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ARTICLE

CADPS functional mutations in patients with bipolar disorder increase the sensitivity to stress

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Abstract

Bipolar disorder is a severe and chronic psychiatric disease resulting from a combination of genetic and environmental risk factors. Here, we identified a significant higher mutation rate in a gene encoding the calcium-dependent activator protein for secretion (*CADPS*) in 132 individuals with bipolar disorder, when compared to 184 unaffected controls or to 21,070 non-psychiatric and non-Finnish European subjects from the Exome Aggregation Consortium. We found that most of these variants resulted either in a lower abundance or a partial impairment in one of the basic functions of CADPS in regulating neuronal exocytosis, synaptic plasticity and vesicular transporter-dependent uptake of catecholamines. Heterozygous mutant mice for $Cadps^{+/-}$ revealed that a decreased level of CADPS leads to manic-like behaviours, changes in BDNF level and a hypersensitivity to stress. This was consistent with more childhood trauma reported in families with mutation in *CADPS*, and more specifically in mutated individuals. Furthermore, hyperactivity observed in mutant animals was rescued by the mood-stabilizing drug lithium. Overall, our results suggest that dysfunction in calcium-dependent vesicular exocytosis may increase the sensitivity to environmental stressors enhancing the risk of developing bipolar disorder.

Introduction

Bipolar disorder (BD) is a chronic psychiatric mood disorder with a lifetime cumulative risk of 4.4% in the worldwide population [1]. Twin and family studies have demonstrated a genetic component of BD with an estimated heritability ranging between 60% and 80% [2]. These results are supported by many genome-wide linkage

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analyses that identified a vulnerability locus for BD on chromosome 3p14-p21 [3, 4], mainly in the early-onset form of the disease [5]. Recent large-scale genome-wide association studies further suggested several candidate genes in this region [6–11].

Some studies have linked BD to perturbations in molecular mechanisms that regulate neurotransmitter release [12–15]. Neurotransmitter release is achieved by a physical attachment of synaptic vesicles to the presynaptic plasma membrane and membrane fusion is triggered by action potential-dependent influx of Ca^{2+} ions into the presynaptic nerve terminal. Growing evidence suggests a major role of presynaptic Ca^{2+} and Ca^{2+} channels in the onset of BD [9, 12, 16]. In this study, we explored the role of a new candidate gene on chromosome 3p14, which encodes the Ca^{2+}-dependent activator protein for secretion (*CADPS*). CADPS is a member of the CADPS protein family that comprises two isoforms, namely CADPS and CADPS2 [17]. CADPS proteins are essentially involved in priming secretory vesicles in neurons and neuroendocrine cells [18–21]. In addition, CADPS and CADPS2 have also been suggested to play a role in the loading of catecholamines into dense core vesicles [21–23]. *CADPS2* dysfunction has been linked to several psychiatric diseases, including autism spectrum disorder and schizophrenia, and is located in a BD susceptibility locus [24–26]. In contrast, no mutation has been identified yet in *CADPS*. However, the observation that CADPS is the most dominantly expressed isoform in the brain and directly interacts with proteins of the neuronal soluble *N*-ethylmaleimide sensitive factor–associated protein receptor (SNARE) complex [27], which have been widely associated with psychiatric disorder vulnerability [13, 14, 28, 29], makes this gene an interesting candidate for vulnerability to psychiatric disorders.

Here we report that common and rare genetic variants in *CADPS* are more frequently observed in individuals with early-onset BD than in control populations. We explored the consequences of missense variants identified in patients on the multiple functions of the protein and showed that most of these variants affect CADPS functions and neurotransmission. In addition, we showed that down-regulation of CADPS in mice results in manic-like behaviours and a higher sensitivity to stress, and hyperactivity was reduced with lithium. We finally showed that patients with mutations in *CADPS* reported more childhood trauma than unmutated family members or than other patients with BD.

Materials and methods

See Supplementary Information for details. Methods are briefly descried as follows.

Subjects

This study combines data from a previously published cohort [9, 30] of 452 individuals with BD (189 males and 263 females) and 1636 control individuals (696 males and 940 females) of French origin for genotyping analyses and child trauma assessment [31, 32] from whom 132 individuals with early-onset BD (56 males and 76 females) have been included in sequencing analyses (see Supplementary Materials and Methods for details). In addition, 184 unaffected controls (105 males and 79 females) with neither personal nor family history of psychiatric disorder or suicidal behaviour have been collected for sequencing analyses.

The research ethics board of the Pitié-Salpêtrière Hospital approved protocols and procedures and written informed consent was obtained from all subjects prior to participation in the study.

Mice

All experiments were conducted in accordance with the European Community Council Directive (86/609/EEC) regarding the housing, care, and experimental procedures on mice.

Deletion of *Cadps* in mice have been generated by homologous recombination and maintained on C57BL/6 J background [21]. $Cadps^{+/-}$ heterozygous mice and their wild type littermates were weaned at 4 weeks and housed two to six per cage by sex and litter regardless of the genotype under standard conditions, with food and water available ad libitum.

Genetic analyses in humans

Genotyping data and copy number variations have been detected using HumanHap550 BeadArrays (Illumina, San Diego, CA, U.S.A.) and analysed using the PLINK software v1.07 [33] as previously described [30]. The *CADPS* coding region and exon-intron boundaries were sequenced by Sanger's method on a 16-Capillary ABI PRISM 3130xl genetic analyser as previously described [14]. All primers and PCR conditions are available on request.

Vesicular exocytosis in PC12 cells

Two million of PC-12 cells were electroporated with the plasmids containing wild type and mutant *CADPS* DNA using Amaxa® Cell Line Nucleofector™ Kit V and Nucleofector™ device according to manufacturer's protocol (Lonza, Basel, Switzerland). The day after, cells were incubated with 20 µM of Fluorescent False Neurotransmitter 511 (FFN511) [34] diluted in 100 µl of Krebs-

Ringer buffer: 10 mM HEPES, 140 mM NaCl, 5 mM KCl, 1.5 mM $MgCl_2$, 2 mM $CaCl_2$, 14.3 mM $NaHCO_3$, 5 mM Glucose, 0.1 mM EGTA, for 1 h at room temperature. Cells were washed three times and exocytosis was triggered with the addition of Krebs-Ringer buffer supplemented with 60 mM KCl, 80 mM NaCl and 1 mM EGTA. Supernatants were collected after 1 min exocytosis and read at 501 nm with Infinite® 200 PRO microplate reader (Tecan, Männedorf, Switzerland). The transfection efficiency was checked for each experiment by quantifying the fluorescence level of the enhanced green fluorescent protein (eGFP) at 504 nm.

