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Research progress in molecular biology of fish immunoglobulin D

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Keywords: IgD; teleost; signaling pathway; B lymphocytes

Abstract

Immunoglobulins are the primary mediators of the humoral immune response in fish. Studies of immunoglobulins in fish are particularly important for the immunological control of fish diseases. While immunoglobulin D (IgD) was first discovered in 1965, it remains the least understood member of the antibody family. During evolutionary development from fish to humans, IgD has developed critical immunological functions. However, these immunological functions are not well understood. There are two forms of IgD, membrane IgD (mIgD) and secreted IgD (sIgD). sIgD and mIgD are formed by B lymphocytes through different splicing modes. In this paper, IgD's structure and formation process in fish, the distribution characteristics of IgD on B cells, the mediated signaling pathways, and IgD functions are reviewed.

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Introduction

Drugs remain the primary means for disease control in aquaculture (Solem and Stenvik, 2006). However, overuse of medicines can result in drug-resistant strains, drug residue, and damage to the microecological balance of aquatic animals; this makes drug control in aquaculture increasingly difficult and jeopardizes the quality and safety of aquatic products. Therefore, fundamental studies on the immune system, immune responses, and immune mechanisms of fish have essential theoretical and practical significance for the effective immunological control of fish diseases. Such studies are fundamental for controlling disease outbreaks and the sustainable healthy development of the aquaculture industry (Xia et al., 2019). Since immunoglobulins are the primary mediators of the humoral immune response in fish, studies of immunoglobulins in fish are particularly important for the immunological control of fish diseases.

Three types of immunoglobulins have been discovered in teleosts, including IgM, IgD, and IgZ/IgT. IgM was the first discovered immunoglobulin in Osteichthyes and is known to produce effective specific humoral antibody responses to various antigens. IgZ/IgT is the most recently discovered immunoglobulin in Osteichthyes. Research suggests that IgZ/IgT plays a primary role in mucosal immunity (Yu et al., 2020). For example, Zhang et al. demonstrated that IgZ/IgT in *Oncorhynchus mykiss* were the same as those of IgA in mammals, with these functions contributing to mucosal immunity (Zhang et al., 2010). Although IgD is an essential antibody in the immune system of Osteichthyes, its mechanism of action remains unknown.

1. Structure of IgD

Despite being first discovered in 1965, immunoglobulin D (IgD) remains the least understood member of the antibody family (Wu et al., 2014). During evolutionary development from fish to humans, IgD has developed critical immunological functions. Initially, IgD was thought to be generated soon after evolution and only expressed in some mammals. In 1997, Wilson et al. discovered a type of chimeric gene in catfish similar to the IgD δ chain of mammals and indicated that IgD is an ancient immunoglobulin that occurs early in the evolutionary stage of vertebrates (Wilson et al., 1997) (**Figure 1**). Subsequently, similar genes were discovered in *Salmo salar* (Hordvik et al., 1997), *Gadus morhua* (Stenvik et al., 2000), *Paralichthys olivaceus* (Hirono et al., 2003), *Tkaifugu rubripes* (Saha et al., 2004) and *Megahbrama amblycephala* (Xia et al., 2015) (**Figure 1**).

