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Zebrafish: A Translational Model System for Studying Neuropsychiatric Disorders

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With the advent of next-generation sequencing technologies, there has been considerable progress in our ability to identify genes that are strongly associated with neurodevelopmental disorders, particularly autism spectrum disorders (ASDs).[1] Despite the challenges of clinical and genetic heterogeneity, which complicate the identification of susceptibility genes, whole-exome sequencing of large cohorts of affected individuals has led to a rapidly expanding list of reliable risk genes that are beginning to illuminate basic neurobiological processes involved in ASDs, such as synaptic dysfunction.[1] Moreover, the application of emerging genomic technologies to other neuropsychiatric disorders, such as schizophrenia, is beginning to provide deeper insights into the pathophysiology of these disorders. However, we now face the challenge of translating findings from large-scale genetics studies into a mechanistic understanding of neuropsychiatric disorders as a prerequisite for developing improved, target-driven pharmacotherapies.

The zebrafish is emerging as a highly tractable model system to address this challenge. The growing popularity of this system is due in large part to key advantages of zebrafish over more traditional preclinical models. For example, zebrafish have rapid, external development of transparent embryos, which enables the visualization of basic neurobiological processes, such as neuronal migration and axon outgrowth, in real time in a living organism. In this way, zebrafish offer an invaluable window into the cell types and developmental stages that are affected when a risk gene is not functioning properly. Moreover, zebrafish larvae are small, easy to handle, and display a range of simple, quantifiable locomotor behaviors that provide a readout of circuit-level activity in a live, behaving organism. Because zebrafish may have progenies containing hundreds of embryos, such simple behavioral assays can be harnessed as a platform for conducting high-throughput small molecule screens and identifying pharmacological candidates that affect a simple behavior or circuit.[2,3] Finally, the recent availability of genetic technologies for the rapid and cost-effective generation of zebrafish “knockouts,” in which the function of a gene of interest has been disrupted, will enable the application of the zebrafish model to a range of neuropsychiatric disorders

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(Figure 1A). Given these advantages, zebrafish have the potential to emerge as a key player in the path from risk gene discovery to the development of mechanism-based pharmacological interventions (Figure 1).

CONSERVED NEURAL PATHWAYS

With any preclinical model system, there is a trade-off between how readily researchers can address fundamental questions regarding neurodevelopmental processes using the model system and how well these findings translate to humans. As McCammon and Sive observe, zebrafish offer a reasonable compromise in this regard, "balanc[ing] experimental tractability and conservation with humans."[4] Despite the obvious evolutionary divergence between humans and zebrafish, there is a striking degree of conservation in neural pathways. While zebrafish lack a neocortex, which is a clear limitation of its translatability compared to rodents, the general organization of the zebrafish central nervous system is similar to that of mammals.[4,5] Specifically, zebrafish are vertebrates and possess the same major subdivisions of the brain as mammals: forebrain, midbrain, hindbrain, and spinal cord[5] (Figure 1B). The patterns of early gene expression in the developing zebrafish brain are also conserved.[5] In addition, the major neurotransmitter systems in the human brain are present in zebrafish, including GABA, glutamate, dopamine, norepinephrine, serotonin, histamine, and acetylcholine.[5] Finally, there is a considerable degree of conservation between the zebrafish and human genomes. In fact, over 80% of genes associated with human disorders have an orthologous version in zebrafish.[4] Moreover, there are experiments that can be easily conducted in zebrafish, such as visualizing early brain development in live embryos and screening hundreds of psychoactive compounds for their neural circuit-modifying effects, which are simply not feasible in rodents due to their in utero development, greater size, and complexity. At the same time, there are features that limit the translatability of zebrafish as a model system. For example, the telencephalon of zebrafish and mammals forms through distinct processes, which complicates direct comparisons of anatomical structures.[4,5] In addition, there are differences at the genomic level between zebrafish and mammals that may present a challenge in identifying the zebrafish genes that correspond to human risk genes. Also, while complex behaviors, such as social behaviors and learning and memory, have been studied in adult zebrafish, these behaviors are less amenable to high-throughput approaches.[4] Nonetheless, given the range of experimental applications that are possible with zebrafish, coupled with a reasonable degree of conservation with humans at the genetic, neurochemical, and structural levels, zebrafish hold tremendous promise as a model system for investigating neural pathways with relevance to human neuropsychiatric disorders.

