The Dopamine D5 Receptor Is Involved in Working Memory

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Pharmacological studies indicate that dopamine D1-like receptors (D1 and D5) are critically involved in cognitive function. However, the lack of pharmacological ligands selective for either the D1 or D5 receptors has made it difficult to determine the unique contributions of the D1-like family members. To circumvent these pharmacological limitations, we used D5 receptor homozygous (−/−) and heterozygous (+/−) knockout mice, to identify the specific role of this receptor in higher order cognitive functions.

We identified a novel role for D5 receptors in the regulation of spatial working memory and temporal order memory function. The D5 mutant mice acquired a discrete paired-trial variable-delay T-maze task at normal rates. However, both D5+/− and D5−/− mice exhibited impaired performance compared to D5+/+ littermates when a higher burden on working memory faculties was imposed. In a temporal order object recognition task, D5+/− exhibited significant memory deficits. No D5-dependent differences in locomotor functions and interest in exploring objects were evident. Molecular biomarkers of dopaminergic functions within the prefrontal cortex (PFC) revealed a selective gene-dose effect on Akt phosphorylation at Ser473 with increased levels in D5−/− knockout mice. A trend toward reduced levels in CaMKKbeta brain-specific band (64 kDa) in D5−/− compared to D5+/+ was also evident. These findings highlight a previously unidentified role for D5 receptors in working memory function and associated molecular signatures within the PFC.

Keywords: dopamine, D5 dopamine receptor, working memory, prefrontal cortex, Akt, cognition, recency memory, mice

INTRODUCTION

Dopaminergic signaling in the brain serves a critical role in cognitive functions (Nicoullon, 2002; Papaleo et al., 2008, 2012; Detrait et al., 2016). This is especially evident in higher order executive functions modulated by the prefrontal cortex (PFC) such as attentional control, working memory, cognitive flexibility, and decision-making (Robbins and Arnsten, 2009; Floresco, 2013; Papaleo et al., 2014). In particular, consistent evidence indicates that the mesocortical dopaminergic system modulates these different cognitive processes by distinct receptor mechanisms. Specifically, activity of the D1-like (D1 and D3) receptor family has a strong impact on the regulation of working
memory, attention, and recency memory across multiple species (Sawaguchi and Goldman-Rakic, 1994; Müller et al., 1998; Aultman and Moghaddam, 2001; Lidow et al., 2003; Managò et al., 2016). In contrast, both D1-like and D2-like (D2, D3, D4) receptor families seem to be implicated in mediating the ability to shift between attentional sets (i.e., cognitive flexibility) (Floresco et al., 2006). Unfortunately, currently available D1-like agonists and antagonists do not have significant selectivity for either the D1 or the D2 receptors (Nichols, 2010). Moreover, in the cortex there is significant overlap between D1 and D3 receptor localization, and the D1 receptor is much more prevalent compared to the D3 receptor (Smiley et al., 1994; Khan et al., 2000), further impeding the investigation of the selective role of D3 receptors in cortex-dependent cognitive functions.

The generation of D1 and D3 genetically modified mice has helped elucidate critical functions of the two receptors in multiple physiological processes (Smith et al., 1998; Miyamoto et al., 2001; Montague et al., 2001; Hollon et al., 2002; Karlsson et al., 2008). In particular, D1 receptor null mutants have deficits in higher order cognitive functions such as working memory (Drago et al., 1994; Xu et al., 1994; Holmes et al., 2001; Xing et al., 2012). In contrast, there have been fewer studies on the behavioral effects of selective disruption of the D3 receptor. An early study of D3 knockout mice indicated that the behavioral consequences of the mutation were minimal. These mice showed no alterations in general health, sensory abilities, neurological reflexes, locomotor activity and coordination, prepulse inhibition, anxiety-like states measured with the elevated plus maze and light-dark box (Holmes et al., 2001). In cognitive function, D3 knockout mice were first reported to have no alterations in performing the hippocampal-dependent Morris water maze or fear conditioning (Holmes et al., 2001). However, a more recent study using mice with the same mutation found significant deficits in object recognition memory, object location memory, Morris water maze performance, and reduced locomotor activity (Moraga-Amaro et al., 2016). The discrepancies between these two studies may be due to differences in the experimental procedures or the different genetic backgrounds used (Holmes et al., 2001: F2 129/SvJ X C57BL/6; Moraga-Amaro et al., 2016: C57BL/6). Additionally, there is still no information on how D3 receptor disruption affects PFC-dependent cognitive function such as spatial working memory and recency memory. The goal of the present study was to investigate the potential involvement of the D3 receptor in working memory function using a well-validated discrete paired-trial variable-delay non-match to place T-maze task (Papaleo et al., 2008, 2012) and a temporal order object recognition task (Managò et al., 2016; Papaleo et al., 2016). Both tasks have been shown to rely on medial PFC functioning (Kellendonk et al., 2006; Barker et al., 2007) and are sensitive to dopaminergic modulation (Hotte et al., 2005; Papaleo et al., 2012; Managò et al., 2016).

