Neuroimaging Genetic Risk for Alzheimer Disease in Preclinical Individuals: From Candidate Genes to Polygenic Approaches

Supplemental Information

The Genetics of Alzheimer Disease

Genome-wide association studies (GWASs) published since 2009 have added over 15 genetic loci to the list of genetic risk factors for AD (1–4). This brings the total number of genes implicated as risk genes in AD to 21 (5). These genes vary in their physiological function from synaptic proteins (*PICALM*) to co-chaperones (*CLU*) and mitochondrial transmembrane transporters (*TOMM40*). There are also autosomal dominant forms of AD caused by a mutation in one of three genes, namely *APP*, *PSEN1* and *PSEN2*, or by overexpression of *APP* caused by a duplication event or trisomy 21, in which three copies of the APP gene are present. These familial, inherited forms of AD provide unique opportunities for studying preclinical AD in mutation carriers, but questions remain as to how generalizable findings in familial AD will be to the much more common late-onset AD. Therefore, this review focuses on neuroimaging genetics of late-onset, sporadic AD which accounts for over 95% of total AD cases (6).

The International Genomics of Alzheimer's Project (IGAP) consortium published their first GWAS effort in 2013 (7). The study was the largest GWAS ever on late-onset, sporadic AD. Using the uniquely large cohort of 74,046 subjects amassed from four smaller data consortia the authors were able to confirm the association of previously implicated loci, as well as detect 11 new AD risk loci. Specifically, the first stage of analysis resulted in 15 genomic regions that showed an association to AD. These regions included 10 previously identified AD genetic risk factors, including *APOE*, and 5 newly implicated loci. The other 9 previously identified loci were *CR1*, *BIN1*, *CD2AP*, *EPHA1*, *CLU*, *MS4A6A*, *PICALM*, *ABCA7* and *CD33*. All available neuroimaging genetics findings for these replicated loci are reviewed below (*APOE*, *CR1*, *BIN1*, *EPHA1*, *PICALM*, *ABCA7*, *CD33*) or in the main text (*CLU*). The 5 new loci identified in the first

stage were *HLA-DRB5-HLA-DRB1*, *PTK2B*, *SORL1*, *SLC24A4*, *RIN3* and *DSG2*. Second stage replication analyses revealed 7 additional, novel loci that reached statistical significance for association: *INPP5D*, *MEF2C*, *NME8*, *ZCWPW1*, *CELF1*, *FERMT2* and *CASS4*. Notably, two loci from the first stage did not reach statistical significance in the second stage replication analyses: *CD33* (a previously identified risk locus) and *DSG2* (a novel locus). The authors found a total of 9 fully replicated, previously identified risk loci, including *APOE*, as well as 11 newly identified risk loci.

Variants identified in GWASs are usually commonly occurring and have low odds ratio associations with disease. Rare variants (occurring in less than 5% of the population) of the *TREM2* and the *MAPT* genes with the moderate to high effect size have also recently been associated with AD (8–10).

We now know more than ever before about the underlying genetics of AD. The growing list of AD risk genes serves to highlight the complexity of AD genetics and the need for more sophisticated experimental designs that combine multiple genetic risk loci. Next, we discuss important and valuable findings from neuroimaging genetics studies in the AD literature that focused on a single gene and then transition to polygenic approaches.

Neuroimaging Candidate Genes for AD

APOE in Older, Healthy Adult Cohorts

Hippocampal volumes have been shown to be smaller in *APOEε4* carriers compared to non-carriers in older healthy adults (11; 12). The rate of hippocampal atrophy is also higher in *APOEε4* carriers (13; 14). There is evidence that hippocampal volumes vary in an allele dose-dependent manner, but it is often not possible to amass enough homozygous *APOEε4* carriers to consider them separately (15). Structural MRI (sMRI) can also be used to measure structural changes within specific areas of the hippocampus. High resolution, partial field of view sMRI allows for the segmentation of the hippocampal complex into specific subregions, such as the

