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A search for AMD risk variants in Alzheimer disease genes and pathways

Mark W. Logue, Ph.D.[a,e], **Matthew Schu, MS**[a], **Badri N. Vardarajan, Ph.D.**[a], **John Farrell, MS**[a], **Kathryn L. Lunetta, Ph.D.**[e], **Gyungah Jun, Ph.D.**[a,c,e], **Clinton T. Baldwin, Ph.D.**[a], **Margaret M. DeAngelis, Ph.D.**[f], and **Lindsay A. Farrer, Ph.D.**[a,b,c,d,e,*]

[a]Department of Medicine (Biomedical Genetics), Boston University Schools of Medicine and Public Health, Boston, MA, 02118, USA.

[b]Department of Neurology, Boston University Schools of Medicine and Public Health, Boston, MA, 02118, USA.

[c]Department of Ophthalmology, Boston University Schools of Medicine and Public Health, Boston, MA, 02118, USA.

[d]Department of Epidemiology, Boston University Schools of Medicine and Public Health, Boston, MA, 02118, USA.

[e]Department of Biostatistics, Boston University Schools of Medicine and Public Health, Boston, MA, 02118, USA.

[f]John A. Moran Eye Center, University of Utah, Salt Lake City, UT, 84132, USA.

Abstract

Several lines of inquiry point to overlapping molecular mechanisms between late-onset Alzheimer disease (AD) and age-related macular degeneration (AMD). We evaluated summarized results from large genome-wide association studies (GWAS) for AD and AMD to test the hypothesis that AD susceptibility loci are also associated with AMD. We observed association of both disorders with genes in a region of chromosome 7 including *PILRA*, and *ZCWPW*1 (peak AMD SNP rs7792525, MAF=19%, OR=1.14, $p=2.34\times10^{-6}$), and with *ABCA7* (peak AMD SNP rs3752228, MAF=0.054 OR=1.22, p=0.00012). Next, we evaluated association of AMD with genes in AD-related pathways identified by canonical pathway analysis of AD-associated genes. Significant associations were observed with multiple previously identified AMD risk loci and two novel genes: *HGS* (peak SNP rs8070488, MAF=0.23, OR=0.91, $p=7.52\times10^{-5}$), which plays a role in the

*Corresponding Author: Dr. Lindsay A. Farrer, Boston University School of Medicine, Biomedical Genetics L320, 72 East Concord Street, Boston, MA 02118, Tel: (617) 638-5393, Fax: (617) 638-4275, farrer@bu.edu.

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clathrin-mediated endocytosis signaling pathway, and *TNF* (peak SNP rs2071590, MAF=0.34, OR=0.89, $p=1.17\times10^{-5}$), which is a member of the atherosclerosis signaling and the LXR/RXR activation pathways. Our results suggest that AMD and AD share genetic mechanisms.

Keywords

Alzheimer disease; Age related macular degeneration; genetic association; gene-based test; pathway analysis

1. Introduction

Age-related macular degeneration (AMD) is the most common form of severe blindness and vision loss among those over 60 years of age(Congdon, et al., 2004). The common dry form (i.e. non-neovascular) accounts for approximately 85–90% of AMD cases and the advanced acute wet form (i.e. exudative, neovascular) is responsible for the majority of persons with AMD who are legally blind. AMD pathogenesis is complex—a result of both genetic and environmental risk factors.

There are several well-established common genetic risk factors for AMD. The loci with the most robust replication across multiple populations and identified as having the strongest effect on AMD risk (odds ratio, OR, for a single risk allele > 3) are *CFH* and *ARMS2/HTRA1*(Edwards, et al., 2005,Haines, et al., 2005,Jakobsdottir, et al., 2005,Klein, et al., 2005,Rivera, et al., 2005). Candidate gene association studies identified other AMD risk genes in the complement pathway including *C2/CFB*(Gold, et al., 2006), *C3*(Maller, et al., 2007,Yates, et al., 2007), and *CFI* (Fagerness, et al., 2009) The *APOE* gene has been linked to *AMD*, with the ε2 and ε4 alleles associated with increased risk and decreased risk of AMD, respectively(McKay, et al., 2011). Other studies were unable to confirm these associations(Pang, et al., 2000,Schultz, et al., 2003) that are attenuated or absent when adjusted for age(Adams, et al., 2012). Many other loci of modest effect have also been identified and replicated in genome-wide association studies (GWAS) including *CETP*, *LIPC*, *TIMP3*, *VEGFA*, *TNFRSF10A*, *COL10A1*, *COL8A1/FILIP1L*, *SLC16A8*, *IER3/DDR1*, *TGFBR1*, *RAD51B*, *ADAMTS9/MIR548A2*, and *B3GALTL*(Chen, et al., 2010,Fritsche, et al., 2013,Yu, et al., 2011). Several of these loci, including *CFH*, *C3*, *LIPC*, and *DDR1*, have multiple risk variants that independently contribute to disease risk(Fritsche, et al., 2013,Gold, et al., 2006,Li, et al., 2006,Maller, et al., 2006).

Like AMD, late-onset Alzheimer disease (AD) is a common disorder among the elderly that has a strong but complex genetic basis including a major contribution by *APOE*. The ε4 allele confers increased risk of AD and the ε2 is protective(Corder, et al., 1994,Corder, et al., 1993,Farrer, et al., 1997), effect directions opposite to those reported with AMD (McKay, et al., 2011). A hypothesis-driven study demonstrated that *SORL1* is genetically associated with AD(Rogaeva, et al., 2007), a finding subsequently confirmed by GWAS(Lambert, et al., 2013,Miyashita, et al., 2013). Large-scale consortium GWAS studies have successfully identified 20 other modest effect loci including *PICALM*, *CR1*, *CLU*, *BIN1*, *ABCA7*, *CD2AP*, *CD33*, *EPHA1*, *MS4A4A/MS4A6E*, *HLA-DRB5/HLADRB1*, *SLC24A4/RIN3*, *DSG2*, *INFP5D*, *MEF2C*, *NME8*, *ZCWPW1*, *CELF1*, *FERMT2*, and *CASS4*(Harold, et al., 2009,Hollingworth, et al., 2011,Lambert, et al., 2009,Lambert, et al., 2013,Naj, et al., 2011,Seshadri, et al., 2010).

