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Review article

Developing zebrafish models of autism spectrum disorder (ASD)

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ABSTRACT

Autism spectrum disorder (ASD) is a serious neurodevelopmental disorder with complex symptoms and unclear, multi-factorial pathogenesis. Animal (rodent) models of ASD-like behavior are extensively used to study genetics, circuitry and molecular mechanisms of ASD. The evolutionarily conserved nature of social behavior and its molecular pathways suggests that alternative experimental models can be developed to complement and enhance the existing rodent ASD paradigms. The zebrafish (*Danio rerio*) is rapidly becoming a popular model organism in neuroscience and biological psychiatry to study brain function, model human brain disorders and explore their genetic or pharmacological modulation. Representing highly social animals, zebrafish emerge as a strong potential model organism to study normal and pathological social phenotypes, as well as several other ASD-like symptoms. Here, we discuss the developing utility of zebrafish in modeling ASD as a new emerging field in translational neuroscience and drug discovery.

Contents

1. Introduction

Autism spectrum disorder (ASD) is a serious debilitating mental illness affecting approximately 1–2% of the general population (Evans, 2013; Mayes et al., 2011; Wing et al., 1967). Recently revisited by the American Psychiatric Association (2013), ASD represents a neurodevelopmental disorder characterized by impaired social communication, repetitive behavior and cognitive deficits (see Table 1 and Fig. 1 for details of clinical phenotypes associated with ASD). In addition to these core symptoms, ASD shows high (~90%) heritability, representing one of the most heritable brain disorders (Crawley, 2012; Edvardson et al., 2013). Notably, ASD is a polygenic disorder with multiple genetic determinants

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; AVP, arginine vasopressin; DSM-5, Dianostic and Statistical Manual of mental disorders, 5th edition; FXS, fragile X syndrome; GABA, gamma-aminobutyric acid; MDMA, 3,4-methylenedioxy-N-methylamphetamine; NMDA, N-methyl-D-aspartate; PCP, phencyclidine; SERT, serotonin transporter; SFARI, Simons Foundation Autism Research Initiative; SSRIs, selective serotonin re-uptake inhibitors; V1aR, V1a receptor.

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Table 1
Diagnostic criteria for autism spectrum disorder (ASD), according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) of the American Psychiatric Association (2013).

A. Social deficits
Persistent deficits in social communication and interaction, including: i) deficits in social–emotional reciprocity and social approach, reduced sharing of interests, failure to initiate/respond to interactions; ii) deficits in nonverbal communication, poorly integrated verbal/nonverbal communication, poor eye contact and body language, deficits in understanding/use of gestures; a deficit in facial expressions and recognizing facial affect; iii) deficits in developing, maintaining and understanding relationships, problems with adjusting behavior to various social contexts, making friends and developing interest in peers.

B. Behavioral and cognitive perseverations
Restricted, repetitive patterns of behavior, interests or activities, manifested by: i) stereotyped/repetitive motor movements, use of objects or speech (e.g., simple motor stereotypies, idiosyncratic phrases); ii) insistence on sameness, routines, ritualized patterns in verbal/nonverbal behavior (e.g., distress at small changes, rigid thinking patterns, stable rituals); iii) highly restricted interests abnormal in intensity or focus (e.g., strong attachment to objects, excessively circumscribed or perseverative interest); iv) hyper/hyporeactivity to sensory input or sensory aspects of the environment (e.g., indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling/touching objects, visual fascination with lights or movement).

C. Symptoms' trajectory
Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life); these symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.

and candidate genes. For example, the SFARI Gene database (https://gene.sfari.org) currently lists 546 genes associated with ASD. Thus, ASD has a complex, poorly understood pathogenesis and associated genetic/environmental risk factors, aberrant brain circuits and disordered molecular pathways (Edvardson et al., 2013; Geschwind, 2008; Kesli et al., 2014; Matson et al., 2012).

Despite recent progress in dissecting the neural underpinnings of ASD (Kéïta et al., 2011; Kujala et al., 2013), its pharmacological therapy is complicated by the lack of efficient, disorder-specific and safe medication (Benvenuto et al., 2013; Farmer et al., 2013). For example, the Food and Drug Administration approves two drugs (atypical neuroleptics risperidone and aripiprazole) for treating ASD-associated irritability (Crawley, 2012). Other agents, such as methylphenidate, selective serotonin re-uptake inhibitors (SSRIs), valproate, atomoxetine, α2 adrenergic agonists and olanzapine, can also treat some ASD symptoms, but are not effective in alleviating others (Benvenuto et al., 2013; Myers, 2007).