entorhinal cortex, cornu ammonis (CA) subfields and the subiculum. Based on this approach, several studies have provided evidence for smaller or thinner subregions in healthy *APOEε4* carriers. Specifically, entorhinal cortex and subiculum are thinner in healthy *APOEε4* carriers compared to non-carriers (16). Two additional studies performed at high-field using MR images acquired at 4 Tesla found thinner CA3 and dentate gyrus subfields in *APOEε4* carriers (17; 18). However, there are studies in which no differences in hippocampal volume between healthy *APOEε4* carriers and non-carriers were found, although they are certainly in the minority (19).

It is not clear whether there are alterations to cortical morphology in older healthy adult carriers of the *APOEε4* allele. One published study reported no significant differences in cortical volume between healthy *APOEε4* carriers and non-carriers (19). However, another study found that *APOEε4* carriers had thicker cortex in bilateral frontal and temporal regions, but a steeper longitudinal atrophic trajectory across the whole cortex (20). This supports the emerging theme that individuals with at least one copy of the *APOEε4* allele experience an acceleration of the volume loss seen in normal aging, both in the hippocampus and across the cortex.

A caveat of structural findings in the *APOE* literature is that atrophy or volume loss is generally seen as an indication of disease processes. In contrast, increased volumes or decreased atrophy rates are usually not interpreted as a possible disease feature. Intuitive results, e.g. where *APOEε4* carriers have lower or smaller volumetric measurements, are likely to appear more in the published literature. In contrast, there is no such biasing intuition in fMRI, which may partially explain why results in the fMRI field comparing *APOEε4* carriers to non-carriers are often contradictory.

APOE is a lipoprotein that transports endogenous lipids. Because myelin is composed primarily of lipids, there is interest in better understanding the relationship between *APOE* and myelin maintenance and repair. Diffusion weighted imaging (DWI) can be used to examine the potential relationship between *APOE* and myelination, using ‘white matter integrity’ measured by fractional anisotropy (FA) as a proxy for myelin health. One study found that white matter

integrity in the medial temporal lobe, but not entorhinal thickness, was associated with improved performance on a verbal memory task (21). There is also evidence for a general decrease in FA in older *APOEε4* carriers (22). Diffusion tensor imaging allows tractography algorithms to estimate the white matter pathways present in the brain. These tracts can then be used to apply mathematical concepts from the field of graph theory to DWI data. Brown and colleagues used graph theory to measure global integration and local interconnectivity in healthy, older subjects. *APOEε4* carriers showed an age-related decrease in local interconnectivity that may indicate different aging trajectories in *APOEε4* carriers and non-carriers (23). The application of graph theory to sMRI data as well as resting state fMRI data may help to elucidate the local and global network properties that are altered early in AD, but more research is needed in this area before such measures can be considered as potential biomarkers for AD.

The task-based fMRI-*APOE* literature tells a frustratingly complex and contradictory story. Some studies report increased, putatively compensatory, activity in *APOEε4* carriers (24; 25). Others report decreased activity, putatively caused by diminished function due to disease processes (26; 27). These contradictions may be partially explained by the heterogeneity of task designs used (28). Differences between tasks can be striking. For example, it is not surprising that results from a sematic memory task and a visuospatial memory task may be difficult to summarize in a single effect of the *APOEε4* allele on brain function (26; 25). Other possible confounding factors in task design can be more subtle. A “paired associates” memory task can actually vary widely in several ways including, but not limited to, the method of presentation of stimuli (audio, visual, or both), types of stimuli (images, words, etc.) and instructions (‘pay attention’ versus ‘remember these pairs’) (28). There are also studies in the literature in which investigators used non-episodic memory-based tasks, which complicates interpretation because there is evidence that *APOEε4* may exert a specific effect on episodic memory systems (29). In contrast to the whole-brain approach of the studies cited above, results from studies that examined blood-oxygen-level dependent signal in the hippocampus are more cohesive, often

reporting decreases in signal in *APOEε4* carriers. In one study, with data acquired using a high-resolution fMRI sequence, decreased activity in *APOEε4* carriers was reported in the CA2, CA3 and dentate gyrus subregion of the hippocampus (30). In another, decreased hippocampal activity during encoding was found in *APOEε4* carriers (31).