Vesicular monoamine uptake assays in CHO cell lines

Chinese Hamster Ovary (CHO) cell lines, constitutively expressing the *Slc18a1* gene, encoding the vesicular monoamine transporter VMAT1 (CHOVMAT1), were provided by Prof. Ahnert-Hilger (Charité Center, Berlin, Germany) and cultured as described [22]. Cells were transfected with wild type and mutant *CADPS* DNA using Lipofectamine™ 2000 (Thermo Fisher Scientific) according to manufacturer's instruction. Twenty-four hours after transfection, the transfection efficiency was checked with the fluorescence of the eGFP and one million cells were permeabilized with streptolysin O [35]. Serotonin uptake was measured using 5-Hydroxy-tryptamine,[H^3]-trifluoroacetate (PerkinElmer, Waltham, MA, USA) and liquid scintillation counting by a Packard 1600TR Tri-Carb Liquid Scintillation Analyzer (Perkin Elmer) as described [22].

Electrophysiological recording of autaptic hippocampal neurons

Autaptic hippocampal neurons were prepared from hippocampal neuroblasts of e18 CADPS/CADPS2-double knockout (DKO) embryos, as described previously [19]. Cells were whole-cell voltage clamped at −70 mV under control of a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, U.S.A.). All analyses were performed using AxoGraph 4.1 (Axon Instruments Inc., Foster City, CA, U.S.A.). The readily-releasable pool (RRP) size was determined by a 6 s application of the external saline solution made hypertonic by the addition of 0.5 M sucrose. Recordings of mEPSCs were performed in the presence of 300 nM tetrodotoxin (TTX). EPSCs were evoked by depolarizing the cell from −70 to 0 mV for 2 ms. The effect of high-frequency stimulation on the amplitude of EPSCs was measured by applying depolarisations at frequencies of 2, 5, 10 and 40 Hz for 100 stimuli.

Statistical analyses

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data are presented as mean ± standard error of the mean or as median ± range. Statistical testing was performed using Prism 6 software (GraphPad Software Inc., La Jolla, CA, U.S.A.). Condition comparisons included parametric tests (Student *t* test / ANOVA) and non-parametric tests (Mann–Whitney *U* test / Kruskal–Wallis test), according to normality of distribution, tested using the Shapiro–Wilk method or Kolmogorov–Smirnov test for electrophysiological data. All statistical tests were two-sided. Differences were considered significant for $p < 0.05$.

Results

CADPS is associated with early-onset bipolar disorder

CADPS is a large gene spanning 477,044 bp on chromosome 3p14. In order to determine whether common polymorphisms in this gene might explain genetic linkages frequently reported on 3p14, we genotyped 176 haplotype-tagging single nucleotide polymorphisms (ht-SNPs) in 452 individuals with BD and 1636 controls. The biggest difference in allele frequencies between patients and controls was observed for rs35462732 ($\chi^2 = 8.84$, $p = 0.003$) (Fig. 1a). This SNP has not been genotyped by the Psychiatric Genomic Consortium (PGC) Bipolar Disorder Group [9] and no information on allele frequency was available for it. However, 4 SNPs (rs9872498, rs1238394, rs833638 and rs17651503) in *CADPS* showed a difference in allele frequencies with a $p < 0.01$ between the 7481 individuals with BD and the 9250 control individuals of the PGC study. These SNPs were located 100 kbp downstream to rs35462732 (lowest *p* value for rs833838, $p = 0.004$). Genetic linkages identified in this region were specific for patients with early-onset BD [5]. We then restricted our sample to 203 patients with an age at onset lower than 22 and showed that the difference in allele frequencies for rs35462732 was even larger ($\chi^2 = 11.05$, $p = 0.0009$, OR = 1.80, 95% CI [1.27;2.55]) (Fig. 1a).

Missense variants in *CADPS* are more frequent in individuals with BD than in unaffected controls

The odds ratio of rs35462732 was not able to explain the multiple genetic linkages previously reported in this region. We thus assumed that rare functional variants in this gene may contribute to increase BD vulnerability. As our linkage and association studies showed a stronger