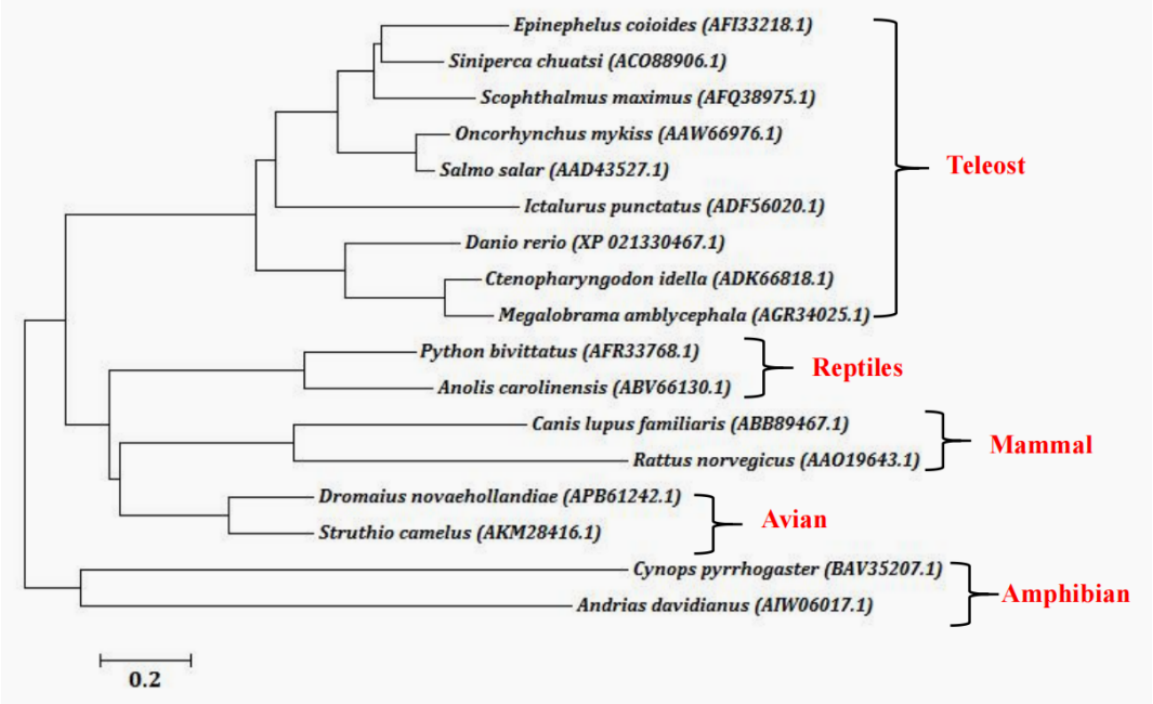

Figure 1 Phylogenetic analyses of IgD using neighboring-joining (NJ) methods. Bootstrap values are calculated from 1000 replicates.

IgD has two forms, membrane IgD (mIgD) and secreted IgD (sIgD) (Xia et al., 2019). sIgD and mIgD are formed by B lymphocytes through different splicing modes. They have similar heavy chain structures, aside from some differences in the carboxyl-terminal sequences in the constant region. Compared with sIgD, mIgD has one section of additional membrane-bound domain. Expression of mIgD on the B cell surface forms the B cell receptor (BCR). It is speculated that the BCR is mainly involved in recognizing and binding to antigens and signal transduction. In fish, sIgD has only been identified in *Oncorhynchus mykiss* and *Ictalurus punctatus*. sIgD mainly exists in serum and humoral liquid and mediates the humoral immune response (Yu et al., 2020; Perdiguero et al., 2019).

Compared with IgD in other vertebrates, IgD in fish has two characteristics. First, the transcript of fish IgD is a chimeric structure, which shares μ1 with IgM as the first constant region. This may be due to the lack of suitable cysteine-mediated L chain binding in δ1 exons and only μ1 can form interchain disulfide bonds to bind with L chains (Wilson et al., 1997). Second, there are many constant domains of IgD in fish and there are usually duplications of exons. Moreover, the number of duplications in the constant domain varies significantly among different fish (**Table 1**).

Table 1 IgD constant regions of different fish.

Fish	*IgD constant region*	*References*
Atlantic salmon		Hordvik et al., 1999
Channel catfish		Wilson et al., 1997
Atlantic halibut	μ1-δ1-(δ2-δ3-δ4)2 -δ5-δ6-δ7	Hordvik, 2002
Grass carp		Xiao et al., 2010
Redfin puffer	μ1-(δ1-δ2-δ3-δ4-δ5-δ6)2-δ7	Saha et al., 2004
Rainbow trout	μ1-δ1-δ2a-δ3a-δ4a -δ2b-δ7	Ramirez-Gomez et al., 2012
Atlantic cod	μ1-δ1A-δ2A -δy-δ1B-δ2B-δ7	Stenvik et al., 2000
Olive flounder		Hirono et al., 2003
Mandarin fish	μ1-δ1-δ2-δ3-δ4-δ5-δ6-δ7	Wang et al., 2010
Bluntsnout bream		Xia et al., 2015

