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

3

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15

16 Running title: CADPS mutations in bipolar disorder and sensitivity to stress

17

1 **Abstract**

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

20

1 **Introduction**

2 Bipolar disorder (BD) is a chronic psychiatric mood disorder with a lifetime cumulative
3 risk of 4.4% in the world-wide population.¹ Twin and family studies have demonstrated
4 a genetic component of BD with an estimated heritability ranging between 60% and
5 80%.² These results are supported by many genome-wide linkage analyses that
6 identified a vulnerability locus for BD on chromosome 3p14-p21,^{3,4} mainly in the early-
7 onset form of the disease.⁵ Recent large-scale genome-wide association studies
8 further suggested several candidate genes in this region.⁶⁻¹¹

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

1 complex,²⁷ which have been widely associated with psychiatric disorder vulnerability,^{13,}
2 ^{14, 28, 29} makes this gene an interesting candidate for vulnerability to psychiatric
3 disorders.

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

13

14 **Materials and Methods**

15 See supplementary information for details. Methods are briefly described as follows.

16

17 *Subjects*

18 This study combines data from a previously published cohort^{9, 30} of 452 individuals with
19 BD (189 males and 263 females) and 1,636 control individuals (696 males and 940
20 females) of French origin for genotyping analyses and child trauma assessment^{31, 32}
21 from whom 132 individuals with early-onset BD (56 males and 76 females) have been
22 included in sequencing analyses (see Supplementary Materials and Methods for
23 details). In addition, 184 unaffected controls (105 males and 79 females) with neither
24 personal nor family history of psychiatric disorder or suicidal behaviour have been
25 collected for sequencing analyses.

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

4

5 *Mice*

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

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

14

15 *Genetic analyses in humans*

16 Genotyping data and copy number variations have been detected using
17 HumanHap550 BeadArrays (Illumina, San Diego, CA, U.S.A.) and analysed using the
18 PLINK software v1.07³³ as previously described.³⁰ The *CADPS* coding region and
19 exon-intron boundaries were sequenced by Sanger's method on a 16-Capillary ABI
20 PRISM 3130xl genetic analyser as previously described.¹⁴ All primers and PCR
21 conditions are available on request.

22

23 *Vesicular exocytosis in PC12 cells*

24 Two million of PC-12 cells were electroporated with the plasmids containing wild type
25 and mutant *CADPS* DNA using Amaxa® Cell Line Nucleofector™ Kit V and

1 Nucleofector™ device according to manufacturer's protocol (Lonza, Basel,
2 Switzerland). The day after, cells were incubated with 20µM of Fluorescent False
3 Neurotransmitter 511 (FFN511)³⁴ diluted in 100µl of Krebs-Ringer buffer: 10mM
4 HEPES, 140mM NaCl, 5mM KCl, 1.5mM MgCl₂, 2mM CaCl₂, 14.3mM NaHCO₃, 5mM
5 Glucose, 0.1mM EGTA, for 1 hour at room temperature. Cells were washed three times
6 and exocytosis was triggered with the addition of Krebs-Ringer buffer supplemented
7 with 60mM KCl, 80mM NaCl and 1mM EGTA. Supernatants were collected after 1
8 minute exocytosis and read at 501nm with Infinite® 200 PRO microplate reader
9 (Tecan, Männedorf, Switzerland). The transfection efficiency was checked for each
10 experiment by quantifying the fluorescence level of the enhanced green fluorescent
11 protein (eGFP) at 504nm.