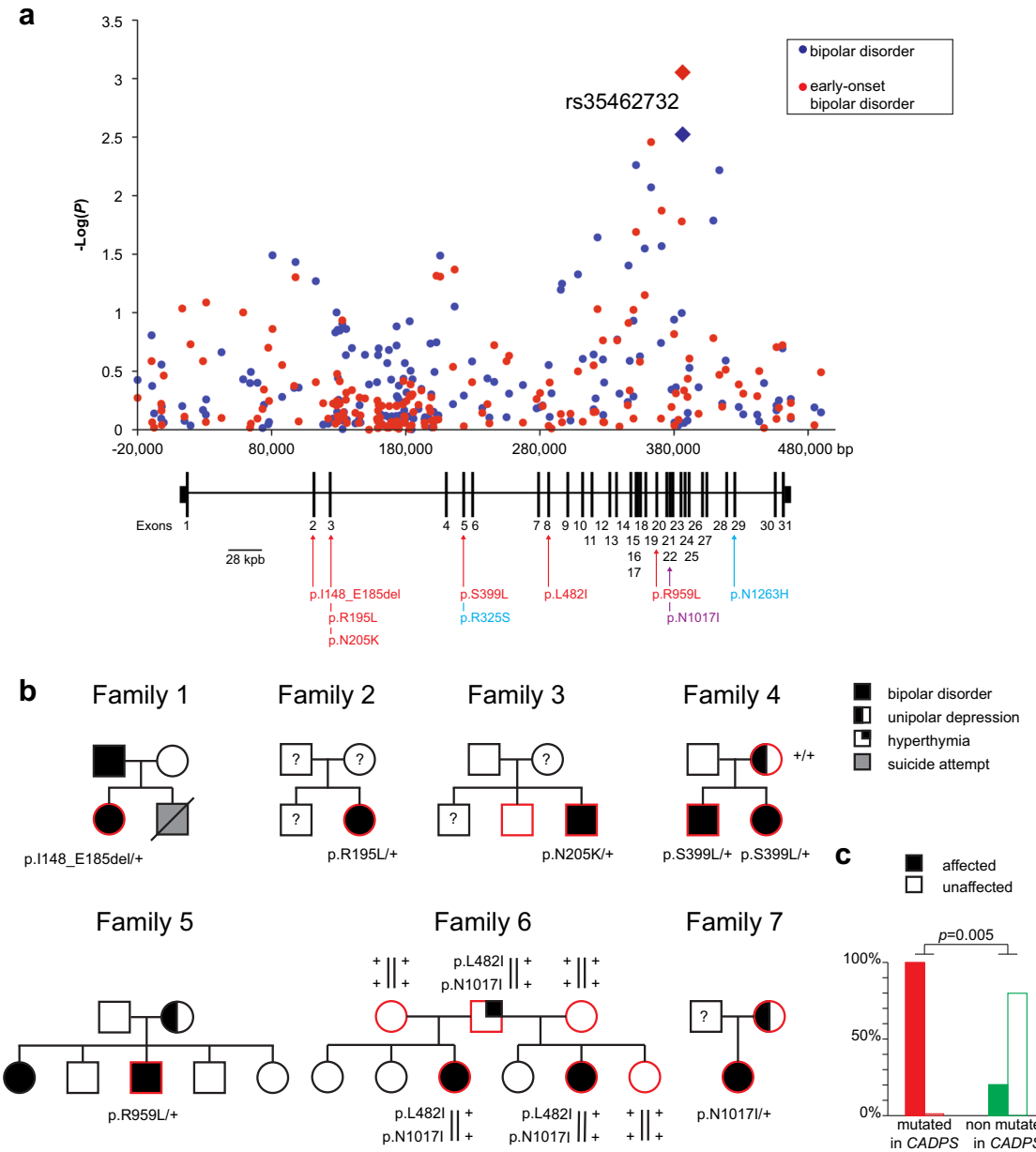


Fig. 1 Genetic exploration of *CADPS* in patients with BD. a Manhattan plot showing results of association studies conducted with 176 ht-SNPs spanning *CADPS* on 456 patients with BD (blue) or 203 patients with early-onset BD (red) and 1636 control individuals. Diamonds represent the most significantly associated SNP. The *x* axis is the gene position according to NM_003716.3 and the *y* axis is the $-\log_{10}P$ value. The missense variants identified in individuals with an early-onset BD, unaffected controls or both are shown in red, blue and purple, respectively. **b** Family pedigrees of early-onset BD patients with missense variants in *CADPS*. Affected individuals are shown in black, with filled symbol for BD, half-filled symbol for unipolar depression and quarter-filled symbol for hyperthymia. Individuals for whom DNA was available are shown with a red border. **c** Comparison of disease probability in mutated and unmutated individuals in the six families in which rare variants were identified. See also Supplementary Fig. S1.

signal for early-onset BD, we sequenced the 31 coding exons as well as regulatory regions of the 3 RefSeq isoforms of *CADPS* (NM_003716.3, NM_183393.2 and NM_183394.2) in a subgroup of 132 patients with early-onset BD. We identified six missense and four synonymous variants (Supplementary Table 1). One of the missense variants (p.N1017I) has been found twice in patients and twice in controls and should be considered as a polymorphism not associated with the disorder. Note that one of the two patients, who carried the p.N1017I variant had another missense variant on the same allele (p.L482I) (Fig. 1b). In order to focus on variants that might be causative for the disorder, we then selected only rare variants with a minor allele frequency lower than 0.001 in

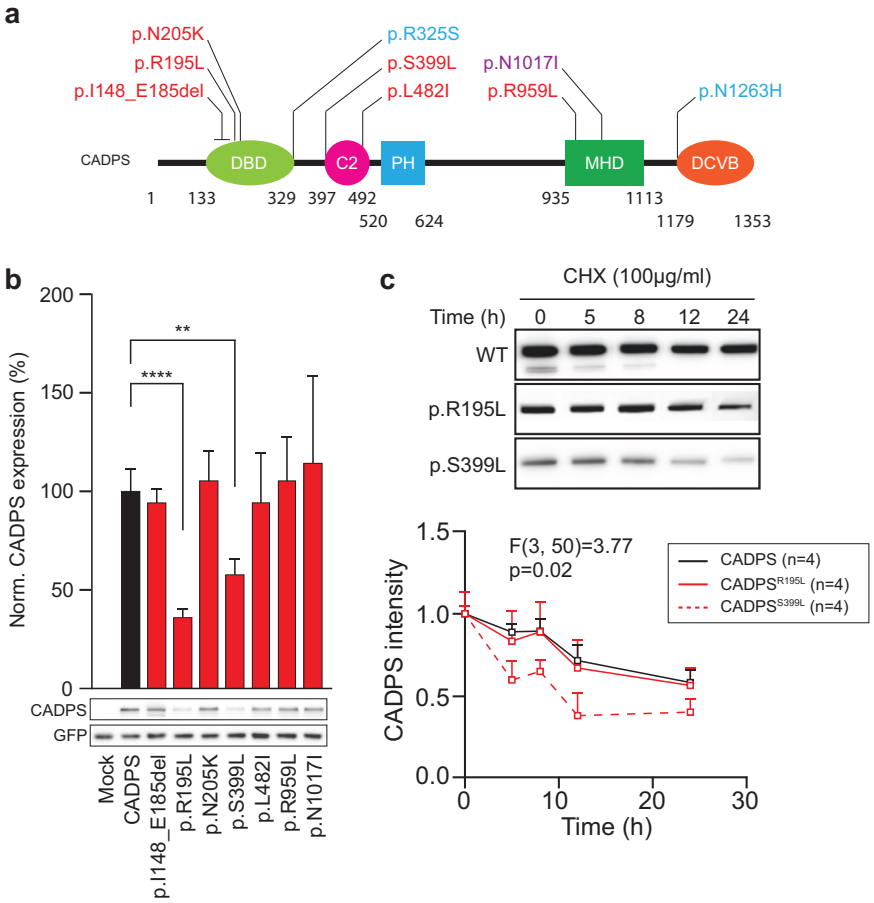


Fig. 2 Characterization of missense variants identified in *CADPS*. **a** Protein location of missense variants identified in individuals with an early-onset BD (red), unaffected controls (blue), or both (purple). (DBD) dynactin binding domain; (C2) C2 domain; (PH) pleckstrin homology domain; (MHD) munc homology domain; (DCVB) dense core vesicle binding domain. **b** Protein expression level of wild type and mutant isoforms of CADPS. Protein level has been evaluated by Western-blot assay in transiently transfected COS cells using antibody directed against CADPS and TUBA. **c** Two under-expressed mutant isoforms of CADPS containing either the p.R195L or the p.S399L mutations were evaluated for protein stability. Transfected COS cells were treated with 100 µg/ml cychloheximide (CHX) over a 24 h time period. Cell lysates were subjected to Western blot analysis with anti-CADPS and quantified before to be plotted on graph. The value of untreated cells was set as 1. The p.S399L mutation significantly decreases the half-life of the protein by a factor 3. All data are presented as mean ± s.e.m. $^{**}p < 0.01$; $^{****}p < 0.0001$. *WT* wild type. See also Supplementary Fig. S2.

the Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org/) for further analyses. In the six families in which rare variants were identified, 100% of individuals with a missense variant in *CADPS* had a mood disorder, whereas this frequency decreased to 20% in unmutated subjects (Fisher's exact test, OR = $+\infty$, 95% CI [2.00;$+\infty$], $p = 0.005$) (Fig. 1c). Only three missense variants were found in 184 unaffected controls, including p.N1017I. Moreover, the missense variant frequency was three times higher in patients than in controls (Fisher's exact test, OR = 3.53, 95% CI [0.57;37.32], $p = 0.13$) as well as in the non-psychiatric and non-Finnish European population from the ExAC database (Fisher's exact test, OR = 3.38, 95% CI [1.08;8.11], $p = 0.02$) (Supplementary Fig. S1). In contrast, no difference was observed for synonymous variants between these three populations (Supplementary Fig. S1). We looked for copy number variations in the 452 patients with BD. Although, any insertion or deletion were found in *CADPS* using DNA chip screening, a deletion was identified by serendipity using real-time PCR. This amplification was used as control to validate another deletion identified in *GRIP1* in one female with late-onset BD. The *GRIP1* deletion was not confirmed, but further exploration around the *CADPS* region confirmed a 9kbp-deletion, removing the exon 2 (p.I148_E185del). As exon 2 is composed of 114 nucleotides, this deletion resulted in a protein shortened of 38 amino acids in the dynactin-binding domain, which has been shown to be necessary for a proper localisation of CADPS2 in neurons [26]. No similar deletion was reported in the Database for Genomic Variants (http://dgv.tcag.ca/) or in the Genome Aggregation

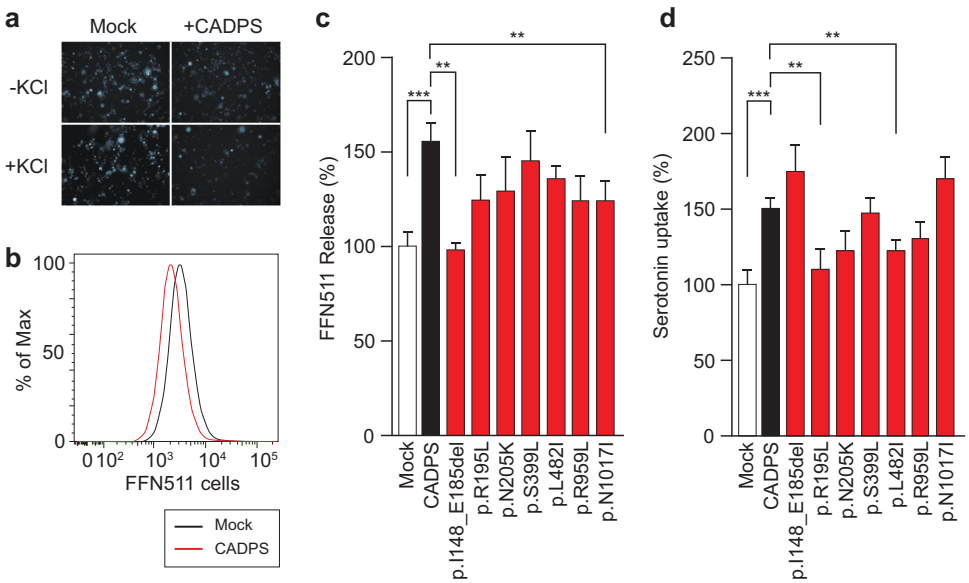


Fig. 3 Mutations in *CADPS* decreased the FFN511 release and monoamine uptake. PC12 cells were electroporated with vector containing CADPS mutations. In the evoqued-exocytosis Krebs-Ringer buffer containing 90 mM KCl, release of FFN511 after 1 min is higher for cells containing CADPS than the mock, as seen in cells by microscopy (**a**) or by FACS (**b**). **c** Release of FFN511 in PC12 cells has been compared wild type and mutated CADPS. Exocytosis of FFN511 was significantly decreased for p.I148_E185del, p.N1017I and the double mutant p.L482I_N1017I, indicating that these mutations altered the released of vesicular monoamines. **d** VMAT1 expressing CHO cells were transfected either with wild type or mutant CADPS and serotonin transport with VMAT1 was compared. Serotonin uptake occurred for 10 min a 37 °C using a solution of potassium-glutamate-ATP containing 40 nM[H3]serotonin and 100 µM of no-labeled serotonin. Serotonin uptake was significantly reduced for p.R195L and p.L482I mutants, suggesting these mutations altered the CADPS ability to promote monoamine uptake. All data are presented as mean ± s.e.m. $^{**}p < 0.01$; $^{***}p < 0.001$. See also Supplementary Fig. S3 and Supplementary Table 1.

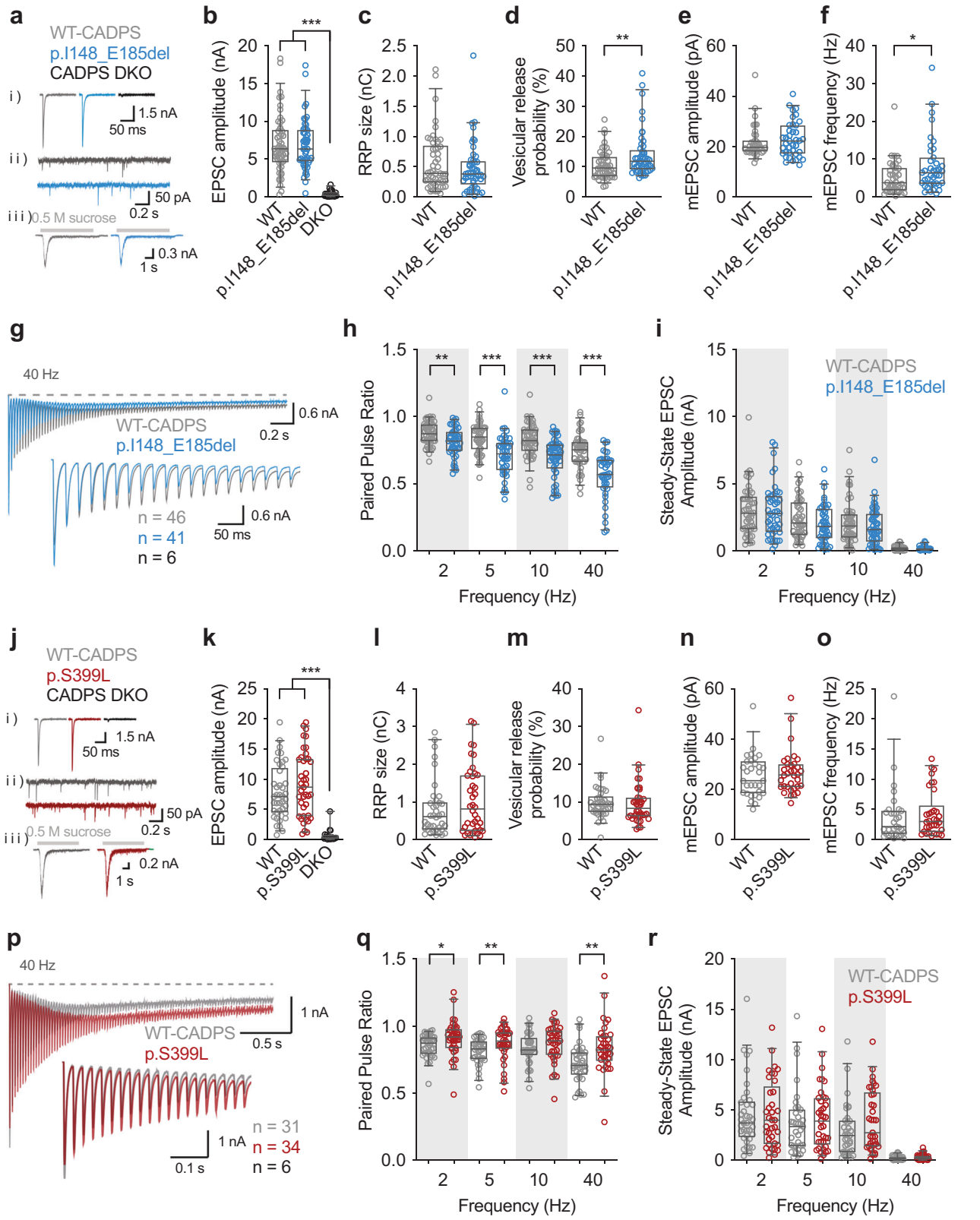
Database of Structural Variants v2.1 (https://gnomad.broadinstitute.org).

