ABSTRACT

Better characterization of the preclinical phase of Alzheimer’s disease (AD) is needed to develop effective interventions. Neuropathologic changes in AD, including neuronal loss and the formation of proteinaceous deposits, can begin 20 years before the onset of clinical symptoms. As such, the emergence of cognitive impairment should not be the sole basis used to diagnose AD or to evaluate individuals for enrollment in clinical trials for preventive AD treatments. Instead, early preclinical biomarkers of disease and genetic risk should be used to determine the most likely prognosis and to enroll individuals in appropriate clinical trials. Neuroimaging-based biomarkers and genetic analysis together present a powerful system for classifying preclinical pathology in patients. Disease-modifying interventions are more likely to produce positive outcomes when administered early in the course of AD. This review examines the utility of the neuroimaging genetics field as it applies to AD and early detection during the preclinical phase. Neuroimaging studies focused on single genetic risk factors are summarized. Particular focus is on the recent increased interest in polygenic methods, and the benefits and disadvantages of these approaches are discussed. Challenges in the neuroimaging genetics field, including limitations of statistical power arising from small effect sizes and the overuse of cross-sectional designs, are also discussed. Despite the limitations, neuroimaging genetics has already begun to influence clinical trial design and is expected to play a major role in the prevention of AD.

Keywords: Alzheimer’s disease, Clinical trials, Genetics, Neuroimaging, Polygenic risk score, Preclinical

NEUROIMAGING OF PRECLINICAL AD

A long prodrome precedes the emergence of the clinical symptoms of Alzheimer’s disease (AD) (1–3). Increasingly, the time between the first silent pathologic changes in the brain and the earliest stages of cognitive impairment is understood to be a critical window during which prevention and treatment strategies may be most effective (4). This preclinical phase of AD pathogenesis that occurs before clinical symptoms emerge is not well characterized. By definition, individuals with preclinical AD are unaware that they are affected by any neurologic pathology, and their deficits are not detectable with cognitive testing. Preclinical AD is distinct from mild cognitive impairment, which is characterized by subtle cognitive decline and sometimes can progress to a clinical diagnosis of AD (5,5). In the absence of detectable cognitive decline, investigators have access to a limited set of research tools to explore preclinical AD in humans. These tools include neuroimaging, genetic testing, and biochemical assays of the blood and cerebrospinal fluid. Neuroimaging genetics research is poised to play a critical role in improving the characterization of the earliest phases of AD pathophysiology. In this article, we discuss the important role of neuroimaging genetics in AD prevention and treatment with a particular focus on the preclinical phase of the disease. Specifically, we review findings resulting from candidate gene and polygenic approaches to neuroimaging genetics studies in AD. The goal of this review is to educate readers on the status of the field, including its many limitations, and to argue that neuroimaging genetics research using polygenic approaches will lead to better characterization of preclinical AD, which is necessary to achieve effective AD prevention.

A common approach for studying preclinical AD is to use a group at increased risk for AD as a potential preclinical cohort and compare them with a cohort of controls without the risk factor. Increased risk can be defined by the presence of a particular genetic risk variant, such as the apolipoprotein E ε4 (APOE:4) allele; a positive family history of AD; subjective memory impairment; and the presence of an early neuroimaging or cerebrospinal fluid biomarker. Well-validated neuroimaging-based biomarkers for AD in these types of cohorts include hippocampal volume loss or thinning, cortical thinning of key AD-related cortical regions, β-amyloid (Aβ) positivity measured by positron emission tomography (PET), and default mode network dysfunction measured by resting state functional magnetic resonance imaging (fMRI) (7–16). There is evidence from patients with familial AD that these biomarkers...
NEUROIMAGING AND AD CANDIDATE GENES

In 2000, the first study to combine neuroimaging and genetic risk for AD in healthy subjects found that carriers of the APOE-ε4 allele had higher activation across several cortical regions during a memory task compared with noncarriers (Figure 1A) (23). This approach, examining a selected variant within a single gene and the association of that variant with brain structure and function, is a type of candidate gene study. Candidate gene studies in neuroimaging are common, but they are controversial because of difficulties in interpretation and replication of results (24). The now common practice of restricting candidates to genes for which a disease association has already been demonstrated has helped to make findings more robust. Still, a gene with a relatively large effect on disease incidence in a genome-wide association study (GWAS) is not necessarily related to neuroimaging phenotypes to the same degree. APOE is the most commonly studied candidate gene for AD. Because of the large proportion of the variance in AD heritability that is accounted for by APOE, investigators have been successful in identifying differences in many neuroimaging modalities based on APOE genotype (Figure 1) (17–19,21); for an updated review including recent findings, see Supplement 1.

