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CFTR acts as a potential therapeutic target for attention deficit-hyperactivity disorder

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The prevalence of attention deficit-hyperactivity disorder (ADHD) symptoms among individuals with cystic fibrosis (CF) is significantly elevated compared to the general population. Given that the cystic fibrosis transmembrane conductance regulator (CFTR) is the causative gene for cystic fibrosis, this raises the possibility of CFTR playing a crucial role in ADHD. In our study, three heterozygous missense variants (p.E217G, p.F316L, and p.T1220I) were detected in the CFTR gene, which co-segregate with ADHD in two consanguineous families, impacting a total of six family members. Through the utilization of a zebrafish model, it was observed that the *cftr* knockout line exhibited behaviors akin to hyperactivity, impulsivity, and attention deficits, mirroring the symptoms seen in human ADHD patients. Single-cell RNA sequencing performed on 7 dpf larvae revealed clusters of neuron cells that exhibited sensitivity to *cftr*, particularly noting a reduction in the number of dopaminergic neuron cells within the *cftr* mutant fish. Additionally, bulk RNA sequencing and proteomic analysis conducted during the early gastrulation stage demonstrated abnormal expression levels of nervous system genes. Notably, we attempted to employ CFTR modulators Lumacaftor (VX-809) and Ivacaftor (VX-770) to ameliorate the ADHD zebrafish model (generated via *per1b* mutant), and it was found that enhanced CFTR activity could mitigate ADHD-like behaviors. In summary, our findings shed light on the potential involvement of CFTR in the pathogenesis of ADHD and pave the way for exploring novel diagnostic approaches and therapeutic strategies for ADHD by targeting CFTR.

Keywords CFTR, ADHD, Zebrafish, Treatment

Attention deficit hyperactivity disorder (ADHD) is one of the most prevalent and heritable debilitating neurodevelopmental disorders characterized by inattention, excessive motor activity, impulsivity, and distractibility, affecting 2–6% of school-age children[1,2]. Studies have found that at least 60% of children diagnosed with ADHD still exhibit some symptoms into adulthood[3]. Current studies remain poorly understood on the neurobiological pathologic mechanism of ADHD. Of note, classical genetic studies indicate that ADHD is strongly heritable, with an estimated heritability for childhood ADHD on an average of 75%[4].

Importantly, a systematic review shows that the reported prevalence rates of ADHD in cystic fibrosis (CF) ranged from 5.26–21.9%[5]. Malena et al. propose that ADHD should be recognized as a co-morbidity of CF, because the occurrence of ADHD symptoms in CF patients is substantially higher than in the general population[6]. It is well-known that cystic fibrosis transmembrane conductance regulator (CFTR) is a pathogenic gene in cystic fibrosis, CFTR variants have been shown to cause CF[7]. Therefore, these findings suggest that CFTR may be associated with ADHD.

The zebrafish (Danio rerio) is becoming an important tool to investigate genetic and pathophysiological mechanisms of various neuropsychiatric disorders. Zebrafish is widely used as an animal model to study neurodevelopment, including the ADHD animal model[8]. Using zebrafish to explore the level of gene knockout may be a way to understand the cause of ADHD further.

In this work, we found CFTR variants in ADHD families, and used the *cftr* mutant zebrafish model to identify the critical role of CFTR in ADHD. Multi-omics analysis elucidated the molecular mechanisms by which CFTR regulates dopamine neurons. In $cftr^{+/-}$ zebrafish larvae, the ratio of dopamine neurons was reduced to 0.42-fold that of wild-type (WT) larvae. These neurons play a crucial role in regulating complex behaviors such as cognition, motivation, learning, and motor activity[8]. Finally, we explored the possibility that the CFTR modulators was able to improve ADHD in the zebrafish model. Hyperactivity and impulsivity of ADHD model ($per1b^{-/-}$ mutants) can be improved by CFTR modulators lumacaftor and ivacaftor. Overall, these findings suggest that CFTR may play a role in the pathogenesis of ADHD through its regulation of dopamine signaling.

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CFTR may be a novel potential therapeutic target for ADHD, and CFTR modulators has the potential possibility to improve ADHD.

Methods

Ethical approval and ethics statement

All experiments in this study were in accordance with the "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.) and were approved by the Animal Care and Use Committee of Second Affiliated Hospital of Chongqing Medical University.

Zebrafish lines and embryos

Wildtype (WT) AB strain, *cftr*^{scu102} (http://zfin.org/action/feature/view/ZDB-ALT-190307-1)[7] and *per1b* mutant[8] fish lines were utilized. Staging of the embryos was carried out as previously described[9].

Zebrafish behavioral analysis

Locomotor activity, active-avoidance conditioning paradigm for learning and memory ability analysis, evaluation of retention, reward-mediated impulsivity evaluation and mirror-image attack test were performed as previously described by Huang et al.[8].