We show that partial reduction (D3+/-) as well as the complete absence (D3-/-) of D3 receptors produces working memory and recency memory deficits suggesting a previously undetected direct role for D3 receptors in PFC-dependent higher order cognitive functions. Finally, we unraveled subtle, but selective alterations in molecular biomarkers within the mPFC of D3 knockout mice. These initial data identify a previously unknown role for the dopamine D3 receptor in cognition and related PFC functioning.

**MATERIALS AND METHODS**

**Mice**

The D2+/- and their D2+/+ and D2-/- littermates were produced as previously described (Holmes et al., 2001). The mice from this mutant line were backcrossed with C57BL/6 mice for 10 generations before testing. We utilized a heterozygous breeding scheme in order to produce mixed litters with all three genotypes. Mouse genotypes were confirmed by PCR. Mice were weaned at P28 and group housed except in the T-maze experiments where mice were single housed starting 1 week before testing. Mice used for testing were male and between P63 and P126 days of age. All procedures were approved by the National Institute of Mental Health Animal Care and Use Committee and followed the National Institutes of Health Using Animals in Intramural Research Handbook.

**Discrete Paired-Trial Variable-Delay T-Maze Task**

The procedure for this T-maze task was similar to one previously used in our laboratory (Papaleo et al., 2008). Mice were habituated to single housing for 1 week and were then food restricted to a level of 85% of their free-feeding weight. The mice were given 8 days for their weight to stabilize and received access to 10 reward pellets (STUL 14 mg pellets; TestDiet, Richmond, IN, United States) during the last 3 days of this period. Following habituation to single housing and stabilization of body weight, mice were habituated to the T-maze apparatus over the course of two sessions. The T-maze apparatus was made of clear acrylic [dimensions of arms (length x width x height): 40 x 10.2 x 17.5 cm]. A recessed food cup was located at the end of each arm. During habituation sessions mice were allowed to retrieve reward pellets from the food cups. At the beginning of the session, each cup was baited with two reward pellets. The cups were re-baited continuously. Mice were allowed to retrieve 16 reward pellets during Session 1 and 20 reward pellets during Session 2. Each session automatically ended after 10 min if the mouse did not retrieve the maximum number of reward pellets. On the day following habituation, mice were given one session of 10 forced-alternation runs. For this session, one goal arm was blocked and the mouse had 2 min to consume the reward pellet located in the open arm. After an inter-trial interval of at least 15 min, the mouse was returned to the maze for another forced run with the open/closed arms switched. Training for the discrete paired-trial delayed alternation task began on the following day. Training consisted of 10 paired trials each day. A paired trial consisted of a forced run where one arm was blocked and the other arm was baited with a single reward pellet. The mouse was given 4 min to consume the pellet. Following consumption, the mouse was returned to the home cage for a 4-s intratrial delay. After the intratrial delay, the mouse was returned to the maze with access to both arms. The arm blocked on the forced run
was now baited with two reward pellets. Again, the mouse was given 4 min to consume the reward pellets. After an inter-trial interval of at least 15 min, mice were returned for another trial. If the mouse entered the unbaited arm, this was recorded as an error and the mouse was removed from the maze. The normal inter-trial interval followed incorrect trials as well. Each testing session utilized a pseudo-randomly chosen pattern of 10 forced runs. Each day, the same pattern was used for each mouse. Mice were trained using these parameters for 20 days or until they reached 80% accuracy for 3 consecutive days. Mice that failed to reach 80% accuracy for 3 consecutive days within the 20-day training period were excluded from the study. Mice were then tested using variable intratrial intervals (4, 30, 60, and 240 s) and a 20-s inter-trial interval. Mice were given four trials of each inter-trial interval on 4 consecutive days.

