    | 466               | 428    | 382               | 1054   | 333                | 385    |                        |        |                   |        |                   |        |
|      |                  | CC               |                    |                        |                   |                   | 83                 | 83     | 50                     | 29     | 188               | 144    | 132               | 385    | 137                | 125    |                        |        |                   |        |                   |        |
|      |                  | CT               |                    |                        |                   |                   | 58                 | 93     | 48                     | 58     | 280               | 240    | 210               | 620    | 207                | 215    |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 19                 | 38     | 12                     | 23     | 98                | 93     | 88                | 217    | 83                 | 85     |                        |        |                   |        |                   |        |
|      |                  | A                | 0.429              | 0.379                  | 0.402             | 0.438             | 109                | 182    | 72                     | 102    | 484               | 429    | 387               | 1084   | 358                | 389    |                        |        |                   |        |                   |        |
| 9    | 689021           | G                | 0.571              | 0.821                  | 0.598             | 0.584             | 198                | 242    | 144                    | 120    | 834               | 623    | 473               | 1350   | 482                | 457    |                        |        |                   |        |                   |        |
|      |                  | AA               |                    |                        |                   |                   | 25                 | 38     | 11                     | 23     | 102               | 98     | 89                | 220    | 77                 | 89     |                        |        |                   |        |                   |        |
|      |                  | AG               |                    |                        |                   |                   | 59                 | 110    | 50                     | 58     | 280               | 237    | 209               | 624    | 202                | 211    |                        |        |                   |        |                   |        |
|      |                  | GG               |                    |                        |                   |                   | 70                 | 88     | 47                     | 32     | 187               | 143    | 132               | 383    | 130                | 123    |                        |        |                   |        |                   |        |
|      |                  | C                | 0.571              | 0.828                  | 0.542             | 0.719             | 210                | 260    | 148                    | 128    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 10   | 641120           | T                | 0.429              | 0.374                  | 0.458             | 0.281             | 114                | 188    | 88                     | 102    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CC               |                    |                        |                   |                   | 74                 | 74     | 51                     | 35     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CT               |                    |                        |                   |                   | 82                 | 112    | 44                     | 58     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 28                 | 37     | 12                     | 23     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | A                | 0.808              | 0.743                  | 0.599             | 0.808             | 139                | 228    | 112                    | 149    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 11   | 4935775          | C                | 0.392              | 0.257                  | 0.401             | 0.194             | 137                | 158    | 100                    | 87     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AA               |                    |                        |                   |                   | 38                 | 88     | 34                     | 55     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CA               |                    |                        |                   |                   | 83                 | 90     | 44                     | 39     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CC               |                    |                        |                   |                   | 37                 | 34     | 28                     | 14     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | A                | 0.981              | 0.884                  | 0.910             | 0.821             | 309                | 424    | monomeric              |        | 1041              | 907    | 810               | 2267   | 788                | 814    |                        |        |                   |        |                   |        |
| 12   | 12285394         | T                | 0.039              | 0.119                  | 0.090             | 0.179             | 13                 | 22     |                        |        | 51                | 39     | 48                | 119    | 58                 | 30     |                        |        |                   |        |                   |        |
|      |                  | CC               |                    |                        |                   |                   | 149                | 201    |                        |        | 498               | 435    | 383               | 1081   | 358                | 382    |                        |        |                   |        |                   |        |
|      |                  | TC               |                    |                        |                   |                   | 11                 | 22     |                        |        | 49                | 37     | 44                | 115    | 54                 | 30     |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 1                  | 0      |                        |        | 1                 | 1      | 1                 | 2      | 1                  | 0      |                        |        |                   |        |                   |        |
|      |                  | A                | 0.039              | 0.108                  | 0.091             | 0.171             | 12                 | 22     |                        |        | monomeric         |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 13   | 2298813          | G                | 0.961              | 0.892                  | 0.909             | 0.829             | 294                | 404    |                        |        | monomeric         |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AA               |                    |                        |                   |                   | 1                  | 1      |                        |        |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AG               |                    |                        |                   |                   | 10                 | 20     |                        |        |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GG               |                    |                        |                   |                   | 142                | 192    |                        |        |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | C                | 0.128              | 0.177                  | 0.107             | 0.139             | 32                 | 31     | 38                     | 43     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 14   | 11600231         | T                | 0.872              | 0.823                  | 0.893             | 0.891             | 240                | 349    | 184                    | 183    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CC               |                    |                        |                   |                   | 1                  | 1      | 5                      | 8      |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CT               |                    |                        |                   |                   | 30                 | 29     | 28                     | 31     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 105                | 180    | 78                     | 78     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | G                | 0.687              | 0.773                  | 0.840             | 0.856             | 200                | 289    | 127                    | 185    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 15   | 2278346          | T                | 0.313              | 0.227                  | 0.380             | 0.144             | 124                | 145    | 73                     | 83     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GG               |                    |                        |                   |                   | 80                 | 83     | 43                     | 80     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GT               |                    |                        |                   |                   | 80                 | 103    | 41                     | 45     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 22                 | 21     | 16                     | 9      |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | A                | 0.938              | 0.905                  | 0.884             | 0.980             | 320                | 389    | 208                    | 218    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 16   | SORL1-183T31     | T                | 0.062              | 0.095                  | 0.116             | 0.020             | 8                  | 31     | 12                     | 10     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AA               |                    |                        |                   |                   | 158                | 179    | 97                     | 104    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AT               |                    |                        |                   |                   | 8                  | 31     | 12                     | 5      |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 0                  | 0      | 0                      | 1      |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | G                | 0.723              | 0.512                  | 0.850             | 0.326             | 180                | 267    | 148                    | 130    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 17   | 558349           | T                | 0.277              | 0.488                  | 0.350             | 0.674             | 98                 | 115    | 74                     | 88     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GG               |                    |                        |                   |                   | 58                 | 93     | 51                     | 32     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GT               |                    |                        |                   |                   | 98                 | 81     | 44                     | 88     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 14                 | 17     | 15                     | 18     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | A                | 0.933              | 0.943                  | 0.903             | 0.879             | 249                | 380    | 208                    | 201    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 18   | 11218340         | T                | 0.067              | 0.057                  | 0.097             | 0.121             | 21                 | 20     | 14                     | 23     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AA               |                    |                        |                   |                   | 114                | 180    | 97                     | 80     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | AT               |                    |                        |                   |                   | 21                 | 20     | 12                     | 21     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 0                  | 0      | 1                      | 1      |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | G                | 0.207              | 0.245                  | 0.277             | 0.175             | 97                 | 88     | 49                     | 58     | 257               | 202    | 229               | 584    | 201                | 188    |                        |        |                   |        |                   |        |
| 19   | 2070045          | T                | 0.793              | 0.755                  | 0.723             | 0.825             | 177                | 288    | 171                    | 108    | 837               | 752    | 631               | 1850   | 629                | 670    |                        |        |                   |        |                   |        |
|      |                  | GG               |                    |                        |                   |                   | 27                 | 15     | 8                      | 5      | 30                | 21     | 29                | 83     | 28                 | 30     |                        |        |                   |        |                   |        |
|      |                  | GT               |                    |                        |                   |                   | 61                 | 78     | 49                     | 68     | 197               | 180    | 171               | 438    | 149                | 178    |                        |        |                   |        |                   |        |
|      |                  | TT               |                    |                        |                   |                   | 87                 | 115    | 87                     | 81     | 320               | 298    | 230               | 708    | 240                | 272    |                        |        |                   |        |                   |        |
|      |                  | C                | 0.803              | 0.755                  | 0.734             | 0.826             | 245                | 320    | 157                    | 189    |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
| 20   | 3824986          | G                | 0.197              | 0.245                  | 0.268             | 0.174             | 83                 | 84     | 47                     | 59     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CC               |                    |                        |                   |                   | 89                 | 127    | 59                     | 80     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | CG               |                    |                        |                   |                   | 67                 | 83     | 39                     | 49     |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |
|      |                  | GG               |                    |                        |                   |                   |                    |        |                        |        |                   |        |                   |        |                    |        |                        |        |                   |        |                   |        |

Supplementary Table 3B.

| Gene  | RS Number | Forward primer (Reverse primer )                              | Detection Primer *             |
|-------|-----------|---------------------------------------------------------------|--------------------------------|
| SORL1 | 4935774   | CGGGGTAGGAATCGGGCTA<br>(TGCTGTTTCAGGGGAAGGA)                  | U1-ATGGTCAGTTCAATTTTCTAAAAAC   |
| SORL1 | 578506    | ACCACTGGGGACTTCCCG<br>(TAATACGCACCTGCACCCG)                   | U6-GTTTTTTTCCCTTCACGAGTACAACC  |
| SORL1 | 582446    | TTGGCTCAATAAAGGACGT<br>(AAAGCCTCCACCTTCTGTG)                  | U7-ATGCAAGCTATTTATGAACATAAAAG  |
| SORL1 | 661057    | TAATTAAGTTTTTGCTCAGGTGAATATT<br>(TGCCCTTCCCTGAATACATTT)       | U7-AAAATGAGTTTGCAATCTTTTGATCTA |
| SORL1 | 11218304  | ATCTTTGTAGCATGACTGGTGG<br>(TCCTTGGCCTGCCTGATT)                | U5-CAGAGACCAACCCCTGAGAACAGCAG  |
| SORL1 | 560573    | TCATCTAAGAAATCTTGATATATGTAAC<br>(AATGTATTCTCTGAATAAATGCTG)    | U2-TCCTTTCTCCAGTTCTAGCTCCTTC   |
| SORL1 | 12364988  | ATTGATCCCTATGACAAACCAA)<br>(TCAAGGATCACTTCCTGGTTT)            | U5-AGACAGTGGAGTAGCCAGAGGGTTC   |
| SORL1 | 668387    | ATACACAGGAATTGGAAAGGATG<br>(CCTCTCCACTCCTCTGACTTC)            | U12-ACATTGACAACATTGACGAACATTC  |
| SORL1 | 689021    | AAAGACATGATCTGTGGACTTGA<br>(TTTCTAGCATCCACTCTAGA)             | U4-CCTTACAGATGATGCAGCCACCCAC   |
| SORL1 | 641120    | TAAAAATTTAAATTGTGGGACAGTAAG<br>(ATTTTATTTCTAGTCTATTACCAGCAAC) | U8-TAATATAGTTCTAAACTGACAGAGG   |
| SORL1 | 4935775   | TTTGGTGGGGCTCCTAGT<br>(ATCAACCATCTCCACACAATCT)                | U12-CACCTCAGACAGAAAGACCCAAAACA |
| SORL1 | 12285364  | TCTGGTTTCCCTGGGCC<br>(CAGTGTGACGACATGATACCA)                  | U1-TCAGCTGCACTSCCCTCACACAGGC   |
| SORL1 | 2298813   | ATAATTATTCTCTTGCAATTTTAGGC<br>(TGGAGGGGACGGGATTGG)            | U6-CGTGTACATCTCTAGCAGTGCTGGA   |
| SORL1 | 11600231  | CATAGGAGCAGTGGCTTTT                                           | U7-CGTCAGAGAAAGGGGCTGTTGCATTT  |

|       |            |                                                                |  |                                 |
|-------|------------|----------------------------------------------------------------|--|---------------------------------|
|       |            | (TATCATCATGCTGACAAAGCAA)                                       |  |                                 |
| SORL1 | 2276346    | AGGTCAATGCCACGGATG<br>(AATGAATGTCAGGCTCTTCTGA)                 |  | U7-CTTGGMCCTTTTCACATGGATATGC    |
| SORL1 | T833T      | ATGGAAGCACAGGGCAAG<br>(AGTAAAGCAGCTGGCTGAGG)                   |  | U5-TGATCATCAATTCTGGCCTGGAGAC    |
| SORL1 | 556349     | AAGGCCAGTGTGTCAGAT<br>(TATTACCATTATTGTTATTAGCTAAGCTTC)         |  | U8-GGAGAGAAAAGGGGTAAGTACCTG     |
| SORL1 | 11218340   | TTTGCTGATTCTCCTTTATTGAC<br>(TAACAATGATATCTATAACAGTATTATTTCCTC) |  | U1-GGTTCTTGGCTGACCCCTATACTAGG   |
| SORL1 | 2070045    | CTCCTCCTGGGTATGTGAC<br>(TCAACTGAGTCCAGGAAGTACTG)               |  | U10-GGGACAACGACTGCAGGGGACTGGTC  |
| SORL1 | 3824966    | CAACTGTACCGGTCAGTACTTC<br>(TCTTTGAGAAAGCATGAAACTC)             |  | U11-GGTTAACATTATAGCTTTTAAACGAT  |
| SORL1 | SorL1-ex26 | CCTTGGACAGTTGGTGGG<br>(ACTGGAAGTTGGAGGCCT)                     |  | U9-GGAGATGAGGGGTGTGCGACTGTGT    |
| SORL1 | 1699102    | TTTCCTCCTCGTCAATCTTCT<br>(TTCATCGGAGCCATCAGA)                  |  | U6-AGTGCAATGGATTCCCGCTGCCCAA    |
| SORL1 | 3824968    | AAAGTACAGAAATCTTCAGTGGACAG<br>(AATGAAGACATCATTTTTCATCTCC)      |  | U3-TGTAAGTACATTTATATACACAAGA    |
| SORL1 | 2282649    | AGCATTTGCAGATTTAGAGAAAATA<br>(AAAGTTGTGTGATGTTTTTCTAACTAC)     |  | U4-CCTTTTCAAATGACTTTCAGTATGAC   |
| SORL1 | 1010159    | TAAGAAATGAGATACCCAAAGGAAAG<br>(ATTCACCTGCCGAGAACCC)            |  | U3-TCCTGCCCTTTTGTATTATTAGTGTGCC |
| SORL1 | 1784933    | AATGAAGACTTCTCTGTGAGCTCT<br>(AGGACATCAGCTCATGGG)               |  | U1-AAGCAGTTCAGGGGTCTTAAATTGG    |
| SORL1 | 1614735    | AAAAGCATTTTAAATGAAAGCAGG<br>(TCAACAAGCTCATCTGTGAAAC)           |  | U6-GCATTTCATTAATAAATTTCCAAAAGTC |
| SORL1 | 1133174    | AAGAAGCAGTGAGGTCTGGG<br>(ATGCCTCGCACCTAAGCG)                   |  | U5-ATGTCTCTGAATATGTGTCTTGTGAC   |
| SORL1 | 1131497    | AATAGAAAATGAAAAACAAATGCCT                                      |  | U4-TACAGGCAGCCTCTGAAGGTGACCA    |