Locomotor activity analysis was performed on the 7 dpf, a single larva was placed in each of 48 wells of a 48-well plate (24 WT and 24 *cftr*$^{+/-}$ mutants), which allowed simultaneous tracking of each larva. Locomotor activities of larvae were monitored for 1 h under the light conditions using an automated video-tracking system (DanioVision Tracking System, Noldus Information Technology). The movement of each larva was recorded and analyzed using Ethovision 10.0 software (Noldus Information Technology). 48-well plates were placed inside the Zebrabox/DanioVision Observation Chamber where white light was illuminated for 1 h. Instruments were placed in the chamber to maintain a constant temperature of 28.5 °C. The test was performed 3 times. Data were further analyzed using GraphPad Prism 8.0 software.

For the locomotor activity assay in adult fish, male or female adult fish were placed in tanks (30 × 30 × 15 cm) and transferred to the experiment room. All sides of the tank were made by opaque panel to prevent external interference. After 1 h of acclimation, fish were placed in tanks containing system water to a depth of 8 cm, and activities were measured. Using the same procedure for larval locomotor activity, swimming activities were monitored for 30 min by counting the swimming distance continuously.

Active-avoidance conditioning paradigm for learning and memory ability analysis was performed using adult male zebrafish, with 6 months of age and ~ 3 cm average length. The detailed methods of train and test of fishes using a testing tank were same to Huang's description[8]. In addition, experimental procedure of evaluation of retention, reward-mediated impulsivity evaluation and Mirror-image attack test was also carried out according to previous study[8].

Single-cell RNA-seq library preparation, sequencing and analysis

Approximately 200 *cftr* mutant or WT zebrafish embryos at 7 dpf were transferred to 2 mL tubes, then the collected embryos were sent to Beijing Biomarker Technologies (BMKGENE) for single-cell RNA-seq library preparation, sequencing and analysis.

Treatment of CFTR modulators

Lumacaftor (cat: HY-13262) and Ivacaftor (HY-13017) were purchased from MedChem Express. The stock solution of two chemicals was prepared by dissolving it in DMSO. We immediately collected zebrafish embryos of *per1b* mutant into egg water after the zebrafish mating. 10 μm Lumacaftor and Ivacaftor with 0.01% DMSO were added into egg water early when embryos were at one-cell stage. 0.01% DMSO solution was used as negative control. We renew egg water with two drugs every 24 h. Lumacaftor and Ivacaftor (2 mg/kg) were injected intraperitoneally (IP) into anesthetized adult zebrafish using a syringe with a 34 G needle. Detailed method refers to Wang's description[10]. The fish with 6 months of age were positioned in the cavity of a sponge with their abdomen up. The needle penetrated the ventral midline between the pectoral fins, and with the needle pointing posteriorly, towards the tail, entered the abdomen and then continued to move under the silver skin. Because the skin is partially transparent, it is possible to closely monitor the movement of the needle, avoiding any physical damage to intestines and/or other internal organs. Behavior tests was performed 1 day after injection as described above.

Results

Identification of CFTR variants in 2 ADHD family trios

To explore whether variants in genes contribute to Attention-deficit/hyperactivity disorder (ADHD), we collected 25 Chinese parents–offspring trios. We analyzed the variations through whole-genome sequencing, each consisting of a child diagnosed with ADHD and his/her unaffected parents[11]. All patients included had only ADHD symptoms tested by Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) and no other diseases, such as cystic fibrosis, according to the diagnosis. According to the whole-genome sequencing data, we identified three *CFTR* (NM_000492.4) missense variants with the following predicted amino acid substitutions: p.E217G (RS ID: rs121909046), p.F316L (RS ID: rs78742051), and p.T1220I (RS ID: rs1800123) in two family trios (Fig. 1). Compound heterozygous variants (p.E217G and p.F316L) were found in one patient inherited from heterozygous carrier parents; heterozygous variant (p.T1220I) was found in another patient inherited from heterozygous carrier mother. The detailed tests scores of two families are shown in Table S1.

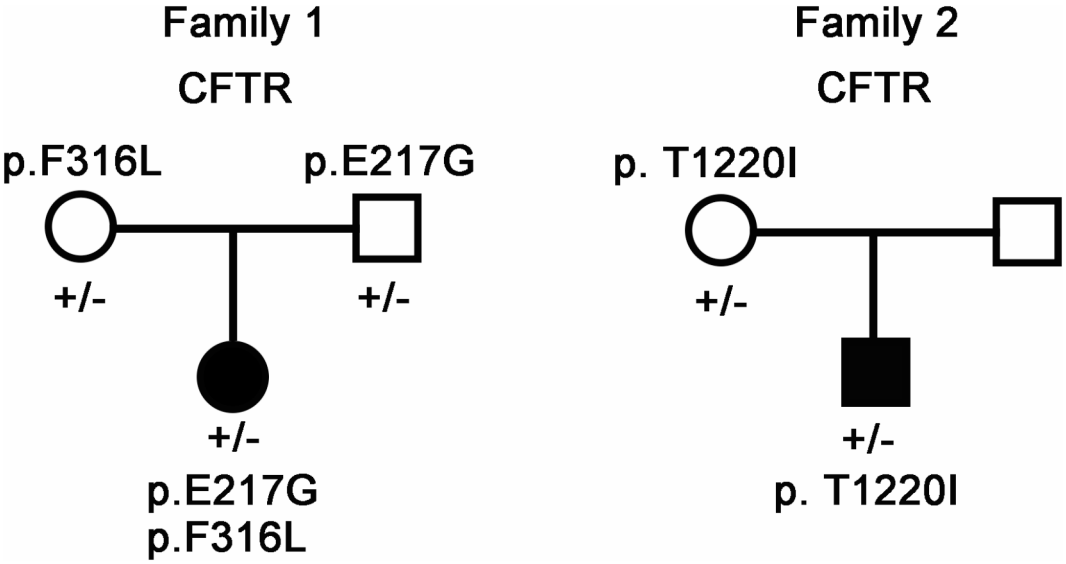


Fig. 1. CFTR mutants observed in ADHD families. Pedigrees of families with ADHD with their CFTR amino acid changes indicated. Filled symbols represent affected individuals.

