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Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: Genetics core aims, progress, and plans

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Abstract The role of the Alzheimer's Disease Neuroimaging Initiative Genetics Core is to facilitate the investigation of genetic influences on disease onset and trajectory as reflected in structural, functional, and molecular imaging changes; fluid biomarkers; and cognitive status. Major goals include (1) blood sample processing, genotyping, and dissemination, (2) genome-wide association studies (GWAS) of longitudinal phenotypic data, and (3) providing a central resource, point of contact and planning group for genetics within the Alzheimer's Disease Neuroimaging Initiative. Genome-wide array data have been publicly released and updated, and several neuroimaging GWAS have recently been reported examining baseline magnetic resonance imaging measures as quantitative phenotypes. Other preliminary investigations include copy number variation in mild cognitive impairment and Alzheimer's disease and GWAS of baseline cerebrospinal fluid biomarkers and longitudinal changes on magnetic resonance imaging. Blood collection for RNA studies is a new direction. Genetic studies of longitudinal phenotypes hold promise for elucidating disease mechanisms and risk, development of therapeutic strategies, and refining selection criteria for clinical trials.

Keywords: Alzheimer's Disease Neuroimaging Initiative (ADNI); Alzheimer's disease; Mild cognitive impairment (MCI); Genome-wide association studies (GWAS); Copy number variation (CNV); Magnetic resonance imaging (MRI); Cerebrospinal fluid (CSF)

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1. Introduction

1.1. Genetic factors in late onset Alzheimer's disease and mild cognitive impairment

Genetic factors play an important role in late-onset Alzheimer's disease (LOAD) as demonstrated by twin data indicating heritability in the range of 58%–79% [1]. The epsilon 4 allele of *APOE* is the strongest known genetic risk factor for AD with a two- to three-fold increased risk for AD in people with one epsilon 4 allele rising to about 12-fold in those with two alleles. Many other genes have also been identified, but until very recently only *APOE* had been consistently replicated. The *APOE* ε4 allele is associated with an earlier age of onset of AD [2], and may account for up to 50% of AD heritability [3]. If AD is ~60%–80% heritable [1], then approximately 30% of the genetic variance is presently unexplained after accounting for *APOE*. Other genes associated with LOAD have been difficult to find. An up-to-date online encyclopedia of all genetic association studies in LOAD, including systematic meta-analyses across datasets investigating overlapping polymorphisms, can be found at http://www.alzgene.org/ [4]. Genetic studies have also been performed in patients with amnestic mild cognitive impairment (MCI) [5,6]. Many approaches are now being applied to identify genes that play a role in the development and progression of AD and MCI, as briefly discussed in the following sections. See Appendix for full names and chromosomal locations of genes discussed in this report.

1.2. Genome-wide association studies

Genome-wide association studies (GWAS) employ tests of association between markers, called single nucleotide polymorphisms (SNPs), distributed across the genome and a phenotype of interest, which could be dichotomous (affected, unaffected) or quantitative (fluid biomarker levels, rate of longitudinal change on imaging metrics, etc.). This approach has identified susceptibility loci in several diseases (see http://www.genome.gov/26525384). GWAS of AD, listed on http://www.alzgene.org/ [4], have confirmed the strong influence of *APOE*, but evidence implicating other genes has been less convincing, despite many biologically plausible and interesting candidates. Other susceptibility genes appear to have modest effects and require very large samples to detect them using a case-control design. Two large GWAS recently implicated several new genes (*CLU*, *PICALM*, and *CR1*) [7,8]. The National Institute on Aging (NIA) sponsored Alzheimer's Disease Genetics Consortium (G. Schellenberg, Principal Investigator; http://alois.med.upenn.edu/adgc/) is attempting to replicate and extend these findings. The AlzGene database provides a continuously updated summary of these findings [4]. By design, its content and meta-analysis results are dynamically changing and reflect the continuing evolution of leading candidate genes for AD and the biological pathways they may represent, regardless whether they emerged from genome-wide or candidate-gene designs [9]. The most robust findings from case-control GWAS and other types of genetic association studies can provide targets for examining quantitative phenotypes derived from Alzheimer's Disease Neuroimaging Initiative (ADNI) imaging and other biomarker data sets.