|        |          |                                                                  |                                 |  |
|--------|----------|------------------------------------------------------------------|---------------------------------|--|
|        |          | (AAGGTACAAAAGCCCTTCTT)                                           |                                 |  |
| SORCS3 | 7084834  | TAGTACATAAGCAATACCAAAGTGC<br>(AGATGAAAGCCCGCCATCG)               | U6-GTAGCCCATGCTGCTTTGAAAGGAC    |  |
| SORCS3 | 4532962  | TTTCAGCATCCCTCAATCTG<br>(AGTGTTAATTATATTGTATGAACACTGTTG)         | U2-CACTGCAAAACCGGTTTGGAAAGAGC   |  |
| SORCS3 | 10884054 | TTTTCCGTGTTGACCAATATC<br>(CCTTTCCTCAGTCACACTCTATC)               | U3-TCAGGAGGTTCAITGGACCTGATAAA   |  |
| SORCS3 | 10884064 | ATCAAAATTGGGGGATCGT<br>(AATCTTTCTCCTTGCCCTTTCTAC)                | U1-TGTATGAGCCACAGCATTTGGTGTGA   |  |
| SORCS3 | 791125   | TTCCATCTTCTTTCAGGACATG<br>(AGAGTGAAGTAAATCCCACGC)                | U9-CTGCGGTCCAAGAATGGAACCAAGAA   |  |
| SORCS3 | 4259767  | TTGATGGCTGGAGTTGGG<br>(TCCCTTTCTCTTTTACCTTACCA)                  | U1-GGATTACAGATACTGTCTCTAAGCAT   |  |
| SORCS1 | 1269918  | TATTACCATCTCTGAGACATACAGAAAGT<br>(ATAAATGCTTATAGAGAGAGACTACGTAA) | U3-TGAGTAAACAAAGCCAAAGATTGAAAC  |  |
| SORCS1 | 7082289  | ATAGGGTAGGACAAAGAGTTCA<br>(TGTCCTCAGGCCACAGG)                    | U5-CAGTGGTGGGTAAAGCCAGGGAGAC    |  |
| SORCS1 | 4917481  | AAAGAACAAAGAGGTCAATGC<br>(ATGTGGAAGGTGAACCCAC)                   | U5-CCGTGTTGTTTCCGTACCACACAC     |  |
| SORCS1 | 3802553  | AATACTAACTCATTTCCCTCTGATGTTG<br>(TCCATGGAGCTGAGATTGA)            | U8-AGCGGACACTCATCCAAAGTGGACTT   |  |
| SORCS1 | 11192993 | TCAAAATGATCTCTCCACCT<br>(ACAGTTGAGACAAATAGAAAACAAATAC)           | U2-GCCTCCCAAAAATGTTGGGATCCACC   |  |
| SORCS1 | 7893643  | ATATTGAAGACTTTGAGAACTGAGATC<br>(TTTCCGTATTGAACACAGTAAAGTATGC)    | U2-ACCTCCCAAAAGCATTTTCAGCAGCG   |  |
| SORCS1 | 10736189 | AAAGAGATTTCTTTTGTCCCCA<br>(ATTTTTCATTTATTAGGATCACAGTG)           | U4-AGGCTCCATGGCACAGGTGGGCTTT    |  |
| SORCS1 | 10884373 | TTCTTTTCTGAGGTTTGAGGAG<br>(TTATTTCACTATTCCATCCTTTTG)             | U10-AATAGCCTTGCAGAGAGAAGGAAGAGA |  |

|            |         |                                                                 |                                        |
|------------|---------|-----------------------------------------------------------------|----------------------------------------|
| SORCS1     | 2993097 | ATGGTCCACTGGTCTGGC<br>(ATGTCCAGAAACCCTATTTC)                    | U7-AGCGTGAGTAGAATGGGACTGAGGG           |
| VPS26      | 4572029 | CTCAGCACATATTCGATTAGTAAATG<br>(AAATACTGTAGTACATTTCTCTAAGGG)     | U6-GCTGCATGATTTTAAAGCTGCTATAC          |
| VPS26      | 4746819 | TTAATATATTTCATGTGTGTAGACAGTTTTG<br>(AACAAATAGAGGAAAAAGTATGCTG)  | U3-AATTCACTGTAAAAATTGGGATGTG           |
| VPS26      | 2394522 | GGTCCAAATTACTTCTGTTGT<br>(TATTCACGTAACACATCATGC)                | GACTGACTGACTGATCTGTATCTTCCCTATCTTATAAC |
| VPS26      | 951718  | GCAGAACATAAATACCAGCCCA<br>(GGGGCAACTTCACCTCACAAAT)              | TTGTGGGAGGGTCCCTATCCTTTG               |
| VPS26      | 2135591 | GGCCAGTCTCTGAGATTCTT<br>(TTCAGTCAAAAAGTCAGCCAGC)                | GACTGACTATGCAGCCAGGGTTGAGAACCAAT       |
| VPS35      | 794279  | TTTTCTACGTCTCCTAGGATGG<br>(TTTAACAATGTAGAGAAATTGATGAAGT)        | U3-TGATTGCCTAATTAAATGCTCAAAA           |
| VPS35      | 700582  | CAACAGAGTAAGTGATTTTCTTTCTTAA<br>(AAAGGAAATCTATATACAAATGCTACCTA) | U1-GCAATATTTCTTTTCATTGTAGAATG          |
| Sortilin 1 | 370088  | ACAAGGGTGCACAGAAAG<br>(ATGAGGGACTGACAAAGGGG)                    | U11-CTCGCCCCCTTACCAATACACACAC          |
| Sortilin 1 | 2228606 | AAGATAATTCTATCCAGACCATGATC<br>(ATGCTGATGGAAGCATGAAT)            | U5-AAGGTGGAGCGCACCTGAGGAAGCCT          |
| Sortilin 1 | 2228604 | ACTTGGGAAAAAGCTTCAAAA<br>(TCCATGTGTCCCCTTGAT)                   | U9-CCTCTGTGTATGGCTGATAAAGGATAC         |
| Sortilin 1 | 111142  | AAGGGAGATCTGTTTGCACA<br>(TAACAGCAGAGGTGTCTGGAG)                 | U3-AATTCTTCGCAAAATCTGATGATCT           |
| Sortilin 1 | 2140924 | TGACAGGCACACTCAGTCC<br>(TTGTATGGGGTGTCAAGTCT)                   | U5-GTCAGCTTGGACTCAACCCCTGCCAG          |
| Sortilin 1 | 1880670 | CCAAACGTATGGAAGACTATTCT<br>(CATTGTCTGCTAAGCAGCTG)               | U12-CCTGATGTCTGTCTCCGTAGGATGT          |
| SORCS2     | 4689630 | TACTGGCCTCTGGTCACTG<br>(TCCCGAAATCTGCGGGAA)                     | U1-TACTTGGGGAGAGGTCCTCAGATTC           |

|                                          |         |                                                     |                               |
|------------------------------------------|---------|-----------------------------------------------------|-------------------------------|
| SORCS2                                   | 7676923 | TAATGTGCAAGAGAGAAATGTG<br>(TTTCTAACTCCTGCTCATTTGAC) | U3-CTCCTTACTAGGAAACAAACAACAA  |
| SORCS2                                   | 7694823 | ATTTGGAGTCAGAAAGGACCTG<br>(AGGAGAGGAGACTGGGCTC)     | U7-GATCCTAGCTCTGGACCTAAGCTCC  |
| SORCS2                                   | 2285789 | AGAACACGTGGTCTTTTGGTC<br>(TGAAGACATCACCCCTCTCTCA)   | U8-AAGTCAGATGACACGTAGAGCTTTA  |
| SORCS2                                   | 4689833 | TAATGAAGGTGGTTTTCTCTCC<br>(ACTGCCTGGAGAATGAGG)      | U4-TTGGCCACATCAGCTTCCGCTCCGA  |
| SORCS2                                   | 2285781 | ATTAGGCAGGGAGAGGC<br>(AACTTCTGGTTTAACCCATTGTC)      | U4-TGTCCCCCGCCTGACGACTGTGCCCT |
| *SNP Tag Sequence used for hybridization |         |                                                     |                               |
|                                          |         | ACGCACGTCCACGGTGATTT (U1)                           | AGGGTCTCTACGCTGACGAT (U7)     |
|                                          |         | GGATGGCGTTCGTCCTATT (U2)                            | GTGATTCTGTACGTGTCGCC (U8)     |
|                                          |         | CGTGCCGCTCGTGATAGAAT (U3)                           | GACCTGGGTGTCGATACCTA (U9)     |
|                                          |         | AGCGATCTGCGAGACCGTAT (U4)                           | AGATAGAGTCGATGCCAGCT (U10)    |
|                                          |         | GCGGTAGGTTCCCGACATAT (U5)                           | AGAGCGAGTGACGCATACTA (U11)    |
|                                          |         | GGCTATGATTGCAATGCTT (U6)                            | CGACTGTAGGTGCGTAACTC (U12)    |

Supplementary Table 3C.

| Primer name    | # exon | Primer Sequence (5'-3')   | Product size (bp) |
|----------------|--------|---------------------------|-------------------|
| 3308 - Forward | 1      | CAGAAAGTGC GCGAAAGG       | 691               |
| 3239 - Reverse |        | GATCAATGCGATTCAAAGTGC     |                   |
| 3309 - Forward | 2      | TGCTTGACAAAAGGAGGAAAAGA   | 299               |
| 3310 - Reverse |        | ACCCACTACTTTTTC CAGTATGAT |                   |
| 3242 - Forward | 3      | CGGGCAAAAAGAGTGAAACT      | 391               |
| 3243 - Reverse |        | ACAAATGCC CAGGTTAGCAC     |                   |
| 3244 - Forward | 4      | GTAAGGCTGTGGTGCC TTGT     | 392               |
| 3245 - Reverse |        | AGGAGGCGAGGTCACTCA        |                   |
| 3246 - Forward | 5      | ACAGACAGCGTGTCATGGAA      | 353               |
| 3247 - Reverse |        | CCAACTCTCTCC CACAGTGA     |                   |
| 3193 - Forward | 6      | CACCATGGTAGGCCTAAACTC     | 396               |
| 3194 - Reverse |        | GCACATCCTTCC AATTCTCTG    |                   |
| 3248 - Forward | 7      | GATGGCAGACATTGCAAAAA      | 370               |
| 3249 - Reverse |        | GAGGCGCCATAAAAATATCCA     |                   |
| 3250 - Forward | 8      | GGCTTTAGGCCAACATTTCAT     | 391               |
| 3251 - Reverse |        | TGTATCCACATGGGCAGCTA      |                   |
| 3195 - Forward | 9      | GAATGTGCTTAATGCCACAAAA    | 483               |
| 3196 - Reverse |        | GAGCAGAGTTTGGGAACTGC      |                   |
| 3306 - Forward | 10, 11 | GGGTTCAGATGATCCTCTCTGT    | 694               |
| 3307 - Reverse |        | GTCTCTGCCATGGACCATTT      |                   |
| 3197 - Forward | 12     | ATGCTGTGGGCATGTGTA        | 346               |
| 3198 - Reverse |        | CTGGGGGAGAGAGCAAGACT      |                   |
| 3254 - Forward | 13     | AGGAGCCCTGGTTCCTGTT       | 500               |
| 3255 - Reverse |        | TGCATGAGGAAAATAAGATACAAA  |                   |
| 3199 - Forward | 14     | TAGACAGGAAGTGGGGTTG       | 390               |
| 3200 - Reverse |        | CCTTCCTTTGCTCAGGGAAA      |                   |
| 3311 - Forward | 15, 16 | TGAAAAGTAGTAAGGTAAGTCAGCA | 880               |
| 3312 - Reverse |        | CCCGGACTTGGGTGAGAT        |                   |

|                |    |                            |     |
|----------------|----|----------------------------|-----|
| 3258 - Forward | 17 | TCACTTGGTCCACACCAT         | 390 |
| 3259 - Reverse |    | CCCATTTCCATAGGCCATT        |     |
| 3260 - Forward | 18 | ACATCTCATCCCTTGCCAGT       | 390 |
| 3261 - Reverse |    | AGCTGGTCCATTCTCTGGAA       |     |
| 3262 - Forward | 19 | TGGGTCAGGTCAGGGGAAATA      | 396 |
| 3263 - Reverse |    | GGAGTTACCTGACCCACGATGA     |     |
| 3264 - Forward | 20 | GGAATTTCCAGGTGAAAGGAA      | 588 |
| 3265 - Reverse |    | CATCCCTCCATAGGCTGAAA       |     |
| 3266 - Forward | 21 | AAAATTGCTGTCTCTTGCATT      | 498 |
| 3267 - Reverse |    | CAAAAGCATGCTGTCTTCCA       |     |
| 3201 - Forward | 22 | GAGAGCTTCTCGCTGTCTCT       | 400 |
| 3202 - Reverse |    | CAATAACACGAAAAAGGTTAGGAA   |     |
| 3268 - Forward | 23 | CACCCGATATCTGGCTGAAT       | 479 |
| 3269 - Reverse |    | AGTGGTACTTCTGGGGTGGTT      |     |
| 3270 - Forward | 24 | TGCCCTTATCTCTCTTGGTTG      | 478 |
| 3271 - Reverse |    | ATGACTCAACCCACCATGT        |     |
| 3272 - Forward | 25 | AGCCACCTGTAAACCCATT        | 498 |
| 3273 - Reverse |    | TGGCACAGGTTCTCTTATCTCA     |     |
| 3274 - Forward | 26 | AATGGCCTGGGTCATGTTTA       | 485 |
| 3275 - Reverse |    | TGGGCTTATTGGTTTCAGA        |     |
| 3276 - Forward | 27 | AAGTAGAATTACTGGGTCAAAGTGTG | 448 |
| 3277 - Reverse |    | TGCTCTCTGCCACGATCAG        |     |
| 3278 - Forward | 28 | GCTCTAGGCTTGGCAGAAAA       | 476 |
| 3279 - Reverse |    | AAAAGGCAAGCTTGGATGC        |     |
| 3203 - Forward | 29 | TTTCAAGGCATGGTTTAGCC       | 392 |
| 3204 - Reverse |    | GCAACTGCTGCTGAGTTACG       |     |
| 3280 - Forward | 30 | CATTGGGTCCATGAAGCTG        | 499 |
| 3281 - Reverse |    | GCCCTGCAAAATACGGAATAA      |     |
| 3313 - Forward | 31 | GCCTCTTCTGACAACCCATT       | 496 |
| 3314 - Reverse |    | CCAGAAGGTCCTCTCTCCACAA     |     |

|                |        |                             |     |
|----------------|--------|-----------------------------|-----|
| 3205 - Forward | 32     | TGCTCTCTATCCGAACTCG         | 386 |
| 3206 - Reverse |        | CGTGTGCTGATCCAGAAATG        |     |
| 3284 - Forward | 33     | CACTCTTCCCGAATGCAGAT        | 496 |
| 3285 - Reverse |        | CACTTGGGGCTCCAAAACCTA       |     |
| 3207 - Forward | 34     | CCAGTCTCGAACTCCTGAGC        | 691 |
| 3208 - Reverse |        | GGCACACATATGTTCCACACA       |     |
| 3286 - Forward | 36, 37 | TGTGACGACCAAGCTAGCAG        | 785 |
| 3287 - Reverse |        | CAAGCTCTTTGTGGTGTCCA        |     |
| 3288 - Forward | 38     | AGCTCCTTCCCTTGGCTTT         | 398 |
| 3289 - Reverse |        | CGTGTGTTGGGAGAGAAAGAA       |     |
| 3290 - Forward | 39     | GTTCAACCAAGCAACTGTGGA       | 500 |
| 3291 - Reverse |        | ACCAGGCTGAGGCAGAAATAA       |     |
| 3292 - Forward | 40     | TGGTAGCACAAAGTAGAACCTGTTT   | 447 |
| 3293 - Reverse |        | TCTGCCCTAGAAATCAGCAGCA      |     |
| 3304 - Forward | 41     | CCGTCATCATCAGTACATAAACG     | 494 |
| 3305 - Reverse |        | GCCTATATCCAAGAGAGCAGCAG     |     |
| 3294 - Forward | 42     | GCCCAAGTGCCTTCTCTGTA        | 399 |
| 3295 - Reverse |        | TCACACACATCAATCCTGGAG       |     |
| 3296 - Forward | 43     | GATTTGGGGTAGGTGGGAAA        | 452 |
| 3297 - Reverse |        | GGAGAGAGACCTCCTGCTGA        |     |
| 3209 - Forward | 44     | GCTGTGGGTCCCAGCTATTA        | 393 |
| 3210 - Reverse |        | TTGCACTCCCTGTGAAACAA        |     |
| 3298 - Forward | 45     | CTCAGCAATCTATGTGTTTATTTT    | 400 |
| 3299 - Reverse |        | TGGTCCTTTAATTCTCAAGTTCA     |     |
| 3300 - Forward | 46     | TGGTAATGTAAAAAGAAACAAGGAGAA | 498 |
| 3301 - Reverse |        | CATCACTACCTGACCACAACTGA     |     |
| 3211 - Forward | 47     | GCTATCGCCCCAGCTTTTT         | 400 |
| 3212 - Reverse |        | CTGAAGGTGGGTGTTTCCT         |     |
| 3302 - Forward | 48     | CTGGCTATGGCAATTGACCTT       | 587 |
| 3303 - Reverse |        | TTTCAAGGAGAAAGGGGTTC        |     |