The structural form of IgD in *Salmo salar*, *Ictalurus punctatus, Atlantic halibut,* and grass carp is VDJ-μ1-δ1-(δ2-δ3-δ4)2 -δ5-δ6-δ7-TM1-TM2; all these fish have one duplication of "δ2-δ3-δ4" (Hordvik et al., 1990; Bengtén et al., 2006; Xiao et al., 2010). In *Takifugu rubripes*, there is duplication of "δ1-δ6" with a structural form of VDJ-μ1-(δ1-δ2-δ3-δ4-δ5-δ6)2-δ7-TM1-TM2 (Saha et al., 1997). Two constant domains of "δ5-δ6" are missing in *Oncorhynchus mykiss*, but there is duplication of δ2 behind δ4. Its structural form is VDJ-μ1-δ1-δ2a-δ3a-δ4a -δ2b-δ7-TM1-TM2 (Hansen et al., 2005). In *Gadus morhua*, four constant domains of δ3-δ6 are missing and there is duplication of "δ1-δ2", which is separated by δy. The unique formula is VDJ-μ1-δ1A-δ2A -δy-δ1B-δ2B-δ7-TM1-TM2 (Stenvik et al., 2000). There are seven δ exons in *Paralichthys olivaceus* and *siniperca chuatsi*, among which there are no serial duplication forms (Wang et al., 2010). Significant structural differences are observed in δ chain genes between Osteichthyes and mammals. Moreover, the structures of the δ chain genes are obviously different among different fish (**Figure 2**).

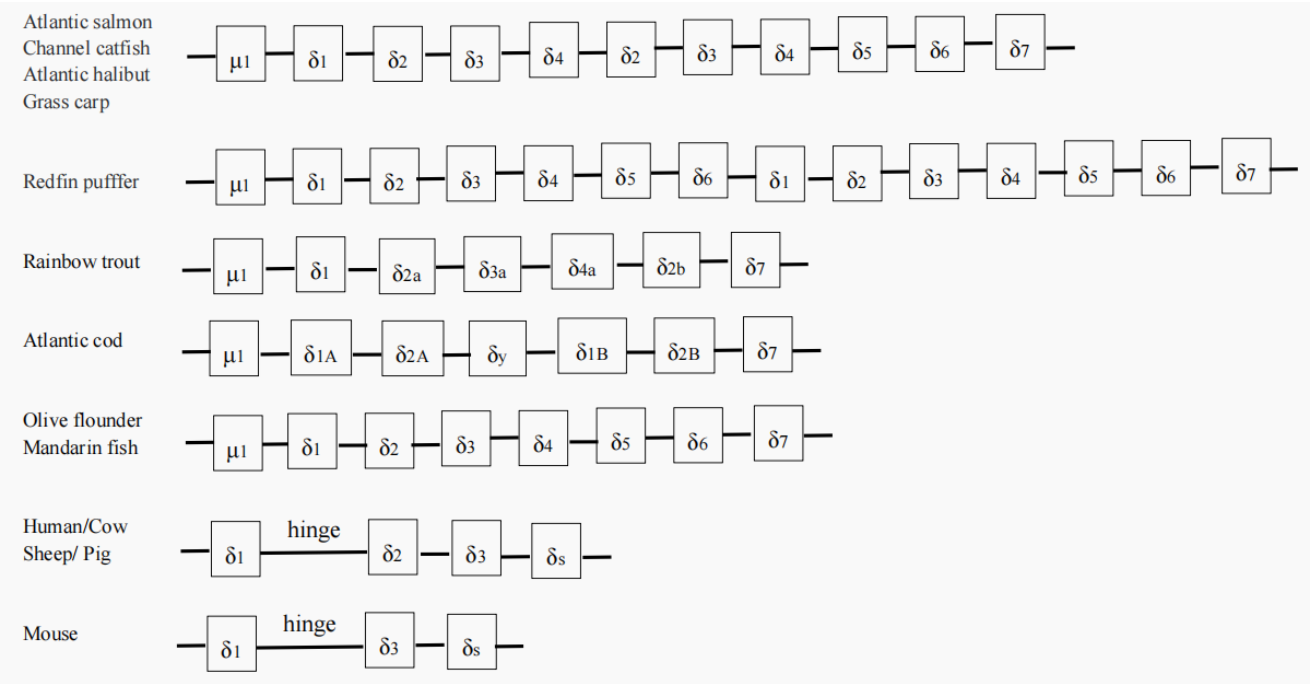

Figure 2 Gene structure of IgD constant region in teleost (Hansen et al., 2005; Hirono et al., 2003; Hordvik et al., 1999; Stenvik et al., 2000; Saha et al., 2004; Solem et al., 2006; Wilson et al., 1997; Wang et al., 2010; Xia et al., 2015; Xiao et al., 2010).

