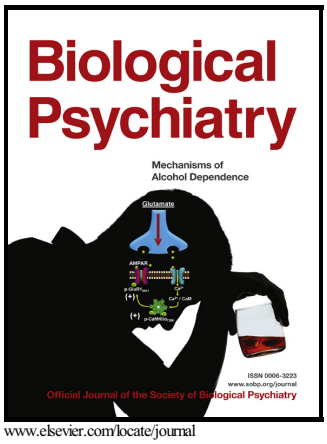

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Imaging Genetics and Genomics in Psychiatry: A Critical Review of Progress and Potential

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Abstract

Imaging genetics and genomics research has begun to provide insight into the molecular and genetic architecture of neural phenotypes and the neural mechanisms through which genetic risk for psychopathology may emerge. As it approaches its third decade, imaging genetics is confronted by many challenges including the proliferation of studies using small sample sizes and diverse designs, limited replication, problems with harmonization of neural phenotypes for meta-analysis, unclear mechanisms, and evidence that effect sizes may be more modest than originally posited, with increasing evidence of polygenicity. These concerns have encouraged the field to grow in many new directions including the development of consortia and large scale data collection projects as well as the use of novel methods (e.g., polygenic approaches, machine learning), which enhance the quality of imaging genetic studies, but also introduce new challenges. Here, we critically review progress in imaging genetics and offer suggestions and highlight potential pitfalls of novel approaches. Ultimately, the strength of imaging genetics and genomics lies in its translational and integrative potential with other research approaches (e.g., non-human animal models, psychiatric genetics, pharmacologic challenge) to elucidate brain-based pathways that give rise to the vast individual differences in behavior as well as risk for psychopathology.

By linking genetic and epigenetic variation to brain structure, function, connectivity, and chemistry via neuroimaging measures (1), imaging genetics and genomics can inform the neural mechanisms through which genetic and molecular differences impact cognition, emotion, and behavior in health and disease. Since being pioneered nearly 20 years ago by candidate gene studies of receptor ligand binding (2-6); **Supplemental Material**), imaging genetics has incorporated a host of allied neuroimaging techniques, most frequently, structural and functional magnetic resonance imaging (sMRI, fMRI) and has been integrated with traditional psychiatric genetics (7-9) and non-human animal models (10-13). More recently, this approach has been extended to epigenetics (14,15), and, as imaging genomics, to discovery-based (16,17) and polygenic (18,19) approaches.

Accompanying an exponential increase in publications, imaging genetics and genomics has also been confronted by several qualitative concerns including the proliferation of studies with small sample sizes, limited replication, unclear mechanisms relating genes to brain and brain to behavior, and evidence that effect sizes may be smaller than originally thought, and perhaps no larger than effects for traditional psychiatric diagnoses (9,20). Such concerns, and the desire to find new genes and pathways via genomic approaches, have led to the formation of consortia and large-scale projects to increase sample size (21-26) as well as the adoption of methodological and technological innovations in genetics (e.g., GWAS, epigenetics), neuroimaging (e.g., multimodal PET/fMRI), and psychiatric genomics (e.g., polygenic risk scores, LD score regression) (9,14,18,27-30), all of which enhance the quality of imaging genetic studies, and each of which is also subject to new potential pitfalls.

Here, we critically review the current state of imaging genetics and genomics highlighting unique strengths, considerations, and limitations of distinct approaches while

considering their utility for psychiatry going forward. We suggest that some criteria to evaluate the usefulness of intermediate phenotypes according to an endophenotype conceptualization are retrograde and counterproductive when applied to imaging genetics in some instances. We argue that single variant analyses remain informative in the context of a polygenic architecture that underlies the majority of imaging phenotypes. Further, we discuss the lack of replication in imaging genetics and what has been learned, and not learned, from meta-analytic efforts. Next, we review the use of candidate and discovery-based polygenic methods that aim to better characterize the complex polygenic architecture of imaging phenotypes and consider pitfalls that these techniques may face and how they may be minimized. We highlight the potential of molecular genomic methods to verify and mechanize relationships between the dynamic genome and neural phenotypes. Finally, we consider how imaging genetics and genomics hold their greatest potential not in isolation, but as methods that can be used alongside other techniques (e.g., pharmacologic challenge), levels of analysis (e.g., the transcriptome, psychiatric genetics), and non-human animal research (e.g., genetic models) in the search for mechanistic consilience (**Table 1**). As imaging genetics and genomics further integrate with molecular genetics, basic neuroscience, and psychiatric genetics, and begins to accumulate not only large but also longitudinal samples, it will be able to more adequately model and test the complex interplay between genes, the brain, body, environment, and behavior and expand these pathways (**Figure 1**). It is hoped that such mechanistic characterization will ultimately improve the nosology, treatment, and prevention of mental illness.

Is the Endophenotype Conceptualization of Intermediate Phenotypes Useful?

Theoretically, intermediate phenotypes, such as imaging phenotypes, lie along a mechanistic pathway through which genetic variation and/or environmental experiences contribute to clinical phenotypes (**Figure 1A**)(31). Here, we refer to the traditional pathway from the static genome to neural intermediate phenotypes and behavior, although modern genetics regularly challenges such unidirectionality (**Figure 1B**). Within the theoretical discussion of intermediate phenotypes, the greatest attention has often focused on the endophenotype conceptualization, which stipulates that endophenotypes are associated with psychiatric disease and heritable, among other considerations (32).