Supplementary Table 3D.

| Fragment # | Primer's name  | Primer Sequence (5'-3') | Primer's Position | PCR annealing Temperature | Fragment size (bp) |
|------------|----------------|-------------------------|-------------------|---------------------------|--------------------|
| 1          | 3213 - Forward | CGCTGCACATTCTCTCCTG     | 5'UTR             | 60                        | 821                |
|            | 3214 - Reverse | TCAATTCCCCAAGAAAGGA     | exon 5-6          | With 1xQ buffer           |                    |
| 2          | 3215 - Forward | CAACCTTCTCTTGGGCTTTG    | exon 4            | 59                        | 989                |
|            | 3216 - Reverse | CGTGGTCTCCCCATGTGT      | exon 12           |                           |                    |
| 3          | 3217 - Forward | AGAGGCACCTTCCTGGACCTC   | exon 11-12        | 59                        | 970                |
|            | 3218 - Reverse | TTTGAAAGCCTGCATCTACCC   | exon 18           |                           |                    |
| 4          | 3219 - Forward | GGCCTGGAGACAGTAGAAGC    | exon 18           | 59                        | 1000               |
|            | 3220 - Reverse | ACTCGTCACTCCGGCACT      | exon 25           |                           |                    |
| 5          | 3221 - Forward | GACCTTGAGGATGACTGTGGA   | exon 24           | 59                        | 990                |
|            | 3222 - Reverse | AAGTTGGTGGGAGTGGAG      | exon 32           |                           |                    |
| 6          | 3223 - Forward | TGCGACGGGTACCGAGAT      | exon 31           | 59                        | 1000               |
|            | 3224 - Reverse | TCTCCATGGTCAGGGTGAAG    | exon 39           |                           |                    |
| 7          | 3225 - Forward | ATCCCACCAACCAGATATCCA   | exon 39           | 59                        | 987                |
|            | 3226 - Reverse | ATTCCCCAAGGTAAGCTGTGA   | exon 46           |                           |                    |
| 8          | 3227 - Forward | CAGCACCTGATGCCCTTAAAAA  | exon 45           | 59                        | 670                |
|            | 3228 - Reverse | CATATTGCAACTCAAAGTGCATC | 3' UTR            |                           |                    |

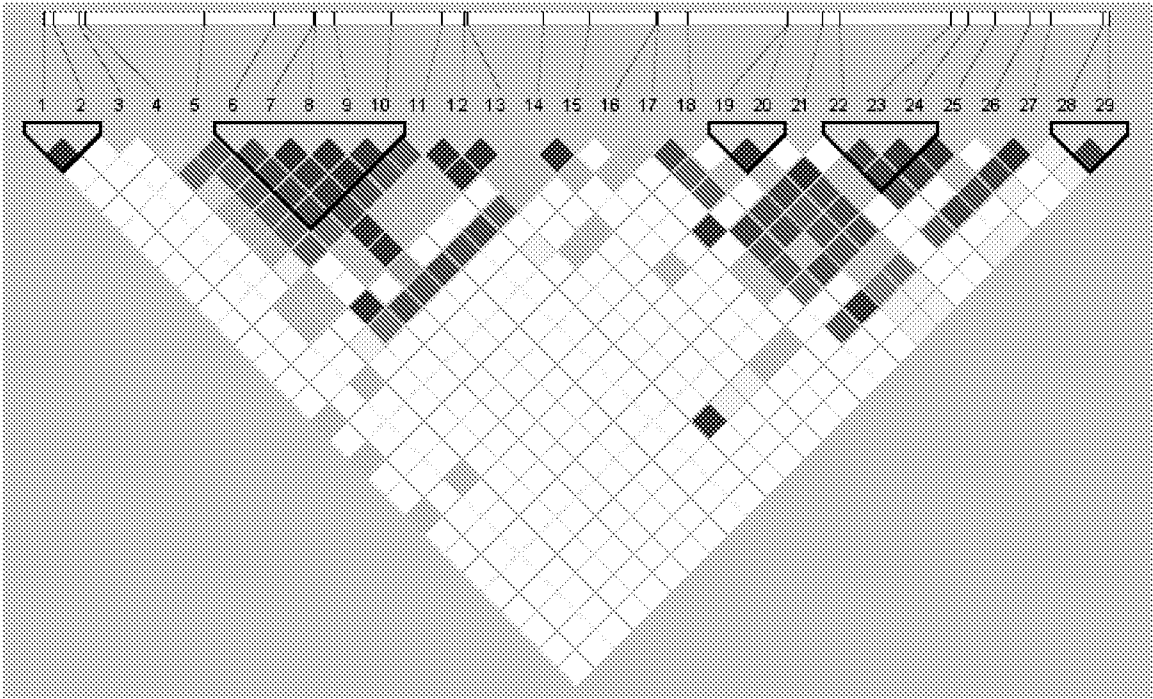
**Supplementary Table 3E****The siRNAs for SORL1 are:**

5'-AAACAACCGCACCAAUUUAUA-3' (termed LR1222),  
5'-AAGUGACACCUUGGUGAGGUA-3' (termed LR1318), and  
5'-AAAGACGGUCAUUGUCAGUAA-3' (termed LR5806).

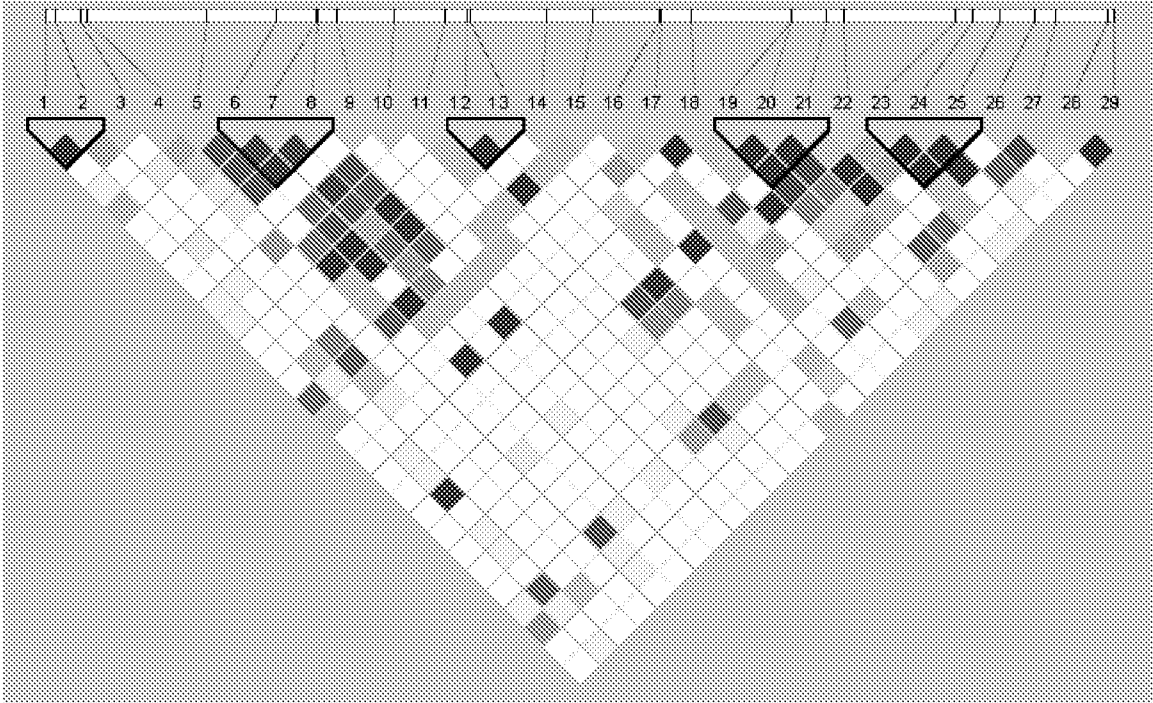
**The siRNAs for VPS26 are:**

5'-AAACAAUCGCCAAAUUGAAA-3' (termed V764); and  
5'-GAAGACCGGAGGUACUUCAAA-3' (termed V925).

**LD structure of North European family data set.**

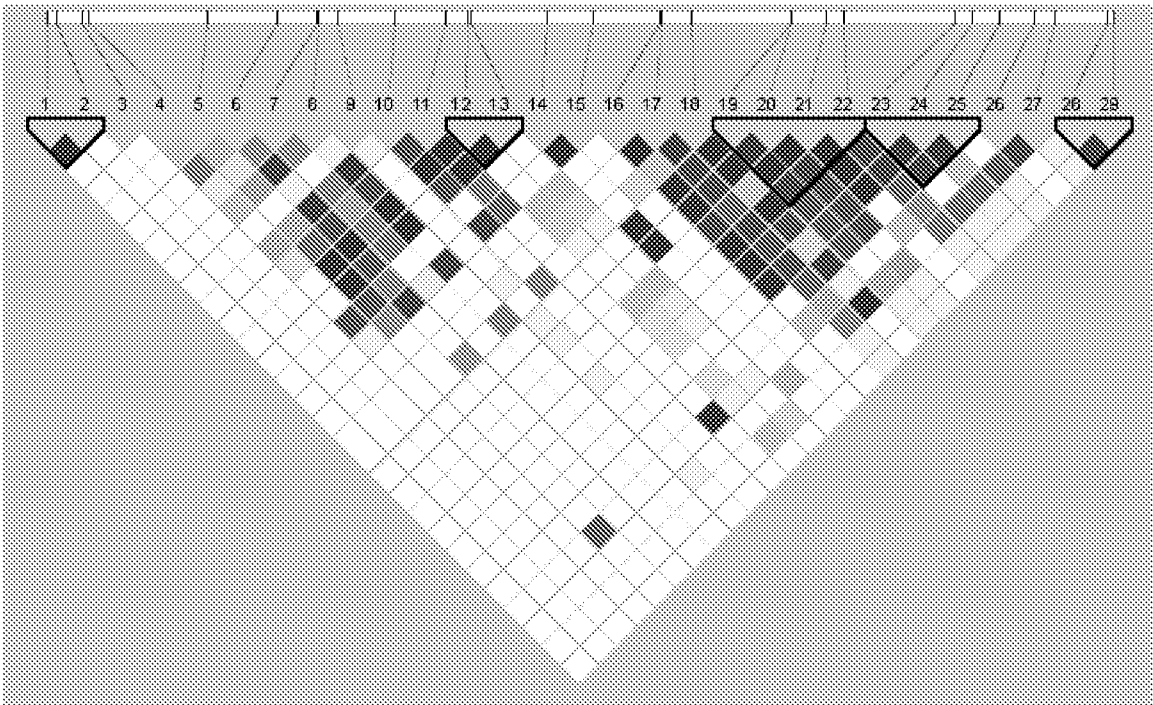


**LD structure of MIRAGE African American family data set.**



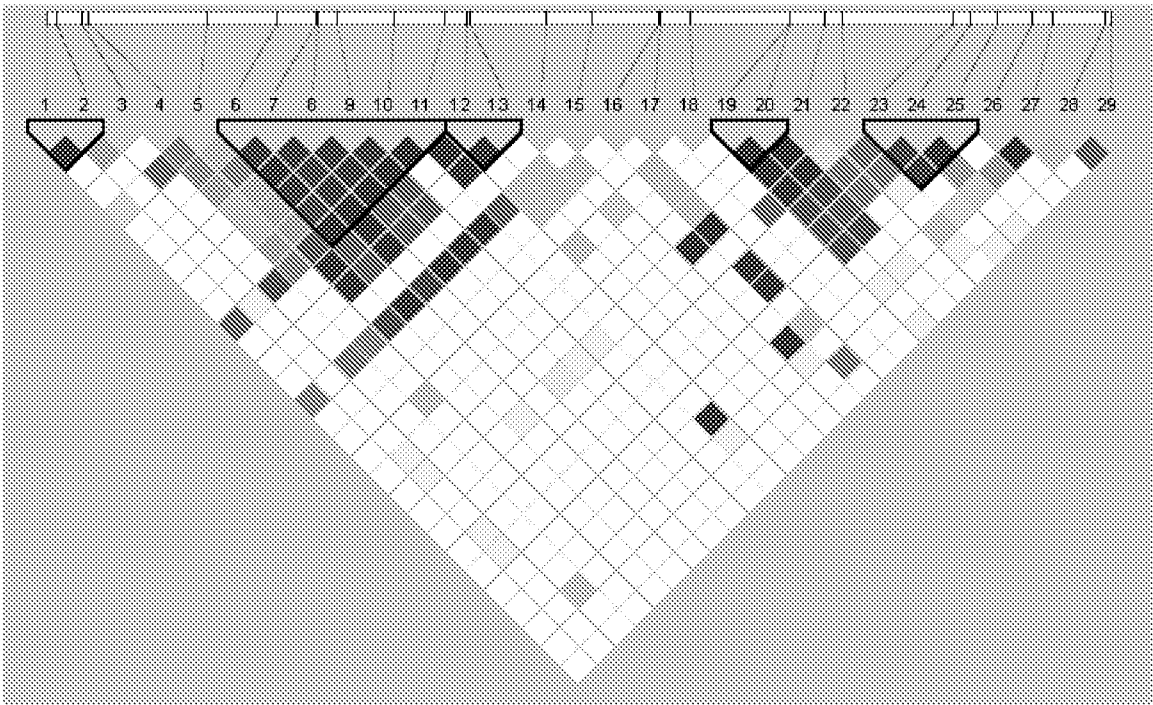
**Supplementary Table 3F-1**

**LD structure of MIRAGE Caucasian family data set.**



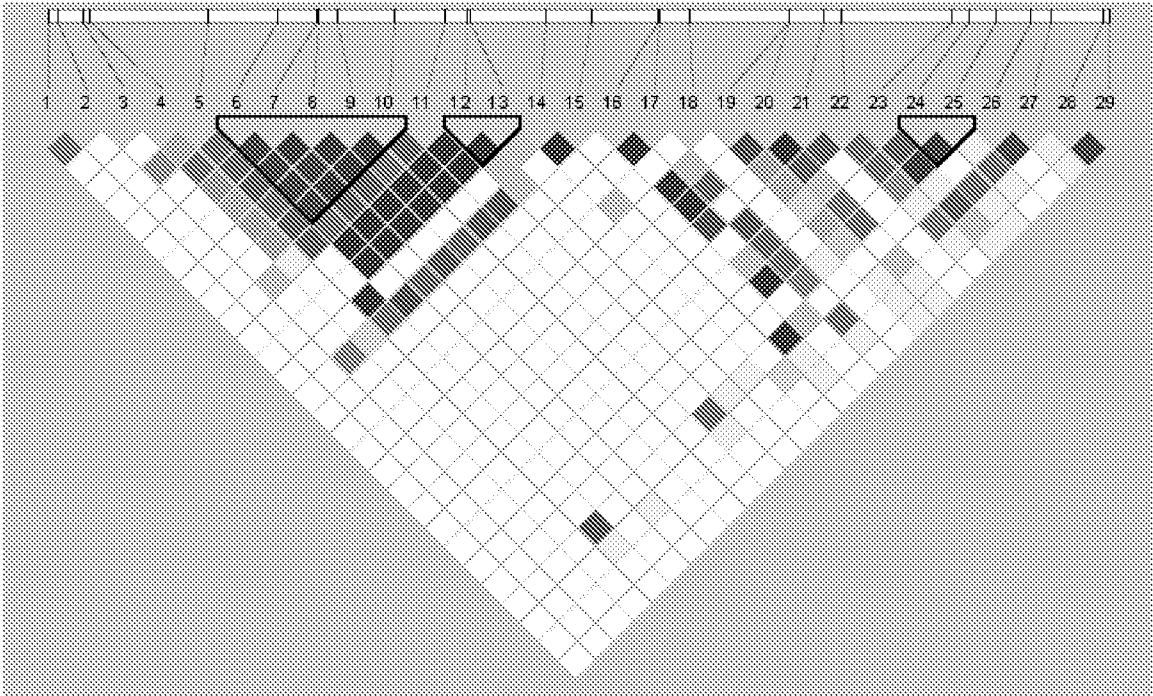
Supplementary Table 3F-2

**LD structure of Hispanic family data set.**



Supplementary Table 3F-3

**LD structure of North European case-control data set.**



**Supplementary Table 3F-4**

**Supplementary Table 4: Single SNP results for all 29 SORL1 SNPs in the six primary datasets**

**A:** Single SNP association results (FBAT p-values) generated for the primary analyses in two independent “discovery datasets” consisting of families with multiple cases of late-onset AD (*North European FAD* and *Caribbean-Hispanic FAD* datasets). After adjustment for multiple testing with an FDR level of 0.1, the cutoff p-values for significant association are 0.024 in the North European family dataset and 0.003 in the Hispanic family dataset. MAF = minor allele frequency. Supplemental Table 2A also shows allele and genotype frequencies. Nominally significant p-values are in bold; \* = association is significant after correction for multiple testing. The alleles *putatively* associated with AD are depicted only for SNPs generating nominal p-values of  $p \leq 0.10$ .