Two missense variants in *CADPS* identified in individuals with BD affect the protein level

In silico analysis of the genetic variants identified in patients with early-onset BD suggested that five out of the six missense variants were predicted to have a damaging impact on the protein function by at least two programs (Supplementary Table 1). In order to determine whether these variants affect the cellular stability of CADPS, we transfected either the wild type or mutant *CADPS* in multiple cell lines. Quantification of the protein level in COS-7 cells was performed by western blot analysis and showed a 40% and 33% decrease in protein expression level for p.R195L and p.S399L, respectively (Fig. 2a, b). This was confirmed both in CHO and PC12 cells with a similar reduction of the protein level, whereas no difference in RNA level was observed for these variants (Supplementary Fig. S2). We checked the protein stability using 100 µg/ml of cycloheximide for 24 h on COS cells transiently transfected with mutated and non-mutated *CADPS* and measured the protein level over time. This showed a 3-fold faster degradation of the protein with the p.S399L mutation (Fig. 2c).

CADPS variants exhibit normal activity in vitro but affect CADPS functions ex vivo

Multiple functions have been shown for CADPS in vesicular exocytosis mechanisms. Missense variants in CADPS were previously found to decrease the Ca^{2+}-triggered exocytosis in a permeable cell assay [36–39]. Thus, we assessed activity in this assay of human CADPS proteins with patients' mutations in HEK293 cells. All proteins tested exhibited activities similar to those of the wild type protein (Supplementary Fig. S3). Although in vitro experiments did not show significant difference between wild type and mutant proteins, we transiently transfected neuroendocrine PC12 cells in order to determine whether the genetic variants identified in individuals with BD may affect directly or indirectly the neurotransmitter release, using FFN511 (Fig. 3a, b). We showed a 50% increase in vesicular exocytosis when wild type CADPS was expressed in PC12 cells as compared with empty vector (paired Student t test, $t = 11.88$, $df = 25$, $p < 0.0001$, Fig. 3c). Interestingly, we showed that the FFN511 release was totally impaired when CADPS lacked exon 2 (paired Student t test, $t = 5.03$, $df = 7$, $p = 0.001$) or when CADPS carried the p.N1017I (paired Student t test, $t = 5.50$, $df = 5$, $p = 0.003$). Release was also abolished when the 2



mutations p.L482I and p.N1017I were present (paired Student t test, $t = 6.39$, df = 5, $p = 0.001$, not shown), as observed in one individual with BD.

As no significant difference was observed for other variants, we checked whether those might affect other functions of CADPS. Indeed, former studies demonstrated

◀ **Fig. 4 Cultured neurons expressing $CADPS_{p.I148\_E185del}$ or $CADPS_{p.S399L}$ exhibit altered short-term plasticity characteristics.** **a**, **j** Sample traces of action potential (AP)-evoked excitatory post-synaptic currents (EPSCs) (i) spontaneously occurring miniature EPSCs (mEPSCs) (ii) and sucrose-evoked EPSCs (iii) recorded in cultured CADPS-CADPS2 double knock-out hippocampal neurons (DKO), which expressed indicated CADPS cDNA. **b**, **k** Lentiviral expression of the p.I148_E185del (blue) and the p.S399L mutation (red) rescued the AP-triggered EPSCs amplitude. No difference has been observed neither in the readily releasable pool (RRP) charge measured in presence of 0.5 M sucrose solution (**c**, **l**), nor in the median amplitude of spontaneously occurring mEPSCs (**e**, **n**) for none of the mutants. An increased vesicular release probability (**d**) and an increased frequency of spontaneously occurring mEPSCs (**f**) has been observed for p.I148_E185del but not for p.S399L (**m**, **o**). **g**, **p** Averaged EPSC responses during a 40 Hz AP train. **h**, **i**, **q**, **r** Paired-pulse ratio during trains of APs at indicated frequencies was decreased in p.I148_E185del (**h**) but increased in p.S399L (**q**). No difference was observed for steady-state EPSC responses for none of the mutants (**i**, **r**). Bars in plots depict median and 5–95 percentile. $N = 6$ cultures for each of the two comparisons. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. See also Supplementary Fig. S4.

that CADPS was also able to promote vesicular monoamine uptake and storage in cell lines and brain [21–23]. We thus transiently transfected CHOVMAT1 cell lines with wild type and mutant forms of *CADPS* and measured serotonin uptake in vitro. We observed a 50% increase of serotonin uptake when CHOVMAT1 cells were transfected with wild type CADPS when compared with empty vector expressing cells (unpaired Student *t* test, $t = 4.26$, $df = 24$, $p = 0.0003$; Fig. 3d), thereby confirming the ability for CADPS to potentiate the vesicular monoamine uptake in vitro. Two mutations, p.R195L and p.L482I, impaired the ability of CADPS to promote uptake (unpaired Student *t* test, $t = 2.95$, $df = 20$, $p = 0.008$ and $t = 2.81$, $df = 24$, $p = 0.01$ for p.R195L and p.L482I, respectively; Fig. 3d). For the p.R195L mutant, loss of function in this assay might be attributed to decreased protein level (see Fig. 2b).