Open Field Locomotor Activity

The experimental apparatus consisted of a novel Plexiglas open field arena (42 × 42 × 30 cm) under red light illumination (5 ± 2 lux). Each mouse was allowed to freely explore the open field alone for 60 min. Horizontal locomotor activity was recorded using infrared photobeam sensors and the VersaMax Open Field Activity Monitoring system (AccuScan Instruments, Inc., Columbus, OH, United States).

Temporal Order Object Recognition Task

Temporal order object recognition testing was conducted as previously described (Managò et al., 2016). The apparatus and lighting conditions were identical to those used for the open field locomotor activity test. On day 1, mice were allowed to freely explore the open field for 60 min. Day 2 consisted of three 5-min sessions. During the first session, mice were allowed to explore two identical objects within the open field arena. The objects were either rectangular boxes (3 × 3 × 6 cm) or Erlenmeyer flasks (4 × 6 cm). The objects could either be white or black. During the second session, 1 h after the first session, mice were allowed to explore two objects of a different shape and color with respect to the objects from the first session. During the third session, 3 h after the second session, mice were allowed to explore one copy of each of the objects presented during the first and second sessions. The sessions were videotaped and scored offline by a reviewer blind to genotype. Mice were considered to be exploring an object when they faced the object and were ≤ 2 cm from the object. Discrimination between the objects in the third session was calculated using a discrimination index that accounts for individual differences in total exploration time. The index was calculated as the difference between the time spent exploring the object from the first session and the object from the second session divided by the total exploration time. Any mice that did not explore objects for more than 4 s during all of the sessions were excluded from the final analysis. One D5+/+, one D5+/−, and four D5−/− mice were excluded because of low total exploration.

Immunoblotting

Frontal cortex tissue was obtained from naive D5+/+, D5+/−, and D5−/− mice. Briefly, mice were killed by decapitation and

Statistical Analysis

The habituation and training phases of the T-maze task were analyzed using one-way ANOVAs with post hoc Bonferroni’s tests. The variable delay portion was analyzed by a two-way ANOVA with genotype as the between-subjects factor and retention interval as the within-subjects factor and post hoc analyses utilized Bonferroni’s tests at each of the retention intervals. Data from the temporal order object recognition task were analyzed using a one-way ANOVA with post hoc Bonferroni’s tests. Protein quantification data for each mouse was first normalized to GAPDH levels (actin for TH protein levels) and then normalized to the mean D5+/+ value for each protein. Group means were then compared using one-way ANOVAs with post hoc Bonferroni’s tests. All data are shown as the mean ± SEM.

RESULTS

D5 Genetic Disruption Did Not Alter Locomotor Functioning or Approach Responses to Food Reward

To identify any potential confounding effects of D5 receptor disruption, we measured open field locomotor activity and approach responses to food reward in the T-maze apparatus. There were no D5 genotype differences in total locomotor activity (F(2,30) = 0.66, p = 0.9420; Figure 1A) or any genotype × time interactions during any of the 5-min time bins within the 60-min test (F(22,330) = 0.63, p = 0.9000; Figure 1B). As expected there was a significant decrease in activity over time for all groups (F(11,330) = 35.68, p < 0.0001; Figure 1B).
TABLE 1 | Primary antibodies.

| Antigen      | Type (clone) | Dilution | Product number | Manufacturer                  |
|--------------|--------------|----------|----------------|------------------------------|
| pAkt (Thr308)| RbM (C31E5E) | 1:1000   | 2965           | Cell Signaling Technology    |
| pAkt (Ser473)| RbP          | 1:1000   | 9271           | Cell Signaling Technology    |
| tAkt        | MM (4D4)     | 1:2000   | 2920           | Cell Signaling Technology    |
| Camkkbeta   | GP           | 1:1000   | sc-9629        | Santa Cruz Biototechnology   |
| Camk2       | MM (G-1)     | 1:5000   | sc-6306        | Santa Cruz Biototechnology   |
| Camk4       | GP           | 1:200    | sc-1541        | Santa Cruz Biototechnology   |
| Drd2        | MM (B-10)    | 1:1000   | sc-6303        | Santa Cruz Biototechnology   |
| Comt        | MM (4/COMT)  | 1:10,000 | 611970         | BD Biosciences               |
| pTH Ser40   | RbP          | 1:800    | A13935         | Millipore                    |
| TH           | RbP          | 1:2000   | AB152          | Millipore                    |
| GAPDH       | MM (mAbcam 9484) | 1:10,000 | ab9484         | Abcam                        |
| Actin       | RbP          | 1:5000   | A20666         | Sigma–Aldrich                |