**B:** Separate confirmatory analyses were performed in four independent “replication cohorts” including two case:control datasets (of *North European* origin or from an *Israeli-Arab* inbred population isolate), and two familial datasets (siblings) from the MIRAGE study (Caucasian Americans and the other of African-Americans). Corrections for multiple testing were not applied in these directed replication analyses. Boxes highlight identical alleles that are nominally associated with disease in at least two independent datasets. ND – not determined. NA – not available. The p-values in the case:control cohort are for allelic association. Alleles *putatively* associated with AD are depicted only for those SNPs generating nominal p-values of  $p < 0.10$ .

Supplementary Table 4A

| Table 4B     |      |                         |      |         |             |     |                             |          |        |                    |             |                           |         |                    |   |            |                             |       |          |          |        |                       |     |      |          |   |                              |  |  |  |  |
|--------------|------|-------------------------|------|---------|-------------|-----|-----------------------------|----------|--------|--------------------|-------------|---------------------------|---------|--------------------|---|------------|-----------------------------|-------|----------|----------|--------|-----------------------|-----|------|----------|---|------------------------------|--|--|--|--|
| SNP          |      | Discovery Datasets      |      |         |             |     |                             |          |        |                    |             | Replication Datasets      |         |                    |   |            |                             |       |          |          |        |                       |     |      |          |   |                              |  |  |  |  |
|              |      | North European Families |      |         |             |     | Caribbean Hispanic Families |          |        |                    |             | Israeli Arab case control |         |                    |   |            | North European case control |       |          |          |        | MIRAGE Caucasian Sibs |     |      |          |   | MIRAGE African-American Sibs |  |  |  |  |
| Minor Allele | SNP# | MAF                     | Info | P       | Risk Allele | MAF | Info                        | Families | P      | OR (95%CI)         | Risk Allele | MAF                       | Info    | Families           | P | OR (95%CI) | Risk Allele                 | MAF   | Info     | Families | P      | Risk Allele           | MAF | Info | Families | P | Risk Allele                  |  |  |  |  |
| G            | 1    | 0.250                   | 39   | 0.204   | 0.394       | 71  | 0.777                       | 0.205    | 0.111  | 1.46 (0.91 - 2.34) | A           | 0.232                     | 0.182   | 1.26 (0.90 - 1.79) | A | 0.256      | 65                          | 0.634 | 0.463(A) | 50       | 0.015  | G                     |     |      |          |   |                              |  |  |  |  |
| C            | 2    | 0.497                   | 54   | 0.072   | 0.338       | 71  | 0.331                       | 0.391    | 0.105  | 1.37 (0.94 - 2.02) | T           | 0.453                     | ND      | ND                 | A | 0.480      | 69                          | 0.574 | 0.158    | 27       | 0.583  |                       |     |      |          |   |                              |  |  |  |  |
| G            | 3    | 0.138                   | 29   | 0.324   | 0.239       | 67  | 0.830                       | 0.197    | 0.794  | 1.06 (0.67 - 1.70) | T           | 0.123                     | 0.082   | 1.51 (0.95 - 2.40) | A | 0.173      | 46                          | 0.197 | 0.504(A) | 45       | 0.447  |                       |     |      |          |   |                              |  |  |  |  |
| C            | 4    | 0.381                   | 46   | 0.119   | 0.408       | 80  | 0.407                       | 0.339    | 0.0026 | 1.84 (1.24 - 2.75) | T           | 0.377                     | 0.070   | 1.32 (0.98 - 1.77) | T | 0.455      | 75                          | 0.307 | 0.311    | 42       | 0.617  |                       |     |      |          |   |                              |  |  |  |  |
| C            | 5    | 0.377                   | 51   | 0.272   | 0.278       | 67  | 0.964                       | 0.350    | 0.0012 | 1.94 (1.30 - 2.90) | C           | 0.412                     | 0.046   | 1.38 (1.01 - 1.90) | C | 0.396      | 72                          | 0.185 | 0.202    | 34       | 0.578  |                       |     |      |          |   |                              |  |  |  |  |
| A            | 6    | 0.379                   | 53   | 0.643   | 0.349       | 82  | 0.167                       | 0.329    | 0.050  | 1.49 (1.00 - 2.22) | T           | 0.341                     | 0.287   | 1.18 (0.87 - 1.61) | T | 0.422      | 61                          | 0.988 | 0.293    | 48       | 0.856  |                       |     |      |          |   |                              |  |  |  |  |
| A            | 7    | 0.474                   | 55   | 0.149   | 0.499       | 73  | 0.174                       | 0.442    | 0.0078 | 1.68 (1.15 - 2.47) | G           | 0.440                     | 0.041   | 1.36 (1.01 - 1.83) | G | 0.489(G)   | 78                          | 0.225 | 0.495(G) | 54       | 0.407  |                       |     |      |          |   |                              |  |  |  |  |
| T            | 8    | 0.429                   | 51   | 0.515   | 0.388       | 80  | 0.013                       | 0.399    | 0.002  | 1.84 (1.25 - 2.71) | C           | 0.392                     | 0.021   | 1.46 (1.06 - 2.01) | C | 0.449      | 72                          | 0.859 | 0.305    | 49       | 0.831  |                       |     |      |          |   |                              |  |  |  |  |
| A            | 9    | 0.429                   | 54   | 0.708   | 0.379       | 78  | 0.017                       | 0.397    | 0.007  | 1.70 (1.15 - 2.50) | G           | 0.398                     | 0.040   | 1.37 (1.01 - 1.86) | G | 0.402      | 76                          | 0.636 | 0.436    | 51       | 0.885  |                       |     |      |          |   |                              |  |  |  |  |
| T            | 10   | 0.429                   | 55   | 0.565   | 0.374       | 79  | 0.021                       | 0.385    | 0.0051 | 1.74 (1.18 - 2.56) | C           | 0.390                     | 0.067   | 1.32 (0.98 - 1.77) | C | 0.458      | 64                          | 0.506 | 0.281    | 42       | 0.630  |                       |     |      |          |   |                              |  |  |  |  |
| C            | 11   | 0.392                   | 55   | 0.225   | 0.257       | 66  | 0.780                       | 0.390    | 0.0006 | 1.99 (1.34 - 2.95) | C           | 0.447                     | 0.030   | 1.41 (1.03 - 1.92) | C | 0.401      | 73                          | 0.586 | 0.194    | 29       | 0.369  |                       |     |      |          |   |                              |  |  |  |  |
| T            | 12   | 0.039                   | 12   | *0.014  | 0.116       | 27  | 0.800                       | 0.000    | 1.000  | NA                 | A           | 0.046                     | 0.557   | 1.23 (0.61 - 2.49) | A | 0.090      | 22                          | 0.587 | 0.179    | 26       | 0.278  |                       |     |      |          |   |                              |  |  |  |  |
| A            | 13   | 0.039                   | 12   | *0.012  | 0.108       | 28  | 0.952                       | 0.000    | 1.000  | NA                 | A           | 0.046                     | 0.431   | 1.33 (0.65 - 2.74) | A | 0.091      | 22                          | 0.399 | 0.171    | 24       | 0.479  |                       |     |      |          |   |                              |  |  |  |  |
| C            | 14   | 0.128                   | 18   | 0.687   | 0.177       | 48  | 0.062                       | 0.177    | 0.462  | 1.20 (0.74 - 1.96) | T           | 0.097                     | 0.124   | 1.50 (0.89 - 2.53) | T | 0.107      | 21                          | 0.177 | 0.139    | 21       | 0.466  |                       |     |      |          |   |                              |  |  |  |  |
| C            | 15   | 0.313                   | 47   | 0.614   | 0.227       | 68  | 0.821                       | 0.318    | 0.049  | 1.51 (1.00 - 2.27) | T           | 0.364                     | 0.363   | 1.15 (0.85 - 1.56) | T | 0.360      | 72                          | 0.203 | 0.144    | 20       | 0.188  |                       |     |      |          |   |                              |  |  |  |  |
| T            | 16   | 0.062                   | 25   | 0.406   | 0.095       | 19  | 0.910                       | 0.050    | 0.600  | 1.26 (0.53 - 2.98) | T           | 0.052                     | 0.0026  | 3.19 (1.45 - 7.03) | A | 0.116      | 30                          | 0.090 | 0.020    | 1        | NA     |                       |     |      |          |   |                              |  |  |  |  |
| T            | 17   | 0.277                   | 51   | *0.0057 | 0.488       | 91  | 0.170                       | 0.384    | 0.042  | 1.49 (1.01 - 2.18) | G           | 0.321                     | 0.205   | 1.24 (0.89 - 1.72) | G | 0.350      | 80                          | 0.437 | 0.326(G) | 56       | 0.645  |                       |     |      |          |   |                              |  |  |  |  |
| T            | 18   | 0.067                   | 22   | 0.075   | 0.057       | 23  | 0.817                       | 0.083    | 0.137  | 1.68 (0.84 - 3.36) | T           | 0.061                     | 0.141   | 1.60 (0.85 - 3.02) | T | 0.097      | 19                          | 0.836 | 0.121    | 14       | 0.428  |                       |     |      |          |   |                              |  |  |  |  |
| G            | 19   | 0.207                   | 42   | 0.031   | 0.245       | 84  | 0.617                       | 0.236    | 0.499  | 1.16 (0.75 - 1.80) | G           | 0.287                     | 0.0082  | 1.79 (1.27 - 2.53) | G | 0.277      | 58                          | 0.765 | 0.175    | 22       | 0.352  |                       |     |      |          |   |                              |  |  |  |  |
| G            | 20   | 0.197                   | 42   | 0.154   | 0.245       | 79  | 0.883                       | 0.245    | 0.494  | 1.17 (0.75 - 1.81) | G           | 0.228                     | 0.148   | 1.29 (0.91 - 1.82) | G | 0.266      | 60                          | 0.780 | 0.174    | 24       | 0.318  |                       |     |      |          |   |                              |  |  |  |  |
| C            | 21   | 0.069                   | 26   | 0.412   | 0.200       | 49  | 0.467                       | 0.093    | 0.239  | 1.48 (0.77 - 2.83) | C           | 0.096                     | 0.0991  | 2.01 (1.18 - 3.42) | C | 0.117      | 36                          | 0.107 | 0.299    | 44       | 0.787  |                       |     |      |          |   |                              |  |  |  |  |
| C            | 22   | 0.302                   | 56   | 0.056   | 0.468       | 88  | 0.386                       | 0.377    | 0.051  | 1.47 (1.00 - 2.17) | T           | 0.312                     | 0.907   | 1.02 (0.73 - 1.43) | T | 0.367      | 59                          | 0.820 | 0.473(T) | 37       | 0.254  |                       |     |      |          |   |                              |  |  |  |  |
| T            | 23   | 0.264                   | 53   | *0.0031 | 0.298       | 78  | 0.513                       | 0.345    | 0.672  | 1.09 (0.74 - 1.61) | T           | 0.125                     | 0.00073 | 2.16 (1.37 - 3.40) | T | 0.333      | 81                          | 0.680 | 0.193    | 26       | 0.818  |                       |     |      |          |   |                              |  |  |  |  |
| T            | 24   | 0.244                   | 48   | *0.020  | 0.249       | 80  | 0.266                       | 0.268    | 0.902  | 1.03 (0.67 - 1.56) | C           | 0.260                     | 0.637   | 1.08 (0.78 - 1.50) | C | 0.304      | 70                          | 0.889 | 0.163    | 22       | 0.104  |                       |     |      |          |   |                              |  |  |  |  |
| C            | 25   | 0.282                   | 55   | 0.086   | 0.460       | 83  | 0.190                       | 0.440    | 0.365  | 1.19 (0.81 - 1.74) | C           | 0.328                     | 0.915   | 1.02 (0.75 - 1.38) | C | 0.375      | 79                          | 0.438 | 0.484(T) | 55       | 0.0069 |                       |     |      |          |   |                              |  |  |  |  |
| G            | 26   | 0.075                   | 12   | 0.069   | 0.184       | 51  | 0.291                       | 0.082    | 0.728  | 1.13 (0.57 - 2.23) | G           | 0.054                     | 0.796   | 1.09 (0.57 - 2.09) | G | 0.085      | 16                          | 0.372 | 0.388    | 47       | 0.648  |                       |     |      |          |   |                              |  |  |  |  |
| A            | 27   | 0.452                   | 50   | *0.010  | 0.356(C)    | 88  | 0.600                       | 0.46(C)  | 0.139  | 1.33 (0.91 - 1.94) | A           | 0.457                     | 0.964   | 1.01 (0.74 - 1.38) | A | 0.486      | 81                          | 0.867 | 0.188(C) | 31       | 0.818  |                       |     |      |          |   |                              |  |  |  |  |
| A            | 28   | 0.443                   | 57   | 0.068   | 0.451(G)    | 89  | 0.903                       | 0.491    | 0.004  | 1.74 (1.19 - 2.54) | G           | 0.422                     | 0.087   | 1.29 (0.96 - 1.73) | G | 0.452      | 82                          | 0.306 | 0.292(G) | 40       | 0.731  |                       |     |      |          |   |                              |  |  |  |  |
| G            | 29   | 0.428                   | 56   | 0.201   | 0.316       | 69  | 0.564                       | 0.378    | 0.017  | 1.60 (1.09 - 2.36) | G           | 0.368                     | 0.065   | 1.34 (0.98 - 1.83) | G | 0.442      | 82                          | 0.192 | 0.172    | 29       | 0.344  |                       |     |      |          |   |                              |  |  |  |  |

**Supplementary Table 5: Three-SNP haplotypes for all SORL1 SNPS**

Haplotypes for all three-SNP windows that have a global p-value for association with or against AD of  $p \leq 0.05$  in at least one dataset. In this table all p-values of  $p \leq 0.05$  are in bold. ND = not done due to a deviation from HWE in control samples in this dataset. Haplotypes that show increased risk for AD in at least two independent datasets are highlighted in black. Haplotypes showing increased risk in one dataset and reduced risk in a dataset with different ethnic/racial origins are in dark grey. Haplotypes that show reduced risk in at least two independent datasets are highlighted in light grey.

| Supplementary Table 5A |  |  |  |  |  |  |  |  |  | Table 5B     |  |  |  |  |  |  |  |  |  | Table 5B     |  |  |  |  |  |  |  |  |  |
|------------------------|--|--|--|--|--|--|--|--|--|--------------|--|--|--|--|--|--|--|--|--|--------------|--|--|--|--|--|--|--|--|--|
| SNP#                   |  |  |  |  |  |  |  |  |  | SNP#         |  |  |  |  |  |  |  |  |  | SNP#         |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |
| Family Score           |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  | Family Score |  |  |  |  |  |  |  |  |  |
| p-value                |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  | p-value      |  |  |  |  |  |  |  |  |  |
| HAP                    |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  | HAP          |  |  |  |  |  |  |  |  |  |
| Frequency              |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  | Frequency    |  |  |  |  |  |  |  |  |  |
| Info Z                 |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  | Info Z       |  |  |  |  |  |  |  |  |  |

**Supplementary Table 6: Two SNP haplotypes for all SORL1 SNPs**

Haplotype tables using a two SNP sliding window. Black highlights represent haplotypes that are significantly associated with AD, while gray highlights represent haplotypes that are protective. As was evident with the 3 SNP window analyses (see main text and Table 3) there was a general clustering of significant results near SNPs 8-10 at the 5' end in the Caribbean Hispanics, and near SNPs 22-25 at the 3' end in the North Europeans and MIRAGE African American cohorts. Part A refers to the discovery cohorts, Part B refers to the replication cohorts.

