Biological Psychiatry: Celebrating 50 Years

Assessing the Candidates

Anil K. Malhotra

In a bygone era, a plethora of studies assessed the relationship between single, or perhaps several, polymorphisms in key "candidate" genes, and various clinical phenotypes including disease susceptibility (1), brain imaging measures (2), cognitive function (2), and treatment response (3), among others. The strength of the candidates was established—not through a series of crowded debates but via evidence of preexisting biological plausibility or, in some cases, limited functional data derived from in vitro or in vivo assays. The dopamine system was of significant interest, given the neurotransmitter's role in diverse brain functions, as well as the detection of seemingly functional (4) and frequently occurring (minor allele frequencies >20%) polymorphisms that could be reasonably expected to influence behavioral phenotypes.

The advent of psychiatric genome-wide association studies (GWASs) that began in 2007 (5) perhaps heralded the end of this approach. In a relatively short period of time it became feasible to assess hundreds of thousands of variants distributed across the genome instead of a few variants localized to within or near a single gene, and to do so at a modest cost. With the concomitant collection of the necessarily large sample sizes, GWASs rapidly became the workhorse approach to gene discovery, with some notable successes (6). Of note, for many disorders, including a recent GWAS of attention-deficit/hyperactivity disorder (ADHD), previously hypothesized candidate genes (e.g., the dopamine transporter gene, *DAT1*) have not been observed to significantly influence risk for the disorder (7). These results have led several authors to conclude that all candidate gene studies are unreliable and that GWASs have rendered candidate gene studies "obsolete" (8).

Despite the general disregard for this line of inquiry, studies that use the general framework of the candidate gene approach are still being conducted. In this issue of *Biological Psychiatry*, two articles adopt variants of a candidate gene strategy to assess the impact of dopaminergic genes. By extending the classical candidate gene approach, they provide new data toward understanding the relationship between dopaminergic genetic variation and clinical phenotypes.

Miguel *et al.* (9) focus on the oft-studied *DAT1* and its relationship to cognitive flexibility and gray matter density as a means to understand the genetic and environmental underpinnings of ADHD. Multiple previous studies have reported significant associations between specific *DAT1* polymorphisms, most notably a 40–base pair variable number of tandem repeats polymorphism, and risk of ADHD; this study extends this approach to focus on an expression-based polygenic risk score of the *DAT1* network (ePRS-*DAT1*), based on the hypothesis that genes involved in the same network have similar expression profiles. To complement the expression work, Miguel *et al.* (9) also construct environmental risk scores based upon hypoxic–ischemic events in utero (HICs) and examine the interactions between the ePRS-*DAT1* and HIC scores in two groups of children from Canada (n = 139) and Singapore (n = 312) with complete birth records, genotype data, and cognitive flexibility assessments at 6 and 4.5 years of age, respectively.

This study is notable for its more comprehensive assessment of both gene expression and in utero environmental factors that may play a role in cognitive and brain development. They report that the environmental factors only influence cognitive flexibility in the group of children with higher ePRS-*DAT1* scores, suggesting that it is the interplay between multiple genetic factors (the ePRS-*DAT1* is comprised of 269 genes) and several environmental events (7 events comprise the HIC score) that is critical for cognitive development. A small magnetic resonance imaging component (n = 40) of the study also suggests that these factors may influence gray matter density, but the modest sample size and number of components assessed render these data less compelling.

A critical strength of the non–magnetic resonance imaging component of the study is the use of two cohorts to assess replicability of results. Clearly, cohorts of children with complete birth records and follow-up cognitive assessments are difficult to collect, and the use of two with similar metrics enhances confidence in the results. The argument for replication, however, is tempered by the use of different cognitive tasks (the Intra-Extra Dimensional Set Shift task vs. the Dimensional Change Card Sort) in developmentally different age groups (4.5 vs. 6 years of age) in the two cohorts. Moreover, the ePRS by HIC interaction was not consistent across both cohorts for an important outcome variable (errors committed) on these two tasks, suggesting some instability of results. Interestingly, specificity of the results for the DAT system was assessed by development of an ePRS for *SLC6A4*, the target of action of the serotonin reuptake inhibitors, with no interaction observed between this ePRS score and HICs on the cognitive measures in the Canadian cohort.

These data, therefore, suggest that the *DAT1* network may be important to understanding dopamine function and its relationship to clinical phenotypes. Tunbridge *et al.* (10), however, conducted a systematic meta-analysis of 14 dopamine-related genes, including *DAT1*, looking for evidence of functional effects of key polymorphisms within these genes. Functionality was broadly defined, with assessment of evidence for effects on several multilevel parameters including gene expression, isoform abundance, protein levels, and protein activity. In this work, Tunbridge *et al.* (10) report that several polymorphisms in genes related to dopamine synthesis and clearance, such as the now (in)famous *COMT* Val158Met variant, and three *DBH* polymorphisms have sufficient support

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to be deemed functional, but that no variants within *DAT1* were found to have meta-analytic support for functionality. It should be noted that the *COMT* and *DBH* variants have been more extensively studied than most other variants, and that the outcome metrics vary considerably by gene from basic cellular assays (e.g., induced pluripotent stem cell–derived neurons) to human assays from imaging studies (e.g., positron emission tomography); nevertheless, the lack of association of any metric to *DAT1* genetic variation is somewhat surprising given the number of studies that have focused on this gene over the past 3 decades.

What is the role for studies focused on dopamine system genetics and clinical phenotype, such as ADHD, which seem to be robustly linked to dopamine function? Studies like those by Miguel *et al.* (9) suggest that incorporation of genetic factors, in this case a polygene-based expression score, can explain some of the variance in behavioral phenotypes. However, it seems critical to complement these analyses with measures of additional factors, which Miguel *et al.* (9) accomplish by the development of a polyenvironmental risk score. Selection of specific candidate gene variants for analysis, however, may be less fruitful given the mixed picture of functionality that Tunbridge *et al.* (10) report. Even for the genes that harbor putatively functional variants, such as *COMT*, incorporation of additional units of analyses may be needed to better understand the overall complexity of most behavioral phenotypes, regardless of their apparent relationship to a specific single variant or gene product.

Moreover, the results from these articles also suggest that any blanket statement about a research approach may not be the most appropriate. Researchers should endeavor to use the best approach to answer the research question at hand. More focused techniques may be the best applicable approach in studies with more restricted research aims, and no strategy should be ruled out a priori. It is our hope that complementary approaches, such as illustrated in these two articles, will continue to be considered in the search for a better understanding of the genetic and nongenetic underpinnings of psychiatric illness.

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Article Information

From The Zucker Hillside Hospital, The Feinstein Institutes for Medical Research, Zucker School of Medicine at Hofstra/Northwell, Glen Oaks, New York.

Address correspondence to Anil K. Malhotra, M.D., The Zucker Hillside Hospital, 75-59 263rd Street, Glen Oaks, NY 11004; E-mail: amalhotra@northwell.edu.

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