12

13 *Vesicular monoamine uptake assays in CHO cell lines*

14 Chinese Hamster Ovary (CHO) cell lines, constitutively expressing the *Slc18a1* gene,
15 encoding the vesicular monoamine transporter VMAT1 (CHOVMAT1), were provided
16 by Prof. Ahnert-Hilger (Charité Center, Berlin, Germany) and cultured as described.²²
17 Cells were transfected with wild type and mutant *CADPS* DNA using Lipofectamine™
18 2000 (Thermo Fisher Scientific) according to manufacturer's instruction. Twenty-four
19 hours after transfection, the transfection efficiency was checked with the fluorescence
20 of the eGFP and one million cells were permeabilized with streptolysin O.³⁵ Serotonin
21 uptake was measured using 5-Hydroxy-tryptamine,[H³]-trifluoroacetate (PerkinElmer,
22 Waltham, MA, USA) and liquid scintillation counting by a Packard 1600TR Tri-Carb
23 Liquid Scintillation Analyzer (Perkin Elmer) as described.²²

24

25

1

2 *Electrophysiological recording of autaptic hippocampal neurons*

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

14

15 *Statistical analyses*

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

25

1

2 **Results**

3 *CADPS is associated with early-onset bipolar disorder*

4 *CADPS* is a large gene spanning 477,044bp on chromosome 3p14. In order to
5 determine whether common polymorphisms in this gene might explain genetic linkages
6 frequently reported on 3p14, we genotyped 176 haplotype-tagging single nucleotide
7 polymorphisms (ht-SNPs) in 452 individuals with BD and 1,636 controls. The biggest
8 difference in allele frequencies between patients and controls was observed for
9 rs35462732 ($\chi^2=8.84$, $p=0.003$) (Figure 1a). This SNP has not been genotyped by the
10 Psychiatric Genomic Consortium (PGC) Bipolar Disorder Group⁹ and no information
11 on allele frequency was available for it. However, 4 SNPs (rs9872498, rs1238394,
12 rs833638 and rs17651503) in *CADPS* showed a difference in allele frequencies with a
13 $p<0.01$ between the 7,481 individuals with BD and the 9,250 control individuals of the
14 PGC study. These SNPs were located 100kbp downstream to rs35462732 (lowest p-
15 value for rs833838, $p=0.004$). Genetic linkages identified in this region were specific
16 for patients with early-onset BD.⁵ We then restricted our sample to 203 patients with
17 an age at onset lower than 22 and showed that the difference in allele frequencies for
18 rs35462732 was even larger ($\chi^2=11.05$, $p=0.0009$, OR=1.80, 95%CI[1.27;2.55])
19 (Figure 1a).

20

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

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

1 studies showed a stronger signal for early-onset BD, we sequenced the 31 coding
2 exons as well as regulatory regions of the 3 RefSeq isoforms of *CADPS*
3 (NM_003716.3, NM_183393.2 and NM_183394.2) in a subgroup of 132 patients with
4 early-onset BD. We identified six missense and 4 synonymous variants
5 (Supplementary Table 1). One of the missense variants (p.N1017I) has been found
6 twice in patients and twice in controls and should be considered as a polymorphism
7 not associated with the disorder. Note that one of the two patients, who carried the
8 p.N1017I variant had another missense variant on the same allele (p.L482I) (Figure
9 1b). In order to focus on variants that might be causative for the disorder, we then
10 selected only rare variants with a minor allele frequency lower than 0.001 in the Exome
11 Aggregation Consortium (ExAC) database (<http://exac.broadinstitute.org/>) for further
12 analyses. In the 6 families in which rare variants were identified, 100% of individuals
13 with a missense variant in *CADPS* had a mood disorder, whereas this frequency
14 decreased to 20% in unmutated subjects (Fisher's exact test, $OR=+\infty$,
15 $95\%CI[2.00;+\infty]$, $p=0.005$) (Figure 1c). Only 3 missense variants were found in 184
16 unaffected controls, including p.N1017I. Moreover, the missense variant frequency
17 was three times higher in patients than in controls (Fisher's exact test, $OR=3.53$,
18 $95\%CI[0.57;37.32]$, $p=0.13$) as well as in the non-psychiatric and non-Finnish
19 European population from the ExAC database (Fisher's exact test, $OR=3.38$,
20 $95\%CI[1.08;8.11]$, $p=0.02$) (Supplementary Figure S1). In contrast, no difference was
21 observed for synonymous variants between these three populations (Supplementary
22 Figure S1). We looked for copy number variations in the 452 patients with BD.
23 Although, any insertion or deletion were found in *CADPS* using DNA chip screening, a
24 deletion was identified by serendipity using real-time PCR. This amplification was used
25 as control to validate another deletion identified in *GRIP1* in one female with late-onset