The requirement of disease-association presupposes the research value of psychiatric nosology. This is problematic because many, if not all, psychiatric diagnoses are heterogeneous amalgamations of symptoms, with the same diagnosis having distinct putative etiologies, as is becoming more clear following RDoC (33,34). Such diagnostic heterogeneity may dilute, and even obliterate intermediate phenotype–disease association. For example, although anhedonia is a cardinal symptom of depression, it is not amongst the most common symptoms (35). As such, anhedonia-related neural circuitry may not be identified or minimized in a general patient/control study (36,37). Indeed, some reports have associated depression with blunted reward-related activity in the ventral striatum (38,39), while others have not (40). Or consider that despite the polygenic nature of psychosis (41), some patients presenting with psychosis have a genetic variation in Huntingtin(42), or velocardiofacial syndrome(43). Thus, it is possible that distinct etiologies associated with unique presentations could be lost or minimized by a reliance on diagnosis (44). The positive results of a recent GWAS study on melancholic depression, a more severe and homogenous form of the disorder further reinforce this concern (33),but see(45). This

is not to imply that understanding variability in disease associated intermediate phenotypes is not important, but rather that constraining imaging genetics research to intermediate phenotypes or genes previously linked to a disorder may stifle research on etiologic brain-based associations by generating an intellectual *file drawer problem* where only hypotheses satisfying endophenotype-disease correspondence are evaluated impeding the development of etiologically-based classification.

Heritability is on a scientific basis a more logical endophenotype criterion. Twin studies have largely focused on the heritability of morphological measures, which approach the upper end of psychiatric estimates, ranging from 60-80% (46,47). The few studies of brain function suggest more modest estimates (~40%)(46). However, *intermediate phenotypes* that are not heritable can still have genetic origins and mediate relationships between genes and behavior. For example, Williams Syndrome, which is characterized by a host of physical and personality characteristics, including excessive sociality is attributable to a microdeletion that typically occurs during the formation of germline cells in people with no history of the disorder (48,49). Common variation within the genes (e.g., *GTF2I*) spanning the microdeletion region have become candidates that are informing phenotypes related to sociability (22,50). Intermediate phenotypes can also represent stable trait differences that while not entirely heritable per se, are dependent upon experience arising as the product gene by environment interactions (e.g., *FKBP5* (20,51,52); **Supplemental Material**). Additionally, within genetic studies, non-heritable intermediate phenotypes may characterize individuals who are part of distinct, non-genetic subgroups, and who would otherwise be indistinguishable diagnostic phenocopies; such insight may contribute to subgroup classification, and diagnostic refinement. Finally, heritability refers to loci shared identical-by-descent representing the static genome. However, genes may traverse

an imaging phenotype on their pathway to behavior, even when the intermediate phenotype is not heritable. For example, a new wave of epigenetic research (14,53-55), examining markers among discordant monozygotic twins is poised to take advantage of non-heritable intermediate phenotypes. The validity of a result that would depend on the phenotype being highly heritable (e.g., polygenic risk) would be suspect if the phenotype was not. However, the widespread application of this criterion could unintentionally impede important etiologic insight generated from genomic research on non-heritable neural phenotypes.

Single Variant Approaches

The majority of imaging genetics research has been conducted within a candidate gene framework. Most studies have focused on a limited number of functionally characterized polymorphisms (e.g., *COMT* rs4680(56), *SLC6A4* 5-HTTLPR(57) within genes coding for products that influence particular neural systems. Most of these variants have been inconsistently associated with neural phenotypes and psychopathology with both positive and null associations reported (58-61). Recently, the unprecedented success of genomewide association studies (GWAS) has identified new candidate genes (e.g., *KTN1(16)*) and corroborated the role of prior suspects (e.g., *SIRT1(33,62,63)*). Polymorphisms discovered in psychiatric GWAS are now being investigated within a candidate framework with promising results emerging (64-66), though other evidence suggests limited overlap between polymorphisms associated with clinical and neural phenotypes (9,67).

The Controversy of Candidate Associations

As in psychiatric genetics(60,61), the intuitive mechanistic and interpretable appeal of candidate imaging genetics findings have led to many replication and extension studies, and as many contradictory findings. Several meta-analyses have concluded that effect sizes are likely smaller than originally reported, may represent false positive associations, and that publication bias may promote false confidence in the robustness and biological importance of these effects (58,68,69). However, the utility of meta-analysis for some imaging phenotypes is questionable.

Meta-analysis tends to work best under two conditions. First, when constructs are measured in a standard fashion (e.g., obesity and type 2 diabetes (70), they estimate effects with great precision. Second, study design differences across studies can be modeled *with* a large number of studies using each design, allowing meta-analyses to examine whether design differences influence associations. Within neuroimaging, the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) consortium has successfully harmonized imaging data across studies to meta-analyze structural phenotypes in a GWAS context(16). However, because many imaging genetics studies, such as those probing task-related activity, do not use standardized methodology (e.g., task and analysis), they present unique challenges.