Previous reports showed that E217G, a mutation with misfolding and functional defects, drastically impairs the maturation of the fully glycosylated CFTR and alters CFTR's activity at the cell surface, and can be stabilized by small-molecule corrector Lumacaftor[12,13]. T1220I mutation can be classified as PM1, PM2, PP3, PP5, and BP1 according to ACMG/AMP classification, suggesting that the variant is possibly pathogenic on the CFTR gene[14,15]. The F316L variant is located in coding exon 8 of the CFTR gene, this amino acid position is well conserved in available vertebrate species. Since supporting evidence is limited at this time, the clinical significance of this alteration remains unclear. Taken together, CFTR variants with functional loss detected in ADHD families suggest the association of CFTR with ADHD.

cftr mutant zebrafish larvae and adults display hyperactive behavior

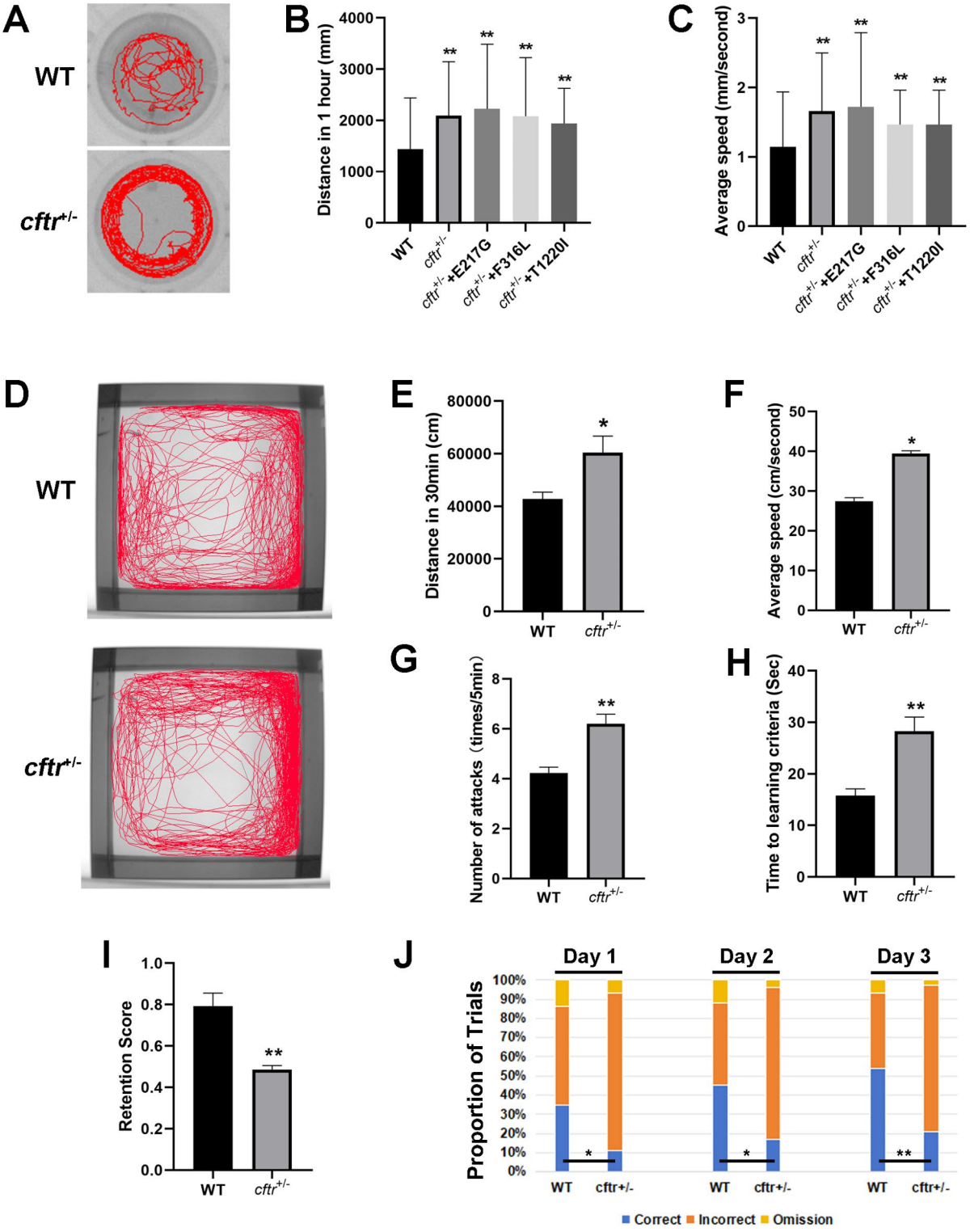
Because human activities mostly happen in the daytime, we examine phenotypes of ADHD in the zebrafish model during the daytime. To identify the association of CFTR with ADHD, we then analyzed the locomotor activities of the zebrafish *cftr* mutant model[7] and found that 7 dpf *cftr* mutant larvae were more active than their wild-type (WT) counterparts without any stimulation (Fig. 2A–C and Table S2). To explore whether CFTR variants found in families lose functions, we synthesized E217G, F316L, T1220I and WT mRNAs and performed rescue experiments by microinjecting human CFTR mutation capped mRNAs into one-cell zebrafish $cftr^{+/-}$ embryos. Results showed that hyperactivity of 7 dpf $cftr^{+/-}$ mutant larvae was rescued by human CFTR WT functional mRNAs. However, E217G, F316L and T1220I mutation mRNA fail to recover hyperactivity (Fig. 2B,C). Thus, these results suggest that the variants of the CFTR gene we found are responsible for the behavioral abnormality and the function of CFTR is evolutionally conserved between human and zebrafish.