1 BD. The *GRIP1* deletion was not confirmed, but further exploration around the *CADPS*
2 region confirmed a 9kbp-deletion, removing the exon 2 (p.I148_E185del). As exon 2
3 is composed of 114 nucleotides, this deletion resulted in a protein shortened of 38
4 amino acids in the dynactin-binding domain, which has been shown to be necessary
5 for a proper localisation of CADPS2 in neurons.²⁶ No similar deletion was reported in
6 the Database for Genomic Variants (<http://dgv.tcag.ca/>) or in the Genome Aggregation
7 Database of Structural Variants v2.1 (<https://gnomad.broadinstitute.org>).

8

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

11 *In silico* analysis of the genetic variants identified in patients with early-onset BD
12 suggested that 5 out of the 6 missense variants were predicted to have a damaging
13 impact on the protein function by at least two programs (Supplementary Table 1). In
14 order to determine whether these variants affect the cellular stability of CADPS, we
15 transfected either the wild type or mutant *CADPS* in multiple cell lines. Quantification
16 of the protein level in COS-7 cells was performed by western blot analysis and showed
17 a 40% and 33% decrease in protein expression level for p.R195L and p.S399L,
18 respectively (Figure 2a and 2b). This was confirmed both in CHO and PC12 cells with
19 a similar reduction of the protein level, whereas no difference in RNA level was
20 observed for these variants (Supplementary Figure S2). We checked the protein
21 stability using 100µg/ml of cycloheximide for 24h on COS cells transiently transfected
22 with mutated and non-mutated *CADPS* and measured the protein level over time. This
23 showed a 3-fold faster degradation of the protein with the p.S399L mutation (Figure
24 2c).

25

1 *CADPS variants exhibit normal activity in vitro but affect CADPS functions ex vivo*
2 Multiple functions have been shown for CADPS in vesicular exocytosis mechanisms.
3 Missense variants in CADPS were previously found to decrease the Ca²⁺-triggered
4 exocytosis in a permeable cell assay.³⁶⁻³⁹ Thus, we assessed activity in this assay of
5 human CADPS proteins with patients' mutations in HEK293 cells. All proteins tested
6 exhibited activities similar to those of the wild type protein (Supplementary Figure S3).
7 Although *in vitro* experiments did not show significant difference between wild type and
8 mutant proteins, we transiently transfected neuroendocrine PC12 cells in order to
9 determine whether the genetic variants identified in individuals with BD may affect
10 directly or indirectly the neurotransmitter release, using FFN511 (Figure 3a and 3b).
11 We showed a 50% increase in vesicular exocytosis when wild type CADPS was
12 expressed in PC12 cells as compared with empty vector (paired Student t test, t=11.88,
13 df=25, p<0.0001, Figure 3c). Interestingly, we showed that the FFN511 release was
14 totally impaired when CADPS lacked exon 2 (paired Student t test, t=5.03, df=7,
15 p=0.001) or when CADPS carried the p.N1017I (paired Student t test, t=5.50, df=5,
16 p=0.003). Release was also abolished when the 2 mutations p.L482I and p.N1017I
17 were present (paired Student t test, t=6.39, df=5, p=0.001, not shown), as observed in
18 one individual with BD.

19
20 As no significant difference was observed for other variants, we checked whether those
21 might affect other functions of CADPS. Indeed, former studies demonstrated that
22 CADPS was also able to promote vesicular monoamine uptake and storage in cell lines
23 and brain.²¹⁻²³ We thus transiently transfected CHOVMAT1 cell lines with wild type and
24 mutant forms of *CADPS* and measured serotonin uptake *in vitro*. We observed a 50%
25 increase of serotonin uptake when CHOVMAT1 cells were transfected with wild type

1 CADPS when compared with empty vector expressing cells (unpaired Student t test,
2 $t=4.26$, $df=24$, $p=0.0003$; Figure 3d), thereby confirming the ability for CADPS to
3 potentiate the vesicular monoamine uptake *in vitro*. Two mutations, p.R195L and
4 p.L482I, impaired the ability of CADPS to promote uptake (unpaired Student t test,
5 $t=2.95$, $df=20$, $p=0.008$ and $t=2.81$, $df=24$, $p=0.01$ for p.R195L and p.L482I,
6 respectively; Figure 3d). For the p.R195L mutant, loss of function in this assay might
7 be attributed to decreased protein level (see Figure 2b).