Results from resting state fMRI work in healthy older *APOEε4* carriers suggest that there may be a convergence on the DMN and connectivity therein, by which *APOEε4* carriers and non-carriers differ. In a recent study, connectivity between the posterior cingulate cortex and the hippocampus, two major nodes of the DMN, was found to be diminished in *APOEε4* carriers (32). Another study, which focused on women, reported significantly reduced DMN connectivity in carriers of the *APOEε4* compared to non-carriers (33). Lastly, a pattern of decreased DMN connectivity along with increased connectivity of another, opposing cognitive network, the salience network, have been described (34; 35). One theory that may explain DMN dysfunction reported in *APOEε4* carriers is that genetic vulnerability for AD may result in a loss of appropriate hippocampal decoupling from cortical DMN regions during an active state, such as when completing a task (36). This theory is supported by a study in which a negative correlation between hippocampus-DMN synchronization and performance on a memory test was reported (36). However, more work is needed to further test this theory.

PET imaging has helped further elucidate the relationship between *APOE* and beta-amyloid (Aβ) accumulation. While the relationship is still far from fully understood, we know the protein products of *APOE* play a role in Aβ clearance, with *APOEε4* performing worse than the ε3 or ε2 alleles (37). This idea is supported by PET imaging studies in which the relationship between Aβ deposition (measured by Pittsburg compound B (PiB) or florbetapir) and *APOEε4* carrier status is examined. Most of these studies report that healthy, older *APOEε4* carriers have increased amyloid load compared to non-carriers (38–41). There are also differences in brain glucose metabolism, measured by fluorodeoxyglucose (FDG), between healthy *APOEε4* carriers and non-carriers. A large study with 806 cognitively normal, PiB-PET negative subjects

recently showed that glucose metabolism in *APOEε4* carriers is lower in the posterior cingulate, precuneus, lateral parietal and inferior temporal regions (42). The raw magnitude of this difference was small but it was similar to differences observed between cognitively normal *APOEε4* carriers and those with mild cognitive impairment. There was also a negative correlation between average FDG uptake across the brain and age across the whole cohort, with the posterior cingulate and precuneus showing a unique vulnerability to both age and *APOEε4* carrier status (42). Hypometabolism in AD vulnerable regions in healthy *APOEε4* carriers has been reported before (43). However, a study of 600 cognitively healthy older subjects found no FDG-PET metabolism differences in *APOEε4* carriers and non-carriers (44). This discrepancy may be due to the inclusion of PiB-PET positive subjects in the latter report, who were stratified based on tracer uptake. Perhaps when subjects are binned by amyloid burden, the power to detect *APOEε4* differences in metabolism, especially in the amyloid positive groups, is reduced.

Magnetic resonance spectroscopy (MRS) is a technique that can be used to measure the relative concentrations of different hydrogen containing metabolites, each with a different peak resonance that can be plotted and quantified. Recent work using MRS in the posterior cingulate, a region particularly vulnerable to AD, has revealed that both GABA and glutamine/glutamate metabolites are reduced in individuals with MCI (45). However, the authors did not detect an association between the metabolites they measured and *APOEε4* status or amyloid deposition, which limits their usefulness as AD-specific biomarkers. In contrast, another recent study that also focused on the posterior cingulate examined choline/creatine and myoinositol/creatine ratios and found that they were significantly higher in older adult carriers of *APOEε4* compared to non-carriers (46). This finding supports earlier work in this field that found that myoinositol/creatine ratio is associated with neurodegenerative disease, as opposed to normal age related cognitive decline (47). Examining creatine levels alone, another study observed significantly lower creatine in *APOEε4* carriers compared to non-carriers (48). There is

also evidence that healthy older individuals with smaller hippocampal volume have a lower N-acetylaspartate/myoinositol ratio, which has been associated with AD, compared to their peers with larger hippocampal volume (49). Taken together, these results indicate that some metabolite measures and ratios may be useful biomarkers in individuals already at increased risk for AD.