We also measured the swimming distance and velocities of adult fish. Similar to larvae, the distance and mean swimming speed of *cftr* mutant adult fish were approximately 1.5 times those of wild-type fish (Fig. 2D–F and Supplementary video). Thus, we conclude that *cftr* mutant larvae and adult fish both exhibit hyperactivity. In addition, we also observed that the *cftr* mutants were more centrophobic than wild-type fish (Fig. 2A and D). In other words, the *cftr* mutants displayed a dramatically decreased exploratory behavior, implicating an increased anxiety phenotype similar to CF patients[16].

cftr mutant adult fishes show perseveration, learning/memory-deficit behaviors and impulsivity-like phenotype

According to Huang's descriptions[8], we also examine several phenotypes of ADHD on $cftr^{+/-}$ adult fishes. An image-attack assay was used to examine "perseveration", a key diagnostic criterion for hyperactivity in ADHD. Camera monitoring showed that wild-type fishes attacked their image intermittently in a 10 min interval. In contrast, $cftr^{+/-}$ fishes attacked their mirror image nearly continuously in a 10-min period, failing to break off the attack as wild-type fish did. Thus, the adult $cftr^{+/-}$ fish displays perseveration-like behavior, shown by their persistently swimming toward the mirror side of the tank during the test (Fig. 2G).

We next measured learning and memory behaviors to investigate the attention-deficit phenotype of $cftr^{+/-}$ adults. During the training period, $cftr^{+/-}$ fishes needed ~ 23 ± 3.2 trials to learn to avoid the electric stimulus to criterion, while the control WT needed obviously less trials (~ 15 ± 2.8 trials). This results indicate that $cftr^{+/-}$ mutants are not able to learn as well as WT fishes (Fig. 2H). Meanwhile, we observed that $cftr^{+/-}$ mutant fish showed a decreased ability to acquire and remember the AAC stimulus compared with WT fish during daytime (Fig. 2I). These results suggest that $cftr^{+/-}$ mutant fish have a lower ability to form long-term memories than wild-



type fish ($n = 20$, t-test, $p<0.05$). Therefore, we conclude that *cftr*$^{+/-}$ mutant fish show a clear deficit in learning and memory ability, which mimics an important symptom in ADHD psychiatric disorders.

To investigate whether zebrafish *cftr* mutants show cognitive impulsivity similar to ADHD patients, we also performed a two-choice serial reaction-time task in adult *cftr* mutant fish as Huang's previous description[8]. Results showed that during the 4 min waiting period, ~ 35% of WT fish maintained their attention to the task and stayed near the green light at the first day of test, and the proportion of WT fish remaining near the green light in the waiting period increased with subsequent trials. In contrast, only ~ 10% of *cftr* mutants stayed near the green light on the first test day, and although the proportion of fish remaining near the green light in the waiting period gradually increased in the following days as in wild types, this proportion was always significantly lower than that for wild types (Fig. 2J), suggesting that *cftr* mutant fish line display impulsivity. Additionally, similar to the *per1b* mutant, as expected for an attention-deficit phenotype, *cftr* mutants also could not maintain their attention