8

9 *Partial Truncation of the Dynactin-Interacting-Domain of CADPS leads to enhanced*
10 *short-term synaptic depression*

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

1

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

3 *In vitro* and *ex vivo* analyses showed that most of the genetic variants identified in
4 individuals with early onset BD resulted in functional abnormalities of CADPS
5 (Supplementary Table 1), suggesting that mutations in this gene may result in vesicular
6 exocytosis dysfunction and thus increase the risk of developing BD. These variants
7 were mainly loss of function mutations at heterozygous state in patients. The *Cadps*
8 homozygous mutant mice (*Cadps*^{-/-}) died at birth. We thus conducted behavioural
9 studies on heterozygous mutant animals (*Cadps*^{+/-}) and their wild type littermates. As
10 observed for two mutations (p.R195L and p.S399L), these animals had a lower
11 expression level of the protein. In addition, they showed a significant decrease of the
12 readily releasable pool in chromaffin cells.²¹ This reduction might limit large dense core
13 vesicle priming reaction and catecholamine secretion in mutant animals. In order to
14 determine if such alterations affect behavioural responses, we used a battery of tests
15 to characterize manic or depressive-like behaviours. During a 9min period, *Cadps*^{+/-}
16 mice covered a significant longer distance in an open field than wild type littermates
17 (Mann-Whitney U test, U=11, p=0.008; Figure 5a). Locomotor activity was also
18 assessed in home cages for three weeks where heterozygous mice showed similarly
19 a higher activity, mainly during activity periods, i.e. nights (Mann-Whitney U test, U=9,
20 p=0.01), suggesting that this hyperactivity was not due to the new environment or to
21 stress generated by moving to the open field (Figure 5b). We then assessed whether
22 mutant mice had depressive-like behaviours using forced swimming test (FST) and tail
23 suspension test (TST), which are both classically used to measure resignation-based
24 antidepressive drug effects. Consistent with hyperactivity, we observed an increased
25 swimming duration (Mann-Whitney U test, U=34, p=0.08) and a longer latency before

1 immobility (Mann-Whitney U test, $U=7$, $p=0.0002$) in FST (Figure 5c). Although not
2 significant, we observed a smaller number of immobility episodes during TST (Mann-
3 Whitney U test, $U=22$, $p=0.10$; Figure 5d). No difference was observed between mutant
4 animals and wild type littermates for the other tests assessed, including startle
5 reactivity, pre-pulse inhibition, food intake, and sucrose preference (Supplementary
6 Figure S5).

7

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

9 It has been widely demonstrated that individuals with BD are more sensitive to stressful
10 events and more specifically that early stress can influence the onset and course of
11 the disorder.^{40, 41} Interestingly, functional studies revealed defects in catecholamine
12 loading or storage in embryonic chromaffin cells from *Cadps*^{-/-} adrenal gland. In adult
13 *Cadps*^{+/-} mice, these cells showed a reduced exocytosis and a lower number of
14 morphologically docked granules.²¹ Adrenal glands are part of the hypothalamo-
15 pituitary-adrenal (HPA) axis, a major part of the neuroendocrine system that controls
16 reactions to stress and regulates digestion, mood, anxiety and emotions, sexuality,
17 and appetite, reminiscent of features of BD. Corticosterone is produced in the cortex
18 of the adrenal gland where CADPS is very weakly expressed.^{42, 43} However, *Cadps*^{+/-}
19 mice showed lower corticosterone plasma levels than wild type littermates in basal
20 conditions (Mann-Whitney test, $U=7$, $p=0.008$; Figure 5e), suggesting that the absence
21 of CADPS may have an impact on the HPA axis and stress response. In order to
22 estimate the impact of stress in *Cadps*^{+/-} mice, we measured plasma corticosterone
23 concentration in our animal model exposed to unpredictable chronic mild stress
24 (UCMS) for four months as well as in animals exposed to an acute stress. Whereas no
25 difference in corticosterone secretion was observed for wild type mice between