Partial truncation of the dynactin-interacting-domain of CADPS leads to enhanced short-term synaptic depression

We next assessed the ability of the 7 *CADPS* cDNA mutants to reverse the secretory deficits of CADPS/CADPS2 DKO hippocampal neurons [19]. All mutant cDNA constructs were able to rescue the dramatic decrease of EPSC amplitudes observed in CADPS/CADPS2 DKO neurons when expressed via lenti viruses (Fig. 4 and Supplementary Fig. S4). However, a deeper exploration of these variant proteins showed physiological differences for two of them (Fig. 4). The p.I148_E185del mutation resulted in a higher vesicular release probability (Fig. 4d), as well as an increase in mEPSC frequency when compared to wild type CADPS expressing neurons (Fig. 4f). In addition, when applying an action potential train of stimuli at frequencies of 2, 5, 10 or 40 Hz, expression of $CADPS^{I148\_E185del}$ in CADPS/CADPS2 DKO neurons resulted in stronger depression than did CADPS expression (Fig. 4g, h). These findings were consistent with an increased release probability. In contrast, expression of the p.S399L mutant cDNA led to a less pronounced short-term synaptic depression (Fig. 4p, q) during trains of action potentials at frequencies of 2, 5, 10 or 40 Hz.

A decreased expression of CADPS increase manic-like behaviours in mice

In vitro and ex vivo analyses showed that most of the genetic variants identified in individuals with early onset BD resulted in functional abnormalities of CADPS (Supplementary Table 1), suggesting that mutations in this gene may result in vesicular exocytosis dysfunction and thus increase the risk of developing BD. These variants were mainly loss of function mutations at heterozygous state in patients. The *Cadps* homozygous mutant mice ($Cadps^{-/-}$) died at birth. We thus conducted behavioural studies on heterozygous mutant animals ($Cadps^{+/-}$) and their wild type littermates. As observed for two mutations (p.R195L and p.S399L), these animals had a lower expression level of the protein. In addition, they showed a significant decrease of the readily releasable pool in chromaffin cells [21]. This reduction might limit large dense core vesicle priming reaction and catecholamine secretion in mutant animals. In order to determine if such alterations affect behavioural responses, we used a battery of tests to characterize manic or depressive-like behaviours. During a 9 min period, $Cadps^{+/-}$ mice covered a significant longer distance in an open field than wild type littermates (Mann–Whitney *U* test, $U = 11$, $p = 0.008$; Fig. 5a). Locomotor activity was also assessed in home cages for three weeks where heterozygous mice showed similarly a higher activity, mainly during activity periods, i.e. nights (Mann–Whitney *U* test, $U = 9$, $p = 0.01$), suggesting that this hyperactivity was not due to the new environment or to stress generated by moving to the open field (Fig. 5b). We then assessed whether mutant mice had depressive-like behaviours using forced swimming test (FST) and tail suspension test (TST), which are both classically used to measure resignation-based antidepressive drug effects. Consistent with hyperactivity, we observed an increased swimming duration (Mann–Whitney *U* test, $U = 34$, $p = 0.08$) and a longer latency before immobility (Mann–Whitney *U* test, $U = 7$, $p = 0.0002$) in FST (Fig. 5c). Although not significant, we observed a smaller number of immobility episodes during TST (Mann–Whitney *U* test, $U = 22$, $p = 0.10$; Fig. 5d). No difference was observed between mutant animals and wild type littermates for the other tests assessed, including startle

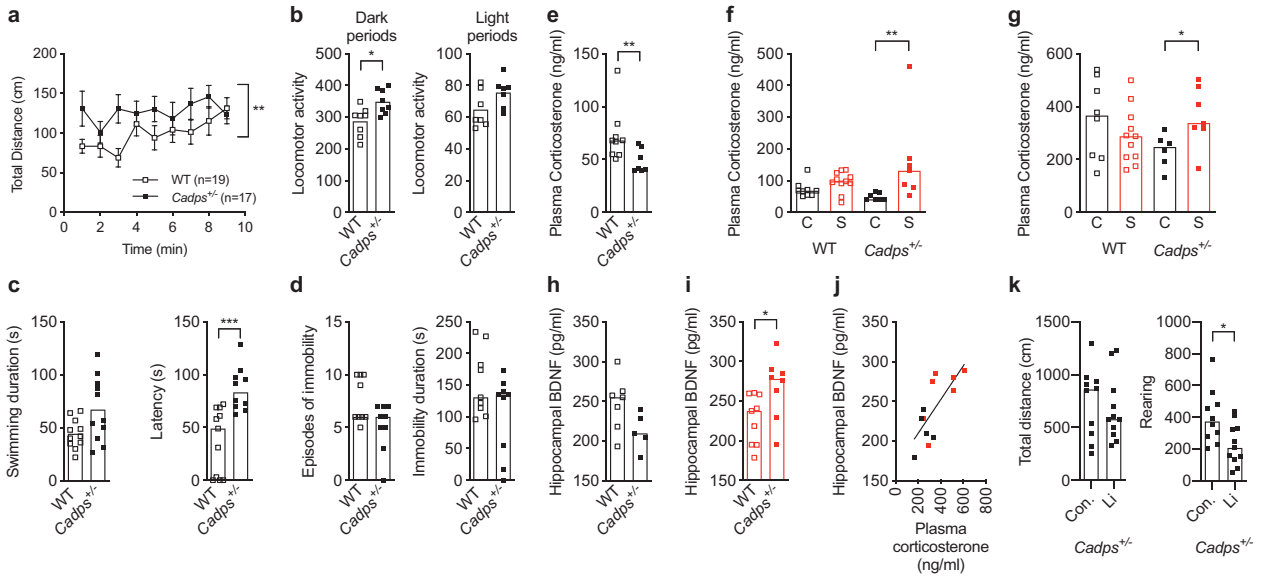


Fig. 5 Heterozygous mutant mice for *CADPS* display manic-like behaviours and a higher sensitivity to stress. *Cadps*$^{+/-}$ mice showed an increased locomotor activity in the open field (**a**), in home cage (**b**), and during the forced swim test (**c**), and a tendency has been observed in the tail suspension test (**d**). *Cadps*$^{+/-}$ mice showed a lower corticosterone level in basal condition (**e**), but are hypersensitive to chronic (**f**) and acute stress (**g**). Animals unexposed (C) and exposed (S) to unpredictable chronic mild stress are indicated in black and red, respectively. Unstressed *Cadps*$^{+/-}$ mice had a lower hippocampal BDNF level (**h**). Unpredictable chronic mild stress (red) increased the BDNF level only in *Cadps*$^{+/-}$ mice (**i**), for which the hippocampal BDNF level was correlated with the plasma level of corticosterone (**j**). **k** Basal locomotor hyperactivity of *Cadps*$^{+/-}$ mice was rescued by lithium (Li) when compared with non-treated *Cadps*$^{+/-}$ animals (Con.). Data in (**a**) are presented as mean ± s.e.m. Bars represent median (**b**–**k**) $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. *WT* wild type. See also Supplementary Fig. S5.

reactivity, pre-pulse inhibition, food intake, and sucrose preference (Supplementary Fig. S5).