Fig. 2. cftr mutant zebrafish display ADHD-like behaviors. (**A**) Representative accumulative total tracks of the *cftr* mutant and WT larvae in 10 min at 7dpf. (**B**) Distances that WT and *cftr* mutant larvae ($n = 15$) traveled in 1 h. (**C**) Mean average swimming velocities of *cftr* mutant and WT larvae in 1 h. (**D**) Representative accumulative total tracks of the *cftr* mutant and WT adult with 6 months of age in 30 min. (**E**) Distances that WT and *cftr* mutant adult ($n = 15$) traveled in 30 min. (**F**) Mean average swimming velocities of *cftr* mutant and WT adult in 30 min. (**G**) Hyperactivity and impulsive-like behavior of the *cftr* mutant adult fish, shown by the mirror-image attack assay. *cftr* mutant, $n = 15$; wild type, $n = 15$. (**H**) The cftr mutant fish required more trials than WT to learn to avoid electricshocks in the daytime, showing significant learning problems. (**I**) The performance of *cftr* mutant fish in the memory test was inferior to that of WT during the daytime. *cftr* mutant, $n = 12$; WT, $n = 12$. (**J**) *cftr* mutant adult fish showed impulsivity-like symptoms determined by a food reward assay with the two-choice serial reaction-time task. Correct (blue bars), incorrect (orange bars), and omission (yellow bars) results were marked with different colors. *cftr* mutant, $n = 18$; wildtype, $n = 18$. Statistical analysis was performed using Student's t-test. Error bars represent mean ± SD. ** and **** represent $p \leq 0.01$ and $p \leq 0.0001$, respectively. A p-value < 0.05 was considered significant, and a $p > 0.05$ was taken as non-significant (ns).

on the task at hand despite having learned the task. The above-described persistent mirror-attacking behavior of the *cftr* mutant fish also can be construed as a trait like increase of impulsivity or aggressiveness, which makes the fish seemingly unable to break off ineffective behaviors.

Taken together, these results show that $cftr^{+/-}$ mutant fish clearly display ADHD-like behaviors, including hyperactivity, forgetfulness, and impulsivity, similar to $per1b^{-/-}$ mutant fish line.

cftr$^{+/-}$ changes cell composition in zebrafish larva

To further investigate the molecular mechanism of ADHD like behavior induced by *cftr* deficiency. We collected the 7 hpf larval samples of WT and $cftr^{+/-}$ line, and carried out single-cell RNA-sequencing analysis to explore the molecular regulation of the nervous system. We obtained high-quality single-cell transcriptomes (8399 and 8409 for WT and $cftr^{+/-}$ larva, respectively) and 43 distinct cell clusters from larva (Fig. 3A) based on the known marker genes of cell clusters[17]. These cell clusters related to Neurons were identified as follows: Sensory, Olfactory/Hair-Cells, NCcranial, NCneural, MHBNeurGlutAll, RetDiff, CranGangAllb, MHBNeurGABA/GlutAll, SCDiff, NCneural25/RetNeuron5/HBProg1a, Dopaminergic Fig. S1).

Of note, the cell proportions for neuron clusters changed significant in $cftr^{+/-}$ zebrafish larva compared with WT. The ratio of neurons (Sensory, Olfactory/Hair-Cells) increased in $cftr^{+/-}$ zebrafish larvae. On contrast, $cftr^{+/-}$ decreased the ratio of RetDiff, CranGangAllb, MHBNeurGABA/GlutAll, SCDiff, NCneural, MHBNeurGlutAll neuron compared with WT. Important, in $cftr^{+/-}$ zebrafish larva, the ratio of dopaminergic neurons decreased to 0.42 fold of WT. Besides, $cftr^{+/-}$ also changed the ratio of muscle and immune cells in zebrafish larvae compared with the control (Fig. 3B and Table S3).

Transcriptional response of dopaminergic neuron toward cftr$^{+/-}$ larva

We then investigated the cell-specific transcriptional changes occurred in zebrafish larvae of $cftr^{+/-}$ fishline by performing differential gene expression analysis across cell types. Especially, abnormal DA system of dopaminergic neurons has been indicated be associated with etiology and pathogenesis of ADHD[8]. Obviously, dopaminergic neuron cell clusters showed a strong transcriptional response toward $cftr^{+/-}$ mutant. We found 145 upregulated and 103 downregulated differentially expressed genes (DEGs) within the dopaminergic neuron cell cluster (Fig. 3C and Table S4). After annotation of the DEGs, by KEGG enrichment analysis, we found that genes involved signaling pathway of p53, Notch, Hedgehog, FoxO, mTOR, Wnt, MAPK etc. were altered by $cftr^{+/-}$ mutant (Fig. 3D).

Embryonic transcriptomics and proteomics at the onset of gastrulation showed significant expression alteration of neural factors

Previous studies performed transcriptomics and proteomics to analyze mRNA and protein expression alteration of *cftr* mutant at onset of gastrulation (5hpf)[18,19]. Of note, the development of many systems in the body, including the nervous system, originates from the onset of gastrulation[20]. So we reanalyze the data of previous transcriptomics and proteomics to uncover the expression change of neural factors. As shown in Fig. S2, we found the expression of a lot of genes and proteins related nervous system changed significantly at the onset of gastrulation, suggesting the ADHD phenotype of *cftr* mutant model originated from early embryo development.

Hyperactivity and impulsivity of ADHD model (per1b$^{-/-}$ mutants) can be rescued by CFTR modulators

Since *cftr* deficiency zebrafish shows ADHD behavior, we asked whether ADHD can be treated by activation of CFTR. Lumacaftor (VX-809) has been shown to correct CFTR mutant protein misprocessing and increase the amount of cell surface localization in vitro. Ivacaftor (VX-770), a CFTR potentiator, can increase the open probability of CFTR channels in vitro and improve clinical outcomes in cystic fibrosis patients. A combination of lumacaftor with ivacaftor has been associated with a greater increase in CFTR activity of chloride transport and cell surface localization than either agent alone[21].