The IgD gene sequence of *Megalobrama amblycephala* is a chimeric structure containing the first constant region μ1 of IgM (Xia et al., 2015). The IgD heavy chain gene structure of *Megalobrama amblycephala* is VDJ-μ1-δ1-δ2-δ3-δ4-δ5-δ6-δ7 (Xia et al., 2015). The mIgD and sIgD gene sequences are highly similar. Compared with sIgD, mIgD has one section of the additional membrane-binding domain (δm) at the carboxyl-terminal of the constant domain (**Figure 3**). The structural form of *Megalobrama amblycephala* mIgD is VDJ-μ1-δ1-δ2-δ3-δ4-δ5-δ6-δ7-δm (Xia et al., 2015).

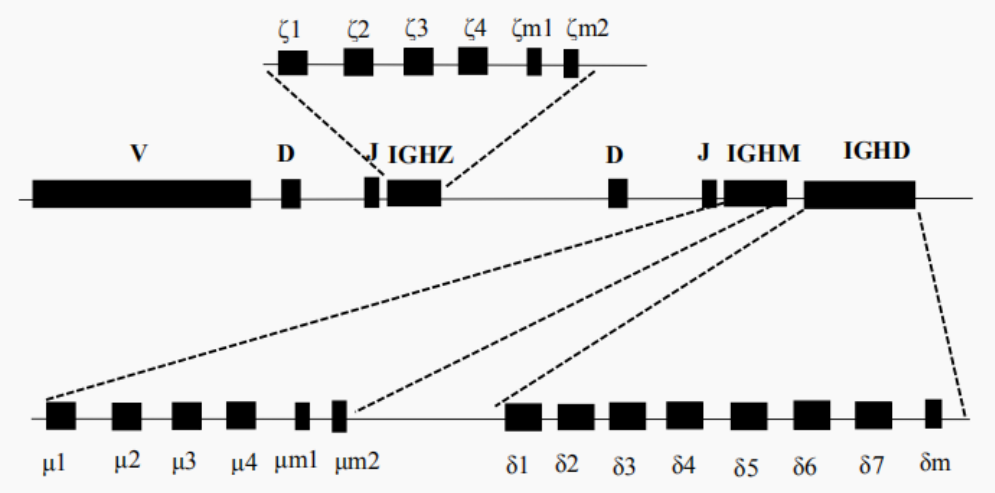

Figure 3 Organization of immunology heavy chain (IgH) gene locus in *Megalobrama amblycephala* (Xia et al., 2015).

2. Production process of IgD

In mammals, IgD and IgM are both antibodies that occur early in the development process of B cells. Intramedullary anterior B cells form IgM through the antigen-independent pathway; IgM is expressed on the B cell surface (Wu et al., 2014). IgD first appears when B cells migrate out from marrow and enter the blood and peripheral lymphoid tissues. At this time, IgD and IgM are expressed simultaneously on the surface of B cells; both are formed by a section of original mRNA through selective shearing of V/D/J, Cu and Cδ exons. IgD and IgM share the same V domain and antigen specificity. Higher expression of IgD than IgM on the cell surface is a sign of mature B cells. After the mature B cells were stimulated by antigen, the expression of IgD on the membrane surface was decreased. A part of B lymphocytes expressing IgM were transformed into plasma cells expressing IgG, IgA and IgE by class switch recombination (CSR). In some human B cells, B lymphocytes expressing IgM transformed into plasma cells expressing IgD (IgM^-IgD^+B cells) through the non-traditional CSR mode, which only expressed IgD. sIgD is responsible for regulating the balance and activity of B cells and strengthening immune system protection. However, the specific CSR mechanism remains unknown.