PICALM

The gene encoding phosphatidylinositol binding clathrin assembly protein (*PICALM*) was identified as an AD risk factor in 2009 (1). The original locus (rs3851179) is located upstream from *PICALM*, but subsequent studies have not only replicated this finding but also identified additional AD-risk single nucleotide polymorphisms (SNPs) within the *PICALM* gene itself (4). *PICALM* is widely expressed in the brain. It is involved in several cellular processes, but especially in the trafficking of proteins and lipids via clathrin mediated endocytosis (50). This process, which is essential to synaptic transmission, has received increased attention in the context of AD in part because of the strong association of AD with *PICALM* uncovered in GWASs (51). *PICALM* ranks third after the *APOE/TOMM40* locus and *CLU* in terms of reproducibility in GWASs (4; 7; 52; 53). Perhaps because of this relatively highly reproduced association, *PICALM*-mediated AD risk has been examined in several neuroimaging genetics studies.

In older adults ranging from cognitively healthy to diagnosed with AD, a significant association between *PICALM* (rs3851179) and hippocampal volume was reported such that carriers of the *PICALM* risk variant had lower hippocampal volume (54). The authors also found a link between *PICALM* risk and reduced entorhinal cortex thickness. The latter finding was replicated in another study that found that the *PICALM* risk allele is associated with a thinner entorhinal cortex in older adults (55). However, *PICALM* was not associated with either hippocampal or entorhinal cortex volume in a cohort of healthy young adults (56).

The relationship between *PICALM* and the functional connectivity of the hippocampus was recently reported in a resting state fMRI experiment. When compared to subjects who were homozygous for the protective allele, risk allele carriers showed weaker negative functional connectivity of the hippocampus to many cortical regions (57). However, this finding from a relatively small cohort is preliminary and needs to be replicated. Lastly, a study examining amyloid deposition as measured by florbetapir-PET found an epistatic effect involving variants of *PICALM* and *BIN1*, another AD risk gene (58). This study is further described below (see *BIN1*).

CR1

Unlike *PICALM*, expression of complement component (3b/4b) receptor 1 (*CR1*) is likely to be low in the brain (59). The CR1 protein's function is complex and varies by cell type. Generally, it is involved in the regulation of the complement cascade, a major component of the innate immune system that helps to amplify the response of the immune system to potential targets. In addition, the CR1 protein is involved in transporting opsonized immune complexes through the circulatory system for efficient removal (60). Neuroinflammation has been associated with AD for many years, but has sometimes been dismissed as a consequence, rather than a cause, of the disease (61). However, this perspective is shifting and inflammatory processes are being studied as potential pathogenic processes in AD (51). One reason, among many, why interest in neuroinflammation and AD has been renewed in recent years is the discovery of an association between a polymorphism in *CR1* and AD in a 2009 GWAS (3).

The *CR1* risk variant was associated with thinner entorhinal cortex in a study including healthy older adults (54). Interestingly, there is also evidence that the *CR1* risk variant is associated with lower entorhinal cortex volume in *young* healthy adults (62). This finding was confirmed in two independent cohorts. Additional research is needed to assess whether the relationship between *CR1* variants and entorhinal cortex is reproducible in larger samples.