RbM, rabbit monoclonal; RbP, rabbit polyclonal; MM, mouse monoclonal; GP, goat polyclonal.

The first phase (two sessions) of the T-maze task is designed to habituate the mice to the testing apparatus and retrieval response required for completion of the task. All groups showed a significant decrease in latency to consume the first pellet from Day 1 to Day 2 ($F_{(1,30)} = 41.71, p < 0.0001$; Figure 2A). Additionally, there were no differences in the raw latency values between the genotypes on either Day 1 or Day 2 ($F_{(2,30)} = 1.20, p = 0.31$). These data support the notion of a negligible impact of $D_5$ on measures of locomotion and motivation.

**D$_5$ Knockout Mice Learn the Non-match to Sample Rule at the Same Rate As D$_{5+}^{+/+}$ Mice**

The next stage of the T-maze task consisted of training required for the mice to learn the non-match to sample rule. There were no significant differences in the number of sessions required to reach the criterion of three consecutive sessions above 80% accuracy ($F_{(2,29)} = 0.70, p = 0.50$; Figure 2B). However, there was a significant difference in accuracy during those last three training sessions ($F_{(2,29)} = 3.63, p = 0.04$; Figure 2C). The genotype effect on accuracy was driven by a slight, but significant, difference between $D_5^{+/+}$ and $D_5^{-/-}$ ($p = 0.04$). The difference between $D_5^{+/+}$ and $D_5^{-/-}$ was not significant ($p = 0.22$). These data indicate a marginal role of the $D_5$ receptor in the ability to acquire working memory rules.

**D$_5$ Knockout Mice Have Working Memory Deficits Compared to D$_{5+}^{+/+}$ When Tested in the Variable Retention Version of the Task**

Following successful completion of the training phase, mice were tested in the variable retention delay portion of the task. In addition to the variable retention delays, the inter-trial delay was set at 20 s (Aultman and Moghaddam, 2001). Accuracy decreased as the retention interval increased across all genotypes ($F_{(2,29)} = 11.00, p < 0.0001$). There was also a significant main effect of $D_5$ genotype on performance ($F_{(2,29)} = 4.91, p = 0.01$). $D_5^{-/-}$ mice showed significantly impaired performance compared to $D_5^{+/+}$ on trials with either 4- or 30-s retention intervals ($p = 0.004$ and 0.001, respectively; Figure 2D). $D_5^{-/-}$ mice had impaired performance at the 30-s interval compared to $D_5^{+/+}$ mice ($p = 0.033$; Figure 2D). There were no significant differences between the genotypes on either the 60- or 240-s retention intervals due to a decrease in the choice accuracy of the $D_5^{+/+}$ mice. Interestingly, the $D_5^{-/-}$ mice had an intermediate phenotype suggesting that there is a gene dose effect of $D_5$ dopamine receptor expression. These findings highlight a clear and previously undetected role of $D_5$ receptors in working memory abilities.
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FIGURE 2 | D5+/− and D5−/− mice display spatial working memory deficits. (A) During habituation, there are no genotype differences in latency to consume the first pellet. (B) All mice learn the non-match to sample rule in the same number of days. (C) D5−/− mice had significantly lower accuracy compared to D5+/− mice, but were no different from D5+/+ mice on the last 3 days of training. (D) During testing when the inter-trial interval was decreased to 20 s, D5−/− mice showed decreased performance at the 4 and 30-s retention intervals compared to D5+/+ mice. Additionally, D5+/− mice demonstrated impaired performance at the 30-s retention interval. n = 8 in the D5+/+ group, 15 in the D5+/− group, and 9 in the D5−/− group. ∗p < 0.05, ∗∗p < 0.01 (D5−/− compared to D5+/+), and #p < 0.05 (D5+/− compared to D5+/+).

had a significantly lower discrimination index compared to the D5+/+ group (p = 0.046), but only a tendency was evident for D5−/− mice. Thus, there might be an U-shaped gene-dose effect on temporal order object recognition as the D5−/− group’s discrimination index was not different from the D5+/+ group’s index (p = 0.52). One sample t-tests indicated that both the D5+/+ (t(7) = 3.28, p = 0.01) and D5−/− (t(10) = 2.50, p = 0.03) groups showed significant recency memory while the D5+/− (t(14) = 0.44, p = 0.67) group did not.