Mutant mice for *Cadps* are more sensitive to chronic and acute stress

It has been widely demonstrated that individuals with BD are more sensitive to stressful events and more specifically that early stress can influence the onset and course of the disorder [40, 41]. Interestingly, functional studies revealed defects in catecholamine loading or storage in embryonic chromaffin cells from *Cadps*$^{-/-}$ adrenal gland. In adult *Cadps*$^{+/-}$ mice, these cells showed a reduced exocytosis and a lower number of morphologically docked granules [21]. Adrenal glands are part of the hypothalamo-pituitary-adrenal (HPA) axis, a major part of the neuroendocrine system that controls reactions to stress and regulates digestion, mood, anxiety and emotions, sexuality, and appetite, reminiscent of features of BD. Corticosterone is produced in the cortex of the adrenal gland where CADPS is very weakly expressed [42, 43]. However, *Cadps*$^{+/-}$ mice showed lower corticosterone plasma levels than wild type littermates in basal conditions (Mann–Whitney test, $U = 7$, $p = 0.008$; Fig. 5e), suggesting that the absence of CADPS may have an impact on the HPA axis and stress response. In order to estimate the impact of stress in *Cadps*$^{+/-}$ mice, we measured plasma corticosterone concentration in our animal model exposed to unpredictable chronic mild stress (UCMS) for four months as well as in animals exposed to an acute stress. Whereas no difference in corticosterone secretion was observed for wild type mice between stressed and non-stressed animals, *Cadps*$^{+/-}$ mice showed a significant higher corticosterone level when animals were exposed to UCMS (Mann–Whitney U test, $U = 3$, $p = 0.004$; Fig. 5f). For all animals, acute stress increased corticosterone secretion (Fig. 5g). However, whereas UCMS slightly decreased the acute stress effect in wild type animals, we observed a significant increased plasma level of corticosterone in *Cadps*$^{+/-}$ mice, when exposed to UCMS (Mann–Whitney U test, $U = 5$, $p = 0.02$), suggesting that mutations in *CADPS* may impaired adaptation to stress.

Although few biomarkers have been identified in BD, a lower level of brain derived neurotrophic factor (BDNF) has been repeatedly reported in individuals with unipolar or bipolar depression [44]. Similarly to what is observed in patients, *Cadps*$^{+/-}$ mice had a lower BDNF level in hippocampus than wild type littermates, in basal condition (Mann–Whitney U test, $U = 6$, $p = 0.07$; Fig. 5h). We then measured hippocampal BDNF level in our animal model after the acute stress. Interestingly, we found that chronic stress had no impact on hippocampal BDNF expression in wild type animals, whereas BDNF levels were increased in UCMS-exposed *Cadps*$^{+/-}$ mice (Mann–Whitney U test, $U = 11$, $p = 0.01$; Fig. 5i). Note, when comparing

corticosterone and BDNF levels in these mutant animals, we showed a significant correlation between the two markers (linear regression, $R^2 = 0.55$, $F = 12.15$, $p = 0.006$; Fig. 5j).

Lithium rescues the hyperactivity of mutant mice for *Cadps*

As lithium is one of the most widely used medication to treat BD, we tested whether it may rescue the manic-like behaviours we reported in *Cadps*$^{+/-}$ mice. Wild type and mutant animals were fed with lithium-carbonate containing chow for three weeks before to measure their activity for 2 h. Although the total distance was not different between heterozygous mice fed with lithium and those receiving untreated food (Mann–Whitney U test, $U = 48$, $p = 0.65$), treated animals showed a significant decrease of the number of rearing when compared with non-treated animals (Mann–Whitney U test, $U = 23$, $p = 0.02$; Fig. 5k), suggesting that lithium reversed the baseline hyperactivity of *Cadps*$^{+/-}$ mice.

Individuals with missense variants in *CADPS* reported more childhood trauma

Mutant mice for *Cadps* suggest that a decrease in the expression level of this protein would increase sensitivity to stress. It has been widely reported that individuals with BD experienced more childhood trauma than unaffected control population [40]. Here, we used the childhood trauma questionnaire [31] to assess how childhood traumas were reported in families with mutations in *CADPS* as well as in a group of 355 subjects with BD and 86 unaffected control individuals. Individuals with BD experienced significantly more childhood traumas than unaffected individuals (Mann–Whitney U test, $U = 10{,}723$, $p = 0.00001$; Fig. 6). Individuals with missense variants in *CADPS* reported also more childhood traumas than unaffected controls (Mann–Whitney U test, $U = 23.5$, $p = 0.009$; Fig. 6). Interestingly, their median score to the childhood trauma questionnaire ($\text{median}_{\text{CTQ\_CADPS}} = 49$) was even higher than the one of the general population of individuals with BD ($\text{median}_{\text{CTQ\_BD}} = 39$, Mann–Whitney U test, $U = 266.5$, $p = 0.14$), whereas no significant difference was not observed when comparing unmutated subjects of the families with controls (Mann-Whitney U test, $U = 76$, $p = 0.06$) or the general population of individuals with BD (Mann–Whitney U test, $U = 626$, $p = 0.69$; Fig. 6).

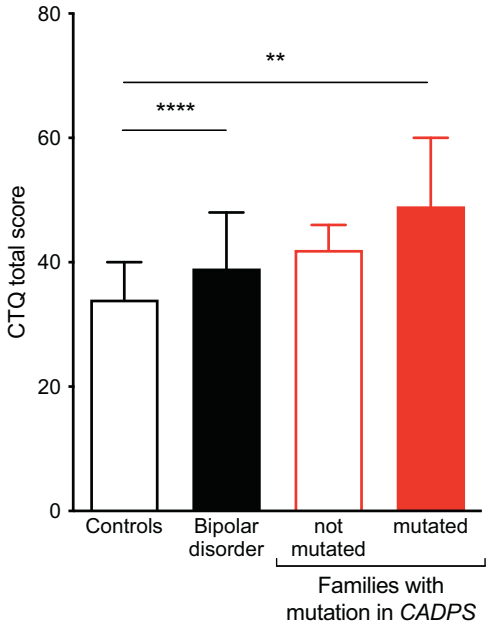


Fig. 6 Assessment of childhood trauma in families with mutations in *CADPS*, 355 independent patients with BD and 86 unaffected controls. Patients with BD experienced more childhood trauma than unaffected controls. Patients with mutation in *CADPS* experienced more childhood trauma than both unaffected controls and the general population of patients with BD. In families with *CADPS* mutations, mutated subjects have a higher score than unmutated subjects. Data are presented as median ± interquartile range. $^{**}p < 0.01$; $^{****}p < 0.0001$.