1.3. Imaging genetics

ADNI provides a unique opportunity to combine imaging and genetics. Advances in brain imaging and high throughput genotyping enable new approaches to study the influence of genetic variation on brain structure and function [10–12]. As a result, imaging genetics has become an emergent transdisciplinary research field, where genetic variation is evaluated using imaging measures as quantitative traits (QTs) or continuous phenotypes. Imaging genetics studies have advantages over traditional case-control designs. An important consideration is that QT association studies have increased statistical power and thus decreased sample size requirements [13]. Additionally, imaging phenotypes may be closer to the underlying biological etiology of AD, making it easier to identify underlying genes.

SNPs and other polymorphisms in several genes, including *APOE*, have been related to neuroimaging measures in brain disorders such as MCI and AD and also in nondemented carriers (eg, [14,15]). However, analytic tools that relate a single gene to a few imaging measures are insufficient to provide comprehensive insight into the multiple mechanisms and imaging manifestations of these complex diseases. Although GWAS are increasingly performed, effectively relating high density SNP data to large scale image data remains a challenging task. Prior studies typically make significant reductions in one or both data types [16]. Whole brain studies usually focus on a small number of genetic variables, whereas genome-wide studies typically examine a limited number of imaging variables [11,17,18]. This restriction of target genotypes and/or phenotypes greatly limits the capacity to identify important relationships. ADNI is contributing new methods that begin to address the high dimensionality of both the imaging and genomic data [6,19,20]. Important challenges in imaging genomics include identifying optimal statistical modeling approaches and addressing power limitations and multiple comparison issues. Given the nature of these challenges, multidisciplinary teams and methods are needed, and ADNI is an ideal test bed for development of new analytic methods.

1.4. Copy number variation

Copy number variations (CNVs) are segments of DNA, ranging from 1 kilobase (kb) to several megabases (Mb), for which differences in regional copy number have been revealed by comparing two or more genomes. These differences can be gains (duplications), losses (deletions), or other more complex rearrangements. CNVs have been implicated in autism, schizophrenia, bipolar disorder, and cancer [21,22]. In addition, duplications of the *APP* gene on chromosome 21 have been shown to cause rare, early-onset familial forms of AD that

follow Mendelian transmission. Only one study [23] has performed a genome-wide CNV scan in non-Mendelian LOAD, with 331 cases and 368 controls, but no new SNP CNVs were significant at a genome-wide threshold. However, a duplication in *CHRNA7* was found, which warrants further investigation. Preliminary analysis of CNVs in the ADNI sample has suggested several genomic loci worthy of follow-up exploration and molecular verification [24,25]. CNV analysis may reveal variations in genomic microarchitecture associated with AD risk. Because CNVs may lead to increased or decreased expression of genes involved in AD, they might be useful in predicting response to particular treatments.

2. Genotyping the ADNI cohort

2.1. ADNI background, sample sources, and informed consent

Data discussed in this report were uploaded to and/or obtained from the ADNI database (www.loni.ucla.edu/ADNI). Detailed information about ADNI can be found at www.adni-info.org, and throughout the other papers in this Special Issue on ADNI. Clinical characteristics and methods are presented in References [26,27], magnetic resonance imaging (MRI) methods are in Reference [28], and those for cerebrospinal fluid (CSF) are in Reference [29]. The ADNI studies described later in the text were approved by institutional review boards of all participating institutions, and written informed consent was obtained from all participants or authorized representatives.

2.2. Brief history of the core and GWAS planning

Genetic assessment beyond *APOE* genotyping was not included in the original ADNI grant. Therefore, a Genetics Working Group (Members of the working group included: Andrew Saykin [Chair], Bryan DeChairo [Pfizer, industry representative, replaced by Elyse Katz in 2009], Lindsay Farrer, Tatiana Foroud, Robert Green, Steven Potkin, Eric Reiman, Gerard Schellenberg, Rudolph Tanzi, John Trojanowski, Christopher van Dyck, Michael Weiner and Kirk Wilhelmsen) was tasked in 2005 to develop plans for genetic studies. The working group considered potential data collection and analysis options, and recommended completing a GWAS using an Illumina Human BeadChip panel (Illumina, Inc, San Diego, CA). A proposal was generated and funds were obtained through the Foundation for National Institute of Health from Merck and Gene Network Sciences, and Pfizer contributed DNA extraction (by Cogenics) and other assistance to the project. A supplement from the National Institute of Biomedical Imaging and Bioengineering provided initial support for data analysis. The Illumina arrays were processed by TGen (Phoenix, AZ), a National Institute of Health Neuroscience Microarray Consortium site. Sample processing, storage, and distribution were provided by the National Institute on Aging-sponsored National Cell Repository for Alzheimer's Disease (NCRAD). Quality control bioinformatics and sample identity confirmation were performed at Indiana University. This group worked with the ADNI Informatics Core to format, document, and post the final genotyping results on the ADNI Laboratory of Neuro Imaging (LONI) web site. With the ADNI Grand Opportunities (GO) grant in 2009, genetics efforts in ADNI were organized as a core to facilitate genetics research related to ADNI biomarkers in an integrated manner.