We treated ADHD model (*per1b* mutant) larvae[8] with 10 μm concentrations of lumacaftor and ivacaftor by directly adding the drug to the breeding egg water at 0 hpf stage and then measured mean swimming speed at 7 dpf. We found that treatment with lumacaftor and ivacaftor was sufficient to reduce the hyperactivity of ADHD

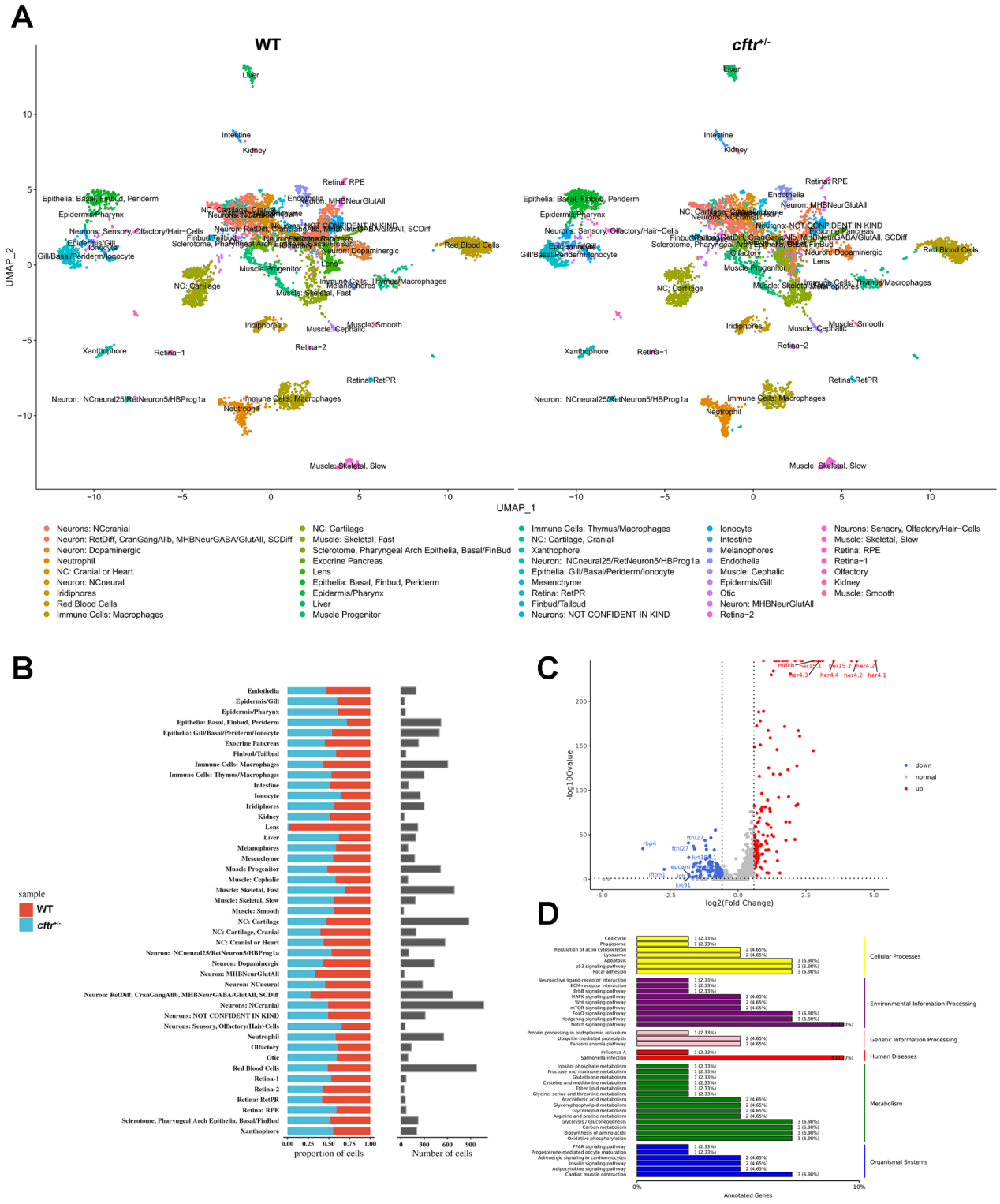


Fig. 3. Single-cell RNA-seq analysis of WT and cftr mutant embryos at 7 dpf. (**A**) Unsupervised clustering of cells in the *cftr* mutants and WT embryos at 7 dpf. Cells are colored according to their cell type annotations inferred from expressed marker genes and published datasets. (**B**) Bar plot shows the percentages of each cell type in *cftr* mutants (blue) or WT embryos (red). (**C**) Volcano plot of differentially expressed genes are depicted in dopaminergic neuron cells cluster. (**D**) KEGG analysis plot and genes included in the dot plot are based on genes significantly enriched within dopaminergic neuron cells cluster.

model (*per1b* mutant) larvae to approximately the activity level of wild-type controls (Fig. 4A,B). These results show that treatment with CFTR modulators was sufficient to rescue hyperactivity in the ADHD model larvae.