In addition to APOE, other GWAS-identified AD risk genes have been studied using a candidate gene approach, including CLU, PICALM, CR1, BIN1, ABCA7, and EPHA1. Of these genes, the one that has received the most attention in the neuroimaging literature is CLU. First linked to AD by May et al. (25) in 1990, the coincident discovery of CLU in two independent GWAS in 2009 renewed interest in CLU and its role in AD (26,27). The association of rs11136000 to AD has been replicated several times (28–30).

Several functional imaging studies have reported an effect of a CLU genotype in task-based and resting functional MRI paradigms. One functional MRI experiment that tested for additive effects of CLU and APOE on blood oxygen level–dependent (BOLD) signal during an executive attention task found a negative correlation between genetic risk and the BOLD signal associated with executive attention in the medial
temporal lobe and other regions (31). In another study, healthy older carriers of the 
CLU risk variant showed decreased coupling of the hippocampus and prefrontal cortex during memory retrieval tasks (recall and recognition) (32). In a resting-state functional MRI experiment, subjects who were homozygous for the 
CLU risk allele had the same general pattern of positive and negative functional connectivity compared with carriers of the protective allele, but the magnitude of the connectivity was stronger in the positive and the negative directions (Figure 2C,D) (33). Taken together, these studies indicate a modulatory relationship between BOLD signal and 
CLU genotype.

PICALM, a gene whose protein product is involved in synaptic transmission, has also been linked to imaging phenotypes in structural and functional imaging (33–36). An epistatic effect of PICALM and 
BIN1, another gene involved in synaptic transmission, on amyloid deposition has been reported (36). 
BIN1 was also linked to smaller entorhinal cortex and temporal pole volume in a structural imaging study (34). 
CR1 has been shown in several studies to be associated with smaller entorhinal cortex volume in younger and older healthy adult subjects (34,37). Finally, a PET study found that there was a relationship between amyloid deposition and polymorphisms in 
ABCA7 and 
EPHA1 such that carrying the risk variant of 
ABCA7 increases the likelihood of amyloid positivity, whereas the low-risk polymorphism of 
EPHA1 decreases the likelihood of amyloid positivity (38). A more complete description of imaging studies focused on these GWAS-identified risk genes is provided in Table 1. See Supplement 1 for more details.

Relatively little genetic variance is accounted for by differentiating experimental groups based on carrier status of a single risk variant. In the next sections, we cover polygenic scores and regression-based polygenic modeling approaches. These efforts aim to measure genetic risk as a continuous metric or as a set of predictors capable of revealing important relationships between genetic risk, brain structure and function, and preclinical AD.

POLYGENIC RISK SCORES

Combining multiple genetic risk loci into a single metric or score is an attractive way to modernize the candidate gene approach by using the metric or score as the “candidate” rather than a single gene. Associations between a risk score and, for example, an imaging endophenotype cannot be attributed to a single gene, but these associations may be clinically useful in the effort to characterize preclinical AD better (39). Such metrics are designed on one of two main theoretical bases: first, that multiple risk polymorphisms in the same disease-related biological pathway will be more likely to disrupt normal functioning of that pathway, or second, that multiple risk polymorphisms affecting various neuronal functions together will predispose or lead to disease. A polygenic...
risk score (PRS) can be calculated in several ways. Unweighted approaches tally the number of known risk alleles carried by a given individual. Weighted risk scores apply a statistic that captures the strength of the relationship between the genetic variant and disease to weight each risk allele differentially. When GWAS data are available, odds ratios are often used to weight risk alleles in a PRS, but other effect size measures can be used (39). Another method of quantifying polygenic risk is assessing genotype patterns and binning subjects by their genotypes at multiple loci. A limitation of this approach is that a large sample is needed to have large enough subgroups for meaningful statistical analysis. Finally, testing for interaction effects, or epistatic effects, between two or more genes is also technically a polygenic approach, although it differs in that risk effects are not additive but rather emerge from specific interactions between loci.

Using a PRS weighted by GWAS-reported odds ratios, Sabuncu et al. (40) showed that increased genetic risk for AD was associated with decreased cortical thickness in AD-vulnerable regions, including entorhinal, lateral temporal, inferior parietal, and posterior cingulate cortices (Figure 3). In another structural imaging study, a large cohort of >8000 cognitively healthy older individuals was used to assess the relationship between a GWAS loci–based weighted PRS and several measures, including intracranial volume, total brain volume, and hippocampal volume (41). The authors reported that higher PRS was associated with smaller hippocampal volume (41). Although the methods are too numerous to review in detail, we highlight a few important perspectives with respect to AD.