2.3. Genotyping methods

Genotyping using the Human610-Quad BeadChip (Illumina, Inc., San Diego, CA) included 620,901 SNP and CNV markers, and was completed on all ADNI-1 participants using the following protocol. A 7 mL sample of blood was taken in ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tubes from all participants, and genomic DNA was extracted at Cogenics (now Beckman Coulter Genomics) using the QIAamp DNA Blood Maxi Kit (Qiagen, Inc, Valencia, CA) following the manufacturer's protocol. Lymphoblastoid cell lines (LCLs) were established by transforming B lymphocytes with Epstein-Barr virus as described in Reference [30]. Genomic DNA samples were analyzed on the Human610-Quad BeadChip according to the manufacturer's protocols (Infinium HD Assay; Super Protocol Guide; Rev. A, May 2008). Before initiation of the assay, 50ng of genomic DNA from each sample was examined qualitatively on a 1% Tris-acetate-EDTA agarose gel to check for degradation. Degraded DNA samples were excluded from further analysis. Of the initial 818 ADNI-1 samples, 731 were analyzed using DNA from peripheral blood and 87 were genotyped using DNA extracted from LCLs at NCRAD. Samples were quantitated in triplicate with PicoGreen reagent (Invitrogen, Carlsbad, CA) and diluted to 50 ng/μL in Tris-EDTA buffer (10mM Tris, 1mM EDTA, pH 8.0). 200ng of DNA was then denatured, neutralized, and amplified for 22 hours at 37°C (this is termed the MSA1 plate). The MSA1 plate was fragmented with FMS reagent (Illumina) at 37°C for 1 hour, precipitated with 2-propanol, and incubated at 4°C for 30 minutes. The resulting blue precipitate was resuspended in a resuspension, hybridization, and wash reagent (Illumina) at 48°C for 1 hour. Samples were then denatured (95°C for 20 minutes) and immediately hybridized onto the BeadChips at 48°C for 20 hours. The BeadChips were washed and subjected to single base extension and staining. Finally, the BeadChips were coated with XStain BeadChip solution 4 (Illumina), desiccated, and imaged on the BeadArray Reader (Illumina).

APOE genotyping was performed at the time of participant enrollment and included in the ADNI database. The two SNPs (rs429358, rs7412) that define the epsilon 2, 3, and 4 alleles, are not on the Human610-Quad BeadChip, and therefore were genotyped using DNA extracted by Cogenics from a 3 mL aliquot of EDTA blood, as described earlier in the text. Polymerase chain reaction amplification was followed by HhaI restriction enzyme digestion, resolution on 4% Metaphor Gel, and visualization by ethidium bromide staining [11].

2.4. Data availability, formats and updated release

BeadStudio 3.2 software (Illumina) was used to generate SNP genotypes from bead intensity data. After performing sample verification and quality control bioinformatics, the genotype data for 818 ADNI participants was uploaded to the ADNI website (http://www.loni.ucla.edu/ADNI) and publicly released in complete form on April 16, 2009. In the first 6 months, there were 42,270 downloads (counted as one per sample) by 94 different users.

GenomeStudio v2009.1 (Illumina), an updated version of BeadStudio, was recently used to reprocess the array data for all 818 samples. GenomeStudio project files containing the genotype information for all samples, as well as new Final Reports will be released in May 2010 on the ADNI LONI web site. In addition, the widely used PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) data format will be provided to facilitate analysis by other groups. A comparison of calls for all 620,901 markers from the two versions of Final Reports for each sample indicated that only nine samples had mismatches in genotypes successfully called in the two versions, and those mismatches comprised <0.01% of all markers. Although the new files are highly similar to the initial release, these data may be helpful to investigators given the new formats and additional information provided. Finally, a new DNA source file is included to identify for each genotyped sample whether peripheral blood (n = 731) or immortalized LCL (n = 87) derived DNA was analyzed.