Supplementary Table 6

| North European Families |      |               |      |        |         |       |               |      |        | Caribbean Hispanic Families |       |                   |                 |        |         |        |                   |                 |        | Arabid Arab case control |        |               |       |        |         |        |               |       |        | North European case control |        |               |       |        |         |        |       |  |  | MIRAGE Caucasians Sbs |  |  |  |  |  |  |  |  |  | MIRAGE African-Americans Sbs |  |  |  |  |  |  |  |  |  |
|-------------------------|------|---------------|------|--------|---------|-------|---------------|------|--------|-----------------------------|-------|-------------------|-----------------|--------|---------|--------|-------------------|-----------------|--------|--------------------------|--------|---------------|-------|--------|---------|--------|---------------|-------|--------|-----------------------------|--------|---------------|-------|--------|---------|--------|-------|--|--|-----------------------|--|--|--|--|--|--|--|--|--|------------------------------|--|--|--|--|--|--|--|--|--|
| Hap                     | SNP# | Hap frequency | Info | Z      | p-value | sin p | Hap frequency | Info | Z      | p-value                     | sin p | Control frequency | Cases frequency | Z      | p-value | sin p  | Control frequency | Cases frequency | Z      | p-value                  | sin p  | Hap frequency | Info  | Z      | p-value | sin p  | Hap frequency | Info  | Z      | p-value                     | sin p  | Hap frequency | Info  | Z      | p-value | sin p  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 1                       | A    | 0.282         | 47   | -1.976 | 0.048   | 0.117 | 0.286         | 60   | -0.869 | 0.503                       | 0.625 | 0.416             | 0.363           | 1.235  | 0.182   | 0.019  | ND                | ND              | ND     | ND                       | ND     | ND            | 0.506 | 67     | 0.564   | 0.592  | 0.885         | 0.065 | 27     | -1.577                      | 0.115  | 0.346         | 48    | -0.946 | 0.046   | 0.012  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 2                       | C    | 0.298         | 47   | 0.932  | 0.351   | 0.281 | 0.310         | 66   | -0.415 | 0.678                       | 0.305 | 0.432             | 0.483           | 2.859  | 0.0078  | 0.019  | 0.416             | 0.363           | 1.235  | 0.182                    | 0.019  | 0.416         | 0.363 | 1.235  | 0.182   | 0.019  | 0.416         | 0.363 | 1.235  | 0.182                       | 0.019  | 0.416         | 0.363 | 1.235  | 0.182   | 0.019  | 0.416 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 3                       | A    | 0.180         | 36   | 1.317  | 0.193   | 0.309 | 0.401         | 66   | 1.019  | 0.379                       | 0.978 | 0.229             | 0.175           | -1.398 | 0.162   | 0.019  | 0.229             | 0.175           | -1.398 | 0.162                    | 0.019  | 0.229         | 0.175 | -1.398 | 0.162   | 0.019  | 0.229         | 0.175 | -1.398 | 0.162                       | 0.019  | 0.229         | 0.175 | -1.398 | 0.162   | 0.019  | 0.229 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 4                       | G    | 0.506         | 97   | 0.033  | 0.973   | 0.978 | 0.468         | 97   | 0.033  | 0.973                       | 0.978 | 0.468             | 97              | 0.033  | 0.973   | 0.978  | 0.468             | 97              | 0.033  | 0.973                    | 0.978  | 0.468         | 97    | 0.033  | 0.973   | 0.978  | 0.468         | 97    | 0.033  | 0.973                       | 0.978  | 0.468         | 97    | 0.033  | 0.973   | 0.978  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 5                       | A    | 0.357         | 54   | 1.676  | 0.043   | 0.283 | 0.273         | 72   | -0.128 | 0.888                       | 0.689 | 0.319             | 0.225           | -2.664 | 0.0062  | 0.019  | 0.319             | 0.225           | -2.664 | 0.0062                   | 0.019  | 0.319         | 0.225 | -2.664 | 0.0062  | 0.019  | 0.319         | 0.225 | -2.664 | 0.0062                      | 0.019  | 0.319         | 0.225 | -2.664 | 0.0062  | 0.019  | 0.319 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 6                       | C    | 0.079         | 17   | -1.188 | 0.235   | 0.141 | 0.126         | 51   | 0.400  | 0.689                       | 0.519 | 0.105             | 0.156           | 1.393  | 0.165   | 0.019  | 0.105             | 0.156           | 1.393  | 0.165                    | 0.019  | 0.105         | 0.156 | 1.393  | 0.165   | 0.019  | 0.105         | 0.156 | 1.393  | 0.165                       | 0.019  | 0.105         | 0.156 | 1.393  | 0.165   | 0.019  | 0.105 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 7                       | T    | 0.307         | 54   | -1.765 | 0.074   | 0.217 | 0.198         | 61   | -0.142 | 0.887                       | 0.988 | 0.249             | 0.398           | 3.242  | 0.0012  | 0.0047 | 0.341             | 0.411           | 2.869  | 0.023                    | 0.108  | 0.341         | 0.411 | 2.869  | 0.023   | 0.108  | 0.341         | 0.411 | 2.869  | 0.023                       | 0.108  | 0.341         | 0.411 | 2.869  | 0.023   | 0.108  | 0.341 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 8                       | T    | 0.360         | 55   | -1.953 | 0.118   | 0.343 | 0.360         | 55   | -1.953 | 0.118                       | 0.343 | 0.360             | 55              | -1.953 | 0.118   | 0.343  | 0.360             | 55              | -1.953 | 0.118                    | 0.343  | 0.360         | 55    | -1.953 | 0.118   | 0.343  | 0.360         | 55    | -1.953 | 0.118                       | 0.343  | 0.360         | 55    | -1.953 | 0.118   | 0.343  | 0.360 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 9                       | T    | 0.343         | 56   | 1.194  | 0.232   | 0.509 | 0.307         | 83   | -0.491 | 0.623                       | 0.424 | 0.249             | 0.265           | -2.005 | 0.042   | 0.0085 | 0.249             | 0.265           | -2.005 | 0.042                    | 0.0085 | 0.249         | 0.265 | -2.005 | 0.042   | 0.0085 | 0.249         | 0.265 | -2.005 | 0.042                       | 0.0085 | 0.249         | 0.265 | -2.005 | 0.042   | 0.0085 | 0.249 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 10                      | T    | 0.364         | 58   | -1.394 | 0.192   | 0.312 | 0.188         | 62   | -0.081 | 0.936                       | 0.246 | 0.372             | 0.279           | -2.132 | 0.033   | 0.044  | 0.372             | 0.279           | -2.132 | 0.033                    | 0.044  | 0.372         | 0.279 | -2.132 | 0.033   | 0.044  | 0.372         | 0.279 | -2.132 | 0.033                       | 0.044  | 0.372         | 0.279 | -2.132 | 0.033   | 0.044  | 0.372 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 11                      | T    | 0.339         | 54   | 1.119  | 0.263   | 0.252 | 0.324         | 87   | -0.141 | 0.298                       | 0.246 | 0.439             | 0.620           | 2.435  | 0.015   | 0.017  | 0.439             | 0.620           | 2.435  | 0.015                    | 0.017  | 0.439         | 0.620 | 2.435  | 0.015   | 0.017  | 0.439         | 0.620 | 2.435  | 0.015                       | 0.017  | 0.439         | 0.620 | 2.435  | 0.015   | 0.017  | 0.439 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 12                      | G    | 0.410         | 57   | -1.883 | 0.085   | 0.251 | 0.315         | 71   | -0.372 | 0.725                       | 0.951 | 0.439             | 0.620           | 2.435  | 0.015   | 0.017  | 0.439             | 0.620           | 2.435  | 0.015                    | 0.017  | 0.439         | 0.620 | 2.435  | 0.015   | 0.017  | 0.439         | 0.620 | 2.435  | 0.015                       | 0.017  | 0.439         | 0.620 | 2.435  | 0.015   | 0.017  | 0.439 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 13                      | G    | 0.426         | 54   | 1.165  | 0.236   | 0.316 | 0.319         | 71   | -0.372 | 0.725                       | 0.951 | 0.439             | 0.620           | 2.435  | 0.015   | 0.017  | 0.439             | 0.620           | 2.435  | 0.015                    | 0.017  | 0.439         | 0.620 | 2.435  | 0.015   | 0.017  | 0.439         | 0.620 | 2.435  | 0.015                       | 0.017  | 0.439         | 0.620 | 2.435  | 0.015   | 0.017  | 0.439 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 14                      | G    | 0.574         | 52   | -0.622 | 0.802   | 0.562 | 0.639         | 75   | 2.123  | 0.037                       | 0.048 | 0.534             | 0.639           | 2.123  | 0.037   | 0.048  | 0.534             | 0.639           | 2.123  | 0.037                    | 0.048  | 0.534         | 0.639 | 2.123  | 0.037   | 0.048  | 0.534         | 0.639 | 2.123  | 0.037                       | 0.048  | 0.534         | 0.639 | 2.123  | 0.037   | 0.048  | 0.534 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 15                      | A    | 0.411         | 52   | 0.915  | 0.358   | 0.348 | 0.411         | 52   | 0.915  | 0.358                       | 0.348 | 0.411             | 52              | 0.915  | 0.358   | 0.348  | 0.411             | 52              | 0.915  | 0.358                    | 0.348  | 0.411         | 52    | 0.915  | 0.358   | 0.348  | 0.411         | 52    | 0.915  | 0.358                       | 0.348  | 0.411         | 52    | 0.915  | 0.358   | 0.348  | 0.411 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 16                      | G    | 0.571         | 54   | -0.194 | 0.854   | 0.181 | 0.639         | 75   | 2.123  | 0.037                       | 0.048 | 0.534             | 0.639           | 2.123  | 0.037   | 0.048  | 0.534             | 0.639           | 2.123  | 0.037                    | 0.048  | 0.534         | 0.639 | 2.123  | 0.037   | 0.048  | 0.534         | 0.639 | 2.123  | 0.037                       | 0.048  | 0.534         | 0.639 | 2.123  | 0.037   | 0.048  | 0.534 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 17                      | G    | 0.437         | 54   | 0.860  | 0.437   | 0.437 | 0.437         | 54   | 0.860  | 0.437                       | 0.437 | 0.437             | 54              | 0.860  | 0.437   | 0.437  | 0.437             | 54              | 0.860  | 0.437                    | 0.437  | 0.437         | 54    | 0.860  | 0.437   | 0.437  | 0.437         | 54    | 0.860  | 0.437                       | 0.437  | 0.437         | 54    | 0.860  | 0.437   | 0.437  | 0.437 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 18                      | A    | 0.410         | 57   | -1.883 | 0.085   | 0.251 | 0.315         | 71   | -0.372 | 0.725                       | 0.951 | 0.439             | 0.620           | 2.435  | 0.015   | 0.017  | 0.439             | 0.620           | 2.435  | 0.015                    | 0.017  | 0.439         | 0.620 | 2.435  | 0.015   | 0.017  | 0.439         | 0.620 | 2.435  | 0.015                       | 0.017  | 0.439         | 0.620 | 2.435  | 0.015   | 0.017  | 0.439 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 19                      | A    | 0.389         | 59   | -1.381 | 0.137   | 0.296 | 0.366         | 66   | -1.167 | 0.259                       | 0.984 | 0.281             | 0.423           | 3.167  | 0.0022  | 0.0037 | 0.281             | 0.423           | 3.167  | 0.0022                   | 0.0037 | 0.281         | 0.423 | 3.167  | 0.0022  | 0.0037 | 0.281         | 0.423 | 3.167  | 0.0022                      | 0.0037 | 0.281         | 0.423 | 3.167  | 0.0022  | 0.0037 | 0.281 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 20                      | A    | 0.411         | 52   | 0.915  | 0.358   | 0.348 | 0.411         | 52   | 0.915  | 0.358                       | 0.348 | 0.411             | 52              | 0.915  | 0.358   | 0.348  | 0.411             | 52              | 0.915  | 0.358                    | 0.348  | 0.411         | 52    | 0.915  | 0.358   | 0.348  | 0.411         | 52    | 0.915  | 0.358                       | 0.348  | 0.411         | 52    | 0.915  | 0.358   | 0.348  | 0.411 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 21                      | C    | 0.131         | 43   | 0.330  | 0.742   | 0.419 | 0.131         | 43   | 0.330  | 0.742                       | 0.419 | 0.131             | 43              | 0.330  | 0.742   | 0.419  | 0.131             | 43              | 0.330  | 0.742                    | 0.419  | 0.131         | 43    | 0.330  | 0.742   | 0.419  | 0.131         | 43    | 0.330  | 0.742                       | 0.419  | 0.131         | 43    | 0.330  | 0.742   | 0.419  | 0.131 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 22                      | A    | 0.556         | 55   | -0.034 | 0.973   | 0.159 | 0.708         | 67   | 0.112  | 0.911                       | 0.948 | 0.280             | 0.362           | -2.346 | 0.0023  | 0.0045 | 0.280             | 0.362           | -2.346 | 0.0023                   | 0.0045 | 0.280         | 0.362 | -2.346 | 0.0023  | 0.0045 | 0.280         | 0.362 | -2.346 | 0.0023                      | 0.0045 | 0.280         | 0.362 | -2.346 | 0.0023  | 0.0045 | 0.280 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 23                      | A    | 0.409         | 54   | -0.827 | 0.408   | 0.376 | 0.213         | 67   | 0.169  | 0.866                       | 0.681 | 0.213             | 67              | 0.169  | 0.866   | 0.681  | 0.213             | 67              | 0.169  | 0.866                    | 0.681  | 0.213         | 67    | 0.169  | 0.866   | 0.681  | 0.213         | 67    | 0.169  | 0.866                       | 0.681  | 0.213         | 67    | 0.169  | 0.866   | 0.681  | 0.213 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 24                      | C    | 0.036         | 13   | 2.067  | 0.039   | 0.081 | 0.036         | 13   | 2.067  | 0.039                       | 0.081 | 0.036             | 13              | 2.067  | 0.039   | 0.081  | 0.036             | 13              | 2.067  | 0.039                    | 0.081  | 0.036         | 13    | 2.067  | 0.039   | 0.081  | 0.036         | 13    | 2.067  | 0.039                       | 0.081  | 0.036         | 13    | 2.067  | 0.039   | 0.081  | 0.036 |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 25                      | C    | 0.962         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 26                      | C    | 0.036         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 27                      | C    | 0.036         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 28                      | C    | 0.962         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 29                      | C    | 0.962         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 30                      | C    | 0.962         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 31                      | C    | 0.962         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 32                      | C    | 0.962         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 33                      | C    | 0.962         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 34                      | C    | 0.962         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24   | 0.351  | 0.725                       | 0.681 | 1                 | 0               | *      | *       | *      | 0.949             | 0.960           | 0.709  | 0.460                    | 0.821  | 0.961         | 21    | -0.795 | 0.462   | 0.471  | 0.942         | 25    | -0.794 | 0.462                       | 0.471  | 0.942         | 25    | -0.794 | 0.462   | 0.471  |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |
| 35                      | C    | 0.962         | 11   | -2.254 | 0.024   | 0.030 | 0.926         | 24</ |        |                             |       |                   |                 |        |         |        |                   |                 |        |                          |        |               |       |        |         |        |               |       |        |                             |        |               |       |        |         |        |       |  |  |                       |  |  |  |  |  |  |  |  |  |                              |  |  |  |  |  |  |  |  |  |

