VIEWPOINT

How fish can help find causes of autism

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> **Developmental window:** Zebrafish embryos are transparent, allowing researchers to label the brain with a dye and study its function in the live animal.

Studies seeking to understand human disorders almost always use a non-human surrogate, or 'model.' The idea is that a model organism can show symptoms of the disorder, just like a human, but allows scientists to perform experiments that are not possible in humans for ethical or practical reasons.

Zebrafish may seem to be an unlikely surrogate for analyzing the causes of autism because fish do not display the complex social behaviors that are abnormal in individuals with autism. However, fish and human genes are very similar, with about 85 percent of all human genes having a fish match, or homolog. Genes also control molecular pathways, and where these have been studied, they are the same in fish and humans.

These shared genes and pathways are the starting point for using fish to understand which genes malfunction in many disorders, including autism[1]. We call the fish a 'tool' rather than a 'model,' to indicate that although it does not develop human symptoms, it is useful for studying aspects of the disorder[2].

How can a fish help identify the genetic contribution to autism? There are several answers, which draw on the unique features of zebrafish.

Although fish don't exhibit autism-like behaviors, they can show certain abnormalities.

Researchers use several assays for these, including brain anatomy, the number of nerve cells and whether these cells find their targets, whether the fish respond to touch, **move normally or show other stereotypic behaviors**, whether too many or too few of their cells die, and whether muscles and other organs form normally.

Good match:

One outstanding advantage is that fish embryos and larvae are transparent, allowing investigators to **observe abnormalities** as they occur in the living animal.

Second, it is relatively easy to measure the function of autism risk genes in fish. Because fish have developed a complex brain only 24 hours after development begins — compared to six weeks in humans and more than a week in mice — assays for fish gene function are rapid. These assays are also simple to perform, because fish embryos develop outside of the mother, and investigators can use a tiny glass needle to inject substances that prevent the function of a target gene.

Because autism may be caused by **several genes malfunctioning together**, researchers may want to test the effects of inhibiting up to five genes simultaneously — easy to do in fish, but difficult in mice. These assays give a measure of gene function, which, although not a direct reflection of human autism symptoms, are very useful in subsequent tests.

The next thing to ask is whether the function of human and fish gene homologs — which have similar, but not identical, DNA sequence — is the same. The test is whether a human gene injected into a fish can reverse abnormalities caused by preventing the function of the matching fish gene. If it does, the fish becomes a test tube that researchers can use to ask whether human gene variants, with small differences in DNA sequence, have normal or impaired rescue ability. There can be hundreds of variants of each human gene, and figuring out which of these change gene function and contribute to autism (or autism spectrum disorders) is once again difficult to do in mammals, but easy in fish.

Researchers can also use the fish to identify genes that contribute to autism when the number of gene copies — also called copy number variations — changes in people from the normal two (one on each chromosome) to one or three.

Table 1. Researchers have used zebrafish to study nearly 30 autism risk genes.

In these ways, fish can identify which autism risk genes are active during brain and body development, which variants alter gene function, and which lead to abnormalities as the number of gene copies changes.

Finally, fish are the only vertebrates in which researchers can perform large-scale whole-animal chemical screens. Researchers can incubate fish larvae with abnormal autism risk genes in sets of chemicals, in order to identify molecules that can overcome the effects of malfunctioning genes and make the fish normal. These screens identify potential therapeutics and are possible because we can obtain thousands of embryos, and because the fish larvae are so small — less than 1 millimeter — allowing many chemicals to be tested in a small space.

Cross collaboration:

In order to use fish for maximum clinical benefit, autism investigators with different expertise must work together. Human geneticists can pass patient DNA sequence information on to fish researchers, who can in turn let geneticists and clinicians know which autism risk genes and variants may be the most important, and which compounds may be worth considering for development as therapies. Mice must also be included, so that tests that assay for behaviorcan be performed in a mammal, on pivotal genes or compounds identified in the fish.

To date, researchers have used zebrafish to study almost 30 autism risk genes (**Table 1**), listed in the **SFARI Gene** database. Our own work, in which we are focusing on a region of human chromosome 16, called 16p11.2, **demonstrates the promise** of fish as an autism tool.

One copy of the 16p11.2 region is **lost in one percent of autism cases**. The region includes 25 genes, but which of those is important in autism has not yet been determined. Strikingly, postdoctoral associates Alicia Blaker-Lee and Sunny Gupta have shown that 20 of 21 genes in the 16p11.2 region tested in zebrafish lead to abnormal development if their function is prevented, demonstrating that this is a very active region of the genome and could include several autism-linked genes.

In order to connect loss of this region with autism spectrum disorders, we have begun to identify genes that lead to abnormal development when their gene function is reduced by 50 percent, as would be expected from the loss of one gene copy. Encouragingly, we have found a single gene, ALDOA, with this characteristic, which we call a 'copy number sensor.' We predict that its function will be important in autism. The gene can now be tested in mice and in individuals with autism.

None of this was known from studying other animals, emphasizing the unique contribution the fish can make to autism research.

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References:

1: Graham S. *Sci. Am.* (2002) **Article**

2: Sive H. *Dis. Model. Mech.***4**, 137-138 (2011) **PubMed**