3. Initial progress in genetic investigations within ADNI

3.1. Genetic analyses of baseline MRI scans

Several baseline MRI GWAS have recently been published on line and are briefly summarized here along with a candidate gene study. Potkin et al. [11] performed the first GWAS of AD cases (n = 172) and controls (n = 209). A case-control analysis identified *APOE* and the adjacent risk gene, *TOMM40* (translocase of outer mitochondrial membrane 40), at the significance threshold of $P < 10^{-6}$. A QT analysis using hippocampal atrophy as the phenotype identified 21 genes or chromosomal regions with at least one SNP with a *P*-value reaching 10^{-6}, including *EFNA5*, *CAND1*, *MAGI2*, *ARSB*, and *PRUNE2*. These genes are involved in regulation of protein degradation, apoptosis, neuronal loss, and neurodevelopment. The *TOMM40* finding is consistent with other recent GWAS results, and a recent sequencing study has generated considerable interest in possible independent influences of this gene [31].

Shen et al. [6] completed a GWAS of 729 ADNI participants on 142 brain-wide MRI phenotypic measures of grey matter density (GMD), volume, and cortical thickness derived using FreeSurfer automated parcellation and voxel-based morphometry methods, as previously described [32]. Data analyzed included genotypes based on 530,992 quality controlled SNPs from 175 AD, 351 MCI, and 203 controls. Cluster analysis of GWAS results ($P < 10^{-7}$) confirmed SNPs in *APOE* and *TOMM40* as strongly associated with multiple brain regions and revealed other novel SNPs proximal to the *EPHA4*, *TP63* and *NXPH1* genes. Detailed analyses of rs6463843 (flanking *NXPH1*) indicated reduced global and regional GMD in TT homozygotes and increased vulnerability to right hippocampal GMD loss in AD patients with the TT genotype. *NXPH1* codes for neurexophilin-1, a protein implicated in synaptogenesis, that forms a tight complex with alpha neurexins, a group of proteins that promote adhesion between dendrites and axons. This adhesion is a key factor in synaptic integrity, the loss of which is a hallmark of AD.

Stein et al. [19] explored the relation between 448,293 quality-controlled SNPs and each of 31,622 voxels of the entire brain across 740 ADNI participants, where GWAS was performed on a tensor-based morphometry metric at each voxel location. A novel method was developed to address the multiple comparison problem and computational burden associated with this unprecedented amount of data. No SNP survived the strict significance threshold, but several genes including *CSMD2* and *CADPS2* were identified. These genes have high relevance to brain structure and are good candidates for further exploration.

Stein et al. [20] in a second GWAS focusing on regional structural brain degeneration, mapped the 3D profile of temporal lobe volume differences using tensor-based morphometry in 742 ADNI participants. This study identified rs10845840, a SNP located in *GRIN2B*, as associated with greater temporal lobe atrophy and with AD. It is particularly notable that *GRIN2B* encodes the N-methyl-D-aspartate glutamate receptor NR2B subunit, which is a target for memantine therapy to decrease excitotoxic damage.

Ho et al. [33] investigated the relationship between an obesity-associated candidate gene (*FTO*) and regional brain volume differences in 206 ADNI control participants. Systematic brain volume deficits were detected in cognitively normal obesity-associated risk allele carriers, as well as in subjects with increased body mass index. The results of this study indicate that a very common susceptibility gene for obesity is associated with detectable deficits in brain structure, which may indirectly influence future risk for neurodegenerative disease.

3.2. Preliminary candidate gene and GWAS of MRI changes at 12-month follow-up

Saykin et al. [34] examined genetic predictors of 12-month change in hippocampal volume and GMD using a candidate and GWAS approach. Scan data included baseline and 12-month 1.5T MRI scans from 627 non-Hispanic Caucasian ADNI participants (141 AD, 60 MCI to AD converters, 241 MCI-stable, 185 healthy controls) that were analyzed as in [6,32] with the additional step of registration of follow-up to baseline scans.