BIN1

Bridge integrator 1 (*BIN1*) was reported as a risk gene for AD in 2010, after a borderline significant association was reported in one of the large 2009 GWASs (1; 52). Like *PICALM*, *BIN1* is involved with the intracellular trafficking of lipids and proteins. *BIN1* encodes a protein that has at least ten known isoforms (60). These isoforms have specific domains that influence their function. In the brain, there is one isoform of BIN1 and it contains the clathrin-associated protein-binding (CLAP) domain, which plays a role in clathrin mediated endocytosis (63). Clathrin mediated endocytosis is an essential process in synaptic vesicle recycling, which is an essential component of efficient synaptic transmission. *PICALM* and *BIN1* are implicated as molecular components of this neuronal process, which may suggest that variability in synaptic transmission efficiency contributes to AD pathology, especially during the early phase characterized by synaptic loss and neuronal death (51).

Because *PICALM* and *BIN1* are both related to synaptic transmission, one group tested for possible epistatic effects between the risk locus for each gene identified in GWASs. Hohman and colleagues used florbetapir-PET scans to test for a possible interaction effect of *BIN1* and *PICALM* on amyloid deposition (58). The authors reported that there was indeed an interaction and that this interaction was reproduced in a second, independent dataset. The *BIN1* risk variant was related to greater amyloid burden, but only in persons who were carriers of the *PICALM* protective variant. This study illustrates a limitation of candidate gene studies because both *BIN1* and *PICALM* were not related to amyloid deposition when examined on their own.

There is, however, evidence that *BIN1* genotype is directly associated with a neuroimaging-based structural biomarker of AD. Based on the preliminary evidence from the 2009 GWASs that *BIN1* may be associated with AD, Biffi and colleagues tested for an association of the *BIN1* risk variant and several neuroimaging phenotypes. The authors showed that the *BIN1* risk variant is associated with thinner temporal pole and entorhinal cortex (54).

Soon after Biffi and colleagues published their paper a *BIN1* locus reached genome-wide significance in a new AD GWAS (52).

ABCA7

ATP-binding cassette, subfamily A, member 7 (*ABCA7*) is one gene in a group of highly conserved transmembrane transporters. These transporters participate in active transport of various substrates across membranes, both at the level of the cell and the organelle (64). *ABCA* transporters are linked to cholesterol and lipid homeostasis, and appear to work directly with *APOE* by transporting lipids out of the cell to be cleared by *APOE* (65). This coordination with *APOE* may be a clue to the mechanism of the association between *ABCA7* and AD. *ABCA7* was first linked to AD in the results of a GWAS in 2011 (2).

The AD-associated locus near *ABCA7* has been studied in one recent neuroimaging study in which the authors were interested in the relationship between cholesterol levels and amyloid deposition (66). Hughes and colleagues described a greater than 2-fold increased risk of amyloid positivity, as measured by PiB-PET, in carriers of the *ABCA7* (rs3752246) risk variant.

EPHA1

EPHA1 is a member of a superfamily of proteins called the receptor tyrosine kinases and is expressed in multiple tissues including the brain (67; 68). The Eph-ephrin family of receptors and ligands are all membrane-bound proteins involved in adhesion and cell-cell contact mediated signaling, like in axonal guidance during development (68). The association between *EPHA1* and AD was first described in two GWASs published in 2011 (2; 4). The neurobiological basis of this link to AD may be related to the high expression of the *EPHA* receptor class in the hippocampus, but the expression and function of specifically *EPHA1* in the hippocampus is not well understood (69).

One study took a neuroimaging genetics approach to describe the relationship between *EPHA1* and amyloid in the brain. Hughes and colleagues found that the *EPHA1* is associated with the likelihood of being amyloid positive, as measured by PiB-PET. In contrast to the findings for *ABCA7* described above, the authors found that risk of amyloid positivity decreased for each C allele of *EPHA1* (rs11767557) (66).