Furthermore, we tested whether CFTR modulators can rescue the mirror-attacking phenotype of ADHD model (*per1b* mutant) adult zebrafish. The mirror-attacking phenotype of zebrafish is an impulsive or aggressive behavior, which also mimics hyperactivity or perseveration of ADHD[8]. Results showed that the persistent mirror-attacking behavior of ADHD model adult fish was significantly reversed with lumacaftor and ivacaftor treatment (Fig. 4C). Then, we measured learning and memory behaviors to investigate the attention-deficit phenotype of ADHD model. As shown in Fig. 4D, the CFTR modulators decreased trials to learn to avoid the electric stimulus to the criterion of ADHD model fish.

To specify the role of CFTR channel function or protein stability in ADHD model improvement, we treated the modulators alone. Results showed that Ivacaftor alone rescued the ADHD-like phenotype (Fig. S3), and Lumacaftor failed (Fig. S4), suggesting that the channel function of CFTR play critical role in ADHD improvement. The observation that CFTR modulators can rescue phenotypes of ADHD model fish suggests that CFTR may be a potential therapeutic target and CFTR agonist may become a new drug for the treatment of ADHD.

Discussion

ADHD symptoms in CF patients are substantially higher than in the general population suggesting the potential critical role of CFTR in ADHD. In this work, we give several evidences to show the association of CFTR with ADHD. Firstly, we find three CFTR variants in 2 ADHD family trios by whole-genome sequencing. Importantly, we demonstrate that these found variants, E217G, F316L and T1220I, fail to recover the hyperactive behavior caused by $cftr^{+/-}$, suggesting functional loss. Secondly, we examined the behavior of the established $cftr^{+/-}$ mutant line, and found ADHD phenotype, including hyperactive behavior, perseveration, learning/memory-deficit behaviors and impulsivity-like phenotype. From the video in supplements, the behavior of the $cftr^{+/-}$ mutant fish line is significantly different from that of the WT. The $cftr^{+/-}$ mutant fish are not moving in circles; instead, they are constantly bumping into the walls of the aquarium. Since they cannot swim out, this creates the observed trajectory. This behavior corresponds with the experimental results that show impulsive mirror-attacking behavior. It aligns well with characteristics of chaos and anxiety[22–25]. Thirdly, Single-cell sequencing results identify that cell proportion and molecular expression of several cell clusters of neurons, dopaminergic neuron especially, alter significantly. Fourthly, CFTR modulators can recover the abnormal behaviors of the known ADHD zebrafish model (*per1b* mutant), suggesting that CFTR may be a potential therapeutic target for ADHD.

The Wnt pathway is one of the most important pathways in developmental embryo and adult tissues. Canonical Wnt signaling is induced mainly by the multifunctional β-catenin protein with downstream activation of transcription factors, such as LEF and TCF. In the nervous system, Wnt signaling play a key role in different neurodevelopmental aspects, including neuron differentiation, synapse formation, neurogenesis, axonal extension and neuroprotection. Evidence demonstrates that Wnt/β-catenin signaling dysregulation is associated with major neurodegenerative disorders. Major findings shown that Wnt/β-catenin signaling in partnership with glial cells is critically involved in each step and at every level in the regulation of dopaminergic neuronal cell health, protection, and regeneration in the Parkinson's disease mouse model[26]. Previous studies show that CFTR binds to Dishevelled protein and maintains Wnt signaling, playing a critical role in early embryo development, organizer formation, hematopoiesis and T lymphopoiesis[9,19,27]. In this work, we also find that Wnt signaling dysregulation in dopaminergic neuronal cell clusters. Taken together, regulation of Wnt signaling by CFTR is critical for development of multi organs, including nervous system.

In conclusion, ADHD phenotype observed in CF patients suggests the relation between CFTR and ADHD. In this work, we found several CFTR variants in ADHD families and established an association of CFTR with ADHD. Based on molecular mechanisms explored by the single-cell sequence, we tried to treat ADHD zebrafish with CFTR modulators and revealed that CFTR may act as a new therapy target for ADHD.

Limitations of the study

While this study provides compelling evidence linking CFTR to ADHD pathogenesis and potential therapeutic avenues, several limitations should be acknowledged. First, the human genetic analysis included a small cohort of 25 Chinese family trios, with CFTR variants identified in only two families. This limited sample size and ethnic homogeneity may restrict the generalizability of the findings to broader populations. Second, the zebrafish model, while valuable for mechanistic insights, cannot fully recapitulate the complex behavioral and cognitive dimensions of human ADHD, such as emotional dysregulation or higher-order executive dysfunction. Third, although CFTR modulators rescued ADHD-like behaviors in zebrafish, translational relevance to humans remains uncertain due to interspecies differences in drug metabolism, neural circuitry, and ADHD pathophysiology. Additionally, while single-cell RNA-seq and proteomic analyses identified molecular changes in dopaminergic neurons and neural pathways (e.g., Wnt signaling), the precise causal mechanisms linking CFTR dysfunction to dopamine dysregulation require further investigation. Addressing these limitations through larger, diverse cohorts, advanced behavioral models, and detailed mechanistic studies will strengthen the clinical relevance of CFTR as a therapeutic target for ADHD.