D5 Knockout Mice Show Selective Gene-Dose Effect on Akt Ser473 Phosphorylation in the PFC

The working memory and temporal order recognition deficits exhibited by D5 mutant mice are similar to those our group has observed in other mouse genetic models of dopamine-related cognitive dysfunction (Papaleo et al., 2008, 2012). Moreover, performance in the discrete paired-trial variable-delay T-maze and temporal order object recognition tasks has been shown to be modulated by alterations in PFC function (Kellendonk et al., 2006; Barker et al., 2007). Thus, we next investigated whether D5 knockout mice might have working memory- and dopamine-related molecular alterations within the mPFC (Papaleo et al., 2008, 2014; Tan et al., 2012; Easton et al., 2013; Managò et al., 2016).

We measured the relative amounts of multiple proteins in the frontal cortex across all three genotypes. These data are presented in Figure 4. Akt is a key intracellular regulatory protein involved in dopaminergic signaling and implicated in psychiatric disorders (Emamian et al., 2004; Beaulieu et al., 2007). There was no significant difference in pAkt Thr308 (F(2,22) = 0.46,
DISCUSSION

In this study, we show that disruption of the dopamine D₅ receptor results in impaired spatial working memory and temporal order memory function. These findings unravel a previously unidentified selective involvement of the D₅ receptor as a critical modulator of higher order cognitive functions associated with the PFC.

The lack of pharmacological agents with selectivity for either the D₁ or D₅ receptor has made it difficult to identify the specific contributions of either receptor to central nervous system function and behavior (Nichols, 2010). Previous research with total D₅⁻/⁻ mice and conditional constructs indicated that the D₅ receptor, in contrast to the D₁ receptor, plays a modest role in dopamine-mediated behaviors (Holmes et al., 2001; Karlsson et al., 2008; Sariñana et al., 2014). However, recent studies utilizing D₅⁻/⁻ mice suggest a role in fear memory consolidation through modulation of phospholipase C signaling (Ouyang et al., 2012) and a role in regulating BDNF and Akt function in the PFC (Perreault et al., 2013). A recent study using the same line of D₅ mutant mice as our current study also identified deficits in spatial and recognition memory in the knockout mice (Moraga-Amaro et al., 2016). Those mice also exhibited reduced locomotor activity, reduced object exploration, and increased anxiety-related states (i.e., increased latency to explore objects), not seen by either Holmes et al. (2001) or us, that may have influenced their cognitive performance. The differences in locomotor activity and object exploration between our study and the report of Moraga-Amaro and colleagues may be due to differences in experimental procedures or genetic background. Nonetheless, loss of the D₅ receptor appears to significantly alter behavior including cognitive function. The spatial working memory deficit we describe is similar to other genetic mouse models characterized by altered dopaminergic function in the PFC (Papaleo et al., 2008, 2012). These findings indicate that dopaminergic signaling through the D₅ receptor may serve a previously underappreciated role in behavior. Our current work is the first report indicating the involvement of the D₅ receptor in spatial working memory function measured with a delayed non-match to place T-maze task.