**REGRESSION APPROACHES TO POLYGENIC RISK**

The use of predictive regression models in clinical biostatistics is common (45). Neuroimaging genetics presents a unique problem with millions of genetic markers (in whole-genome data) that can be used as predictors and many outcome phenotypes of interest. Furthermore, linkage disequilibrium, or the tendency of certain genetic loci to be inherited together, must be considered when using any regression method because many of these models assume that predictors are independent (46). The numerous data reduction or selection methods used in regression analyses can be categorized as follows: stepwise regression, regularized regression, mixed linear modeling, projection, and prior biological knowledge (47–51). Although the methods are too numerous to review in detail, we highlight a few important perspectives with respect to AD.

Stepwise regression optimizes a linear model by successively removing, adding, or alternating between adding and removing predictors. One study specifically demonstrated there is an advantage to using machine learning–based, cross-validated genetic algorithms over stepwise regression to predict conversion from mild cognitive impairment to AD (47). Regularized regression is similar to stepwise regression in that it assumes that a small number of the predictors will be the most informative. These approaches, such as Lasso or sparse regression (e.g., ridge, elastic net), penalize larger models in favor of more parsimonious models. Silver et al. (48) used sparse reduced-rank (Lasso) regression to model

<table>
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<th>Gene</th>
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<tr>
<td>CLU</td>
<td>.86 (.84–.89)</td>
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PICALM | .87 (.85–.89) | | | | Synaptic transmission |
|CR1   | 1.18 (1.14–1.22) | | | | Innate immunity |
|BIN1  | 1.22 (1.18–1.25) | | | | Synaptic transmission |
|ABCA7 | 1.15 (1.11–1.19) | | | | Lipid homeostasis |
|EPHA1 | .90 (.88–.93) | | | | Adhesion and contact mediated signaling |

**Table 1. Risk Genes for Alzheimer’s Disease Identified by Genome-wide Association Studies: Neuroimaging Modalities in the Literature and Representative References**

*From Lambert et al. (61).
groups of SNPs that all are within a single biological pathway and calculate the strength of the relationship of that pathway to AD-related neuroimaging phenotypes. The authors reported that SNPs belonging to insulin signaling, vascular smooth muscle contraction, and focal adhesion pathways were the strongest predictors of structural change over 24 months of follow-up. Another study used an elastic net regularization method to explore genetic risk factors for AD affecting the hippocampal surface and found that APOE and TOMM40 were associated with hippocampal surface differences in anterior and middle regions.

Genome-wide complex trait analysis (GCTA) is an example of an optimized linear modeling approach to polygenic risk for phenotypes. Developed to determine the portion of variability of a given trait that can be explained by all available SNPs rather than SNPs that survive genome-wide significance, GCTA takes advantage of linear mixed effect modeling to combine fixed effects such as age and sex with SNPs as random effects. A recent update to the approach ensures that this procedure can be completed in reasonable time despite the high computational demand of considering millions of SNPs and many phenotypes. The authors of the updated GCTA approach used a cohort of 1320 subjects to compute heritability estimates for several structural neuroimaging measures including whole-brain cortical thickness. Ridge et al. used the GCTA approach to examine the proportion of the variance in AD status explained by 11 known, common genetic risk loci for AD and found that only 8% (SE 0.03) of phenotypic variance was accounted for by these markers, whereas 33% (SE 0.0072) of the variance was due to common known and unknown SNPs. These results suggest that there are many more common AD-associated SNPs that have not been identified yet and that genetic variants that explain a large proportion of phenotypic variance are rare.

To test across many millions of SNP-SNP interactions, it is necessary to apply a method that is capable of performing the computationally intensive task of high-dimensional predictor selection. Hibar et al. used a machine learning approach that was designed to perform well when the number of predictors is greater than the number of observations, as is the case when examining human SNP data, by ranking the normalized predictors by their correlation to the dependent variable. The authors discovered that the volume of a region of the temporal lobe was associated with the interaction between two SNPs across the clinical categories in an Alzheimer’s Disease Neuroimaging Initiative (ADNI) sample. Another study, also using an ADNI sample, reduced the number of SNP-SNP interactions they tested using a linear regression approach by testing only for interactions between SNPs that were members of a common biological pathway, such as calcium signaling or axon guidance, both of which were associated with entorhinal cortex and hippocampal atrophy in their cohort. This approach based on prior biological knowledge has been shown to be an effective method of predictor selection. Similarly, SNP data reduction using projection techniques such as independent component analysis has been used to identify independent groups of genes affecting a given trait. Post hoc pathway analysis of the components can reveal whether they are enriched for genes related to—for example, as Meda et al. found in their ADNI sample—inflammation, diabetes, obesity, and cardiovascular disease.