Multiple lines of evidence indicate that AD and AMD risk may share molecular mechanisms. Analyses of a sample from a cardiovascular-health study(Baker, et al., 2009) found that early AMD was associated with low cognitive functioning. Additionally, late-

stage AMD has been associated with incident AD(Klaver, et al., 1999). Both AMD and AD are characterized by abnormal extra-cellular deposits: amyloid-β (Aβ) plaques in AD and drusen in AMD. These deposits share a similar molecular composition including complement factor proteins(Dentchev, et al., 2003). Aβ has been found in drusen and AMD retinas(Dentchev, et al., 2003). Anti-Aβ substrate reduced pathological features in a mouse model of AMD(Ding, et al., 2008). In addition, drusen contain the AD-related APOE protein (Mullins, et al., 2000). AMD and AD share indicators of poor vascular health as risk factors. Smoking(Anstey, et al., 2007,Klein, et al., 1993), hypertension and/or higher blood pressure(Hyman, et al., 2000,Kennelly, et al., 2009,The Eye Disease Case-Control Study Group, 1992,van Leeuwen, et al., 2003), and atherosclerosis(Casserly and Topol, 2004,van Leeuwen, et al., 2003) are risk factors for both disorders. AMD risk is related to lower levels of high-density lipoprotein cholesterol (HDL-c) and both disorders are associated with higher serum cholesterol(Hyman, et al., 2000,Kivipelto, et al., 2001,The Eye Disease Case-Control Study Group, 1992), although not all studies replicate this(Klein, et al., 2003,Reitz, et al., 2004).

Given the evidence of etiological overlap between these two disorders, we hypothesized that findings from AD genetic studies can inform a search for novel AMD genes. This mirrors examination of AMD risk variants in complement factor genes as possible risk factors for AD(Gatta, et al., 2008,Hamilton, et al., 2007,Le Fur, et al., 2010,Proitsi, et al., 2012,Zetterberg, et al., 2008). These studies have found that complement factor genes with variants which are highly predictive of AMD play at most a modest role in the risk of AD. However, the direction of effect, the genetic models which appear most predictive, and which SNPs in the genes are associated can differ between the disorders(Gatta, et al., 2008,Proitsi, et al., 2012). In this study, we incorporated results from individual SNP, gene-based, and biological pathway analyses of AD in a design to discover additional AMD risk loci.

2. Materials and methods

2.1 Materials

Primary data for this investigation are summarized results for a common set of more than two million HapMap2 imputed SNPs from the Age-related Macular Degeneration Genetics (AMDGene) Consortium GWAS for AMD(Fritsche, et al., 2013) and the Alzheimer Disease Genetics Consortium (ADGC) for AD(Naj, et al., 2011). The ADGC sample includes 11,840 cases and 10,931 controls from 15 different studies(Naj, et al., 2011). Top-ranked findings from the AD GWAS were examined in the AMDGene GWAS reported by Fritsche et al. (2013) which contained data from more than 7,600 cases and 50,000 controls who were enrolled in 14 separate studies.

2.2 Genetic Analyses

Our first line of investigation was an analysis to identify SNPs that are associated with risk to both disorders. Because the focus of our study was on identification of new AMD risk loci, we excluded SNPs within 1 megabase (Mb) of a genome-wide significant ($p<5\times10^{-8}$) SNP from the AMDGene GWAS. In this approach, the AD dataset was used for discovery and the top-ranked SNPs were “replicated” in the AMD dataset. We examined association of AMD with all SNPs meeting a Benjamini–Hochberg false-discovery rate (FDR; (Benjamini and Hochberg, 1995) of < 10% in the AD dataset. These SNPs were considered to be significantly associated with AMD if the p-value exceeded a Bonferroni-adjusted significance threshold of 0.05 divided by the number of examined SNPs. Because the association between SNPs in the *APOE* region and AMD is well documented and the region

over which linkage disequilibrium (LD) extends is very large, we excluded SNPs and genes located between positions 45.3 and 45.8 Mb on chromosome 19.

Next, we employed a genome-wide gene-based approach to identify Alzheimer genes to be tested for association with AMD. This analysis was performed by first identifying the peak SNP within 5 kb of each gene and then applying a multiple-testing correction based on the effective number of independent tests represented by the SNPs in the gene as determined by the Li and Ji method(Li and Ji, 2005) which accounts for linkage disequilibrium (LD). Determining the significance of a gene using the peak SNP adjusted with the Li and Ji approach contrasts with other gene-based methods (e.g., VEGAS(Liu, et al., 2010) which consider information from multiple SNPs in the gene region. LD between SNPs was estimated using information derived from the Caucasian controls in the MMAP AMD sample(Chen, et al., 2010,Fritsche, et al., 2013). These corrected p-values were then used to compute a FDR for each gene(Benjamini and Hochberg, 1995). For this investigation, genes were considered as possible AD loci for subsequent analyses with AMD if the gene-based test statistic exceeded a 10% FDR cutoff. These AD-implicated genes were then evaluated for association with AMD using the peak SNP approach described above. A gene was determined to be significantly associated with AMD if its gene-level corrected significance ($p_{corrected}$) was less than 0.05/k where k is the number of putative AD genes examined.

Finally, we applied a biological pathway approach to identify additional genes which are involved in processes related to AD based on canonical pathway analysis using the Ingenuity pathway analysis (IPA) software; http://www.ingenuity.com). We identified AD-related pathways in three ways. First, we investigated the list of all genes with an FDR of less than 10% in a test of association with AD (i.e., the genes examined for association with AMD as described above). In a separate analysis, we looked for pathways which were enriched for the genes in the AlzGene database (www.alzgene.org) which were significant according to a meta analysis of curated information from the literature using AlzGene's methodology (Lars Bertram Personal Communication, June 16, 2012). The significant genes in the AlzGene and GWAS FDR10% lists are not independent because AlzGene incorporated significant results from published GWAS including the ADGC GWAS. We also conducted a separate pathway analysis of the 10% FDR genes after excluding the genes in high LD with *APOE*. The methodology for these analyses is similar to the gene-enrichment analyses of AD risk performed by Jones et al. (Jones, et al., 2010), however, that study examined enrichment in gene-ontology (GO)(Harris, et al., 2004) and KEGG(Kanehisa, et al., 2006) functional categories, whereas IPA utilizes the Ingenuity Knowledge Base (IKB)—a curated database of gene-gene interactions and functions summarizing the findings of over 200,000 scientific articles. The canonical pathway analysis implemented in IPA entails computation of an enrichment score based on the number of genes in the provided target list that fall into a specific canonical pathway (similar to a GO term) and calculates the probability that this pathway is enriched for the genes using Fisher's Exact Test of association. A Benjamini– Hochberg false discovery rate (FDR) approach was used to adjust the resulting p-values for the multiplicity of INGENUITY pathways examined within each of the three gene sets (FDR). Because this method often yields many highly significant pathways and each pathway may contain hundreds of genes, we restricted our attention to the most significantly and consistently implicated pathways; that is, pathways that are most enriched for AD genes across the three analyses. Genes in each of these pathways were evaluated for association with AMD using a gene-level test as described above. Within each pathway, genes were considered significant if they had a p-value less than 0.05/k where k is the number of genes in the pathway.