Animal (experimental) models of brain disorders are an indispensable tool for drug discovery and dissecting the pathogenic mechanisms of brain disorders (Kalueff et al., 2007; Kas et al., 2013; Silverman et al., 2012, 2013). Complementing traditional rodent models of brain disorders, new experimental approaches using zebrafish (*Danio rerio*) are rapidly gaining popularity in neuroscience research (Gerlai, 2010a, 2011, Kalueff et al., in press). Can zebrafish be used to model complex brain disorders? For decades, zebrafish have been viewed as 'simple' organisms with relatively primitive, instinctively-driven behaviors suitable mainly for screening drugs, genetic mutations or developmental defects (see Gaikwad et al., 2011; Kalueff et al., 2013; Stewart and Kalueff, 2012 for discussion). However, mounting recent experimental evidence shows that zebrafish possess high genetic and physiological homology to mammals and display complex affective, social and cognitive responses which are similar to those observed in rodents and humans (Gerlai, 2010b, 2011; Kalueff et al., 2013 Pather and Gerlai, 2009; Stewart et al., 2013). Taken together, this suggests that zebrafish models can be used extensively in translational neuroscience research (Gerlai, 2010a; Kalueff et al., 2013, in press).

While various experimental (e.g., genetic or pharmacological) manipulations model certain symptoms and/or disordered pathways of ASD, they do not reflect the entire disease state. However, several social, motor and cognitive phenotypes (Table 2) are commonly assessed in rodent models of ASD, providing important mechanistic insights into its neurobiology (Crawley, 2012; Kas et al., 2013); also see discussion of this further in the text. The evolutionarily conserved nature of social behavior and its molecular pathways suggests that novel experimental models can be developed to complement and enhance the existing rodent ASD paradigms. Can we use zebrafish to study ASD-related pathogenesis? Addressing this important question, here we discuss recent advances and outline future promising directions of research in the field of novel zebrafish models of ASD-like states.

2. Traditional experimental models relevant to ASD

2.1. Genetic rodent models relevant to ASD

Laboratory rodents are highly sociable animals, and therefore are useful to study normal and pathological social behaviors (Brodkin, 2007; Crawley, 2012; Fairless et al., 2013; Kas et al., 2013; McFarlane et al., 2008; Moy et al., 2008; Ryan et al., 2010; Silverman et al., 2012). With mouse and rat genomes now being fully characterized, various genetic models of ASD and related disorders have been developed. For example, the Fragile X Syndrome (FXS) is an inherited mental retardation disorder caused by a single mutation in the *FMR1* gene in the X chromosome. FVB and C57BL/6 mice with *FMR1* genetic knockout display some FXS social and behavioral symptoms (Bernardet and Crusio, 2006; Pietropaolo et al., 2011).

Loss-of-function mutations in the *Nlgn4* gene in mice encoding for Neuroligin-4 impair social behavior and vocal communication, establishing *Nlgn4* mice as a genetic model of ASD-related pathogenesis (El-Kordi et al., 2012; Jamain et al., 2008) relevant to human genetic data linking this gene to ASD (Pampanos et al., 2009). Shank proteins are also involved in modulation of synaptic communication and

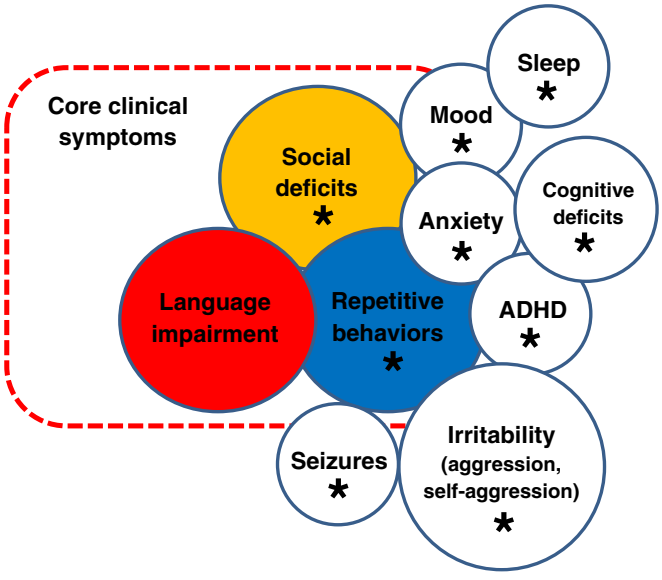

Fig. 1. Summary of key clinical features of autism spectrum disorder (ASD) and related syndromes (based on Kas, Glennon, 2013). Core ASD symptoms in this diagram are marked with color (circle sizes are adjusted for illustration purposes and do not represent a particular aspect of notice). Asterisks denote domains (clusters of symptoms) that can be modeled in zebrafish (also see Table 3 for details; ADHD—attention deficit and hyperactivity). Note that majority of clinical ASD phenotypes can be modeled in zebrafish. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)