In addition to more traditional regression approaches, advanced association models, such as canonical correlation, can be used to analyze large neuroimaging genetics data sets.
efficiently. These methods are outside the scope of this review, but a summary is provided in Supplement 1.

LIMITATIONS

Power: Effect Sizes and Variant Frequency

A major challenge in neuroimaging genetics is sufficiently powering studies to detect hypothesized effects. One problem is the low effect size of common genetic associations with disease in human polygenic disorders \((57,58)\). An exception to this pattern is the APOE locus, where a commonly occurring variant is strongly associated with increased AD risk. APOE accounts for a larger amount of the variance in AD heritability than any single known genetic locus in another human neurobehavioral, polygenic disorder. Theoretically, because APOE accounts for a relatively large proportion of the heritability variance in AD, it is possible that accurately modeling polygenic risk for AD will be simpler than in other common polygenic neurobehavioral diseases. For neuroimaging genetics investigators who are anxious to demonstrate their field is uniquely positioned to identify early, preclinical predictors of disease, AD is an attractive neurologic disorder.

At the present time, it is unclear if the underlying genetics of AD are best described as many high-effect rare variants (e.g., TREM2 or MAPT) that, in different individuals, each leads to clinical AD or many low-effect common variants that together in a single individual can lead to clinical AD. To neuroimaging genetics investigators, there are advantages and disadvantages to a common-variant or rare-variant theory of AD genetics. Rare variants occur in so few individuals that it is difficult to amass a large cohort of carriers. However, with increased emphasis on data sharing and access to continuously expanding reservoirs of pooled data, reasonably sized samples of individuals with specific rare variants may be plausible (given a minor allele frequency of .002, a sample of 20,000 subjects would be needed to identify 40 carriers of the TREM2 risk variant \((59)\)). Often, rare variants associated with a particular disease have a relatively high effect size, which may make differences between carrier groups easier to detect, even at smaller sample sizes. In contrast, carriers of common variants are more easily amassed in large numbers, but investigators need extremely large cohorts to detect the low-effect size association that usually accompanies a disease-related common variant (Figure 4). As discussed in previous sections, methods for modeling multiple genetic risk factors in a single experiment are actively being developed and may help to exploit the synergistic predictive power of many low-effect size common variants. In a thorough analysis of the PRS literature, Dudbridge \((39)\) used heritability estimate, sample size, locus significance threshold, and a PRS weighting method to generate formulas that allow investigators to estimate the likelihood that future studies will be sufficiently powered. The findings indicated that perhaps hundreds of thousands of subjects would be required to make PRS useful at the individual level. Sample sizes are generally not of this magnitude, but they are increasing quickly. Another simulation-based study based on 10,000 cases and control subjects reported that subjects in the top 5% of genetic risk for hypothetical disease are three to seven times more likely to be affected \((56)\). A threefold to sevenfold increase in risk is clinically useful if not conclusive, as it suggests some individuals may be better candidates for clinical trials and that more frequent follow-up assessments are indicated.

Cross-Sectional Versus Longitudinal Designs

Another major challenge in neuroimaging geneticists of AD is the predominant use of cross-sectional experimental designs to uncover the pathophysiological trajectory of AD. In the literature, inferences about the trajectory of AD are overwhelmingly made from cross-sectional studies in which data are collected from each subject only once and all the subjects are randomly distributed across the age range under investigation with equal numbers of male and female subjects. This approach makes it particularly difficult to make conclusions on the subject level because cross-sectional studies confound between-subject and within-subject variation \((61)\). Given this limitation, drawing longitudinal conclusions based on cross-sectional evidence, even from many studies, is precarious and should be done cautiously \((62)\).

The importance of early detection in neurodegenerative diseases such as AD is illustrated by the extensive neuronal loss already present in patients with AD who are only mildly symptomatic \((63)\). In addition, more recent work has established that AD risk genes are associated with differences in brain structure and function even in young people, including children and infants \((64,65)\). In light of these associations in young people, how can investigators optimize experimental
design for the study of AD risk and preclinical AD? Following subjects in longitudinal designs better allows for making inferences about disease trajectory, but these studies are difficult in practice. However, in the modern pro-collaboration atmosphere, multicohort longitudinal designs are feasible because many sites can each collect longitudinal data on a reasonably small number of subjects, and, assuming proper standardization and oversight is in place, these subjects can be combined to create a much larger cohort. The ADNI is a good example of a multicenter effort in neuroimaging genetics of AD (66,67). Optimized longitudinal mapping of AD progression would help identify individuals who are in the preclinical phase of AD. These individuals are likely to benefit the most from intervention, especially a drug that slows or halts disease progression. Such a drug is not available at the present time, but an accurate and precise definition of preclinical AD is an essential component to the success of any candidate.