Figures were generated using R (http://www.r-project.org) and LocusZoom (http://csg.sph.umich.edu/locuszoom/). Presented genomic positions are based on the hg19

(February 2009) human genome assembly. LD estimates and LD-plots were computed using Haploview (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) based on the white non-Hispanic subjects comprising the 1000 Genomes EUR sample. Minor allele frequency estimates were also derived from the EUR sample.

3. Results

A total of 226 SNPs in the summary results for the AD meta-analysis dataset exceeded a 10% FDR cutoff and were more than 1 Mb away from a previously established genome-wide significant AMD association. None of these SNPs survived Bonferroni correction ($p<2.2\times10^{-4}$) when evaluated for association with AMD. In fact, only *ABCA7* SNP rs3752246, which was associated with AD ($p = 5.79\times10^{-7}$), was nominally associated with AMD ($p = 0.038$). To ensure that the lack of correspondence between the two disorders was not an artifact of overly stringent discovery criteria in the AD sample, we also examined all 485 SNPs which were associated with AD at $p<10^{-4}$ and not within one Mb of known AMD loci. The most significant finding among these SNPs was rs12539172 in *C7ORF71* (AD: $p = 6.35\times10^{-5}$; AMD: $p = 0.0016$) which did not exceed the multiple testing correction threshold of 0.05/485= 0.00010. Hence, loosening the criteria for SNPs associated with AD did not yield significant evidence of a shared locus with AMD.

Next, we tested the hypothesis that, even though the same variants may not be shared across the two disorders, other variants in AD-related genes may be associated with AMD risk. Based on a FDR cutoff of 10%, 59 genes were identified which are potentially AD-related, including 24 genes in the *APOE* region and several previously established AD genes: *ABCA7*, *BIN1*, *PICALM*, *CD2AP*, *SORL1*, *EPHA1*and the *MS4A*–cluster genes (Supplementary Table 1). We evaluated the group of 35 genes outside of the APOE region for association with AMD. Three of these were significantly associated with AMD after correcting for the number of genes examined including *PILRA*, *ZCWPW1*, and *ABCA7* (Table 1). *PILRA* and *ZCWPW1* are adjacent genes on chromosome 7q22 whose 3' UTR regions are separated by 773 bp. The most significant associations to AMD within 5 kb of each gene (that is, the peak SNP on which the gene-level significance is based) is rs7792525 for *PILRA* ($p=7.02\times10^{-6}$) and rs11771241 for *ZCWR1P1* ($p=1.61\times10^{-5}$; Table 1). In fact, rs11771241 is less than 5 kb from *ZCWR1P1* but within the boundaries of *PILRA*. Similar evidence of association was observed for other SNPs in both of these loci with AD and AMD (Figure 1). The association peak for both disorders extends across a region of high LD from approximately 99.90 to 100.15 Mb (Figure 1) encompassing several other genes including *PLIRB*, *MEPCE*, *PPP1R35*, *C7ORF61*, *TSC22D4*, and *NYAP1*. The congruence of results for AD and AMD in this region is modest at the SNP level. The most significantly associated AD SNP, rs1476679, is nominally significantly associated with AMD ($p=0.0042$, $OR=0.93$). However, the most-significantly associated AMD SNPs are not associated with AD. For example, the top AMD SNP, rs7792525, is not associated with AD ($p=0.51$). Thus, the functional variants for AMD and AD in this region may not be identical.

Pathway analysis of genes identified as significantly associated with AD by gene-based analyses (FDR<10%) revealed very significant associations with the clathrin-mediated endocytosis signaling ($FDR=6.61\times10^{-6}$), LXR/RXR activation ($FDR=1.10\times10^{-4}$) and atherosclerosis signaling ($FDR=1.10\times10^{-4}$) pathways (Table 2A). These pathways are not entirely distinct; approximately 40% of the genes are common to the atherosclerosis and LXR/RXR activation pathways. The same three pathways were also the most significant based on analyses of genes which were nominally significant using the AlzGene methodology (Table 2B), however their order based on significance level is reversed: LXR/RXR Activation ($FDR = 6.31\times10^{-11}$), atherosclerosis signaling ($FDR = 6.31\times10^{-11}$), and

clathrin-mediated endocytosis signaling (FDR = 1.55×10^{-6}). We repeated the pathway analysis with the top genes identified using the gene-based (FDR< 10%) approach after excluding genes in LD with *APOE* (Table 2C). The three "top" pathway from the previous analyses were again significantly enriched for AD genes. The clathrin-mediated endocytosis signaling pathway remained significant after FDR correction (FDR=0.0053), but the LXR/RXR activation and atherosclerosis signaling pathways did not (FDR=0.11 for each). Therefore, we examined the clathrin-mediated endocytosis signaling, LXR/RXR activation, and atherosclerosis signaling pathways genes for AMD-predisposing variants.

The clathrin-mediated endocytosis signaling pathway has 192 genes (excluding genes in the *APOE* region which were not evaluated for association with AMD). Three of these genes are significantly associated with AMD after correction for multiple testing (Table 3). The two most significant genes, *APOM* and *CSNK2B*, are in moderate LD (r^2=0.275) and located in the major histocompatibility region between 250 kb and 300 kb away from the *C2-CFB* locus (Figure 2). These SNPs are not in LD with the peak *C2-CFB* SNP (rs429608, r^2<0.05), however, several SNPs in the region including *APOM* and *CSNK2B* were genome-wide significant in the large AMD GWAS (Fritsche, et al., 2013). Thus, on the basis of these results alone, it is unclear whether these are veritable independent associations. The third significant gene identified from this pathway was *HGS*. The association peak was observed with rs8070488 ($p=1.88\times10^{-5}$) which is a synonymous coding variant (P595P). Association of AMD with SNPs in this region has not been reported previously. None of the *HGS*-region SNPs were associated with AD (p>0.25).

Six LXR/RXR activation pathway genes were significantly associated with AMD after correction for the 132 genes examined in the pathway (Table 4). Genome-wide significant SNPs were found in three genes in the MHC region (*APOM*, *C4A*, and *C4B*), *CETP*, and *C3*. The *TNF* SNP was also significant after multiple test correction ($p=1.17\times10^{-5}$). *TNF* SNPs were not in LD with the peak *C2-CFB* SNP located more than 330 kb away, but were modestly correlated with *APOM* (r^2=0.173) and *CSNK2B* (r^2=0.083) (Figure 2). We also tested association of AMD with a different set of 132 atherosclerosis signaling genes and obtained significant results with SNPs in four genes including *APOM* and *TNF*, which were identified in the other two pathways studied, and *COL10A1* which is among the previously reported AMD genes (Fritsche, et al., 2013,Yu, et al., 2011). The fourth gene, *PLA2G12A* (Table 5), is located near *CFI*, another previously established AMD risk locus (Arakawa, et al., 2011,Fagerness, et al., 2009).