Methodological differences across neuroimaging studies can meaningfully impact the nature of measured neural phenotypes. For instance, consider the literature on amygdala reactivity to emotional faces in autism. Early studies produced evidence of hypoactivation (71,72). However, eye tracking research has shown that children with autism typically avoid eye contact (73), which conveys important emotional information and robustly recruits amygdala activation(74). Studies directing or measuring participant eye gaze have shown elevated amygdala reactivity in autism that is correlated with eye gaze duration (75-77). A meta-analysis

not considering such design differences, may produce data that autism is not associated with amygdala function (78). This is not to suggest that studies of non-imaging phenotypes are impervious to these challenges (e.g., self-report versus measured weight) or that such differences are responsible for inconsistent findings, but merely, that harmonization challenges are heightened among meta-analyses of some imaging phenotypes.

Meta-analyses have attempted to model differences across studies. For example, in a meta-analysis of the relationship between 5-HTTLPR genotype and amygdala function, Murphy and colleagues (68) examined whether a host of study characteristics influence the association. However, the small number of studies using each design and variability within study groupings may have left this approach unable to adequately model differences. For example, studies were coded according to ethnicity and studies of German (79) and Korean participants (80), where grouped together as "not European/Mixed." Such heterogenous representations of study variability are inadequately powered and conceptualized leaving the conclusions of marginally significant small effects, debatable. What remains unequivocal is that data are inconclusive; whether positive or null associations better represent reality can only be addressed by further research. Overall, meta-analytic approaches have provided road maps for challenges associated with candidate studies (60,81) and identified loci conferring small effects for psychiatric and structural neuroimaging phenotypes (16,82). However, the utility of meta-analyses incorporating studies using diverse methodology when study related differences cannot be systemically evaluated, is questionable.

Much like data on complement component 4 and schizophrenia (83), some imaging genetics phenotypes may presently be better informed by convergence across modes of investigation (e.g., **Table 1**). For instance, the significance of 5-HTTLPR findings may be

weighed alongside observations in non-human animal models, and effects of the 5-HTTLPR polymorphism on serotonin transporter expression (84). For example, work using tissue oxygen amperometry (which measures hemodynamic responses equivalent to BOLD fMRI in freely moving rodents;(85), has shown that serotonin transporter overexpression reduces amygdala responses to aversive cues in mice; a finding remarkably convergent with significant results reported in the human 5-HTTLPR BOLD fMRI amygdala literature (86,87).

Genomewide Association Study Approaches

Much like initial psychiatric GWAS, the first imaging GWAS did not identify any genomewide significant polymorphisms, likely due to inadequate power (17). While other early imaging GWAS have observed genomewide significant results these were not replicated (88). Arguably, within imaging, GWAS did not become particularly informative until the development of large consortia such as ENIGMA (21), through which investigators have pooled effect size estimates to achieve samples large enough to reliably detect loci of small effect (9,16). For example, two GWAS have linked rs7294919 genotype to hippocampal volume (89,90), with subsequent candidate replication (91).

In addition to identifying new polymorphisms, GWAS data invite speculation regarding prior assumptions. Indeed, recent evidence suggests that genetic associations for schizophrenia and subcortical brain volume are similarly small in size and non-overlapping (9). If data accumulate showing that neuroimaging measures are not associated with larger effects than clinical diagnoses, it will be important to consider factors that may contribute to this. For instance, despite being etiologically and descriptively heterogenous, clinical diagnoses have been

well studied psychometrically and have acceptable to excellent reliability, with notable exceptions (depression, generalized anxiety(92). However, other than structural neural phenotypes, which have evidence of robust reliability (93,94), the reliability of many neuroimaging phenotypes has not been rigorously investigated with inconsistent effects reported and conclusions unclear (95-99).

The success of meta-analytic psychiatric GWAS (41) has led to suggestions that GWAS may best inform psychiatry by using large samples with relatively easily assayed phenotypes (100,101). As a consequence, imaging genetics would be most useful to understand the neural mechanisms underlying these associations. Clearly, this approach has utility, as multiple studies are beginning to demonstrate (66). However, much like the endophenotypic conceptualization, this approach presumes the value of our current conceptualization of mental illness, and further, assumes that loci linked to a particular disorder would also be linked to related neural phenotypes. However, unlike data suggesting that depression, subjective well-being, and neuroticism share substantive overlap in associated genetic variation (102), there is no overlap between genetic variation contributing to indices of subcortical brain volume and schizophrenia (9). Broadly, these results suggest that psychiatric and intermediate phenotype GWAS may provide different information that may ultimately lead to refined conceptions of mental illness decades in the future. Immediately, these data suggest that subcortical volume abnormalities observed in schizophrenia may instead arise from rare mutations (e.g., de novo), schizophrenia itself (103), its treatment (104,105), associated risk factors and potential GxE(106). By probing overlap across clinical phenotypes with neural outcomes, imaging genetics may usefully inform the origins of individual differences among psychiatrically-relevant neural phenotypes (e.g., subcortical volume schizophrenia). As larger samples accumulate (Ns>3000), techniques such as

LD score regression (107) may be used to estimate genetic correlations across neural and behavioral phenotypes. Imaging genomics may identify novel loci that do or do not map onto diagnostic categories, but may nonetheless contribute to our understanding of psychiatric conditions and potentially lead to refined nosology and treatment in the future.