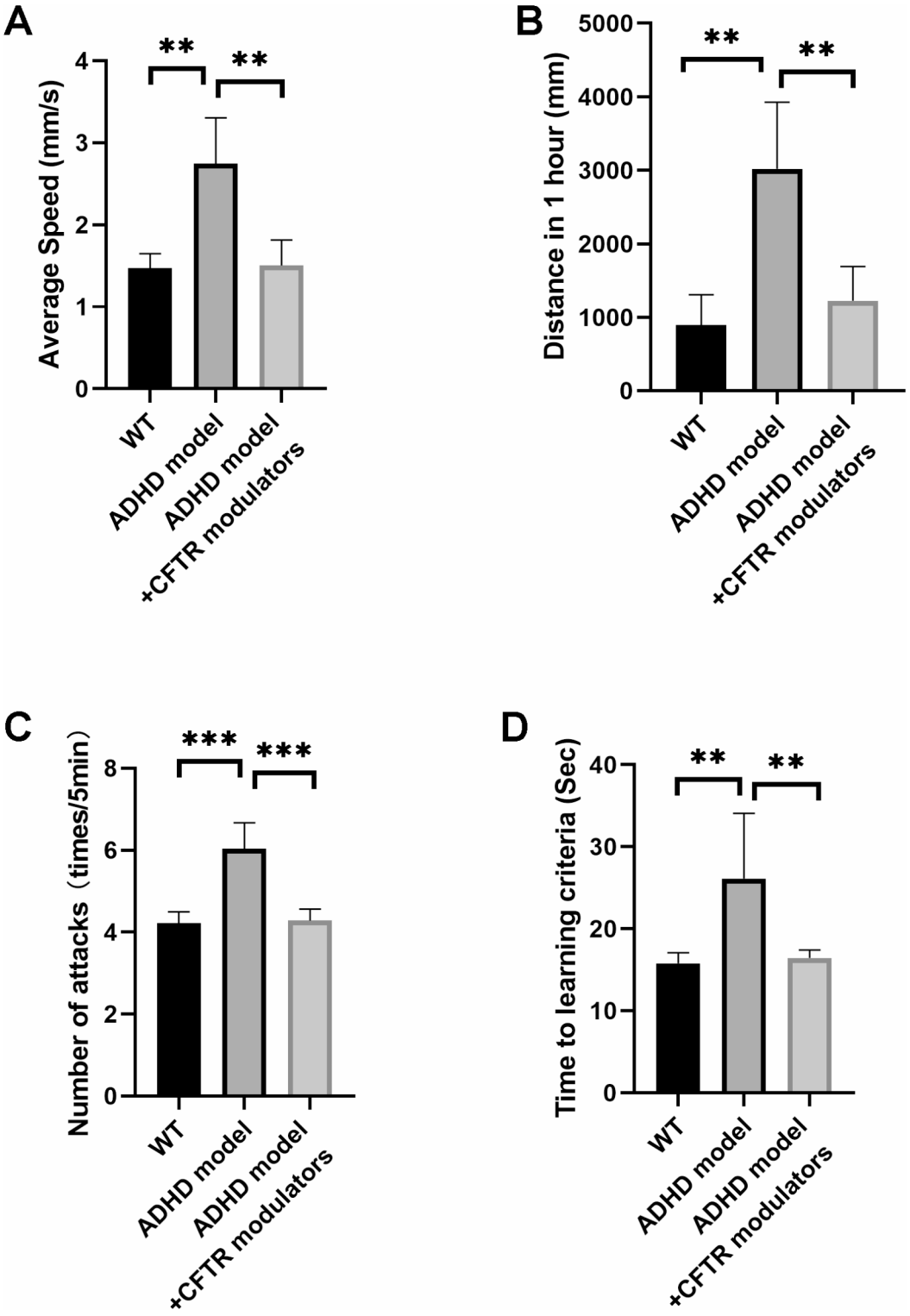


Fig. 4. Treatment of ADHD zebrafish model (per1b mutant) with CFTR modulators. Abnormal swimming velocities (**A**) and traveled distances (**B**) of *per1b* mutant larvae ($n = 15$) can be recovered by CFTR modulators. Impulsive-like behavior (mirror-image attack assay) (**C**) and learning problems (avoiding electricshocks) (**D**) of *per1b* mutant larvae ($n = 15$) can be recovered by CFTR modulators. Statistical analysis was performed using Student's t-test. Error bars represent mean ± SD. ** and **** represent $p \leq 0.01$ and $p \leq 0.0001$, respectively. A p-value < 0.05 was considered significant, and a $p > 0.05$ was taken as non-significant (ns).

Data availability

The datasets generated and/or analyzed during the current study are available in the [GSE284214] repository, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi? acc=GSE284214].

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Author contributions

Q. L. conceived and designed the experiments; Q. L., T. W., J. L. and X. L. performed the experiments; Q. L. and T. W. analyzed the data; J. L. and X. L. coordinated the project. Q. L. and T. W. wrote the paper.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

All experiments in this study were in accordance with the "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.) and were approved by the Animal Care and Use Committee of Second Affiliated Hospital of Chongqing Medical University.

Consent for publication

not applicable.

Additional information

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