4. Discussion

The goal of this study was to identify novel AMD loci in a hypothesis-driven approach based on the idea that AMD and AD have shared genetic underpinnings. Although we did not observe significant overlap in genetic association at the single-SNP level, our gene-based analyses and investigation of biological pathways implicated several novel AMD loci including *ABCA7*, *HGS*, and *PILRA/ZCW1P1*.

ABCA7 is an established risk gene for AD in both Caucasians(Hollingworth, et al., 2011,Naj, et al., 2011) and African Americans(Logue, et al., 2011,Reitz, et al., 2013). It is unlikely that the *ABCA7* association is confounded with variants in the *C3* gene—a known AMD risk locus located approximately 6 Mb away. The peak AMD *ABCA7* SNP (rs3752228) is 5,356 bp away from rs3764650 and 15,328 bp away from rs3752246, which are the peak AD risk SNPs in *ABCA7* identified by Hollingworth et al. (2011) and Naj et al. (2011) respectively. Rs3752228 is not in LD with either of these two AD-associated SNPs (r^2<0.01). It is worth noting that rs3752228 is nominally associated with AD (p=0.011).

Conversely, the AD-associated SNP rs3752246 is nominally associated with AMD (p=0.038). However, the direction of effect for both SNPs differs between AD and AMD.

The region of LD containing *PILRA*, *ZCWPW1* and several adjacent genes (*PLIRB*, *MEPCE*, *PPP1R35*, *C7ORF61*, *TSC22D4*, and *NYAP1*) on chromosome 7q22 has also not been associated previously with AMD. Because of high LD among these genes (Figure 1C), we are not able to conclude with certainty whether the association to AMD is explained by a single variant or multiple variants in *PILRA*, *ZCWPW1*, or perhaps one of the other genes in the LD block. Recently, *ZCWPW1* emerged as a new AD risk locus in the largest GWAS for this disorder to date (Lambert, et al., 2013). The zinc finger CW domain encoded by this gene is a motif of about 60 residues that functions as a histone modification reader and thus is involved in epigenetic regulation(He, et al., 2010). *PILRA*, the paired-immunoglobulin-like type 2 receptor, binds with herpes simplex virus-1 (HSV-1) which is neurotoxic to sensory neurons in the eye and brain(Satoh, et al., 2008). Several but not all studies have shown an increase of HSV-1 in brains of AD subjects compared to controls(Hill, et al., 2007), and particularly among subjects with the *APOE* ε4 allele(Itzhaki and Lin, 1998). Although there are no reports linking HSV-1 to AMD, *PILRA* has a role in the entry of HSV-2 into retinal pigment epithelial cells(Shukla, et al., 2009). Sequencing and molecular experiments will be necessary to determine which of these genes and variants therein are causally linked to AD and AMD.

HGS is a member of the clathrin-mediated endocytosis signaling pathway. It is a zinc-finger protein that is ubiquitously expressed in all tissues and localizes to the surface of early endosomes(Komada, et al., 1997). *HGS* has a role in lysosomal sorting and down-regulation of membrane receptors via the endosomal sorting complex required for transport (ESCRT) pathway (Bache, et al., 2003,Bilodeau, et al., 2002). Recently, it has been demonstrated that *HGS* is a regulator of endosomal cholesterol trafficking(Du, et al., 2012).

Analysis of AD-related pathways led us to previously established AMD-risk loci including *C3*, *COL10A1*, and *CETP*. Our investigation of AD pathway genes also revealed significant associations of AMD with genes near recognized AMD loci. *PLA2G12A* is located 10 kb from the 3'UTR of *CFI* (r^2 between peak *CFI* and *PLA2G12A* SNPs is 0.34) and, thus, may not be a true AMD risk gene. *APOM* and *CNSK2B* (which harbor genome-wide significant SNPs but not considered as independent AMD loci in the AMDGene GWAS; (Fritsche, et al., 2013) and *TNF* span 89 kb of the MHC region on chromosome 6 (Figure 2) and, thus, may represent one association signal. Supporting the idea that there are at least two distinct AMD susceptibility loci in the MHC, we observed that the top *APOM*, *CNSK2B*, and *TNF* SNPs are not in LD with the genome-wide significant association peak at rs429608 in the *C2-CFB* region (r^2<0.05). Nonetheless, further studies are needed to determine whether associations with *APOM*, *CNSK2B*, and *TNF* are tagging functional variants in only one of these genes or in other genes in or near *C2-CFB*.

Pathway analysis of known and potentially related AD genes yielded numerous gene networks, the most significant of which were the clathrin-mediated endocytosis (CME) signaling, LXR/RXR activation and atherosclerosis signaling pathways. There is a growing body of evidence linking these pathways to AD and AMD. These pathways incorporate many of the GO processes identified in a prior gene enrichment analysis of AD risk loci (Jones, et al., 2010) such as reverse cholesterol homeostasis, cholesterol transport, and cholesterol efflux. CME is one of the major mechanisms by which LDL, nutrients, and hormones are internalized into the cell. CME is also central to the processing of Ab (Wu and Yao, 2009). Few studies have investigated the possible role of endocytosis in AMD. Bando et al. (2007) found increased levels of clathrin and adaptin (two key CME proteins) in AMD

donor eye tissue compared to non-AMD donor eyes, and concluded that this may be due to increased endocytosis in AMD tissue, perhaps triggered by high levels of LDL.

The relationship between AMD and atherosclerosis signaling was previously observed in the pathway analysis of AMD genes by Fritsche et al. (Fritsche, et al., 2013). The LXR/RXR activation pathway plays a role in atherosclerosis and in cholesterol homeostasis. Prior work demonstrated that lipid metabolism and lipid processing are involved in AMD (Ebrahimi and Handa, 2011,Kishan, et al., 2011). Similarities in the composition of drusen in AMD maculae, amyloid plaques in the AD brain, and atherosclerotic plaques have been noted (Mullins, et al., 2000). Two of the replicated GWAS-significant AMD loci—namely *LIPC* and *CETP* —are associated with HDL-C levels(Kathiresan, et al., 2009,Willer, et al., 2008), although the *CETP* risk allele is associated with higher levels of HDL-C and the *LIPC* risk allele is associated with lower HDL-C levels(Chen, et al., 2010). *ABCA7*, one of the novel AMD loci identified in this study, has a major role in the enhancement of phagocytosis, and its interaction with apolipoproteins further increases this function (Tanaka, et al., 2011).