Previous studies using D₁/₅ agonists and antagonists have implicated D₁-like receptors in the regulation of working memory (Aultman and Moghaddam, 2001; Mizoguchi et al., 2009). Additionally, the D₅ receptor is widely expressed in the cerebral cortex and hippocampus, regions critically involved in spatial working memory function (Knowlton et al., 1985; Luciana and Collins, 1997; Ciliax et al., 2000; Khan et al., 2000), suggesting there may be a specific role for the D₅ receptor. Interestingly, there appears to be some redundancy in D₁-like receptor modulation of working memory. Indeed, D₁ receptor knockout mice, like D₅ receptor knockout mice, show deficits in working memory function and abnormal regulation of BDNF protein levels ($F_{(2,13)} = 1.04, p < 0.38$; Figures 4A,B). Overall, these findings indicate a selective impact on PFC Akt activation by D₅ receptor while sparing other dopamine-related biomarkers.
in the PFC (Xing et al., 2012). Like spatial working memory, temporal order recognition memory requires intact signaling between the PFC and hippocampus (Barker et al., 2007; Barker and Warburton, 2011). Here we report a potential U-shaped relationship between the degree of D₅ receptor insufficiency and performance in the temporal order recognition task in contrast to the apparent linear gene-dose relationship seen in spatial working memory. Although the underlying cause of the discrepancy between relative performance in the temporal order object recognition task and discrete paired-trial variable-delay T-maze is unknown, previous research has shown that the optimal dopaminergic tone is variable depending on the particular task with which the animal is currently engaged (Floresco, 2013).

In the current experiments, we investigated the protein levels of CaM kinases because previous studies using mouse models of dopaminergic dysfunction suggested a role for this family of kinases in modulating working memory in this particular T-maze task (Papaleo et al., 2012). In particular, our previous studies linked an alteration of overall dopamine levels within the PFC (Papaleo et al., 2008) or altered D₂ trafficking (Papaleo et al., 2012) with CaM kinases expression. In contrast, no major D₅-dependent effect was evident in CaM levels, with the possible exception of the brain-specific CaMKβ isoform. We did not observe any alterations in TH or dopamine D₁ receptor protein levels. Moreover, previous research demonstrated no change in dopamine D₁ receptor function following D₃ inactivation (Hollon et al., 2002). Thus, our findings combined with previous evidence that D₁/D₅ receptor pathways modulate PFC long-term potentiation and intrinsic excitability through the activation of CaMK pathways (Chen et al., 2007) suggest a possible selective role of D₁ receptors in these processes.

The only significant change in our protein assays in the PFC resulting from the loss of the D₅ receptor was an increase in pAkt (Ser473). This might be in agreement with previous pharmacological manipulation suggesting that the D₅ receptor regulates phosphorylation of Akt in the PFC in mice (Perreault et al., 2013). Akt activity has been linked to cell proliferation, growth, survival, and metabolism, and it has been implicated in sex differences and psychiatric disorders (Chen et al., 2004; Emamian et al., 2004; Beg et al., 2017; Sannino et al., 2017). In particular, Akt activity has been proposed as an intracellular key regulatory protein directly linked to the activity of D₂ postsynaptic receptors (Beaulieu et al., 2007). Here we add new evidence implicating D₅ receptors in Akt-mediated signaling that will require further and more focused investigation.

The D₅ receptor is uniquely located to play an important role in modulation of PFC function. Anatomical studies in nonhuman primates show that D₅ receptors are positioned in extrasynaptic microdomains where they can interact with the 1,4,5-triphosphate receptor to mobilize calcium from intracellular stores (Paspalas and Goldman-Rakic, 2004). These microdomains are critical locations for the signaling mechanisms underlying dopaminergic volume transmission in the cortex. The current results point specifically to a critical role for the D₅ receptor in PFC-dependent spatial working memory as well as recency memory. Further studies may serve to define the parameters of D₅ dopamine receptor activity as it relates to other cognitive domains. However, given the relatively precise localization of D₅ receptors, a therapeutic strategy selectively targeting them may improve cognitive function with potentially fewer side effects compared to drugs selectively targeting the D₁ receptor. This would be relevant for many neurological and psychiatric disorders, such as schizophrenia, bipolar disorders, and others (Wu et al., 2012; Narayanan et al., 2013; Laruelle, 2014).

CONCLUSION

D₅⁺/− and D₅⁻/− mice have spatial working memory deficits in a discrete paired-trial variable-delay T-maze task as well as recency memory deficits in a temporal order object recognition task. These data represent new evidence that the dopamine D₅ receptor is directly involved in higher order cognitive functions.

AUTHOR CONTRIBUTIONS

GC, FM, DS, DW, and FP contributed to the conception and design of the reported studies. GC and FM conducted all of the experiments. GC, FM, and FP analyzed the data. GC, FM, DS, DW, and FP contributed to the drafting and revision of the manuscript. All authors approved the final version and agreed to be accountable for all aspects of the work.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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