**Supplementary Table 7: Four SNP haplotypes for all SORL1 SNPs**

Haplotype tables using a four SNP sliding window. Black highlights represent haplotypes that are significantly associated with AD, while gray highlights represent haplotypes that are protective. As was evident with the 3 SNP window analyses (see main text and Table 3) there was a general clustering of significant results near SNPs 8-10 at the 5' end in the Caribbean Hispanics, and near SNPs 22-25 at the 3' end in the North Europeans and MIRAGE African American cohorts. Part A refers to the discovery cohorts, Part B refers to the replication cohorts.

Supplementary Table 7

[illegible]

**Supplementary Table 8: Five SNP haplotypes for all SORL1 SNPs**

Haplotype tables using a five SNP sliding window. Black highlights represent haplotypes that are significantly associated with AD, while gray highlights represent haplotypes that are protective. As was evident with the 3 SNP window analyses (see main text and Table 3) there was a general clustering of significant results near SNPs 8-10 at the 5' end in the Caribbean Hispanics, and near SNPs 22-25 at the 3' end in the North Europeans and MIRAGE African American cohorts. Part A refers to the discovery cohorts, Part B refers to the replication cohorts.

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**Supplementary Table 9: Six SNP haplotypes for all SORL1 SNPs**

Haplotype tables using a six SNP sliding window. Black highlights represent haplotypes that are significantly associated with AD, while gray highlights represent haplotypes that are protective. As was evident with the 3 SNP window analyses (see main text and Table 3) there was a general clustering of significant results near SNPs 8-10 at the 5' end in the Caribbean Hispanics, and near SNPs 22-25 at the 3' end in the North Europeans and MIRAGE African American cohorts. Part A refers to the discovery cohorts, Part B refers to the replication cohorts.

Supplementary Table 9

[illegible]

Supplementary Table 10.

| variation  | genomic nucleotide position* | Exon/intron # | Function               | Comments   |
|------------|------------------------------|---------------|------------------------|------------|
| A>G        | 24381                        | Intron 3      | (+) 38 3' from exon 2  | novel      |
| G>A        | 51138                        | Exon 6        | E270K                  | novel      |
| T>C        | 51170                        | Exon 6        | S280S                  | novel      |
| G>C        | 97863                        | Exon 13       | S594T                  | novel      |
| C>T        | 109362                       | Intron 18     | (-) 45 5' from exon 18 | novel      |
| C>T        | 109392                       | Intron 18     | (-) 15 5' from exon 18 | novel      |
| T>C        | 124448                       | Exon 23       | F1099L                 | novel      |
| C>G        | 137660                       | Intron 26     | (-) 18 5' from exon 26 | novel      |
| G>T        | 142420                       | Intron 29     | (+) 49 3' from exon 28 | novel      |
| deletion C | 143414                       | Intron 30     | (-) 65 5' from exon 30 | novel      |
| C>T        | 144357                       | Exon 30       | N1392N                 | rs2276412  |
| A>G        | 145289                       | Exon 31       | S1428G                 | novel      |
| C>T        | 158425                       | Exon 33       | T1511I                 | novel      |
| G>A        | 159623                       | Exon 35       | V1594M                 | novel      |
| A>T        | 161244                       | Intron 37     | (+) 67 3' from exon 36 | novel      |
| G>A        | 173074                       | Exon 42       | K1895K                 | rs17125548 |

Supplementary Table 10: Rare sequence variants in SORL1

Rare sequence variations found in the SORL1 gene during sequencing analysis (allele frequency is <0.05). These variants were too rare to be used in the present genetic association studies. Typical late-onset FAD family structures were also too incomplete to discern whether these rare SNPs had Mendelian inheritance or whether the segregated with disease. \*Accession number is AP000977.

What is claimed is:

- 1 1. A method for determining if a patient is at risk of developing Alzheimer's Disease,  
2 comprising
  - 3 a. obtaining a DNA sample from the patient,
  - 4 b. determining if the DNA sample shows a nucleotide variant in an SNP that is a  
5 member of the group comprising SNPs rs560573, rs985421, rs593769, rs12364988,  
6 rs668387 [SNPs 8-12], rs4935775, 17 rs12285364, rs2298813 [SNPs 16-18],  
7 rs11600231, rs2276346, rs10502262, SORL1-T833T, rs556349, rs7131432,  
8 rs11218340 and rs10892756.[ SNPs 19-26] of the sortilin-related receptor low density  
9 lipoprotein receptor class A (SORL1) gene, and
  - 10 c. if a high risk nucleotide variant is detected concluding that the patient is at  
11 high risk of developing Alzheimer's Disease, or if a low risk variant is detected  
12 concluding that the patient is at low risk of developing Alzheimer's Disease.
- 1 2. The method as in claim 1, wherein the high risk nucleotide variant is an allele variant  
2 that is a member selected from the group comprising C at SNP, G at SNP rs985421, C  
3 at SNP rs593769, G at SNP rs11600231, T at SNP rs556349, C at SNP rs668387, T at  
4 SNP rs668387, G at SNP rs2276346, and G at SNP s10892756.
- 1 3. The method as in claim 1, wherein the high risk nucleotide variant is a haplotype that  
2 is a member selected from the group comprising
  - 3 CGC at SNP rs560573, rs985421, and rs593769, respectively or other haplotypes in  
4 linkage disequilibrium therewith;
  - 5 CGC at SNP rs985421, rs593769, and rs12364988, respectively or other haplotypes  
6 in linkage disequilibrium therewith;
  - 7 GCC at SNP rs985421, rs593769, and rs12364988, respectively or other haplotypes  
8 in linkage disequilibrium therewith;

9           ATA at SNP CTT at SNP rs560573, rs985421, and rs593769, respectively or other  
10           haplotypes in linkage disequilibrium therewith;

11           CTT SORL1-T833T, rs556349, rs7131432, respectively or other haplotypes in  
12           linkage disequilibrium therewith;

13           TTC at SNP rs556349, rs7131432, rs11218340, respectively or other haplotypes in  
14           linkage disequilibrium therewith;

15           ACT at SNP rs556349, rs7131432, rs11218340, respectively or other haplotypes in  
16           linkage disequilibrium therewith;

17           TGG at SNP rs556349, rs7131432, rs11218340, respectively or other haplotypes in  
18           linkage disequilibrium therewith;

19           CTG at SNP rs7131432, rs11218340 and rs10892756. or at a site in linkage  
20           disequilibrium with it,

21           GGT at SNP rs7131432, rs11218340 and rs10892756, respectively or other  
22           haplotypes in linkage disequilibrium therewith;

23           ATA at SNP rs4935775, rs12285364 and 18 rs2298813, respectively or other  
24           haplotypes in linkage disequilibrium therewith;

25           GAC at SNP rs7131432, rs11218340 and rs10892756, respectively or other  
26           haplotypes in linkage disequilibrium therewith;

27           CCA at SNP rs7131432, rs11218340 and rs10892756, respectively or other  
28           haplotypes in linkage disequilibrium therewith;

29           ATA at SNP rs4935775, rs12285364 and rs2298813 or at a site in linkage  
30           disequilibrium with it,

31           TAG rs12285364, rs2298813 and     rs11600231, respectively or other haplotypes in  
32           linkage disequilibrium therewith;

AGG at SNP rs2298813, rs11600231, and rs2276346, respectively or other haplotypes in linkage disequilibrium therewith;

GGC at SNP rs11600231, 20 rs2276346 and rs10502262, respectively or other haplotypes in linkage disequilibrium therewith;

GCC rs2276346, 21 rs10502262 and SORL1-T833T, respectively or other haplotypes in linkage disequilibrium therewith;

CAT at SNP rs560573, rs985421, and rs593769, respectively or other haplotypes in linkage disequilibrium therewith;

CGT at SNP rs560573, rs985421, and rs593769 or at a site in linkage disequilibrium with it, and

TTC at SNP, rs556349, rs7131432, rs11218340, respectively or other haplotypes in linkage disequilibrium therewith.

4. The method as in claim 1, wherein the low risk nucleotide variant is a haplotype variant that is a member selected from the group comprising:

GCG at SNP rs560573, rs985421, and rs593769, respectively; or another haplotype in linkage disequilibrium with it,

GCG at SNP rs985421, rs593769, and rs12364988, respectively; or another haplotype in linkage disequilibrium with it,

CGG at SNP rs985421, rs593769, and rs12364988, respectively; or another haplotype in linkage disequilibrium with it,

TAT at SNP CTT at SNP rs560573, rs985421, and rs593769, respectively; or another haplotype in linkage disequilibrium with it,

GAA SORL1-T833T, rs556349, rs7131432, respectively; or another haplotype in linkage disequilibrium with it,

13           AAG at SNP rs556349, rs7131432, rs11218340, respectively; or another haplotype in  
14           linkage disequilibrium with it,

15           TGA at SNP rs556349, rs7131432, rs11218340, respectively; or another haplotype in  
16           linkage disequilibrium with it,

17           ACC at SNP rs556349, rs7131432, rs11218340, respectively; or another haplotype in  
18           linkage disequilibrium with it,

19           GAC at SNP rs7131432, rs11218340 and rs10892756, respectively; or another  
20           haplotype in linkage disequilibrium with it,

21           CCA at SNP rs7131432, rs11218340 and rs10892756, respectively; or another  
22           haplotype in linkage disequilibrium with it,

23           TAT at SNP rs4935775, rs12285364 and rs10892756, respectively; or another haplotype in  
24           linkage disequilibrium with it,

25           CTG at SNP rs7131432, rs11218340 and rs10892756, respectively; or another haplotype in  
26           linkage disequilibrium with it,

27           GGT at SNP rs7131432, rs11218340 and rs10892756, respectively; or another  
28           haplotype in linkage disequilibrium with it,

29           TAT at SNP rs4935775, rs12285364 and rs2298813, respectively; or another  
30           haplotype in linkage disequilibrium with it,

31           ATC rs12285364, rs2298813 and rs11600231, respectively; or another haplotype  
32           in linkage disequilibrium with it,

33           TCC at SNP rs2298813, rs11600231, and rs2276346, respectively; or another  
34           haplotype in linkage disequilibrium with it,

35           CCG at SNP rs11600231, rs2276346 and rs10502262 or in linkage disequilibrium  
36           with it,

37 CGGrS2276346, 21 rs10502262 and SORL1-T833T or in linkage disequilibrium with  
 38 it,  
 39 GTA at SNP rs560573, rs985421, and rs593769, respectively; or another haplotype in  
 40 linkage disequilibrium with it,  
 41 GCA at SNP rs560573, rs985421, and rs593769, respectively; or another haplotype in  
 42 linkage disequilibrium with it, and  
 43 AAG at SNP, rs556349, rs7131432, rs11218340, respectively; or another haplotype  
 44 in linkage disequilibrium with it.

1 5. The method as in claim 1, wherein the low risk nucleotide variant is an allele variant  
 2 that is a member selected from the group comprising: G at SNP, C at SNP rs985421,  
 3 G at SNP rs593769, C at SNP rs11600231, A at SNP rs556349, G at SNP rs668387,  
 4 A at SNP rs668387, C at SNP rs2276346, and C at SNP s10892756.

1 6. The method as in claim 1, wherein the patient's DNA sample is derived from any  
 2 patient cell type, preferably cells selected from the group comprising lymphoblasts,  
 3 epidermal cells, and fibroblasts.