Polygenic Approaches

With the exception of ligand-based neuroimaging techniques that target specific receptors, *in vivo* neuroimaging data provide assays of higher-order neural circuit function and structure reflective of thousands of interacting neurons and glia. As such, this resolution may be incongruous with the action of single genetic variants (20) leading imaging genetics and genomics to adopt polygenic techniques to quantify aggregate influence.

Polygenic Scores

Polygenic scoring approaches fall broadly within two categories: *polygenic risk scores (PRS)*, and *biologically-informed multilocus profile scores (BIMPS)* (20). The PRS approach summates "risk" alleles or weighted effects based upon prior GWAS summary statistics (108) and can identify neural mechanisms correlated with genetic risk for psychopathology. For example, depression PRS are associated with reduced ventromedial prefrontal cortex thickness, which is, associated with negative affect(18). For PRS studies to be maximally informative, particularly for phenotypes common in non-ascertained samples, it is important to evaluate whether associations remain after taking into account phenotypic expressions of the disease. Ideally, such relationships could be tested longitudinally to examine whether PRS-based associations with

neural phenotypes precede and predict psychopathology. Notably, the PRS approach allows genomic liability to psychopathology to be evaluated among individuals without disorder expression thereby avoiding confounds of medication and disease process which plague the etiologic insight of psychiatric case-control studies (109). Further, unlike other approaches that estimate bivariate genetic correlations (e.g., LD score) PRS allow for the degree of polygenecity to be examined and are more amenable to smaller samples, as long as the discovery cohort is sufficiently powered. Lastly, the application of Bayesian analytic approaches may have utility for imaging genetics in this context as they have improved observed effect sizes in a psychiatric phenotypes(110).

There are several limitations to the PRS approach however. It assumes additivity alone (which is supported(111)) and neglects potential epistatic effects, which while observed in imaging genetics studies (112,113) have yet to be widely replicated (114). Also, by aggregating across the genome, when used in isolation, PRS provide no insight into potential underlying molecular mechanisms. Further, this approach is constrained by the phenotypes used in the discovery-based sample, which may introduce heterogeneity (34,92) or be unrelated to the neural phenotypes under study (9). It is plausible that PRS are composed of heterogeneous gene sets contributing to distinct aspects of psychiatric diagnoses, wherein brain relationships are not observed within the full set but potentially a subset. Moreover, the predictive utility of PRS are largely based upon the sample size of discovery datasets, which arguably are just beginning to be achieved (115). Lastly, while recent developments in CRISPR/Cas techniques have facilitated multiplex genomic editing (116,117) that may eventually approximate polygenic risk, PRS approaches are not currently amenable to direct translational work in non-human animals.

The BIMPS approach summates functionally characterized polymorphisms across a given neural system to derive a composite of relative signaling within that pathway. For example, Nikolova and colleagues (28), found that BIMPS reflective of genetically-conferred elevations in dopamine signaling are predictive of elevated reward-related ventral striatum activity. Arguably, BIMPS approaches compound concerns regarding higher false discovery rates for candidate genetic association studies because they rely on priors for the genes (and loci in those genes) that constitute the system, assume how individual variants collectively contribute to overall signaling, posit that the action across these loci is additive, and provide multiple plausible profiles to be developed. For example, in light of opposing relationships between prefrontal and subcortical dopamine signaling (118,119), a dopamine BIMPS could reasonably be developed that reverse codes predominantly cortical-based genetic influence (120) as opposed to tonic dopamine regardless of region (28). As a result, it will be critical for future research to attempt replication defined as the same BIMPS and phenotype.

Notably, the integration of PRS and BIMPS may prove particularly efficacious. For example, imaging genetics could use GWAS-based results from psychiatric genetics to prioritize variants within a given system or systems. Using this framework, a recent study discovered common genetic moderators of the transcriptome response to stress hormone activation, that were also associated with depression (8). A PRS/BIMPS polygenic profile of variants associated with both stress hormone transcriptome response and depression was associated with overgeneralized amygdala responsiveness, providing a putative neural mechanism through which the transcriptome response to stress may influence depression risk (8).

System and Pathway-Level Analyses

Multiple methods have been developed to explore genetic variation at a system or pathway level, in either an *a priori* or exploratory fashion. When evidence suggests that a particular protein or connected system contributes to a neural phenotype, yet SNP-based priors are unavailable or limited, candidate gene-level and systems-level analytic approaches may be employed, as has been more commonly done within psychiatry (7,121), but are beginning to be implemented in the context of imaging genetics (27). Clearly such approaches require adequate correction for the multiple exploratory tests conducted within and across sets to reduce Type 1 error rates; permutation-based procedures that keep genetic architecture intact while shuffling an intact phenotypic structure are particularly suited for this. Notably, how genetic variation within a gene/system-set is aggregated is controversial, with averaging being the most common (7). Nonetheless, results emerging from such analysis may prioritize particular sets and polymorphisms for further research interrogating potential function.

Using a more agnostic approach, GWAS data may also be mined to identify enrichment in known systems (122). For example, by using a full-genome pathway analysis (i.e., reducing 909,622 SNPs to 1,658 pathway), calcium responsive pathways were linked to neural activation to a face matching task in the absence of a genomewide significant locus (123). One benefit of pathway enrichment analyses is that it distills genomic data into genetic data representative of defined neural systems leading to data that may be more mechanistically interpretable and allow for greater translation with non-human animal models and pharmacologic challenge studies that can precisely target these systems. A unique concern of this approach is that it is restricted by known protein-protein interaction databases ((124) that may neglect known and unknown functional interactions among proteins.