For the candidate gene analysis, common SNPs with high minor allele frequency (≥20%) in the top 30 genes of the AlzGene database [4] were examined. Regression models were performed using SAS/STAT 9.3 (SAS Institute Inc, Cary, NC) to test the ability of SNPs to predict hippocampal volume and GMD changes. Models including diagnostic group (AD,

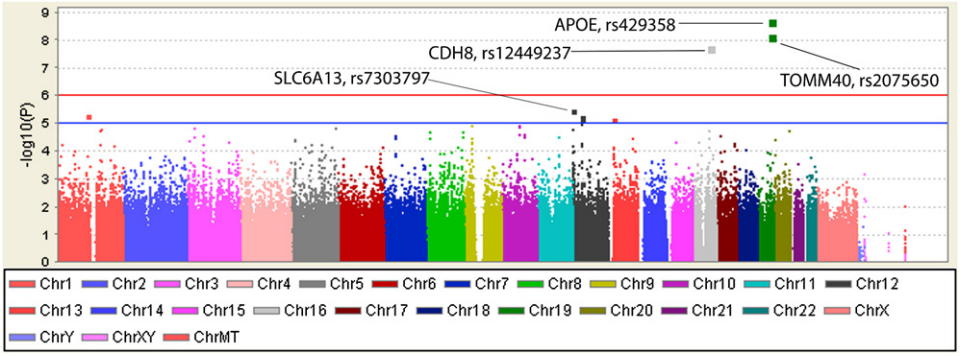

Fig. 1. Manhattan plot for percent change in hippocampal volume over 1 year.

MCI to AD converters, MCI-stable, healthy controls), SNP (0,1,2), parental history of dementia (0,1,2), *APOE* ε4 carrier status (0,1), and interactions were computed. Covariates included baseline age, sex, education, handedness, and total intracranial volume. A total of 732 SNPs were available with minor allele frequency ≥20%. A corrected significance threshold of $P < .01$ was determined using Bonferroni adjustment ($.01/732 = 1.37 \times 10^{-5}$). Using this model and criterion, five AD genes (*NEDD9*, *SORL1*, *DAPK1*, *IL1B*, and *SORCS1*) showed significant SNPs associated with hippocampal volume or GMD changes after accounting for *APOE*, diagnosis, and other factors. In addition, SNPs from several other candidate genes (*MYH13*, *TNK1*, *ACE*, *PRNP*, *MAPT*, *PCK1*, *GAPDHS*, and *APP*) showed less robust indications of possible association that nonetheless encourage further investigation in relation to imaging phenotypes.

The preliminary GWAS was performed using PLINK software release v1.06 (http://pngu.mgh.harvard.edu/~purcell/plink/), which permits modeling of additive main effects for each SNP on a genome-wide basis but not group × genotype interaction effects as described for candidate gene analyses. Results of main effects for SNPs identified associations with hippocampal change measures. Manhattan plots show chromosomal locations for association peaks for hippocampal volume (Fig. 1) and GMD (Fig. 2). As expected, *APOE* (rs429358, the epsilon 4 marker) and *TOMM40* (rs2075650) showed significant genome-wide association with both change measures. In addition, a SNP not previously linked to AD was significantly associated with rate of hippocampal volume loss. This SNP, rs12449237 located at 16q22.1, is intergenic between *CDH8* (cadherin 8, type 2) and *LOC390735*. *CDH8* codes for a calcium-dependent cell adhesion protein implicated in synaptic adhesion and axonal growth and guidance. Neuronal cadherin interacts with presenilin-1 [35], and cell adhesion molecules may be decreased in MCI and AD [36], suggesting the possibility of a mechanistic relationship to AD that warrants investigation. Several other loci or genes labeled in Figs. 1 and 2, while not reaching genome-wide significance, showed association signals worthy of follow-up, including *SLC6A13* with volume change, and *MAD2L2*, *LOC728574*, *QPCT* and *GRB2* with change in GMD.

In sum, initial analyses indicate that common variations in major AD candidate genes, as well as other novel loci, are related to rate of longitudinal hippocampal structural change over 12 months in the ADNI cohort. Replication in independent samples and further characterization of these relationships over time will be important.