One of the genes that emerged from the AD pathway analysis and shown to be associated with AMD is *TNF*. TNF is a pro-inflammatory cytokine that is involved in both initiating and limiting/terminating the inflammatory response to prevent tissue damage (Makhatadze, 1998,Marino, et al., 1997). It is expressed at low levels in the brain, but is up-regulated in AD patients (Fillit, et al., 1991). In animal studies administration of 3,6′-Dithiothaliodmide reduces TNF expression and reduces AD-related traits and behaviors (Tweedie, et al., 2012). Early studies examining the treatment of AD with the anti-TNF agent etanercept—which is approved for use in the treatment of arthritis—were encouraging (Tobinick and Gross, 2008a,Tobinick and Gross, 2008b). *TNF* is a strong candidate AMD gene based on the role of inflammation in AMD. The related tumor necrosis factor receptor superfamily, member 10a (*TNFRSF10A*) gene has been implicated in multiple AMD studies (Arakawa, et al., 2011,Fritsche, et al., 2013). Several anti-TNF agents have been or are being evaluated in conjunction with AMD. A study in monkeys found that anti TNF-a agent ESBA105 reduced the appearance of laser-induced choroidal neovascularization, indicating that it may be preventative of AMD (Lichtlen, et al., 2010). However, a pilot study using the anti-TNF agent infliximab (Remicade) found that the injections were not well tolerated (Giganti, et al., 2010).

By design, novel AMD SNPs identified by testing association with established AD risk variants implicitly have a pleiotropic effect. Solovieff et al. (Solovieff, et al., 2013) recently compared methods for establishing polygenic effects at a locus or region. Accordingly, our two-stage design is “robust” because moderate strength associations will not be missed in the discovery GWAS sample of more than 20,000 cases and controls. Several other approaches discussed in this review could not be applied in our study because they require all traits to be measured in the same individuals, or individual level data, or are effective only when the number of traits being examined is large. The PRIMe method (Huang, et al., 2011), is similar to our gene-level investigation, but PRIMe examines LD-based regions rather than gene regions for association with multiple traits. We also applied a meta-analysis approach to identify loci underpinning both AD and AMD by searching for SNPs having moderately significant signals for each trait which became genome-wide significant after combining results from the analyses of both disorders. The results of these analyses (not presented) were similar to the two-stage analysis of the individual SNPs. The only genome-wide significant SNPs from the meta-analysis were driven by known risk loci of one trait or the other rather than any joint effect.

The strengths of this study include a hypothesis-driven approach, which reduced considerably the number of tests, hence allowing an opportunity for finding AMD-risk loci

that would be over-looked in GWAS, and the availability of very large AD and AMD GWAS datasets. However, our association analyses were performed using summarized results from the constituent datasets, thus precluding evaluation of more complex models to test for gene-gene interaction or effects of dominant alleles. We could also not perform conditional analyses necessary to explore confounding amount loci from the same region (e.g., loci in the MHC region). By design, the novel AMD variants we identified did not achieve genome-wide significance likely because of small effect size or low frequency of risk variants. Thus, the associations with these variants are unlikely to explain a substantial proportion of AMD heritability and will need to be confirmed in independent datasets. However, the documentation of a link between these genes and risk of AMD is useful as it helps more closely delineate the molecular mechanisms leading to AMD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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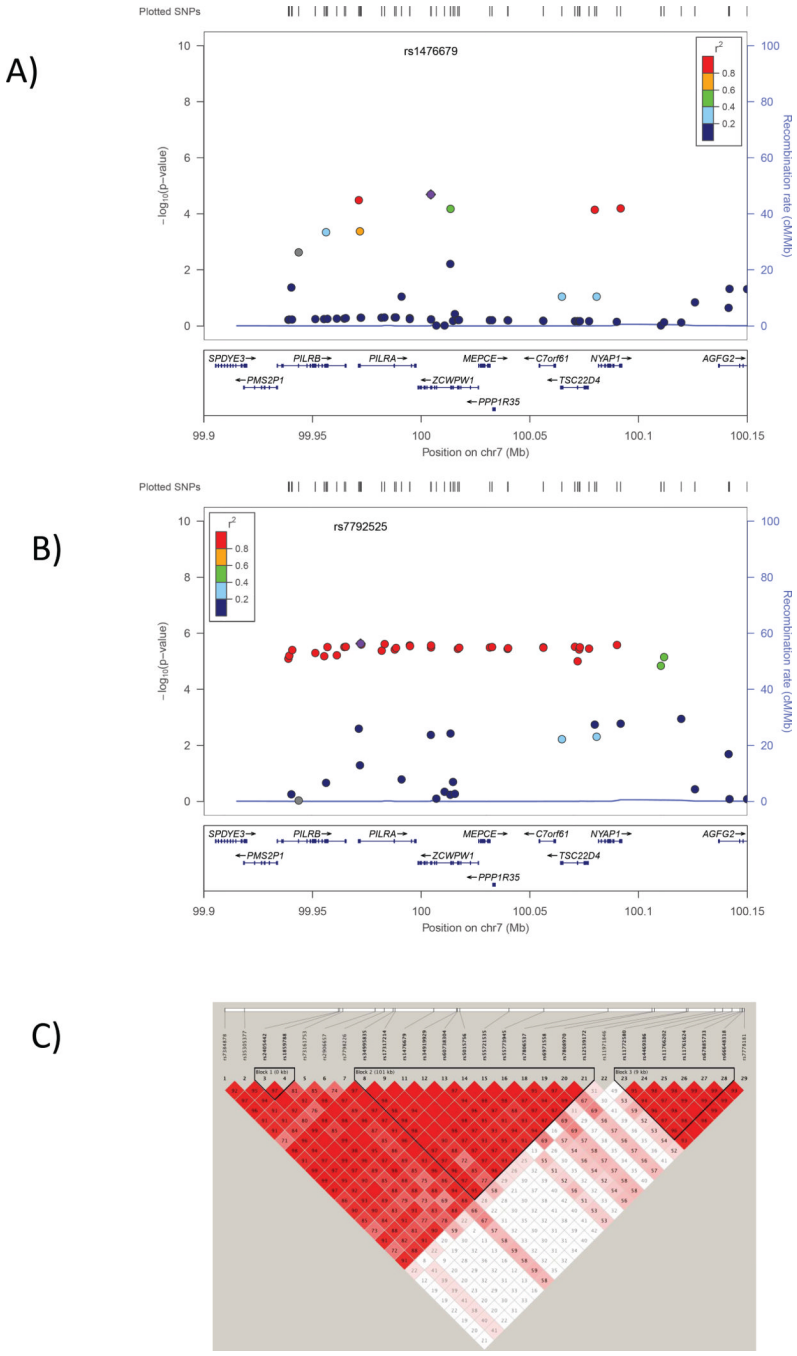

Figure 1.