VISUALIZING BRAIN DEVELOPMENT

One of the main advantages of zebrafish as a model system is that they have relatively small larvae with transparent heads, such that the whole brain of a larval zebrafish can be visualized under the microscope (Figure 1B). In addition, zebrafish embryos exhibit rapid external development. Therefore, it is possible to observe processes such as neurogenesis and axon outgrowth in a developing vertebrate brain in the span of only 24 hours. Importantly, the zebrafish orthologs of many human genes that have been implicated in

neuropsychiatric disorders are expressed in the brain during early developmental stages, providing support for the feasibility of studying their function in zebrafish. Moreover, various genetic tools, which have been used in other model systems, have clear advantages when applied to zebrafish, given the ease of whole-brain imaging. For example, transgenic lines that label specific types of neurons with different fluorescent markers are readily available for use in zebrafish. Because excitatory-inhibitory imbalance has been hypothesized as an underlying mechanism in ASDs, we utilized two previously developed transgenic lines that label excitatory glutamatergic and inhibitory GABAergic neurons to investigate their simultaneous development in zebrafish lacking the function of the ASD risk gene, *Contactin Associated Protein-2* (*CNTNAP2*).[3] Using this approach, we found that these zebrafish mutants have significant deficits in inhibitory neurons, particularly in the forebrain.[3] Furthermore, as the technology for targeting genes associated with neuropsychiatric disorders in zebrafish continues to advance, the ability to image the brains of zebrafish mutants using these experimental tools is likely to play an important role in illuminating common neurodevelopmental pathways involving risk genes.

SMALL MOLECULE SCREENS

Another key advantage of zebrafish as a preclinical model system is their large progenies, which facilitate the conduct of high-throughput small molecule screens (Figure 1C). Because zebrafish larvae are small and easy to handle in a laboratory setting, they can be placed in a 96-well plate and their activity in response to hundreds of psychoactive compounds can be monitored using automated methods, providing a quantitative readout of the behavioral effects of these compounds and insight into the neurochemical pathways underlying specific behavioral patterns. For example, Rihel et al. (2010)[2] utilized this “behavioral profiling” approach to test over 500 psychoactive compounds on sleep behaviors in larval zebrafish. By quantifying different parameters of zebrafish activity at night and during the day, this study found that groups of psychoactive compounds could be correctly classified by their mechanism of action based solely on their behavioral effects in zebrafish larvae. Interestingly, this study provides further evidence for the conservation of neurochemical pathways between zebrafish and mammals, revealing similarities between the effects of psychoactive compounds on zebrafish and their known behavioral effects in mammals. For example, α_2-adrenergic agonists, such as clonidine and guanfacine, which are used to treat attention-deficit/hyperactivity disorder and Tourette’s disorder, have sedating effects in zebrafish larvae and decrease their daytime activity.[2] Moreover, we utilized this experimental approach to identify drugs that reverse an abnormal behavior in zebrafish mutants of the autism risk gene, *CNTNAP2*.[3] By comparing the behavioral profiles of mutants to the profiles of control fish exposed to hundreds of compounds, we found that compounds with estrogenic activity reverse the behavioral abnormality of nighttime hyperactivity in mutants.[3] Therefore, behavioral profiling led to the identification of a class of compounds that was not previously associated with *CNTNAP2*.[3] While an important next step is to confirm candidates identified from zebrafish screens in a rodent model, behavioral profiling reveals the strength of the zebrafish system as a first-pass screening approach to identify small molecules that target neurochemical pathways with relevance to neuropsychiatric disorders (Figure 1D). Further, zebrafish behavioral profiling may emerge

as a complimentary approach to drug discovery along with high-throughput pharmacological screens in cell culture models of neuropsychiatric disorders, such as human-induced pluripotent stem cells (iPSCs), providing insight into the effects of compounds on neural circuits in a live, behaving organism.