3.3. Copy number variation

CNVs are alterations in DNA sequence resulting in gains (duplications) and losses (deletions) of genomic segments. Because CNVs often encompass or overlap genes, they may have important roles in disease. Swaminathan et al. [25] analyzed CNVs in data from 193 AD, 116 MCI, and 131 control participants with blood-derived DNA samples that remained after CNV-specific quality control. Although no excess CNV

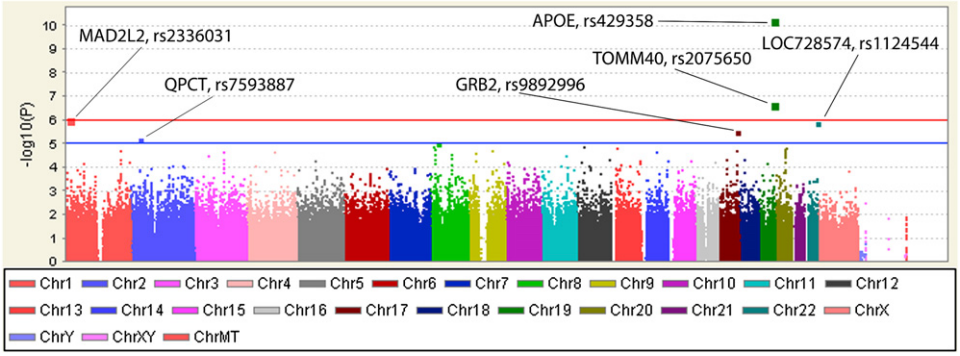

Fig. 2. Manhattan plot for percent change in hippocampal grey matter density over 1 year.

burden was observed in AD and MCI cases relative to controls, gene-based analysis revealed CNVs in cases but not controls in regions of *TP53TG3*, *CSMD1*, *SLC35F2*, *HNRNPCL1*, and *UBTFL2*, as well as the candidate gene, *CHRFAM7A*. Using a different approach and larger sample, Macciardi et al. [24] observed large deletions that could disrupt pathways of biological significance including axonal development.

3.4. GWAS of cerebrospinal fluid biomarkers

Levels and ratios of CSF biomarkers ($A\beta_{1–42}$, t-tau, p-tau_{181p}, p-tau_{181p}/ $A\beta_{1–42}$, and t-tau/ $A\beta_{1–42}$) have been shown to be promising biomarkers for AD diagnosis [27,29]. Kim et al. [37] conducted a GWAS of CSF biomarkers on 374 non-Hispanic Caucasian ADNI participants. This analysis identified SNPs in the regions of *APOE*, *TOMM40*, and *EPC2* that reached genome-wide significance for one or more of the CSF biomarkers. In addition, association signals were noted in *CCDC134*, *ABCG2*, *SREBF2* and *NFATC4*, although these did not reach genome-wide significance. Although *APOE* and *TOMM40* are well-established candidate genes, *EPC2* is a novel finding requiring further characterization and independent replication.

3.5. Imaging associations with PICALM

The recent large case-control GWAS noted earlier in the text [7,8] that identified new promising AD candidates did not include neuroimaging measures or MCI participants. We examined key brain regions implicated in early AD to determine whether variation in these new gene loci were predictive of structural changes. Entorhinal cortex (EC) neurodegeneration is one of the earliest known structural findings in MCI and AD. Fig. 3 shows the association of the previously reported *PICALM* (phosphatidylinositol-binding clathrin assembly protein) SNP and mean bilateral EC thickness at baseline [32]. Covariates included baseline age, sex, education, handedness, and intracranial volume. The number of G alleles of rs3851179 was linearly associated with reduction of EC thickness at baseline across groups ($P < .002$). This remained significant after adding *APOE* status as a covariate ($P = .006$), suggesting *PICALM* may have an independent contribution to neurodegenerative changes. Although there was a main effect of SNP across groups, the pattern of results as shown in Fig. 3 suggests that the combined AD and MCI-converter (probable AD within 12 months) group drive this effect. This finding illustrates the potential synergy between large case-control GWAS and an imaging genetics approach.