3. Distribution characteristics of IgD on B cells

Most studies on IgD expression and function have been performed using human and mouse tissues and cell lines, as well as knockout mice. In humans and mice, IgM and IgD are expressed on the surface of immature B cells through selective splicing of primary RNA transcripts. Double positive B cells (IgM^+/IgD^+) are important components of peripheral B cells and play a role in antigen binding, which down-regulates the expression of IgD. Most memory B cells can transform between different categories and express high-affinity IgG, IgE or IgA through high-density mutations of somatic cells. Category transformation also occurs in specific IgM^-/IgD^+B cells which are found in humans. Cells are transformed using a transformation domain between Cμ and Cδ genes (Perdiguero et al., 2019). Although the contents of IgM-/IgD+B cells in human peripheral blood is very low (about 3.0%), these cells are present in large amounts in the upper respiratory mucosa (20-25%), particularly in tonsil tissue. IgM^-/IgD^+B cells in the human upper respiratory tract are viewed as a special B cell cluster. Compared with traditional IgM^+/IgD^+B cells, IgM^-/IgD^+B cells in the human upper respiratory tract are larger in size, have more cytoplasm, and form plasmablasts. Eva-stina Edholm et al. discovered the IgM^-/IgD^+B cell group and IgM^+/IgD^+B cell group in *Ictalurus punctatus* (Edholm et al., 2010). In this study, the content of IgM^-/IgD^+B cells in peripheral blood was as high as 60-80% (Edholm et al.,

2010). In *Ictalurus punctatus*, IgM^-/IgD^+B cells are larger than IgM^+/IgD^+B cells and have more cytoplasm. IgM^+/IgD^+B cells secrete higher levels of IgM, but lower levels of IgD. IgM^-/IgD^+B cells form a type of special cell group which has a high expression level of IgD but no expression of IgM (Edholm et al., 2010). However, the relationship and interaction mechanism between IgM^-/IgD^+B cells and IgM^+/IgD^+B cells remains unclear. It is speculated that IgM^-/IgD^+B cells and IgM^+/IgD^+B cells might interact to function as an antigen binding receptor, thus facilitating activation, differentiation and proliferation of cells.

4. IgD-mediated signalling pathway

mIgD and mIgM have the same intracellular structure and both are comprised of three amino acids. The process by which downstream mIgD-type BCRs stimulate protein tyrosine kinase (PTK) and release calcium ions is the same as that of mIgM-type BCRs. However, tolerance induction, apoptosis or cytokine secretion differ due to the activation of mIgD or mIgM after B cells are activated. Geisberger et al. proposed that mIgD might trigger the signal threshold for cell activation, incompetence and death by increasing mIgM to regulate the functions of mIgM. Therefore, mIgD might transfer a kind of negative or tolerant signal (Geisberger et al., 2006). Compared with normal lupus mice, lupus mice with IgM defects have been found to have higher antibody levels and suffer serious nephritis due to abnormal T-cell subgroups (CD4-CD8-αβ+) and immune complex renal deposition (Lutz et al., 2006). It is speculated that the downstream signalling pathway mediated by mIgD might provide negative regulation over differentiation of antibody B cells and activation of abnormal T cells.

5. Functions of IgD

The reasons for the expression of IgM and IgD membrane receptors on the surface of B cells remain unclear. One assumption is that IgM and IgD can transduce different signals. It is speculated that IgM and IgD are related to unique BCR-associated proteins. Some studies have suggested that mIgD plays a role in the tolerance or apoptosis signal transfer pathway (Chen et al., 2014). Antigen binding with IgM on immature B cells in vivo may lead to cell apoptosis, while antigen binding with IgD does not. The mIgD expression level in incompetent B cells in mice is higher than that of mIgM. Human B cells that have higher expression of mIgD than mIgM produce weak responses to antigen stimuli. These B cells also express spontaneous reactive mIgD, which can activate the tolerance mechanism, leading to immune anergy.

Moreover, the reaction between mIgD and superantigen genes in mice can cause development stagnation of immature B cells. The degree of stagnation is related to the initial expression level of mIgD in the developmental stage. However, opposing results indicate that mIgD might inhibit B cell tolerance; thus, the above experimental results might be due to different categories of B cell lines, different degrees of B cell maturity, and different receptor densities on the B cell surface (Wu et al., 2014).