Multivariate and Machine Learning Methods

As an alternative to univariate models, imaging genomics has begun to adopt "big data" techniques to facilitate data-driven discovery including the simultaneous modelling of genetic and imaging data to identify components with shared variance (29,125-127)). For example, parallel independent component analysis (p-ICA(29), uses genomewide and whole-brain imaging data to yield clusters of functionally related SNPs that are correlated with phenotypic components. Though traditionally performed agnostically at the whole-genome whole-brain level, modified hybrid approaches allow for the incorporation of prior information while also providing data-driven estimation (128). The multivariate fusion of imaging and genetics data allows for the identification of statistically linked genomic and neural components, which may provide insight into common mechanisms. Additionally, machine learning methods are beginning to be used in imaging genetics and genomics to predict or classify disease outcomes, which is perhaps the most direct clinical application of such methods for psychiatry. The use of these techniques in imaging genetics and genomics have typically relied upon well characterized candidate genes (e.g., (129), though data-driven analyses are also emerging (130). While in its infancy, considering clinical, neural, and genetic features in tandem for disease prediction is a promising future avenue of exploration that may have important clinical ramifications.

Despite their many benefits, multivariate techniques face a variety of unique limitations. Indeed, their use within psychiatric genetics has been controversial (131,132). For example, the high dimensionality of data frequently violates assumptions by including more features (i.e., input variables) than observations (i.e., participants). As such, dimensionality reduction is typically required. Correspondingly, the vast number of inputs, multiple tests performed, and

increased number of parameters being estimated risks overfitting the models and necessitates a heightened reliance on replication to confirm associations. However, the use of proper out-of-sample cross-validation approaches (e.g., leave-N-out), common in the machine learning literature outside of imaging genetics, can maximize the generalizability of a given study and, as such, should be universally adopted within the field.

Imaging Genetics and Genomics Going Forward: Conclusions

As imaging genetics and genomics prepares to enter its third decade, the field has exponentially expanded from its modest candidate gene investigation of ligand binding to include large scale single studies with more than 1,000 participants (perhaps unfathomable to neuroimaging researchers even 10 years ago), longitudinal designs, extensive data sharing, cross-modal investigation, and translation with non-human animal and psychiatric genetics research (21-26). Moreover, the field has begun to adopt novel methodology (e.g., the transcriptome) and analytic approaches (e.g., PRS, pathway analyses). This growth will undoubtedly enhance its ability to generate new etiologic knowledge that may ultimately enhance psychiatric nosology, treatment, and ideally, prevention. However, the same standards of skepticism, interest in replication, and insistence on biological validation apply as have arisen in candidate gene, one-locus at a time, imaging genetics. Arguably, as imaging genetics and genomics data increase in dimensionality and testing (e.g., data sharing), these concerns are only heightened.

Replication: Let's Do It When We Can but Accept When We Can't

As proposed by Carter and colleagues (133), and is applicable to research across fields (134), replication and appropriate correction for multiple testing is critical for confidence in research findings. While direct replication is the sine que non, it is rarely done within an imaging genetics study (e.g.,(16), and we are hesitant to recommend it as a blanket criterion for publication, even when studies are small (133). Often methodological innovations are accompanied by substantive cost and going forward we could envision small samples that could yield formative insight into the genetic architecture of neural phenotypes that could not feasibly be replicated (e.g., recruitment based upon a rare variant; PET studies)(135). Ideally, we would replicate every association before it is published. However, we also must work within practical funding constraints. When replication can be tested, it undoubtedly should and null results should not be discouraged by journals (133). However, when replication cannot be attempted, perhaps it is best to take it for what it is – it might be an exceptionally innovative study that provides formative insight or a false positive, that unfortunately, may bias future research(135). The publication of null results and addressing citation biases within the literature (136), would help combat the development of such biases. When replication cannot be reasonably obtained, a compromise is using within-sample cross validation, which is feasible for small studies and would make inferences more generalizable. Further, it will be important to critically evaluate the properties of imaging phenotypes that may influence replication such as reliability and the factors that may influence this (e.g., time of day) to distill imaging phenotypes into trait-and state-related facets that enhance their research utility. Lastly, in addition to replication, we believe that evidence of consilience should also be considered when evaluating findings, particularly in the context of increasing collaboration between imaging genetics, molecular genetics, non-human-animal

models, as well as psychiatric and behavioral genetics ((84); **Table 1**). Evaluating convergence across methods is particularly useful when meta-analytic approaches may not yet be able to adequately model widespread between study variability. Indeed, it is precisely in the context of such convergence, that single variant candidate polymorphism investigations remain informative.