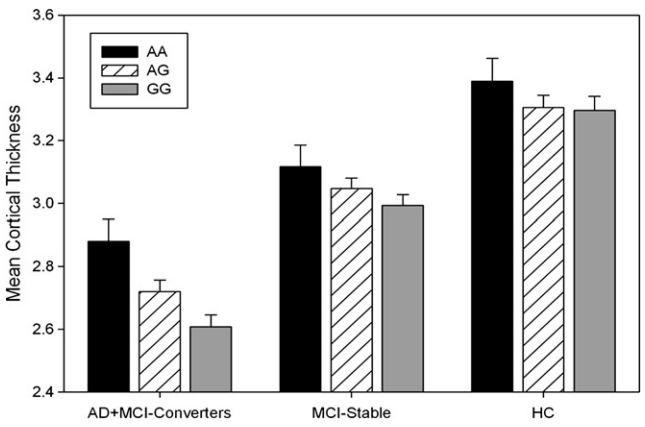

Fig. 3. Mean (± SE) bilateral entorhinal cortex thickness on baseline MRI as a function of *PICALM* (rs3851179) genotype and diagnosis group. Participants who had a baseline diagnosis of MCI but converted to probable AD within a year are included with the AD at baseline group. The MCI-stable group included participants with a stable MCI diagnosis over 1 year. See text for details.

4. Future directions

Several rapidly emerging technologies have the potential to enhance the genetic information yield from ADNI-1 as well as to open new possibilities for investigating the influence of genetic variation in the ADNI-GO and planned ADNI-2 projects which will examine early amnestic MCI in addition to the current diagnostic groups. Here we briefly discuss future resequencing, RNA and replication studies.

4.1. Deep resequencing

Although GWAS have identified genes and chromosomal regions of interest, association does not permit inference of causation. As a result, targeted sequencing or deep resequencing of genes or regions associated with a phenotype is often employed to catalog all sequence variation (SNPs, insertion/deletions, etc), as a first step in determining which variation(s) directly contribute to disease risk. Deep resequencing has helped to determine how sequence variations in genes are associated with several psychiatric disorders [38–40]. These techniques hold promise for uncovering new variants that may be involved in the pathogenesis of LOAD, yet there has been limited application of this approach to date [41]. Promising genes and regions such as those described in this report can be selectively targeted for resequencing in the next few years. In 5–10 years, whole genome sequencing for all ADNI samples is likely to become cost effective.

4.2. Gene expression and RNA studies on peripheral blood samples

Analysis of aberrant gene expression profiles in AD is difficult because tissue samples for expression analysis cannot typically be acquired from living patients. Additionally, postmortem RNA degradation and protein modification may potentially confound the interpretation of data obtained from neuropathological samples. Expression profiling of peripheral blood mononuclear cells (PBMCs) may offer advantages in deciphering gene regulation patterns. Circulating blood is accessible and there is communication between the brain and immune system through multiple mechanisms that may be expressed in blood. In addition, abnormal *APP* expression, altered levels of antioxidant enzymes, oxidative damage to DNA, RNA, and protein, deregulated cytokine secretion, and augmented rates of apoptosis are features shared by

AD brain and blood lymphocytes, and PBMCs have been successfully employed in investigating other neurological diseases [42]. Peripheral blood cells share more than 80% of the transcriptome with other tissues types including brain, suggesting that these cells can be used for molecular profiling in humans [43]. PBMCs have been used for gene expression profiling in AD with encouraging results [42,44,45].

4.3. MicroRNAs and gene regulation

MicroRNAs (miRNAs) are a large family of short (21–25 nucleotide), noncoding regulatory RNAs involved in post-transcriptional gene regulation and silencing. They bind to complementary sites typically located in the 3' untranslated regions (3'-UTR) of target messenger RNA (mRNA) molecules. They repress mRNA expression, which results in gene silencing by interfering with translation or by targeting mRNA for degradation [46]. The miRNA database miRBase (http://www.mirbase.org/; version 13.0) identified 706 validated miRNAs in the human genome, although there may be a thousand or more. There is evidence that miRNAs play a role in synaptic development, plasticity, and long-term memory [47]. Recent studies have shown that miRNAs can regulate expression of genes such as *APP* and *BACE1*, suggesting that miRNAs may play a role in AD pathophysiology [48–50].

4.4. Replication, meta-analyses and collaboration with other cohort studies

It is essential for all genetics findings to undergo replication in independent samples given the heightened risk of false discovery. A challenge for replication studies of findings from ADNI, particularly for imaging genetics analyses, is the lack of comparable data sets. Fortunately, World Wide ADNI efforts will generate excellent replication opportunities in the future. Similarly, collaborative analyses of large pooled data sets with substantial power will become possible. In the interim, possibilities exist for replication and joint analysis studies with existing cohorts that acquired relatively similar neuroimaging and genetics data sets.

