Commentary

Biological Psychiatry: GOS

A Critical Appraisal of Imaging Transcriptomics

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Bridging levels of explanation from disease-associated genotypes to clinical phenotypes has been recognized as a critical objective of biological psychiatry (Figure 1). While neuroimaging has potential as an intermediate phenotype linking clinical manifestations with underlying biological mechanisms, this translation has remained difficult to achieve since the most widely used magnetic resonance imaging (MRI) methods offer limited insight into the cellular and molecular properties of brain tissue. In recent years, investigators have attempted to bridge the gap between the findings from psychiatric neuroimaging studies and the putative molecular drivers behind such findings using transcriptomic data, most commonly maps of whole-brain gene expression from the Allen Human Brain Atlas (AHBA) (1). This cross-fertilization of neuroimaging and genomics has contributed to the burgeoning field of imaging transcriptomics, thoughtfully reviewed by Arnatkeviciute *et al.* (2) in the current issue of *Biological Psychiatry: Global Open Science*.

A major focus of imaging transcriptomics has involved deciphering the biological basis of statistical maps derived from brain imaging studies (i.e., imaging-derived phenotypes [IDPs]) using atlases of whole-brain gene expression. An example of a commonly used study design is to investigate the anatomical correspondence between maps of gene expression and IDPs of case-control differences in regional brain properties such as cortical thickness, myelination, or functional connectivity. Genes are then ranked by how closely their spatial patterns of transcription match the IDP, and this gene list is then tested for enrichment of specific pathways, cell types, and/or biological processes. For example, a study highlighted by the authors showed that regional case-control differences between healthy participants and participants with psychosis related to spatial transcription patterns of genes involved in synaptic signaling and nervous system development (3).

Arnatkeviciute *et al.* (2) highlight several important limitations of imaging transcriptomics studies in their review, only a few of which we will discuss in greater detail here. First, the end point of many imaging transcriptomics studies is a list of genes and associated biological processes that may not be specific to the disease or IDP in question. The authors discuss statistical methods to provide more realistic null models that could improve the specificity of the relationships discovered by imaging genetic studies. For instance, spatial null models can be used to address type I errors caused by spatial autocorrelation present in both IDPs and transcriptomic maps. However, given that any single gene can perform multiple roles (i.e., pleiotropy) and that multiple genes can contribute to a single phenotype (i.e., polygenicity), associations between genes and IDPs are still virtually guaranteed to be nonspecific. This issue is not new to neuroimaging and can be framed by analogy to the problem of reverse inference in functional MRI studies.

Reverse inference in cognitive neuroimaging refers to the logic that a certain mental process underlies a certain pattern of brain activation, based on evidence from prior studies that have associated a putatively relevant functional MRI task with local brain activity (4). For example, a study that finds increased functional MRI activity in the amygdala after subjects are shown pictures of a presidential candidate may be interpreted to suggest that this politician elicits fear among citizens. Reverse inference is problematic in this context since singular brain regions are typically associated with numerous psychological phenomena, such that amygdala activation cannot be interpreted to specifically denote a fear response. By analogy, reverse inference occurs in imaging transcriptomics studies when genes and their associated biological processes are inferred to influence a colocalized IDP. However, a correlation between a gene expression map and an IDP does not necessarily imply that the gene meaningfully influences the IDP under question.

Another challenge relates to the interpretation of gene set enrichment analyses from imaging transcriptomic studies, which often serve as the “headline result” in an imaging transcriptomic analysis. As Arnatkeviciute *et al.* (2) note, commonly used gene enrichment methods were designed for differential expression analyses performed across individual cases and controls, not for tests of anatomical correspondence with spatially embedded transcriptomic maps. Fulcher *et al.* (5) have shown that this leads to inflated false discovery rates for gene categories depending on their degree of spatial autocorrelation, which consequently biases the gene categories that tend to be most frequently reported in the literature. The authors have proposed a solution to this problem by designing a gene enrichment pipeline that compares against spatially autocorrelated null IDP maps, an approach that is similar in spirit to spatial null models proposed by other groups (5). However, it is also worth simply questioning whether gene set enrichment analysis of common biological processes or molecular pathways is an appropriate methodology that provides interpretable results for a given imaging transcriptomics study, depending on the specific experimental context.

As opposed to gene set enrichment analyses that agnostically query molecular pathways or biological processes, imaging transcriptomics results may be better interpreted within the context of cell type enrichment analyses. It is well known that bulk RNA sequencing and microarray technologies, as used in the AHBA, are highly sensitive to the cell type composition of the obtained sample—and that cell type composition varies considerably across the spatial axes of the cortex. Indeed, cell type–specific gene expression patterns