FUTURE DIRECTIONS

In the next several years, a number of emerging technologies are likely to transform our ability to capitalize fully on the advantages of the zebrafish system for elucidating biological mechanisms in neuropsychiatric disorders. For example, activity-sensing transgenic lines that monitor regional changes in brain activity in an awake, behaving larval zebrafish have tremendous promise, when used for the analysis of zebrafish mutants of risk genes, to illuminate the neural circuits underlying simple behavioral tasks that are disrupted when a risk gene is not functioning properly. Moreover, with the recent introduction of the CRISPR (clustered regularly-interspaced palindromic repeats)/Cas9 system, which induces mutations in a gene of interest with a high degree of efficiency, it is now possible to generate mutations in multiple risk genes in zebrafish relatively rapidly and at low cost. Importantly, the ability to study multiple zebrafish mutants in parallel using high-throughput behavioral profiling has the potential to illuminate neurochemical mechanisms underlying neuropsychiatric disorders. While many complex behaviors cannot be fully recapitulated in the zebrafish or any preclinical system, the strength of the zebrafish system is its ability to provide mechanistic insights into the function of risk genes in basic biological processes, as a path towards identifying relevant neurochemical pathways. As a vertebrate model system that combines ease of genetic and experimental manipulation, visualization of brain development, and the potential for high-throughput small molecule screens, zebrafish represent a robust system to elucidate convergent biological processes and novel pharmacological targets with relevance to neuropsychiatric disorders.

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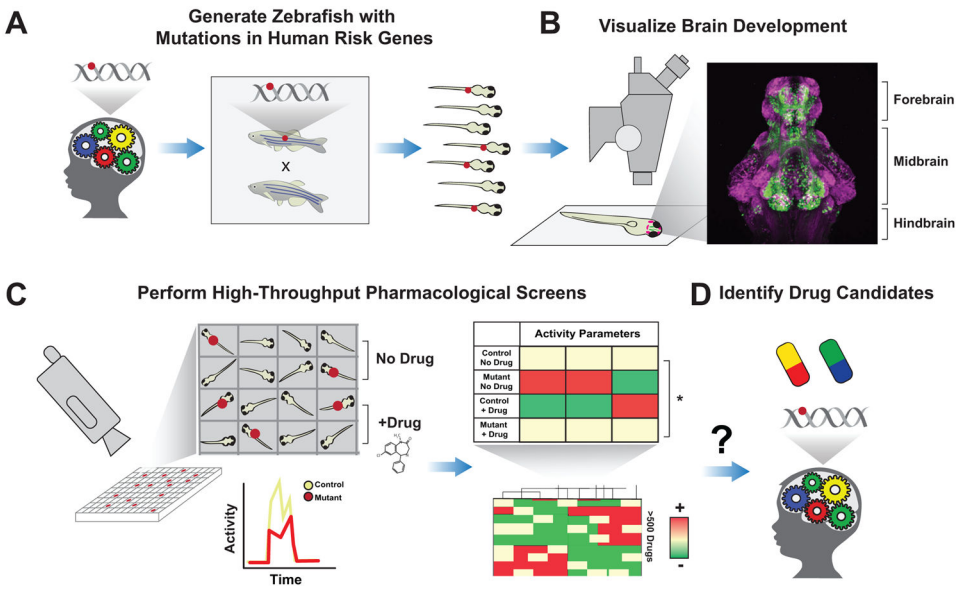

Figure 1.

Schematic representation of the functional analysis of risk genes in neuropsychiatric disorders using a zebrafish model system. (A) Risk genes (red dot in DNA strand) that are strongly associated with neuropsychiatric disorders are first identified in human genetics studies. To elucidate their role in neurodevelopment, zebrafish carrying mutations in these risk genes are generated using molecular approaches, such as the CRISPR (clustered regularly-interspaced palindromic repeats)/Cas9 system. (B) Zebrafish larvae have transparent embryos, which allow for visualization of brain development in zebrafish carrying mutations in risk genes. A representative high-resolution image of a control larval zebrafish brain (ventral view) is shown with labeled excitatory glutamatergic (magenta) and inhibitory GABAergic (green) neurons. The major subdivisions of a vertebrate brain (forebrain, midbrain, hindbrain) are shown. (C) High-throughput behavioral profiling of hundreds of zebrafish larvae, some carrying a mutation in a risk gene (red dot), is conducted by tracking their activity in 96-well plates and monitoring their responses to psychoactive compounds using a video camera. Hierarchical clustering of various behavioral parameters can be used to identify small molecules that reverse abnormal behaviors in zebrafish mutants by restoring their activity to control levels (asterisk in magnified clustergram). The schematic clustergram depicts the effects of >500 drugs (y-axis) on a range of behavioral parameters (x-axis). Relative locomotor activity levels are shown: increased activity (red), decreased activity (green), control activity (cream). (D) The zebrafish system has the potential to serve as a first-pass screening approach to identify molecular pathways involved in neuropsychiatric disorders and potential therapeutic targets for further investigation.