5. Conclusion

There is considerable momentum in genetic studies of AD and MCI. Although it is beyond the scope of ADNI to support longitudinal gene expression and miRNA studies and targeted deep resequencing at this time, the relevant data will be collected and banked and the core will provide a venue to foster new proposals and collaborations so that the most scientifically promising leads and directions are pursued.

The availability of advanced brain imaging techniques such as MRI and positron emission tomography paired with the ability to capture over a million genetic markers by current array technology makes this an exciting time for imaging genetics research, where there is great potential for discovery. Many promising preliminary results have emerged to be followed-up by replication and detailed characterization studies. As the ADNI-1 cohort is followed beyond the present 3 years and augmented by new participants and updated methods (e.g., amyloid positron emission tomography and multiplex CSF biomarkers), the prospects for generation of new knowledge regarding disease mechanisms that can inform treatment development and clinical trials design are compelling. Analyses addressing the role of genetic variation can be expected to enhance the value of this important initiative.

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Appendix

Expanded gene names and chromosomal locations

Gene Abbreviation	Gene Name	Chromosomal Location
ABCG2	ATP-binding cassette, sub-family G, member 2	4q22.1
ACE	angiotensin I converting enzyme 1	17q23.3
APOE	apolipoprotein E	19q13.2
APP	amyloid beta (A4) precursor protein	21q21.3
ARSB	arylsulfatase B	5q14.1
BACE1	beta-site APP-cleaving enzyme 1	11q23.3
CADPS2	Calcium-dependent secretion activator 2	7q31.32
CAND1	cullin-associated and neddylation-dissociated 1; TBP-interacting protein TIP120A	12q14.3
CCDC134	coiled-coil domain containing 134;	22q13.2
CDH8	cadherin 8, type 2	16q21.1
CHRFAM7A	cholinergic nicotinic receptor, alpha 7 subunit, exons 5-10 and family with sequence similarity 7A, exons A-E fusion	15q13.2
CHRNA7	cholinergic nicotinic receptor, alpha 7 subunit	15q13.3
CLU	clusterin, apolipoprotein J	8p21.1
CR1	complement component (3b/4b) receptor 1	1q32.2
CSMD1	CUB and Sushi multiple domains 1	8p23.2
CSMD2	CUB and Sushi multiple domains 2	1p34.3
DAPK1	death-associated protein kinase 1	9q34.1
EFNA5	ephrin-A5	5q21.3
EPC2	enhancer of polycomb homolog 2	2q23.1
EPHA4	ephrin type-A receptor 4	2q36.1
GAPDHS	glyceraldehyde-3-phosphate dehydrogenase, spermatogenic	19q13.12
GRB2	growth factor receptor-bound protein 2	17q25.1
GRIN2B	glutamate N-methyl D-aspartate receptor, subunit 2B	12p13.1
HNRNPCL1	heterogeneous nuclear ribonucleoprotein C-like 1	1p36.21
IL1B	interleukin 1, beta	2q14
MAD2L2	mitotic arrest deficient homolog-like 2	1p36.22
MAGI2	membrane associated guanylate kinase, WW and PDZ domain containing 2; atrophin-1-interacting protein A	7q21.11
MAPT	microtubule-associated protein tau	17q21.31
MYH13	myosin, heavy chain 13	17p13.1
NEDD9	neural precursor cell expressed, developmentally down-regulated 9	6p24.2
NFATC4	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4	14q11.2
NXPH1	neurexophilin 1	7p22
PCK1	phosphoenolpyruvate carboxykinase 1	20q13.31
PICALM	phosphatidylinositol binding clathrin assembly protein	11q14.2
PRNP	prion protein	20p13
PRUNE2	prune homolog 2	9q21.2
QPCT	glutaminyl-peptide cyclotransferase	2p22.2
SLC35F2	solute carrier family 35, member F2	11q22.3
SLC6A13	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	12p13.33
SORCS1	sortilin-related VPS10 domain containing receptor 1	10q25.1
SORL1	sortilin-related receptor containing LDLR class A repeats	11q24.1
SREBF2	sterol regulatory element binding transcription factor 2	22q13.2
TNK1	tyrosine kinase, non-receptor, 1	17p13.1
TOMM40	translocase of outer mitochondrial membrane 40 homolog	19q13.32
TP53TG3	tumor protein p53 target 3	16p13
TP63	tumor protein p63	3q28
UBTFL2	upstream binding transcription factor, RNA polymerase I-like 2	11q14.3