Association of SNPs in the chromosome 7q22 region with AD (**panel A**) and AMD (**panel B**). Significance of the association of each SNP (circles) is plotted as the negative logarithm of the p-value. Correlations (r^2) between tested SNPs with the SNP most significantly associated with AD (*ZCWPW1* SNP rs1476679) and AMD (*PILRA* SNP rs7792525) are indicated using the color scheme shown in the legend. Map positions and direction of transcription of genes in the region are shown in the bottom portion of each panel. **C)** Linkage disequilibrium (LD) across the same region. The measure of disequilibrium (D') between each pair of SNPs is shown in each square. A summary of the strength of LD across

the region is indicated by color such that dark red shading indicates very strong LD and white shading indicates weak or no LD. Discrete blocks of LD are demarcated with black triangles.

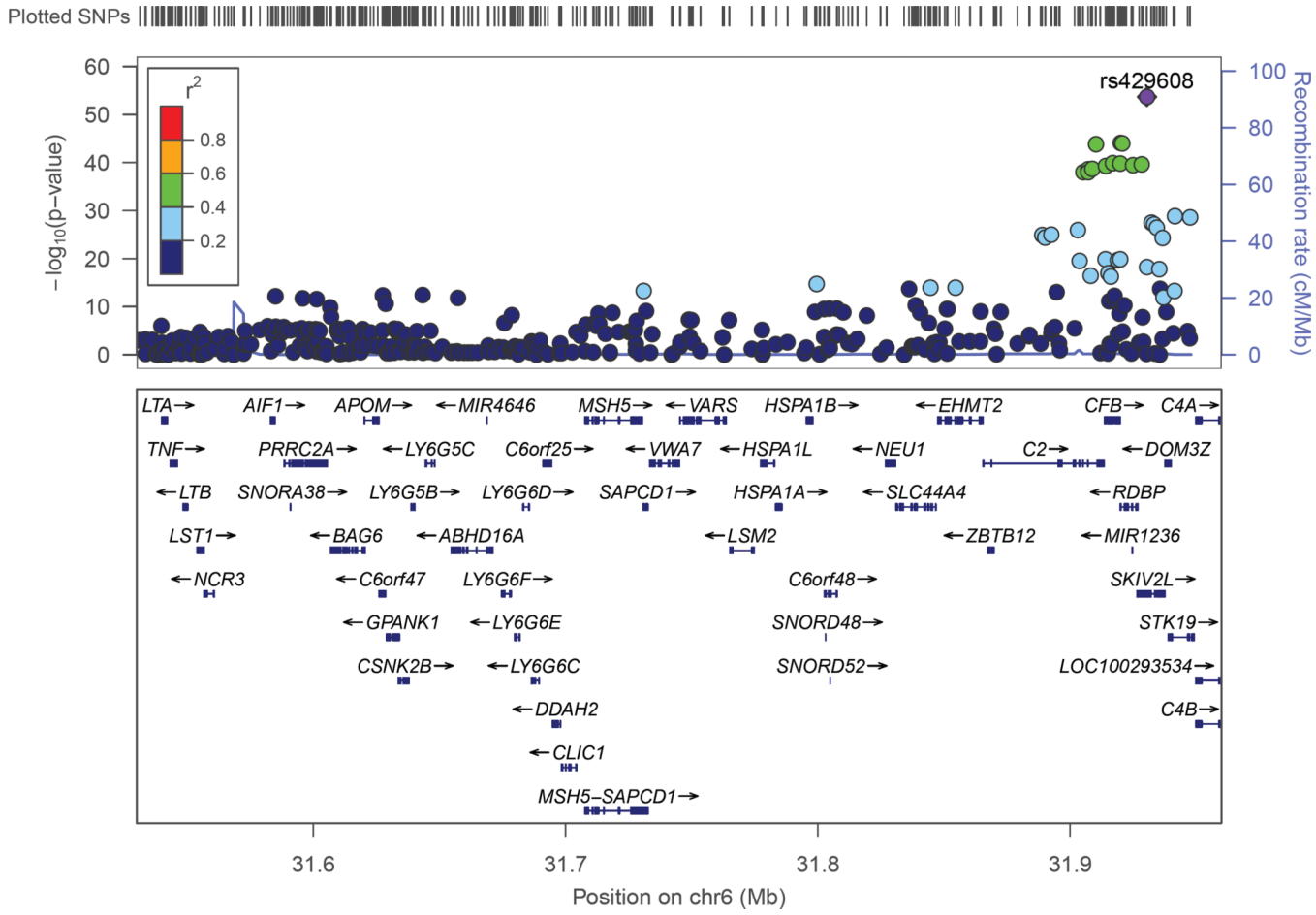

Figure 2.

Association of AMD with SNPs in the major histocompatibility locus region. Significance of the association of each SNP (circles) is plotted as the negative logarithm of the p-value. Correlations (r^2) between tested SNPs with the SNP most significantly associated with AMD in this region (rs429608) are indicated using the color scheme shown in the legend. Map positions and direction of transcription of genes in the region are shown in the bottom portion of the figure.