In Search of Mechanistic Understanding

Much like psychiatric and behavioral genetics, a major constraint on the utility of imaging genetics and genomics is its ability to inform molecular mechanisms which is predicated on the functional characterization of polymorphisms. With few exceptions (e.g., *FKBP5(51)*), polymorphisms have yet to be functionally detailed in a convincing manner. Pairing imaging genetics with molecular genetic and basic neuroscience research tools, holds tremendous potential. For example, in one of the most significant advances in psychiatric genetics, Sekar and colleagues (83) conducted a series of studies distilling the effects of the schizophrenia associated major histocompatibility (MHC) locus to complex variation within complement component 4 and showed that these alleles altered C4A and C4B expression in the brain, that was proportional to schizophrenia risk. Further, because C4 mediated synaptic pruning during postnatal development in mice, it is plausible that this may account for reduced synapses in schizophrenia. Indeed, it is precisely in this context that focused analyses remain relevant in our polygenic world.

In addition to better understanding molecular mechanisms using emergent technologies (e.g., RNA and methylation microarrays, RNA-Seq, bisulfite sequencing, ChIPSeq, mass spectroscopy), available databases of gene expression (BRAINEAC (137), braincloud (138),

GTEx (139)) and bioinformatics tools (e.g., WUSTL epigenome browser (140)) may prove fruitful, particularly when interrogating novel and uncharacterized polymorphisms. Additionally, recent developments that allow for the imputation of the genetically-related transcriptome using GWAS data, such as TWAS (141) and PrediXcan (142), may aid in identification and confirmation of phenotype-related genes. It is also important to highlight that while integrative approaches will undoubtedly lead to greater etiologic insight, unique challenges need to be actively confronted. For example, because methylation is dynamic (143), with evidence that even proximal experiences (e.g., meal consumption), shape its landscape and measurement (144), it will be important for imaging genetics studies to collect DNA samples temporally linked to imaging data.

Summary

In conjunction with *in vitro*, *in vivo*, non-human animal research, pharmacologic manipulation, and psychiatric and behavioral genetics, imaging genetics and genomics can provide unique mechanistic insight into the genetic and experiential differences that contribute to psychiatric risk. We suggest that elements of the endophenotypic research conceptualization (e.g., disease-association) impede progress within imaging genetics and psychiatry and that some conclusions arising from meta-analyses may be premature in light of phenotypic harmonization concerns. Further, we highlight the potential of relatively novel approaches in imaging genetics (e.g., PRS, pathway, multivariate) as well as challenges and limitations that each face while suggesting that single variant candidate gene analyses remain relevant, particularly in large samples alongside convergent evidence and anticipated small effects. Lastly, as knowledge and data continue to grow and are accompanied by methodological advances, data sharing, and prospective data

collection, it becomes increasingly important to extend the traditional unidirectional model of imaging genetics (**Figure 1A**) to explore the complex, and testable, interplay between the genome, brain, body, and experience (**Figure 1B**). Presently, the most prudent manner to begin testing these pathways is through multimodal data convergence – imaging genetics is but one crucial component in the elaborate and multifaceted puzzle surrounding the interface between brain and behavior - integrating across various lines of evidence is likely to provide the most complete picture.

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Figure 1. Imaging Genetics and Genomics Models