CD33

Sialic acid binding immunoglobulin-like lectin-3 (*CD33*) is a membrane-bound receptor expressed on immune cells (70). *CD33* plays a role in the differentiation of immature immune cells, as well as in the signaling of mature immune cells in the innate and adaptive immune system (70). Despite strong evidence from several GWASs that a variant of *CD33* is associated with AD, this association was not fully replicated in the IGAP consortium GWAS (2; 4; 7). This failure to replicate the association in the largest AD GWAS to date casts some doubt on the strength and reproducibility of this gene's association with AD. Perhaps the association is specific to certain regions and ancestries, even within Caucasian populations. A single study has examined the relationship between *CD33* genotype and a neuroimaging phenotype. Bradshaw and colleagues found that the risk variant of *CD33* was associated with greater, more diffuse amyloid deposition as measured with PiB-PET imaging (71).

Advanced Association Models

In addition to more traditional regression approaches, advanced association models can be used to confront the challenges of working with large datasets in neuroimaging genetics. Canonical correlation is a method for interpreting large cross-covariance matrices that maximizes correlation between linear combinations of pairs of vectors within a given matrix. Sparse canonical correlation takes this process a step further by minimizing the number of features used to find the maximum correlation structure using, for example, the well-known least

squares approach (72). Sparse canonical correlation has been used to explore genetic risk factors for AD affecting the hippocampal surface (73). Looking only at AD risk genes as listed in the AlzGene database, the authors of that study found that *APOE* and *TOMM40* were associated with hippocampal surface differences in sparse regions, including anterior and middle areas (73). Variations of the sparse canonical correlation approach have been used in two other studies focused on AD (74; 75). The first used a "knowledge-guided" algorithm that accounted linkage disequilibrium and genetic co-expression networks and examined the relationship between SNPs within *APOE* and amyloid deposition as measured by florbetapir-PET (74). This study identified only a single SNP in *APOE* that was associated with amyloid deposition, but they argue that their method can be scaled up to genome wide studies. The second study that used a similar approach and also examined *APOE* discovered an association between a specific SNP and gray matter density in right hippocampus (75).

Limitations

Clinical Utility of GWAS Loci: The Search for Causal Variants

The causal variants that give rise to the *APOEε4* allele are known polymorphisms at rs429358 and rs7412. Variants at these loci alter the structure and function of the translated APOE protein (76). In fact, *APOEε4* "structure correctors", which make protein products of *APOEε4* behave like the more common protein products of *APOEε3* are currently being developed as a possible treatment for AD (77). In contrast, many of the GWAS-identified AD risk loci are located in intronic (*CLU*, *ABCA7*) or intragenic (*BIN1*, *EPHA1*) regions with no evidence that variants affect protein structure or function. An intragenic region may play some regulatory function, but in the cases of *EPHA1* and *BIN1* there is little evidence of conservation of these intragenic regions, which makes a regulatory role in genetic expression unlikely (60). There is even some debate over whether or not genes are correctly identified when significantly associated loci reside in non-coding regions. The common approach is to report the SNP as related to the nearest gene,

but this is not necessarily the case. The search for the causal variants for these genes and for genes implicated in other common disease by GWASs is still ongoing (78). Ostensibly, the causal variant for one of these genetic risk loci will be a polymorphism in high linkage disequilibrium with the GWAS locus. In addition, the polymorphism should affect the downstream structure or function of the gene's RNA or protein product. The utility of GWAS-identified risk genes as potential drug targets is limited without first identifying the causal variants driving the association at each locus. One important step in this effort is the development of a functionally annotated genome and the tools to explore it, such as ENCODE (https://www.encodeproject.org). Using ENCODE investigators can quickly discover basic functional information about a locus of interest, perhaps one they identified in a GWAS. The functional elements annotated in the ENCODE project help investigators distinguish between, for example, regulatory elements, close-range promoters and genes that are likely to be transcribed (determined using RNA-seq and similar techniques). ENCODE is an excellent example of a large-scale collaborative project that will enhance the scientific community's ability to interpret genetic association signals.