IMPLICATIONS FOR CLINICAL TRIALS

Despite major challenges related to statistical power, polygenic risk modeling, and generalizability, the field of neuroimaging genetics is poised to play a major role in the development of effective treatments for AD. All phase 3 AD treatment trials in humans have had negative outcomes, not meeting end points despite promising data in model organisms and in preceding trial phases (68,69). This high failure rate may be partly the result of heterogeneity across the study participants enrolled in these clinical trials. One source of heterogeneity is neuropathologic variation. The clinical-neuropathologic correspondence of AD (pure AD and AD-vascular mixed pathology) occurs in about 87% of clinical AD cases that come to autopsy (70). Of patients with clinically diagnosed AD, >10% actually have some other neurodegenerative disorder, such as frontotemporal lobar degeneration or corticobasal degeneration. It is reasonable to assume that subjects with each of these diseases, from pure AD and mixed AD pathology to frontotemporal lobar degeneration and corticobasal degeneration, will respond differently, if at all, to potential treatments that target a single molecular species, such as Aβ oligomers or plaques. One way to help minimize neuropathologic heterogeneity is through the use of PET. Although costly, the use PET for Aβ and tau as a prescreening technique in clinical trials would allow investigators to amass a more neuropathologically homogeneous cohort. Neuropathologic prescreening using PET is currently being implemented for the first time as part for the A4 (Anti-Amyloid Treatment in Asymptomatic Alzheimer’s Disease) trial, the protocol of which requires a positive Aβ florbetapir F 18 PET scan for enrollment into the treatment arm (71). Another imaging-based method for neuropathologic prescreening is diffusion-weighted MRI, which can be used to estimate the severity of vascular pathology (72).

Neuropathologic differences are not the only source of heterogeneity in clinical trial subjects. It is also important to consider the heterogeneity of the underlying genetics in each individual subject. Depending on the mechanism of the candidate drug, there may be some variation of response in trial participants with different genetic risk factors for AD (73). Also, it is likely that by examining genetic risk, the ability to identify asymptomatic individuals who will progress to show cognitive decline is improved. Investigators should consider implementing genetic prescreening measures that select for clinical trial participants who have certain genetic risk factors for AD (74). Clinical trials in AD have already started to use carriage of one or two risk variants (APOE, TOMM40) as a prescreening measure (75). Kohannim et al. (76) published a study in which they tested the hypothesis that a polygenic screening protocol would decrease the sample size necessary to detect an effect in a hypothetical trial. The authors ranked 394 cognitively healthy subjects and subjects with mild cognitive impairment from ADNI in order of decreasing PRS, calculated based on multiplying risk alleles for APOE, CLU, CR1, and PICALM by the logarithm of the odds ratios reported for each gene in GWAS. They found that by selecting only the top 15% of subjects with highest genetic risk, the required sample size to show differences in temporal lobe atrophy decreased from 142 to 69 (76). This study is excellent evidence that genetic prescreening would increase statistical power in trials. Binning participants by genetic risk may be the next frontier in AD clinical trial design.

Another important role for neuroimaging genetics in clinical trials is the development of hard, noncognitive end points to assess treatment efficacy (77). Most AD trials to date have used soft end points, such as paper-and-pencil memory measures or composite dementia severity scores (68,69). However, cognitive end points will no longer be appropriate as trials shift their focus to preclinical individuals who are asymptomatic. Neuroimaging-based biomarkers and other markers, such as cerebrospinal fluid analyte levels, that capture pathologic changes that precede cognitive decline must be refined for use as clinical end points (77).

CONCLUSIONS

A neuroimaging genetics approach uses minimally invasive technologies to characterize the earliest pathophysiologic changes in preclinical AD. In the effort to prevent and treat AD, the short-term goal of combining multiple genetic factors, neuroimaging biomarkers, and other measures to estimate AD risk is to preselect clinical trial and research participants. The long-term goal is to provide more detailed prognoses in the clinic during the preclinical phase that can be used to create optimized treatment plans and enroll ideal candidates in specific clinical trials.

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Polygenic Approaches to Neuroimaging of Preclinical AD

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