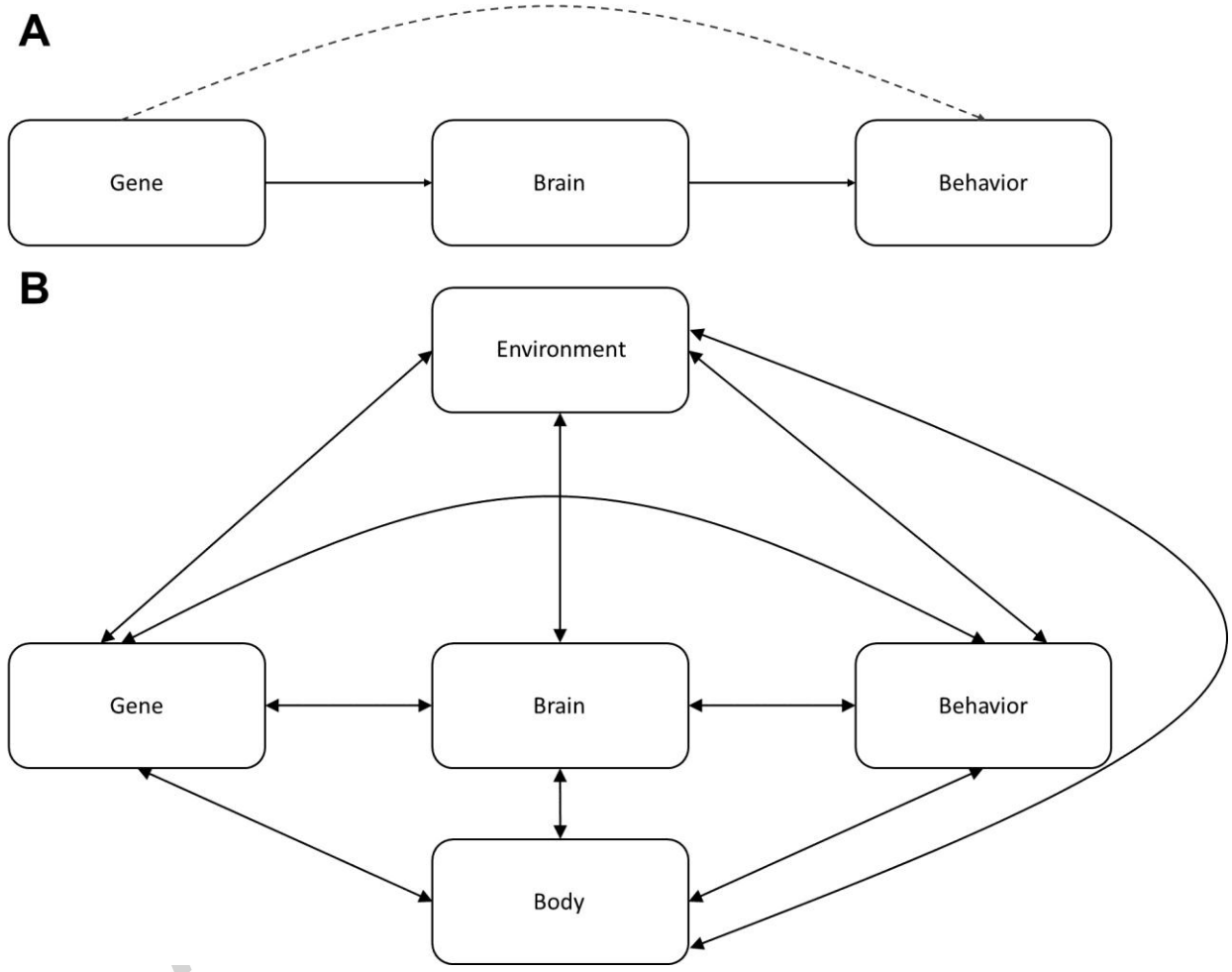
Figure 1. (A) The traditional imaging genetics and genomics model suggests that genetic variation confers risk for psychopathology indirectly through its influence on the brain. This theoretical model is well suited for traditional mediation models estimating indirect associations (demarcated with the dashed line), through which genetic background is linked to behavior through neural phenotypes. **(B)** *Imaging genetics and imaging genomics redux:* In the future, as imaging genetics and genomics expand to include larger and longitudinal samples it will be possible to evaluate a more complete interactive model in which bidirectional relationships between the genome, brain, and behavior may be investigated in the context of environmental experience and peripheral biological markers. For example, socioeconomic status has been associated with epigenetic modifications that are, in turn, related to psychiatrically-relevant brain function (145). Moreover, environmental experience (e.g., trauma experienced during early life) moderates genetic associations with neural phenotypes and associations between neural phenotypes and behavior (20,146). Further, genetic background influences peripheral indices such as gut microbiome (147), which in turn has been linked to neural phenotypes and psychopathology (148). As a result, a more complete mechanistic understanding requires multiple levels of analyses in the context of longitudinal and convergent data. Currently, convergence across multiple methods and studies testing legs separately is attainable. Informed by such studies, in the future, as large multimodal longitudinal studies develop, it is plausible that more complete pathways could be tested in the framework of a single study.

Table 1. Converging Evidence: The Example of Fatty Acid Amie Hydrolase (*FAAH*) rs324420 genotype (C/A; C385A)

Source of Evidence	Findings	Benefits	Limitations
In Vitro Function	A allele homozygosity is associated with less FAAH cellular expression in T-lymphocytes and transfected cells due to post-translation mechanism preceding folding (149).	Controlled functional characterization and isolation of step at which allelic variation impacts function	Unclear if similar function is observed in vivo amongst an interactive system
In Vivo Function	A allele carriers had lower [(11)C]CURB PET binding (FAAH binding) (150).	In vivo functional characterization	Often small samples, unclear links to behavior and other relevant phenotypes (e.g., brain function, structure)
Non-human Animal Manipulation	Knock-in mouse model: A allele associated with forebrain FAAH protein expression, hydrolytic activity, and elevated anandamide. A allele associated with increased projections from infralimbic to basolateral amygdala and enhanced fear extinction, and reduced anxiety (13).	Controlled manipulation of system using a variety of means (e.g., pharmacologic, genetic)	Unclear whether translates to humans and related conditions. Questionable phenotypic convergence across species for some phenotypes.
Human Manipulation (Pharmacologic Challenge)	Human: THC administration associated with reduced anxiety and threat-related amygdala reactivity (151).	Manipulation of a specific system allowing causal inferences to be drawn. For some substances, limitations on who can be exposed for human studies.	Temporary and chronic manipulation unclear translation to genetic risk. Uncertain whether artificial manipulations create other systematic changes.
Imaging Genetics and Genomics	A allele associated with decreased threat-related amygdala reactivity and increased amygdala habituation (152).	Provides a tractable and clinically-relevant phenotype. Offers system-level insight.	Molecular mechanisms of association unclear
Psychiatric/Behavioral Association (Candidate or GWAS)	A allele associated with enhancd fear extinction, reduced anxiety and stress sensitivity (10).	Provides clinical relevance	Unclear biological mechanisms
Treatment	Some evidence that FAAH inhibition improves anxiety in rodent models (153). Most common self-reported reason for using cannabis is anxiety	Evaluation of applicable therapeutic potential	Dependent upon other evidence, ability and safety to manipulate target. Lack of regional specificity in humans

reductions. THC administration reduces anxiety in clinical populations (154).

The endocannabinoid system has been linked to stress recovery, anxiety, and substance use, across a host of models. Fatty Acid Amide Hydrolase (FAAH) in an enzymatic regulator of endocannabinoid signaling. Within the endocannabinoid system, it primarily degrades the endocannabinoid ligand anandamide.