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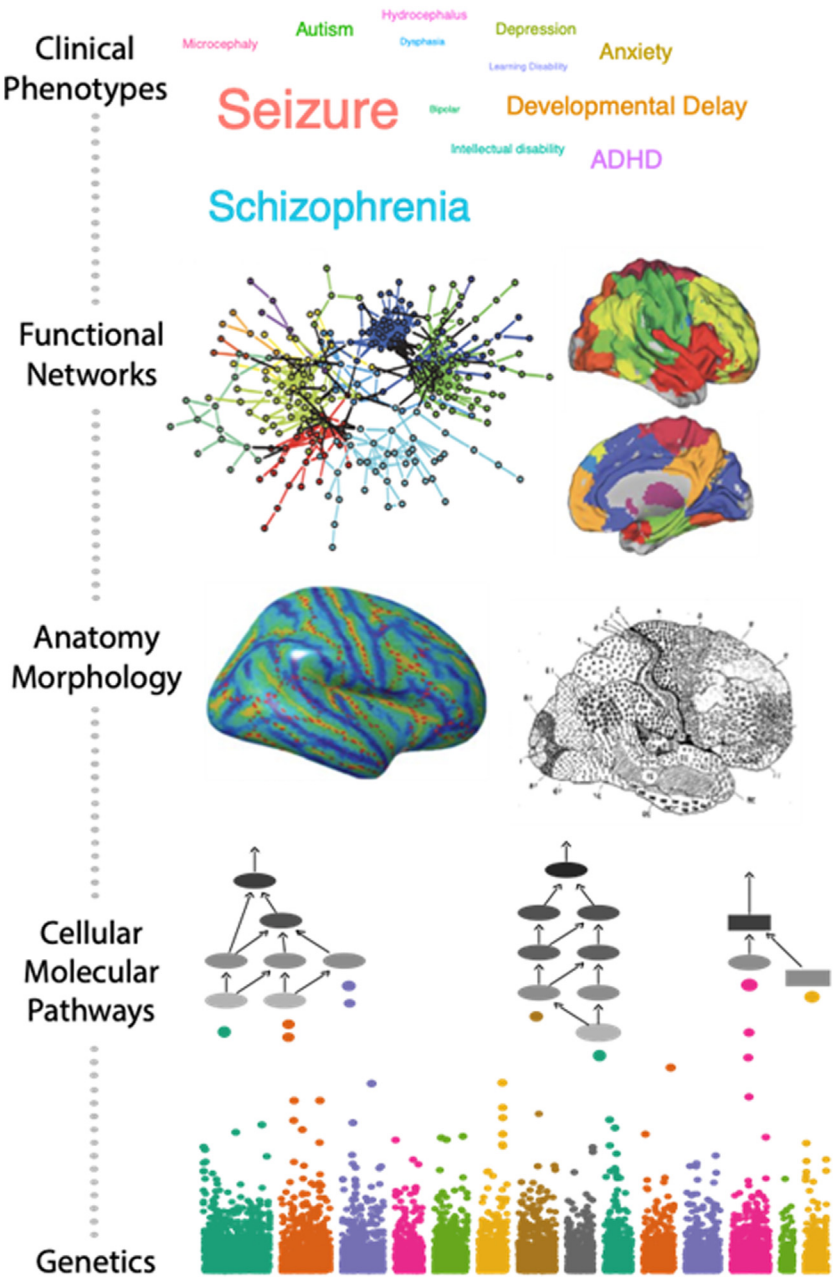

Figure 1. Bridging biological scales to study neuropsychiatric disorders. A major goal of biological psychiatry is to understand how disease-associated genomics lead to clinical phenotypes. Brain imaging reflects an intermediate phenotype that has been proposed as a bridge across biological scales. ADHD, attention-deficit/hyperactivity disorder.

tend to represent the greatest source variation across bulk brain transcriptomic datasets. As such, gene coexpression analyses of these data tend to identify modules of coexpression linked to cell types. In the imaging transcriptomics context, Seidlitz *et al.* (6) developed brain maps of the anatomical distribution of cell types by combining AHBA with genetic markers for specific cell types obtained from single-cell RNA studies. These maps have been used, for example, to show that the spatial distribution of adult diffuse gliomas, derived from in vivo brain MRI scans, follows the distribution of oligodendrocyte precursor cells, a hypothesized cell of origin for this type of cancer (7).

Finally, to reach the goals of imaging genomics, it will be important to design studies that can test which genes and molecular pathways are directly related to IDPs and, ultimately, neuropsychiatric illness. Imaging transcriptomics studies may identify associations between gene expression patterns and case and control differences in IDPs, but this does not imply that alterations in the identified genes are causally implicated in a neuropsychiatric illness. To more directly test the influence of specific genes or gene networks, gene-first studies of individuals with known genetic abnormalities can be useful to determine how genes and their associated pathways affect

brain structure and function—as a complement to phenotype-first studies where individuals are ascertained based on clinical characteristics. For example, Seidlitz *et al.* (6) demonstrated that patients with neurodevelopmental copy number variants show differences in "morphometric similarity networks" that mirror the transcriptional maps of genes affected by a given copy number variant. Nevertheless, current imaging transcriptomics approaches are limited in their ability to identify candidate causal genes for specific IDPs. In the future, it will be important to continue to expand imaging transcriptomics beyond studies of anatomical correspondence based on AHBA. For example, transcriptome-wide association studies of IDPs can identify single nucleotide polymorphisms associated with IDPs that are functionally active and associated with expression of specific genes, by integrating large-scale imaging genetics samples with functional genomics reference panels in the developing and adult brain (8).

To overcome the fundamental limitation that imaging and transcriptomics data are generally obtained in 2 different cohorts of individuals, the next generation of studies should also seek to relate imaging and transcriptomics data obtained within the same cohorts. Such studies could rely on longitudinal designs integrating in vivo imaging with postmortem gene expression (9); however, the lack of sufficient investment in brain banks remains a principal obstacle for such studies. Another possibility is to focus on less invasive approaches, such as combining brain MRI with gene expression data obtained from peripheral blood or cerebrospinal fluid in the same participants. Finally, special attention should be paid to clinical populations where both imaging and brain tissue biopsies are obtained during existing clinical care, such as in subsets of patients with epilepsies or brain tumors (10).

A critical frontier in psychiatric neuroimaging is to integrate across levels of biological explanation, from genomic studies of clinical populations to animal models establishing pathophysiological mechanisms. Imaging transcriptomics can offer crucial pieces of evidence to help triangulate the neurophenotypes of psychiatric disorders with underlying genetic drivers. Insights derived from such studies could inform the development of novel therapies targeted against molecular mechanisms of psychopathology. To realize this potential, psychiatric neuroimaging should embrace study designs that can more directly implicate genes with the clinical manifestations of neuropsychiatric disease.

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Article Information

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