In mammals, knockout mouse experiments have shown that IgD can replace IgM in the early development of B cells (Lutz et al., 1998). The influences of IgD or IgM heavy chain defects had minor effects on mice. Lutz pointed out that IgD can replace the functions of IgM to a very great extent and the number of mature B cells in the peripheral blood of mice with IgD defects was decreased by 30∼50% (Lutz et al., 1998). IgD is superior to IgM in its functions and plays a vital role in regulating immune responses and immune homeostasis (Lutz et al., 1998).

Although the function of IgD has been well studied in mammals, there are very few studies of the function of IgD in fish. Tian et al. stimulated mandarin fish with *Flavobacterium cloumnare* and collected samples on the 1st, 2nd, 3rd, and 4th week, respectively (Tian et al., 2009). qPCR revealed that the expression of IgD in the head kidney was upregulated significantly at the 3rd and 4th week; its expression in the spleen was markedly upregulated at the 3rd week; its expression in the gills was markedly

upregulated at the 1st and 2nd week, but then declined to normal levels (Tian et al., 2009). The expression of IgD in blood did not fluctuate significantly throughout the experiment, except for a small amount of growth in the 3rd week, and it was speculated that IgD transcription does not occur in the blood (Tian et al., 2009). Perdiguero et al. found that IgD+/IgM-B cells are the primary lymphocytes in the intestines and gills of *Oncorhynchus mykiss*, and the combination of IgD symbiotic bacteria in the intestines may stimulate the expression of IgD in intestinal B cells (Perdiguero et al., 2019). In addition, studies have shown that sIgD can combine with symbiotic bacteria on the oral mucosa, pharynx mucosa, gill mucosa, and intestinal mucosa in a teleost, and it was speculated that sIgD might play an essential role in maintaining mucosal homeostasis (Yu et al., 2020). After infection with *Aeromonas hydrophila* by intraperitoneal injection, IgD expression in the kidney of *Megahbrama amblycephala* reached its peak on the 7th day after infection, the expression of IgD in the spleen, liver, and intestine reached the maximum value on the 21st day after infection, and in gills reached the maximum value on the 14th day after infection (Xia et al., 2015). According to the expression pattern of the IgD gene in different tissues after infection with *Aeromonas hydrophila*, it is speculated that IgD may play an essential role in the resistance of *Megalobrama amblycephala* against *Aeromonas hydrophila* infection (Xia et al., 2015).

Using monoclonal antibody 2E5 against secretory IgD, IgD can be detected in the serum of *Ictalurus punctatus* (Bengten et al., 2002). The concentration of secreted IgD in the serum of *Ictalurus punctatus* is 0.04mg/ml, which was detected by the coomassie brilliant blue method (Bengten et al., 2002). At present, it is known that the IgD gene mRNA of rainbow trout exists in all immune tissues, but at the protein level, the concentration of IgD in serum is deficient and its function is still unclear (Ramirez-Gomez et al., 2012). In rainbow trout plasma, the total IgD level is in the range of 2-80ug/ml, but the detection level of mucosal secretion may be very low or lower than this level (Yu et al., 2018). Xu et al. found that the concentration of IgD in serum and gill mucus of rainbow trout had no significant change during infection with *Ichthyophthirius multifiliis* (Xu et al., 2016). Yu et al. detected IgD in the nasal mucus of rainbow trout infected with *Ichthyophthirius multifiliis* and found that there was a small amount of specific IgD in the nasal mucus of rainbow trout (Yu et al., 2018). In addition, some studies have shown that sIgD can combine with symbiotic bacteria on the oral mucosa, pharyngeal mucosa, gill mucosa, and intestinal mucosa, and it is speculated that sIgD may play an essential role in maintaining mucosal homeostasis (Yu et al., 2020).

6. Conclusions and prospects

The transcript of fish IgD is a chimeric structure that shares μ1 with IgM as the first constant region. There are many constant domains of IgD in fish, and there is usually duplication of exons. Moreover, the number of repetitions of constant domains varies significantly among different fish. B lymphocytes form two forms of IgD molecules, mIgD and sIgD, through different splicing modes. There are very few studies of IgD functions in fish, and the IgD-mediated immune signaling pathway is still unclear. Further studies on the roles and functions of IgD in the immune system of fish under pathogenic bacterial infection are still required.

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The authors declare no conflict of interest.

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