Imaging Genetics and Genomics in Psychiatry: A Critical Review of Progress and Potential

Supplemental Information

The Origins of Imaging Genetics

Guided by convergent evidence from *in vitro*, psychiatric, and behavioral candidate gene studies (e.g., **Table 1**), imaging genetics began in 1998 before the draft of the human genome was complete. Characterizing the replication challenge that is inherent to the field, the first two imaging genetics studies (1, 2) reached opposing conclusions on whether the missense ankyrin repeat and kinase domain containing 1 (*ANKK1)* C/T single nucleotide polymorphism (SNP), rs1800497 (also known as Taq1A, previously assigned to *DRD2*[1]), is associated with *in vivo* dopamine receptor type 2 (D2R) availability and density. Pohjalainen and colleagues (1) found that the T allele of rs1800497 is associated with *reduced* dopamine type 2/3 receptor availability in the striatum among 54 healthy volunteers. Contrastingly, Laruelle and colleagues (2) found no difference in binding according to rs1800497 genotype in a sample of healthy controls (n=47) and patients with schizophrenia (n=23); however, a consistent unreported trending association is observed in controls. A meta-analysis of *in vivo* and postmortem studies supports the association between the T allele and reduced D2R availability among healthy individuals (3). The mechanism underlying these functional associations remains controversial; it is plausible that they may emerge as a result of interactions between *ANKK1* and *DRD2* or linkage disequilibrium patterns with nearby SNPs within *DRD2*, or otherwise unknown interactions. Nonetheless,

[1] Notably, this SNP was initially mistakenly believed to be within the dopamine receptor type gene (*DRD2*) but actually resides downstream of *DRD2* within *ANKK1*, which codes for a protein kinase involved in signal transduction.

evidence suggests that this SNP may be associated with psychiatric phenotypes (4, 5) potentially as a result of these functional associations; (but see also (6)) and lack of GWAS significance (7).

These initial imaging genetic findings were followed in 1998 and 2000 by ligand binding studies by Heinz and colleagues in humans and rhesus monkeys that associated SPECT imaging of [I-123]β-CIT binding to polymorphisms within the serotonin transporter (*SLC6A4)* and dopamine transporter (*SLC6A3)* genes (8-11). Alongside *in vitro* studies, these initial imaging genetics studies have been highly influential, inspiring a wealth of research examining associations between these genotypes and individual differences in structural and functional neural phenotypes as well as psychiatric disorders and variability in behavior (e.g., (12)). Thus, from its ligand-based beginnings, imaging genetics has produced findings that converge with data from multiple other modalities providing potential mechanistic pathways through which genetic variation in some of the most well-studied candidate loci may impact psychiatrically relevant behavior and risk. For further historical review please see (13).

Imaging genetics did not become widespread until it employed functional magnetic resonance imaging (fMRI) to examine associations between functional polymorphisms in the apolipoprotein E (*APOE*), catechol-O-methyltransferase (*COMT*) and serotonin transporter (*SLC6A4*) and neural activation during memory and emotion tasks (14, 15). These studies paved the way for the broader adoption of imaging genetics in the context of functional and structural MRI due to its lower cost, wide availability and lack of ionizing radiation exposure. Further, the larger sample sizes that can be obtained using MRI have led to the development of massive datasets through data sharing and large scale studies (16-21). In addition to encouraging new ways of characterizing brain function and structure such as examining interactions within and between large scale brain networks, these large datasets allow for the application of analytic

techniques such as GWAS (22), gene x gene interaction (23), gene x environment interaction (24), and pathway analysis (25) that may link both genes and behavioral phenotypes to brain function in new and interesting ways. This extension to MRI has enabled the rapid expansion of the field and helped popularize the intermediate phenotype approach in psychiatry by helping contextualize gene – behavior relationships through the mediating effects of brain (**Figure 1A**) (26), which has subsequently been refined in the form of the research domain criteria (RDoC) for psychiatric disease (27).

Gene x Environment Interaction

Given large effects of the environment, and in particular childhood maltreatment and poverty, on the expression of psychopathology, a complete etiologic understanding requires the incorporation of environmental factors (28). The interplay between genotype and environmental factors (including adversity and advantage) may occur due to selective environmental exposure due to genotype (i.e., gene-environment correlation) or due to their interaction (i.e., gene x environment interaction, GxE). Inspired by GxE observations in traditional psychiatric genetics that have been profoundly influential (29) but have also grown increasingly contentious (30), imaging genetics has begun to interrogate GxE using single variant and polygenic approaches. For example, studies have linked a functional variant in FKBP5 that has been associated with stress-related psychopathology and disease, to threat-related amygdala responsiveness in the context of prior childhood maltreatment (31, 32). That this association occurs in the context of adversity occurring early in life is consistent with observations in clinical and molecular epigenetic research (33). In another recent example, within 3 independent samples, polygenic risk for schizophrenia was negatively associated with cortical thickness only among male

participants who used cannabis (34). While these findings are intuitively appealing and provide ample mechanistic speculation for psychopathology risk, it is important to highlight that GxE research within an imaging genetics framework is confronted by a host of unique challenges including assessment of the environment in resource intensive studies, the need to appropriately model covariates, as well as power limitations introduced by interactive terms; a more complete discussion of these unique challenges is presented in (24).

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