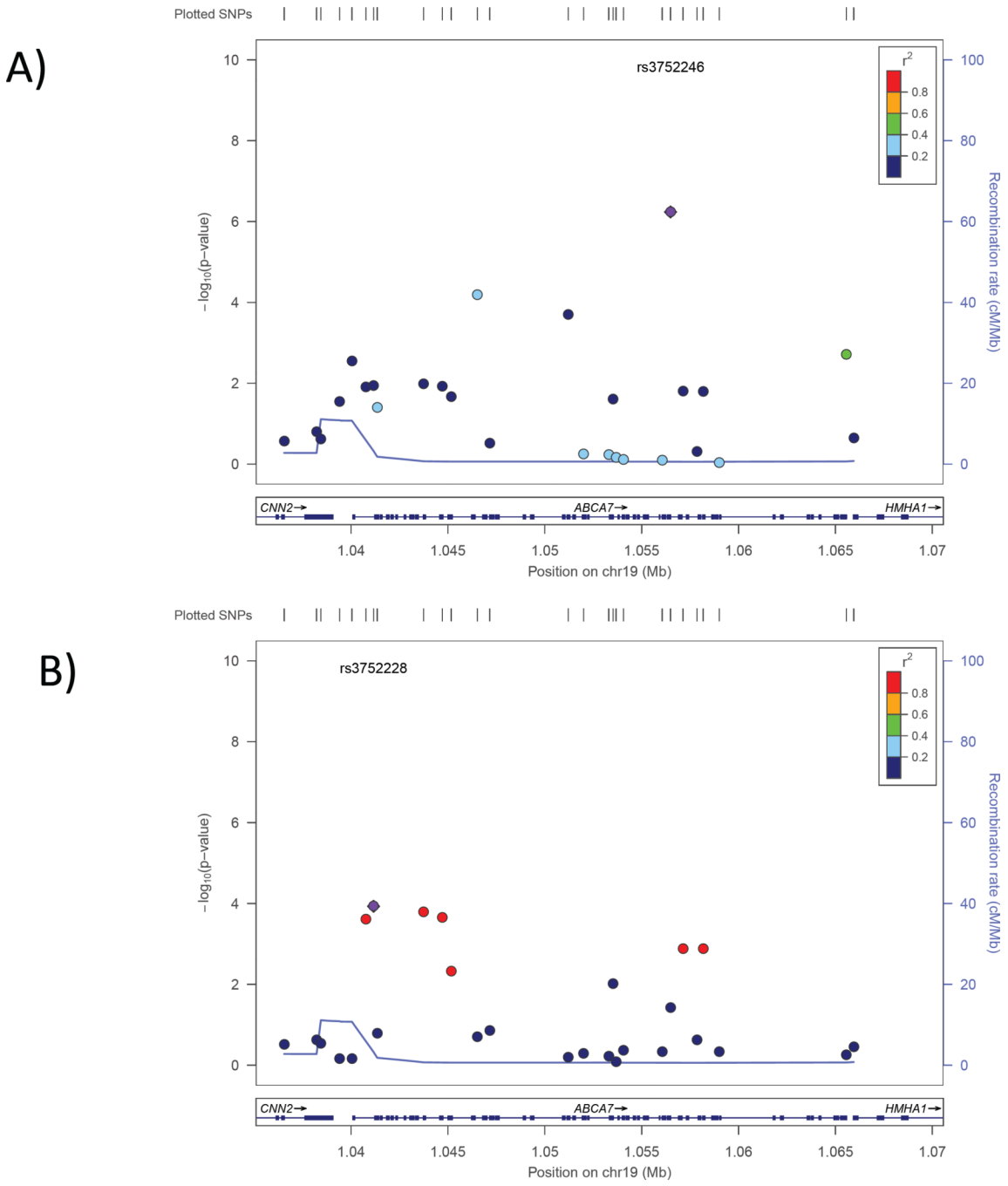

Figure 3.

Association of SNPs in the *ABCA7* region with AD (**panel A**) and AMD (**panel B**). Significance of the association of each SNP (circles) is plotted as the negative logarithm of the p-value. Correlations (r^2) between tested SNPs with the SNP most significantly associated with AD (rs3752246) and AMD (rs3752228) are indicated using the color scheme shown in the legend. Map positions and direction of transcription of genes in the region are shown in the bottom portion of each panel.

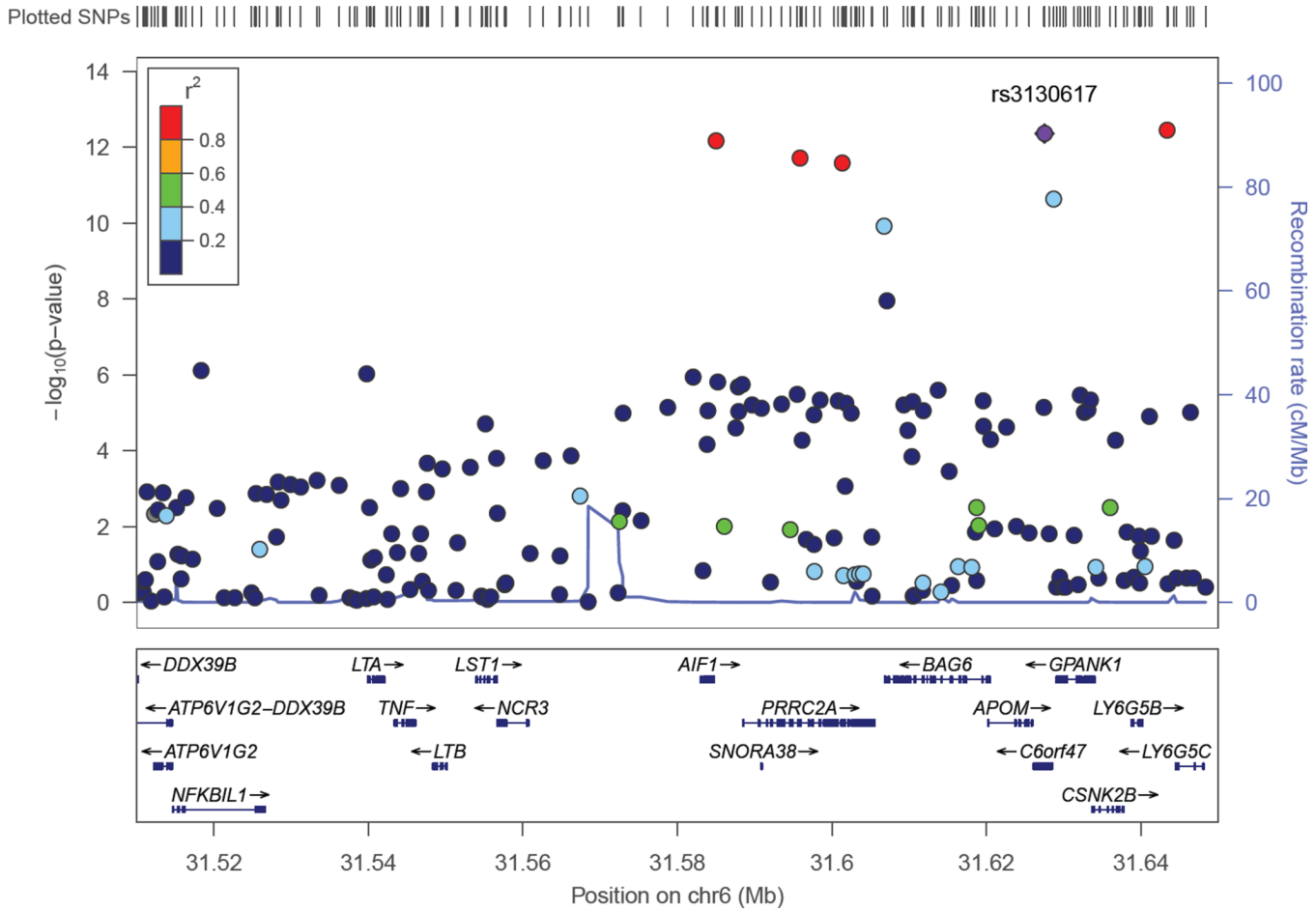

Figure 4.

Association of AMD with SNPs in the portion of the major histocompatibility locus encompassing *TNFAPOM* and *CSNK2B*. Significance of the association of each SNP (circles) is plotted as the negative logarithm of the p-value. Correlations (r^2) between tested SNPs with the SNP most significantly associated with AMD in this region (rs3130617) are indicated using the color scheme shown in the legend. Map positions and direction of transcription of genes in the region are shown in the bottom portion of the figure.