Mechanistic Interpretations and Neuroimaging Genetics

The incorporation of human genetics into neuroimaging studies has identified brain traits that are associated with specific genetic variants or, more germane to this review, with genetic risk scores. However, as described in the text that precedes this section, the genetic loci are often identified via GWAS and thus, the causal variant is not known. This inherently limits the mechanistic insights researchers are able to gain from neuroimaging genetics studies of this kind. For *APOE*, for which there is no ambiguity about causal variants, neuroimaging has revealed that carriage of the *APOEε4* allele is related to increased amyloid deposition as measured by PET imaging which, in turn, is related to neuronal death and a higher rate of cortical thinning in AD-vulnerable regions when compared to matched controls who do not carry

the risk variant. Saykin and colleagues (2015) describe a multi-step process to move from genetic signals to targeted therapeutics in which genetics and neuroimaging intersect at the first step (discovering genetic loci that are robustly associated with a relevant trait) and the final step (identifying individuals most likely to benefit from experimental therapies) (79). The middle steps include identification of causal genes, testing hypothesized mechanisms in model systems and developing therapeutics that act on these mechanisms. Thus, we believe the salient point is that neuroimaging genetics research is essential to the development and execution of therapeutic hypotheses, even if, in isolation, these studies do not always yield new mechanistic insights.

Generalizability Across Ancestries

It is important to recognize that all the largest AD GWASs used large cohorts of Caucasian European or American subjects. This creates a potential problem with generalizability to other ancestry groups, especially that of African ancestry (80). While there are published GWASs examining AD genetics in minority ancestral groups, one only of these, focused on African American participants, has topped 1,000 participants in the case and control groups ((81); see Table 1 in (82)). Thus, these groups remain understudied compared to the very large GWASs with non-Hispanic Caucasian participants. The genetic loci implicated by studies of Caucasians might fail to replicate in a cohort of subjects from a different ethnic background due to population specific variants, differing patterns of linkage disequilibrium or even a heterogeneous genetic basis of AD in different ethnic groups (83). To illustrate this issue, consider that many small GWASs have tried to replicate the association of *CLU* with AD in non-Caucasian cohorts. The results of these studies indicate that there is an association between *CLU* and AD in Chinese cohorts, but not in cohorts of non-white Americans or Europeans (53; 84; 85). The limited generalization of results from large published GWASs in AD is a problem and a greater effort must be made to amass comparably large samples of different ancestral groups for new association studies. This effort may lead to the identification of certain genes that are associated

with AD regardless of genetic background. These genes would be good candidates for increased research resources and drug targeting due to their greater generalizability. Also, importantly, further exploration of the genetic basis of AD in people of African and Hispanic descent may help elucidate any biological bases for the epidemiological differences observed in these ethnic groups, including higher incidence and earlier onset of AD (86).

Small Sample Sizes: Consequences for Neuroimaging Genetics

As eloquently described by Button and colleagues (2013), small sample sizes in neuroimaging studies decrease statistical power which leads to a decreased rate of detectable true positive results while leaving the rate of false positives unchanged (87). This has the effect of increasing the likelihood that a significant result is, in fact, spurious. Small sample sizes also bias studies toward large effect size results, as these are the only results that can be significant given the power limitations. The latter phenomenon has been dubbed the “winner’s curse” and leads to studies that are very difficult to replicate (87). Given these known problems, why are neuroimaging studies with small samples still (albeit less and less so) prevalent? This is related to the relatively high cost of acquisition of neuroimaging data, the currently accepted need to “pilot” and publish new paradigms and techniques before formal funding for large-scale studies can be won and also immense pressure, especially on young investigators, to publish frequently (88). Taken together, it is clear that sample size is a very important consideration when performing a neuroimaging genetics study and robust power analyses are a crucial component of any research program.

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