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# A search for age-related macular degeneration risk variants in Alzheimer disease genes and pathways

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

[a] Department of Medicine (Biomedical Genetics), Boston University School of Medicine, Boston, MA, USA
[b] Department of Neurology, Boston University School of Medicine, Boston, MA, USA
[c] Department of Ophthalmology, Boston University School of Medicine, Boston, MA, USA
[d] Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA
[e] Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA
[f] John A. Moran Eye Center, University of Utah, Salt Lake City, UT, USA

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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 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 *ZCWPW1* (peak AMD SNP rs7792525, minor allele frequency [MAF] = 19%, odds ratio [OR] = 1.14, $p = 2.34 \times 10^{-6}$), 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 2 novel genes: *HGS* (peak SNP rs8070488, MAF = 0.23, OR = 0.91, $p = 7.52 \times 10^{-5}$), which plays a role in the clathrin-mediated endocytosis signaling pathway, and *TNF* (peak SNP rs2071590, MAF = 0.34, OR = 0.89, $p = 1.17 \times 10^{-5}$), which is a member of the atherosclerosis signaling and the LXR/RXR activation pathways. Our results suggest that AMD and AD share genetic mechanisms.



## 1. Introduction

Age-related macular degeneration (AMD) is the most common form of severe blindness and vision loss among those over the age of 60 years (Congdon et al., 2004). The common dry form non-neovascular accounts for approximately 85%–90% of AMD cases and the advanced acute wet form (i.e., exudative or neovascular) is responsible for most of the 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, 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).

* Corresponding author at: Biomedical Genetics E201, Boston University School of Medicine, 72 East Concord Street, Boston, MA 02118, USA. Tel.: +617 638 5393; fax: +617 638 4275.
*E-mail address:* farrer@bu.edu (L.A. Farrer).

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Like AMD, late-onset Alzheimer disease (AD) is a common disorder among elderly individuals 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 allele 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, 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 extracellular 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 pathologic features in a mouse model of AMD (Ding et al., 2008). In addition, drusen contain the AD-related apolipoprotein E (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 etiologic overlap between these 2 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 that 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 single nucleotide polymorphisms (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. Methods

### 2.1. Materials

Primary data for this investigation are summarized results for a common set of more than 2 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 7600 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 ($p < 5 \times 10^{-8}$) 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 used 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 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 Michigan/Mayo Clinic/AREDS/UPENN (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 previously. A gene was determined to be significantly associated with AMD if its gene-level corrected significance ($p_{corrected}$) was <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/; analyses run in May and June of 2013). We identified AD-related pathways in 3 ways. First, we investigated the list of all genes with an FDR of <10% in a test of association with AD (i.e., the genes examined for association with AMD as described previously). 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. (2010), however, that study examined enrichment in gene-ontology (GO) (Harris et al., 2004) and KEGG (Kanehisa et al.,