Table 1

AD-related genes which were significantly associated with AMD at the gene level ($p_{corrected} < 0.0014$) after correcting for the 35 autosomal non-*APOE* region genes examined. The most significant SNP within 5 kb of each gene is shown.

GENE	SNP	CHR	BP	MA	MAF[a]	OR	p	$p_{corrected}$
PILRA	rs7792525	7	99972122	G	17.9%	1.14	2.34E-06	7.02E-06
ZCWPW1	rs11771241	7	99994785	A	19.4%	1.14	2.68E-06	1.61E-05
ABCA7	rs3752228	19	1041164	T	3.6%	1.22	0.00012	0.0012

[a]MAF= Minor allele frequency, based on 1000 Genomes EUR population.; $p_{corrected}$=The gene-level corrected significance based on the peak SNP adjusted for effective number of tests within a gene.

Table 2

The canonical pathways most-significantly enriched for Alzheimer-related genes based on an INGENUITY analysis of the **A**) the 59 genes with < 10% FDR in association with AD, **B**) the 54 nominally significant AD loci using AlzGene's methodology, and C) the 36 genes with 10% FDR after excluding the other *APOE*-region genes such as *TOMM40* and *PVRL2*.

A) Top Pathways from FDR 10% Alzheimer-associated Genes				
Ingenuity Canonical Pathways	**p**	**FDR***	**Ratio**	**Genes**
Clathrin-mediated Endocytosis Signaling	1.20E-7	6.61E-06	0.036	*APOC1, APOE, CD2AP PICALM, APOC4, APOC2, CLU*
LXR/RXR Activation	5.89E-06	1.10E-04	0.037	*APOC1, APOE, APOC4 APOC2, CLU*
Atherosclerosis Signaling	5.89E-06	1.10E-04	0.037	*APOC1, APOE, APOC4 APOC2, CLU*
IL-12 Signaling and Production in Macrophages	9.55E-06	1.35E-04	0.032	*APOC1, APOE, APOC4 APOC2, CLU*
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	3.89E-05	4.37E-04	0.024	*APOC1, APOE, APOC4 APOC2, CLU*

B) Top Pathways from AlzGene Nominally Significant Genes				
Ingenuity Canonical Pathways	**p**	**FDR***	**Ratio**	**Genes**
LXR/RXR Activation	1.00E-12	6.31E-11	0.074	*IL33, APOC1, APOE, IL1A LDLR, TF, APOC4, IL1B, TNF CLU*
Atherosclerosis Signaling	1.00E-12	6.31E-11	0.073	*IL33, APOC1, IL8, APOE, IL1A APOC4, IL1B, CCR2, TNF, CLU*
Clathrin-mediated Endocytosis Signaling	4.07E-08	1.55E-06	0.041	*APOC1, APOE, CD2AP, LDLR, TF, PICALM, APOC4, CLU*
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	1.38E-07	3.89E-06	0.11	*IL33, IL8, IL1A, IL1B, TNF*
Role of Cytokines in Mediating Communication between Immune Cells	4.37E-07	9.77E-06	0.091	*IL33, IL8, IL1A, IL1B, TNF*

C) Top Pathways from FDR 10% Alzheimer-associated Genes- Excluding genesin LD with APOE.				
Ingenuity Canonical Pathways	**p**	**FDR***	**Ratio**	**Genes**
Clathrin-mediated Endocytosis Signaling	1.17E-04	0.0053	0.020	*APOE,CD2AP,PICALM,CLU*
Role of Tissue Factor in Cancer	0.011	0.11	0.017	*PTK2B,HBEGF*
RhoA Signaling	0.012	0.11	0.017	*PTK2B,EPHA1*
LXR/RXR Activation	0.012	0.11	0.015	*APOE,CLU*
Atherosclerosis Signaling	0.012	0.11	0.015	*APOE,CLU*
IL-12 Signaling and Production in Macrophages	0.015	0.11	0.013	*APOE,CLU*

* Significance adjusted for multiple pathways examined using Benjamini–Hochberg false discovery rate (FDR).

Table 3

Clathrin-mediated endocytosis signaling pathway genes that are significantly associated ($p_{corrected}$<0.00026) with AMD after correcting for the 192 tests of genes outside of the *APOE* region.

GENE	SNP	CHR	BP	MA	MAF[a]	OR	P	$p_{corrected}$
APOM	rs3130617	6	31627523	C	22.6%	0.83	4.39E-13	4.39E-12
CSNK2B	rs805262	6	31628733	T	46.3%	1.20	2.44E-11	2.69E-10
HGS	rs8070488	17	79663931	C	25.5%	0.91	1.88E-05	7.52E-05

[a]MAF= Minor allele frequency, based on 1000 Genomes EUR population.; $p_{corrected}$=The gene-level corrected significance based on the peak SNP adjusted for effective number of tests within a gene.

Table 4

LXR/RXR activation pathway genes that were significantly associated ($p_{corrected}<0.00038$) with AMD after correcting for the 132 tests of genes outside of the *APOE* region.

GENE	SNP	CHR	BP	MA	MAF[a]	OR	p	$P_{corrected}$
C3	rs2230199	19	6718387	C	20.4%	1.46	1.74E-26	2.95E-25
C4A	rs389512	6	31947594	C	13.5%	0.69	2.11E-29	8.44E-29
APOM	rs3130617	6	31627523	C	22.6%	0.83	4.39E-13	4.39E-12
CETP	rs1864163	16	56997233	A	26.8%	0.80	7.68E-13	1.23E-11
C4B	rs6472	6	32007849	C	11.2%	0.80	4.52E-09	1.81E-08
TNF	rs2071590	6	31539768	A	36.5%	0.89	9.76E-07	1.17E-05

[a] MAF= Minor allele frequency, based on 1000 Genomes EUR population.; $p_{corrected}$=The gene-level corrected significance based on the peak SNP adjusted for effective number of tests within a gene.

Table 5

Atherosclerosis signaling pathway genes that were significantly associated ($p_{corrected}$<0.00038) with AMD after correcting for the 132 tests of genes outside of the *APOE* region.

GENE	SNP	CHR	BP	MA	MAF[a]	OR	P	$p_{corrected}$
APOM	rs3130617	6	31627523	C	22.6%	0.83	4.39E-13	4.39E-12
COL10A1	rs3812111	6	116443735	A	39.2%	0.89	7.19E-08	5.76E-07
PLA2G12A	rs17586561	4	110648632	A	42.2%	1.12	1.26E-06	5.02E-06
TNF	rs2071590	6	31539768	A	36.5%	0.89	9.76E-07	1.17E-05

[a]MAF= Minor allele frequency, based on 1000 Genomes EUR population; $p_{corrected}$=The gene-level corrected significance based on the peak SNP adjusted for effective number of tests within a gene.