1 7. A method for confirming a diagnosis of Alzheimer's Disease in a patient having a  
 2 diagnosis of probable or possible Alzheimer's Disease comprising:  
 3 a. obtaining a DNA sample from the patient,  
 4 b. determining if the DNA sample shows a nucleotide variant in an SNP that is a  
 5 member of the group comprising SNPs rs560573, rs985421, rs593769, rs12364988,  
 6 rs668387 [SNPs 8-12], rs4935775, 17 rs12285364, rs2298813 [SNPs 16-18],  
 7 rs11600231, rs2276346, rs10502262, SORL1-T833T, rs556349, rs7131432,  
 8 rs11218340 and rs10892756.[ SNPs 19-26] of the sortilin-related receptor low density  
 9 lipoprotein receptor class A (SORL1) gene, and

- 10 c. if a high risk nucleotide variant is detected, concluding that the patient has  
11 Alzheimer's Disease, or if a low risk nucleotide variant is detected concluding that  
12 the patient does not have Alzheimer's Disease.
- 1 8. The method as in claim 7, wherein the patient's DNA sample is derived from any  
2 patient cell type, preferably cells selected from the group comprising lymphoblasts,  
3 epidermal cells, and fibroblasts.
- 4 9. The method as in claim 7, wherein the high risk nucleotide variant is an allele variant  
5 that is a member selected from the group comprising C at SNP rs560573 or tight ld, G  
6 at SNP rs985421, C at SNP rs593769, G at SNP rs11600231, T at SNP rs556349, C  
7 at SNP rs668387, T at SNP rs668387,, G at SNP rs2276346, and G at SNP  
8 s10892756.
- 1 10. The method as in claim 7, wherein the high risk nucleotide variant is a haplotype that  
2 is a member selected from the group comprising:  
3 CGC at SNP rs560573, rs985421, and rs593769, respectively or other haplotypes in  
4 linkage disequilibrium therewith;  
5 CGC at SNP rs985421, rs593769, and rs12364988, respectively or other haplotypes  
6 in linkage disequilibrium therewith;  
7 GCC at SNP rs985421, rs593769, and rs12364988, respectively or other haplotypes  
8 in linkage disequilibrium therewith;  
9 ATA at SNP CTT at SNP rs560573, rs985421, and rs593769, respectively or other  
10 haplotypes in linkage disequilibrium therewith;  
11 CTT SORL1-T833T, rs556349, rs7131432, respectively or other haplotypes in  
12 linkage disequilibrium therewith;  
13 TTC at SNP rs556349, rs7131432, rs11218340, respectively or other haplotypes in  
14 linkage disequilibrium therewith;

15 ACT at SNP rs556349, rs7131432, rs11218340, respectively or other haplotypes in  
16 linkage disequilibrium therewith;

17 TGG at SNP rs556349, rs7131432, rs11218340, respectively or other haplotypes in  
18 linkage disequilibrium therewith;

19 CTG at SNP rs7131432, rs11218340 and rs10892756. or at a site in linkage  
20 disequilibrium with it,

21 GGT at SNP rs7131432, rs11218340 and rs10892756, respectively or other  
22 haplotypes in linkage disequilibrium therewith;

23 ATA at SNP rs4935775, rs12285364 and rs2298813, respectively or other  
24 haplotypes in linkage disequilibrium therewith;

25 GAC at SNP rs7131432, rs11218340 and rs10892756, respectively or other  
26 haplotypes in linkage disequilibrium therewith;

27 CCA at SNP rs7131432, rs11218340 and rs10892756, respectively or other  
28 haplotypes in linkage disequilibrium therewith;

29 ATA at SNP rs4935775, rs12285364 and rs2298813 or at a site in linkage  
30 disequilibrium with it,

31 TAG rs12285364, rs2298813 and rs11600231, respectively or other haplotypes in  
32 linkage disequilibrium therewith;

33 AGG at SNP rs2298813, rs11600231, and rs2276346, respectively or other  
34 haplotypes in linkage disequilibrium therewith;

35 GGC at SNP rs11600231, rs2276346 and rs10502262, respectively or other  
36 haplotypes in linkage disequilibrium therewith;

37 GCC rs2276346, rs10502262 and SORL1-T833T, respectively or other haplotypes  
38 in linkage disequilibrium therewith;

39 CAT at SNP rs560573, rs985421, and rs593769, respectively or other haplotypes in  
40 linkage disequilibrium therewith;  
41 CGT at SNP rs560573, rs985421, and rs593769 or at a site in linkage disequilibrium  
42 with it, and  
43 TTC at SNP, rs556349, rs7131432, rs11218340, respectively or other haplotypes in  
44 linkage disequilibrium therewith.

1 11. The method of claim 7, further comprising:

- 2 d. obtaining a cell sample from the patient,
- 3 e. determining the level of SORL1 expression in the patient cell sample,
- 4 f. comparing the level of expression of SORL1 in the patient cell sample to the  
5 level of expression of SORL1 in a cell sample taken from a normal patient, and
- 6 g. determining that the patient has Alzheimer's Disease if the level of SORL1  
7 expression in patient sample is significantly lower than the level in the cell sample  
8 from the normal patient, and that the patient does not have Alzheimer's Disease if the  
9 level of SORL1 expression in patient sample is normal.

1 12. A method for determining if a patient is at risk of developing AD, comprising:

- 2 a. obtaining a cell sample from the patient,
- 3 b. determining the level of SORL1 expression in the patient cell sample,
- 4 c. comparing the level of expression of SORL1 in the patient cell sample to the  
5 level of expression of SORL1 in a cell sample taken from a normal patient, and

- 6 d. determining that the patient is at risk for Alzheimer's Disease if the level of  
7 SORL1 expression in patient sample is significantly lower than the level in the cell  
8 sample from the normal patient.
- 1 13. The method of claim 12, wherein the cell sample comprises cells selected from the  
2 group comprising lymphoblasts, epidermal cells, fibroblasts, or any other cell.
- 3 14. A method for treating Alzheimer's Disease in a patient, comprising administering a  
4 therapeutically effective amount of SORL1 or a biologically active fragment or  
5 variant or modification thereof.
- 1 15. The method of claim 14, wherein the SORL1 is formulated in a pharmaceutical  
2 composition that crosses the blood brain barrier and the SORL1 is human  
3 recombinant SORL1.
- 1 16. A pharmaceutical composition for treating Alzheimer's Disease comprising human  
2 recombinant SORL1 or a biologically active fragment or variant or modification  
3 thereof.
- 1 17. The pharmaceutical composition of claim 16 formulated to cross the blood brain  
2 barrier.
- 1 18. The method as in claim 7, wherein the low risk nucleotide variant is a haplotype  
2 variant that is a member selected from the group comprising:  
3 GCG at SNP rs560573, rs985421, and rs593769, respectively; or another haplotype in  
4 linkage disequilibrium with it,  
5 GCG at SNP rs985421, rs593769, and rs12364988, respectively; or another haplotype  
6 in linkage disequilibrium with it,  
7 CGG at SNP rs985421, rs593769, and rs12364988, respectively; or another haplotype  
8 in linkage disequilibrium with it,

9 TAT at SNP CTT at SNP rs560573, rs985421, and rs593769, respectively; or another  
10 haplotype in linkage disequilibrium with it,  
11 GAA SORL1-T833T, rs556349, rs7131432, respectively; or another haplotype in  
12 linkage disequilibrium with it,  
13 AAG at SNP rs556349, rs7131432, rs11218340, respectively; or another haplotype in  
14 linkage disequilibrium with it,  
15 TGA at SNP rs556349, rs7131432, rs11218340, respectively; or another haplotype in  
16 linkage disequilibrium with it,  
17 ACC at SNP rs556349, rs7131432, rs11218340, respectively; or another haplotype in  
18 linkage disequilibrium with it,  
19 GAC at SNP rs7131432, rs11218340 and rs10892756, respectively; or another  
20 haplotype in linkage disequilibrium with it,  
21 CCA at SNP rs7131432, rs11218340 and rs10892756, respectively; or another  
22 haplotype in linkage disequilibrium with it,  
23 TAT at SNP rs4935775, rs12285364 and 18, respectively; or another haplotype in  
24 linkage disequilibrium with it,  
25 CTG at SNP rs7131432, rs11218340 and, respectively; or another haplotype in  
26 linkage disequilibrium with it,  
27 GGT at SNP rs7131432, rs11218340 and rs10892756, respectively; or another  
28 haplotype in linkage disequilibrium with it,  
29 TAT at SNP rs4935775, rs12285364 and rs2298813, respectively; or another  
30 haplotype in linkage disequilibrium with it,  
31 ATC rs12285364, rs2298813 and rs11600231, respectively; or another haplotype  
32 in linkage disequilibrium with it,

33 TCC at SNP rs2298813, rs11600231, and rs2276346, respectively; or another  
34 haplotype in linkage disequilibrium with it,  
35 CCG at SNP rs11600231, 20 rs2276346 and rs10502262 or in linkage disequilibrium  
36 with it, CGGs 2276346, 21 rs10502262 and SORL1-T833T or in linkage  
37 disequilibrium with it,  
38 GTA at SNP rs560573, rs985421, and rs593769, respectively; or another haplotype in  
39 linkage disequilibrium with it,  
40 GCA at SNP rs560573, rs985421, and rs593769, respectively; or another haplotype in  
41 linkage disequilibrium with it, and  
42 AAG at SNP, rs556349, rs7131432, rs11218340, respectively; or another haplotype  
43 in linkage disequilibrium with it.

1 19. The method as in claim 1, wherein the patient has a mild cognitive disorder.

1 20. The method as in claim 14 wherein the therapeutically effective amount is an amount  
2 of SORL1 that reduces amyloid beta in the serum, plasma or cerebrospinal fluid of  
3 the patient.

1 21. A method for preventing Alzheimer's Disease in a patient at risk of developing it,  
2 comprising administering a therapeutically effective amount of SORL1 or a  
3 biologically active fragment or variant or modification thereof.

1 22. The method of claim 21, wherein the SORL1 is formulated in a pharmaceutical  
2 composition that crosses the blood brain barrier and the SORL1 is human  
3 recombinant SORL1.

1 23. The method as in claim 21, wherein the therapeutically effective amount is an amount  
2 of SORL1 that reduces amyloid beta in the serum, plasma or cerebrospinal fluid of  
3 the patient.