Discussion

In the present study, we accumulated evidence that genetic variants in *CADPS* may increase the vulnerability to BD. We first reported that common polymorphisms in *CADPS* were more frequent in individuals with BD than in control population and demonstrated that the difference was even greater when only patients with an early age at onset were considered. This association was strengthened by difference in allele frequencies observed in large populations of individuals with BD and controls from the PGC [9]. It was also consistent with previous genetic studies, which reported a genetic linkage between BD and the 3p14 region, mainly with early-onset BD [3–5]. Early-onset BD has long been demonstrated to correspond to a homogeneous subgroup of patients with a higher genetic component than later forms [45–50]. Early-onset BD shares a similar aetiology with late-onset BD with a small effect for common polymorphisms and larger effects for very rare variants [51]. By screening for rare variants in individuals with early-onset BD, we observed that the missense variant frequency was three times higher than in control populations, in contrast to synonymous variants for which no significant difference was observed. This difference between synonymous and non-synonymous substitution rates in patients and controls suggests that most of the rare missense variants may have functional consequences subjected to purifying selection. All missense variants identified in individuals with BD were predicted to alter protein function (Supplementary Table 1).

In addition, we have identified the deletion of a full exon in one patient with BD with a strong impact on the protein sequence. Due to the limited sensitivity of DNA chips, we may have overlooked the copy number variant detection in affected and unaffected individuals. However, such a variation looks rare since no similar deletion has been reported in public databases. Further experiments confirmed for all but two variants (p.R959L and p.N205K) functional alteration of CADPS. Although in vitro experiments showed that the *CADPS* mutations identified in individuals with BD did not appear to affect the protein activity, cellular studies showed that these mutations may affect either the amount of the protein (p.R195L and p.S399L), the Ca^{2+}-dependent exocytosis (p.I148_E185del and p.N1017I) or the ability of CADPS to promote monoamine uptake (p.R195L and p.L482I). Results on monoamine uptake and cellular secretion assays suggest that different domains of CADPS may regulate distinct functions of the protein, as previously shown for both CADPS and CADPS2 [26, 36–38]. Interestingly, the exon 2 deletion (p.I148_E185del) showed a higher vesicular release probability and a higher mEPSC frequency than wild type CADPS. This result is consistent with multiple studies on human induced pluripotent stem cells-derived neurons, which showed that BD neurons are more spontaneously active than control neurons [15, 52, 53]. In addition, this mutation showed a stronger depression of EPSC responses when trains of action potentials were applied, suggesting that this mutation may result in an alteration of synaptic plasticity, which has widely been reported to play a key role in BD pathophysiology and therapeutics [54]. More generally, our results suggest that abnormalities in Ca^{2+}-dependent exocytosis may increase the risk of developing an early-onset form of BD and thereby corroborates the previous report of an association between *SNAP25*, a direct binding partner of CADPS [27], and early-onset BD [14]. Numerous genetic studies have reported calcium signalling as the most associated pathway with BD [9, 12, 16], which may have an impact on the age at onset of the disease [55]. In addition, calcium signalling is among the most affected pathways in cellular models of BD [56].

CADPS has been shown to trigger synaptic and large dense-core vesicle exocytosis [19, 21, 43, 57]. All affected individuals with missense variants in our study were heterozygous for CADPS mutations, which was not surprising since homozygous mutant mice for *Cadps* die at birth [21]. Interestingly, two mutations showed a significant lower protein level in transfected cells. Adult heterozygous mutant mice for *Cadps* showed a significant reduction of CADPS and have a reduction of the readily releasable pool and a reduction of catecholamine secretion in neuroendocrine cells [21]. Variations in catecholamine and monoamines have been widely documented in individuals with BD and are the target of many antidepressant and antipsychotic treatments [58]. This mouse model showed both behaviours and biomarkers that bear resemblance to BD. In addition, lithium reversed their manic-like behaviours. Interestingly, mutant mice for CADPS resulted in over-reactivity to acute stress when animals experienced chronic mild stress. This model perfectly matches with a two-hits model that has been proposed for psychoses [59], in which an early stress interacts with genetic factors to increase the vulnerability to BD. Then further stressors in adolescence or early adulthood trigger the disease in vulnerable subjects. This hypothesis is supported by the observation that individuals carrying mutation in *CADPS* reported more childhood trauma than other individuals with BD. Interestingly, the scores observed for mutated individuals in these families were higher than those observed for unmutated ones. Although there is no doubt that childhood traumas are more frequent in families with *CADPS* mutations, this difference suggests either that mutated individuals experienced more childhood trauma than unmutated ones or that they were more sensitive to trauma and thus scored higher to the questionnaire. Therefore, a functional mutation in *CADPS* may affect the HPA axis, increasing the reactivity to stressful events. This gene-environment interaction may thus result in changes in more central physiological processes, as reflected by changes in BDNF level in our mouse model, increasing the sensitivity to a later stressor that would trigger the disease. Although a gender effect has been reported to interact with childhood trauma [60, 61], our sample was too small to consider this parameter in our study and such analyses would need further investigations in larger sample.

In summary, our genetic studies suggest that functional mutations in *CADPS* may increase the risk of developing early-onset BD. This risk may result from alteration in Ca^{2+}-dependent exocytosis mechanisms that would increase sensitivity to environmental stressors.

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Author contributions SJ designed the study, with the help of JR and DN for electrophysiological experiments, CK and MNB for mouse behavioural analyses and TFJM for CADPS in vitro analysis; SJ, JS, CK, AN, AH and EC generated genetic data and conducted genetic analyses; JS, NP, AH, VL, RT, SM and TFJM performed biochemical experiments; CK, GG and MNB conducted behavioural analyses in mice; DN and JR performed electrophysiological experiments on hippocampal mouse neurons; CH, BE and ML designed, collected and analysed clinical data, with the help of CB and PLC who collected biological samples in patients and controls; SJ, JS, DN, CK, BE, MNB and TFJM wrote the article.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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