FIG. 1A

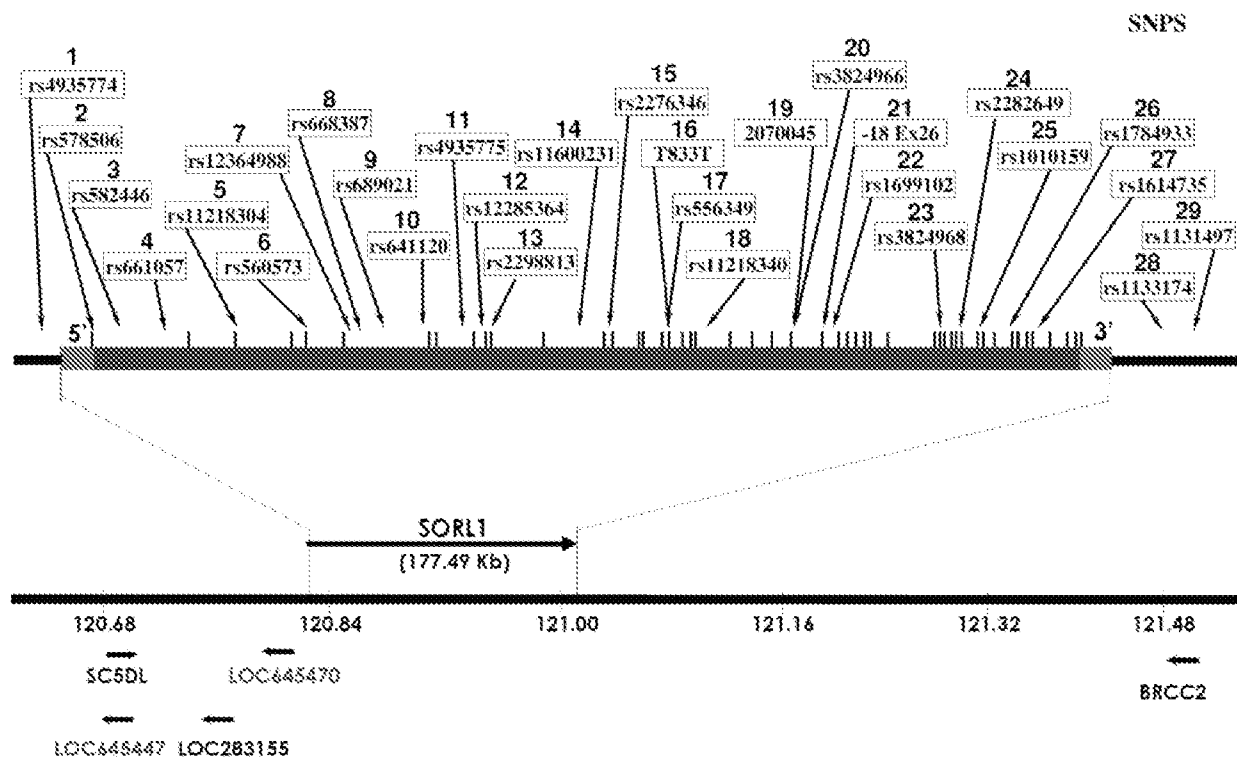


FIG. 1B

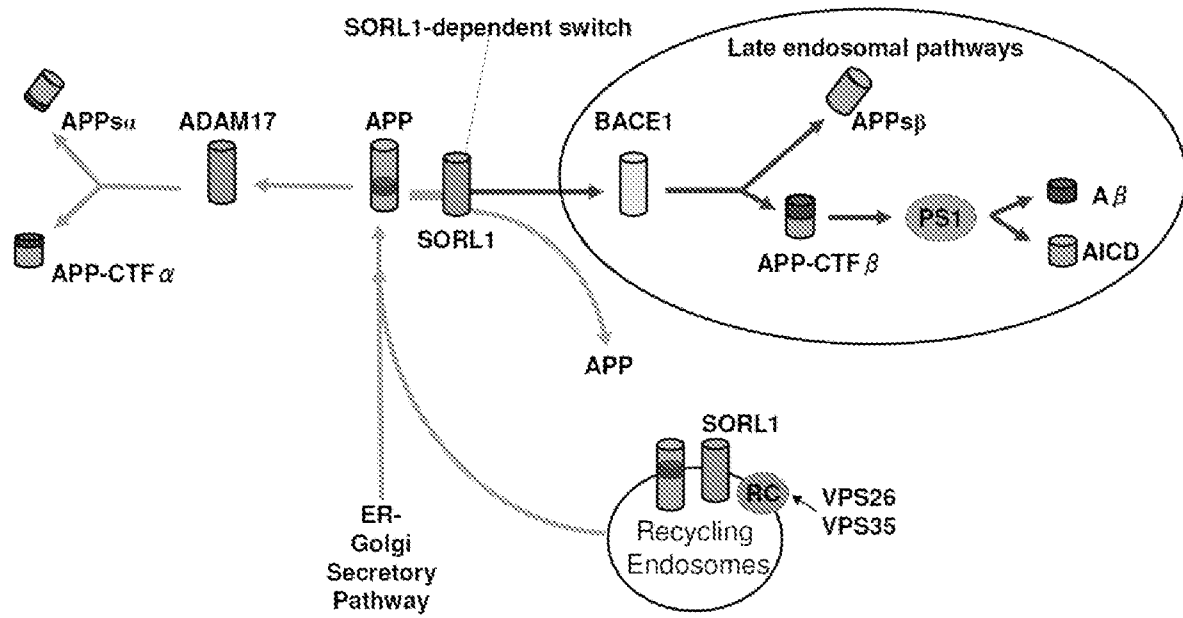


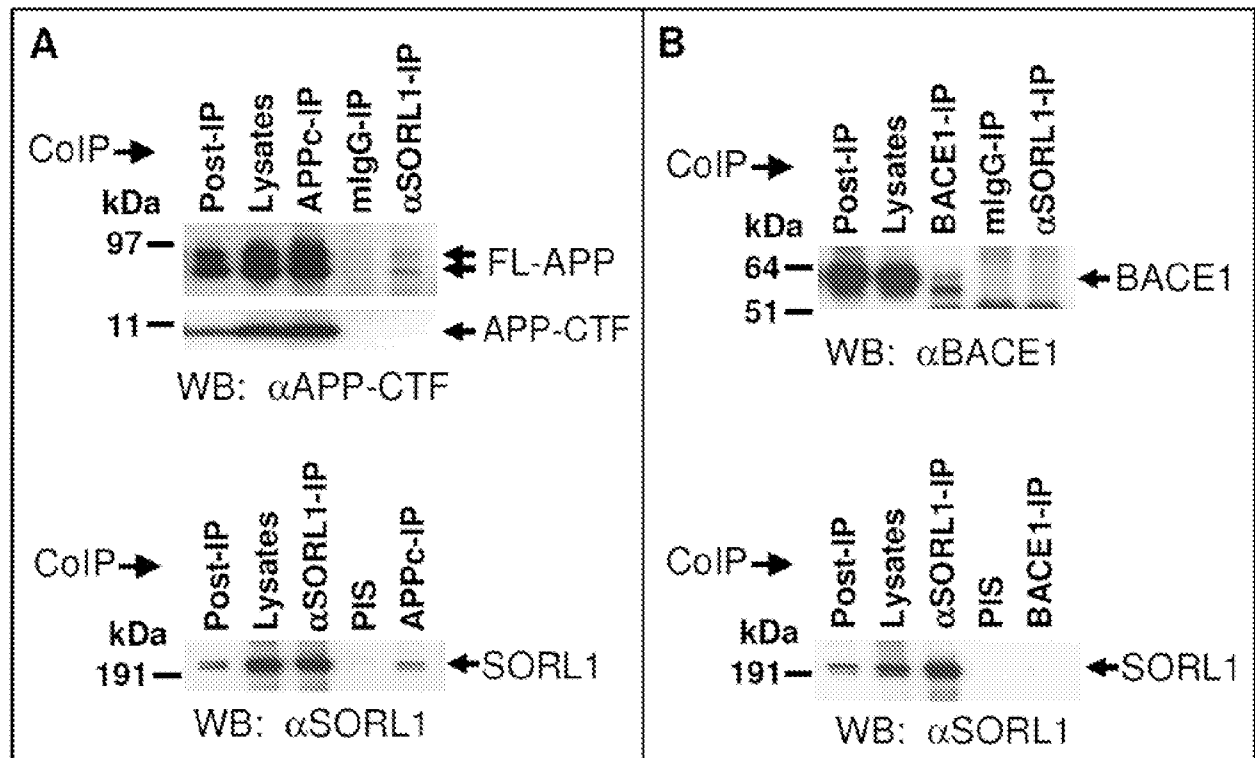
**FIG. 2**

FIG. 3A

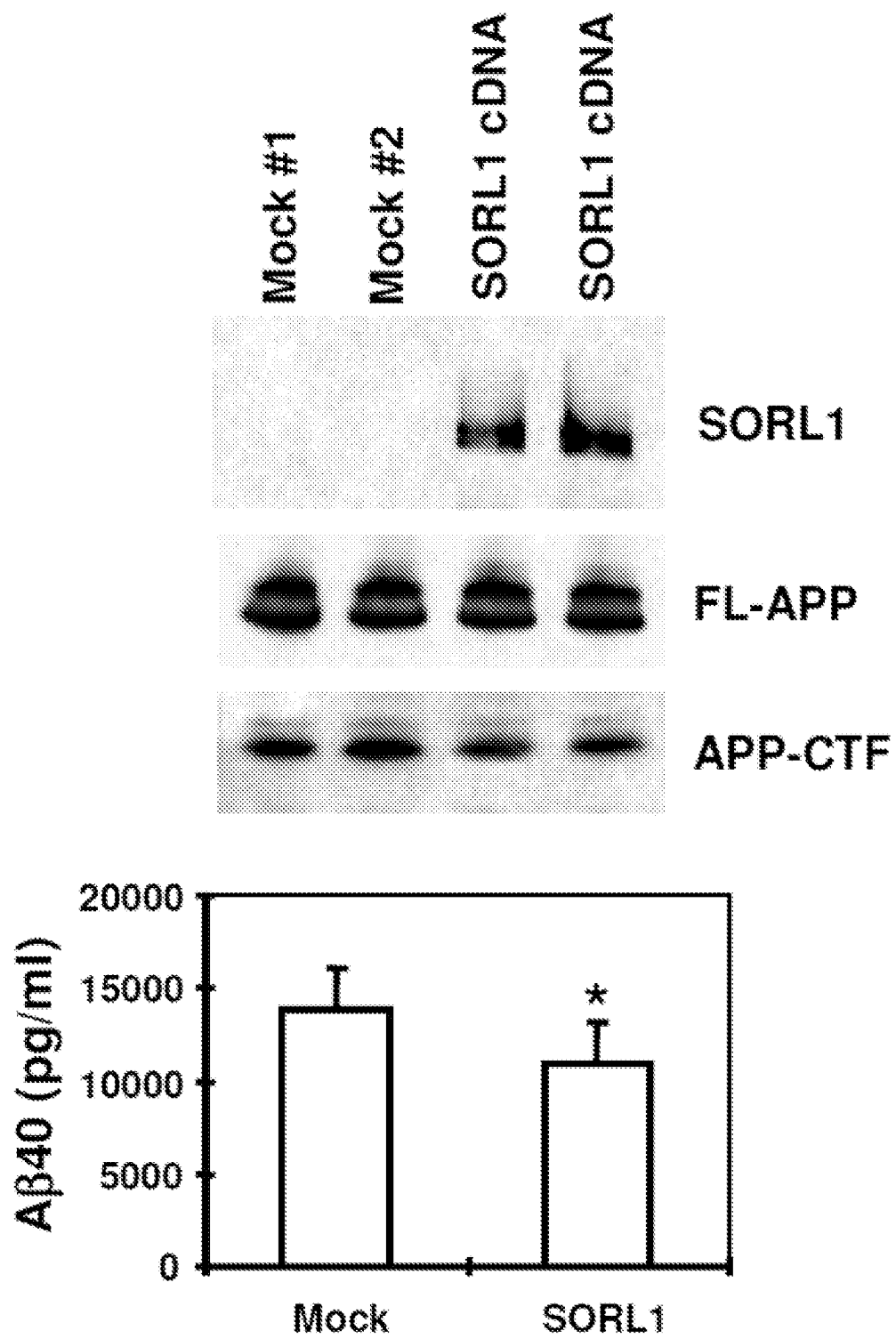


FIG. 3B

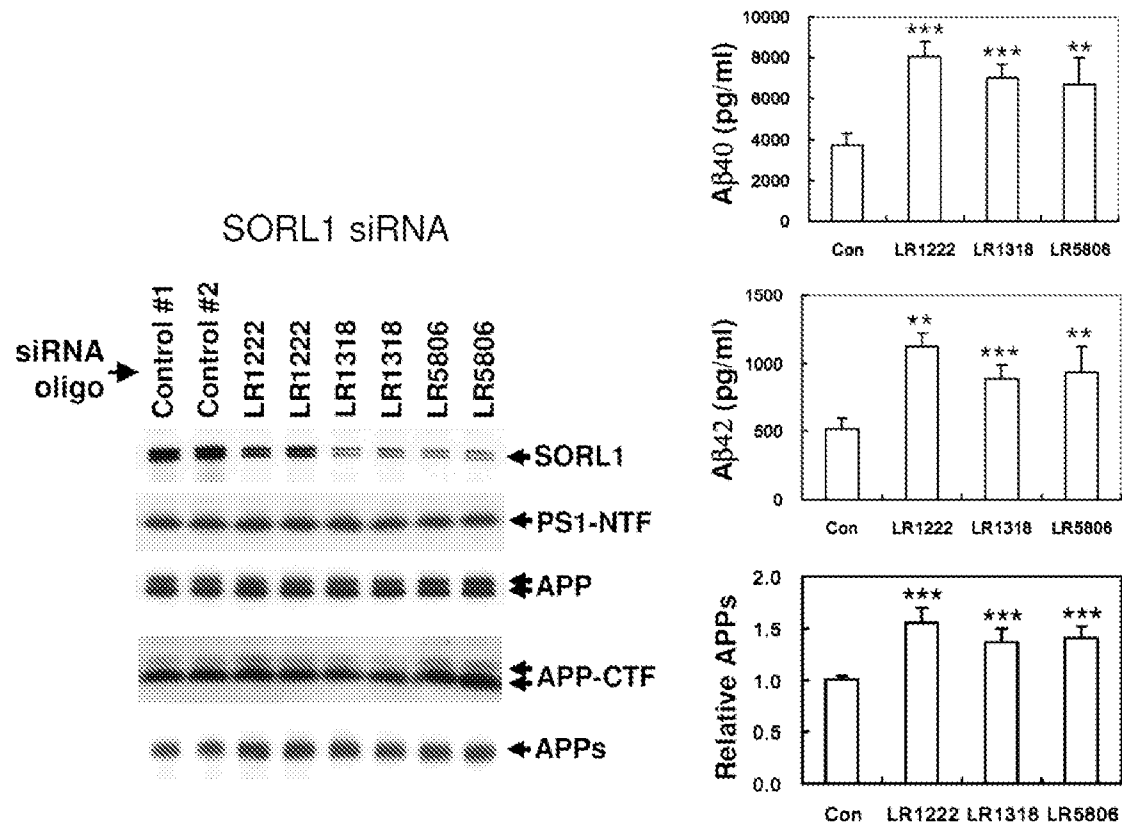


FIG. 3C

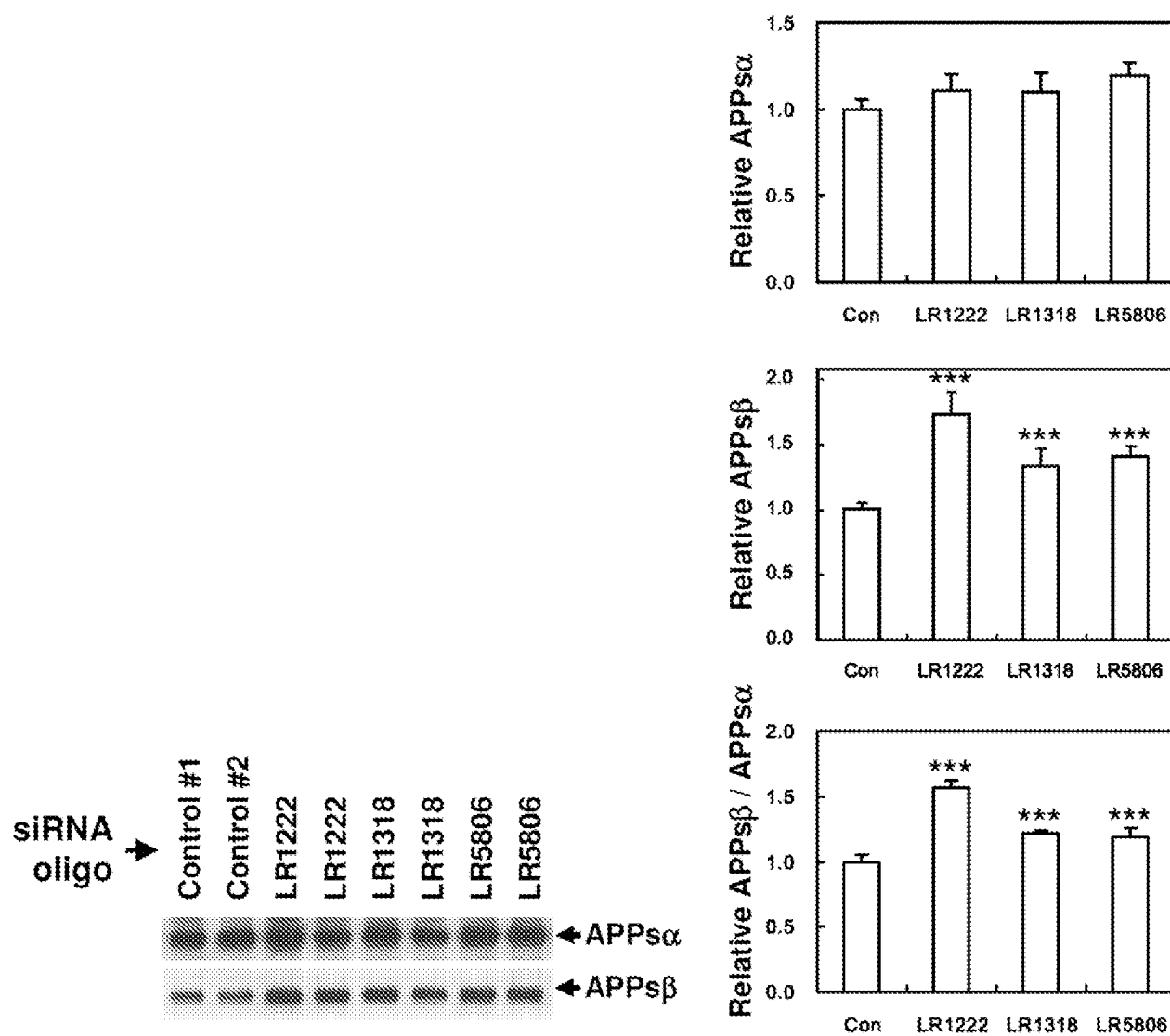


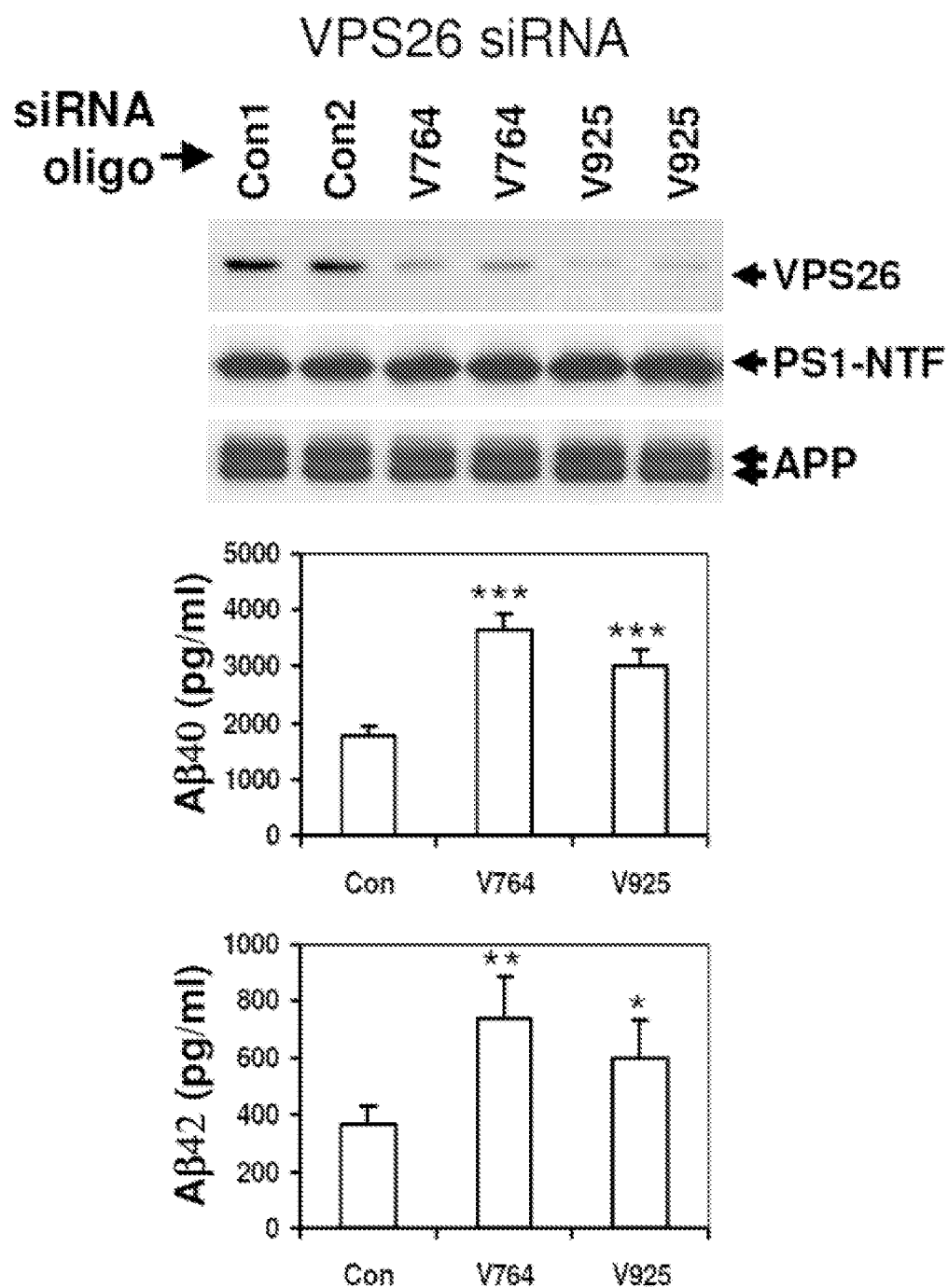
**FIG. 3D**

**FIG. 4**