1 stressed and non-stressed animals, *Cadps*^{+/-} mice showed a significant higher
2 corticosterone level when animals were exposed to UCMS (Mann-Whitney U test, U=3,
3 p=0.004; Figure 5f). For all animals, acute stress increased corticosterone secretion
4 (Figure 5g). However, whereas UCMS slightly decreased the acute stress effect in wild
5 type animals, we observed a significant increased plasma level of corticosterone in
6 *Cadps*^{+/-} mice, when exposed to UCMS (Mann-Whitney U test, U=5, p=0.02),
7 suggesting that mutations in *CADPS* may impaired adaptation to stress.

8
9 Although few biomarkers have been identified in BD, a lower level of brain derived
10 neurotrophic factor (BDNF) has been repeatedly reported in individuals with unipolar
11 or bipolar depression.⁴⁴ Similarly to what is observed in patients, *Cadps*^{+/-} mice had a
12 lower BDNF level in hippocampus than wild type littermates, in basal condition (Mann-
13 Whitney U test, U=6, p=0.07; Figure 5h). We then measured hippocampal BDNF level
14 in our animal model after the acute stress. Interestingly, we found that chronic stress
15 had no impact on hippocampal BDNF expression in wild type animals, whereas BDNF
16 levels were increased in UCMS-exposed *Cadps*^{+/-} mice (Mann-Whitney U test, U=11,
17 p=0.01; Figure 5i). Note, when comparing corticosterone and BDNF levels in these
18 mutant animals, we showed a significant correlation between the two markers (linear
19 regression, R²=0.55, F=12.15, p=0.006; Figure 5j).

20

21 *Lithium rescues the hyperactivity of mutant mice for Cadps*

22 As lithium is one of the most widely used medication to treat BD, we tested whether it
23 may rescue the manic-like behaviours we reported in *Cadps*^{+/-} mice. Wild type and
24 mutant animals were fed with lithium-carbonate containing chow for three weeks
25 before to measure their activity for two hours. Although the total distance was not

1 different between heterozygous mice fed with lithium and those receiving untreated
2 food (Mann-Whitney U test, $U=48$, $p=0.65$), treated animals showed a significant
3 decrease of the number of rearing when compared with non-treated animals (Mann-
4 Whitney U test, $U=23$, $p=0.02$; Figure 5k), suggesting that lithium reversed the baseline
5 hyperactivity of *Cadps*^{+/-} mice.

6

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

8 Mutant mice for *Cadps* suggest that a decrease in the expression level of this protein
9 would increase sensitivity to stress. It has been widely reported that individuals with
10 BD experienced more childhood trauma than unaffected control population.⁴⁰ Here, we
11 used the childhood trauma questionnaire³¹ to assess how childhood traumas were
12 reported in families with mutations in *CADPS* as well as in a group of 355 subjects with
13 BD and 86 unaffected control individuals. Individuals with BD experienced significantly
14 more childhood traumas than unaffected individuals (Mann-Whitney U test, $U=10,723$,
15 $p=0.00001$; Figure 6). Individuals with missense variants in *CADPS* reported also more
16 childhood traumas than unaffected controls (Mann-Whitney U test, $U=23.5$, $p=0.009$;
17 Figure 6). Interestingly, their median score to the childhood trauma questionnaire
18 ($\text{median}_{\text{CTQ_CADPS}}=49$) was higher than the one of the general population of individuals
19 with BD ($\text{median}_{\text{CTQ_BD}}=39$, Mann-Whitney U test, $U=266.5$, $p=0.14$), whereas no
20 significant difference was observed when comparing unmutated subjects of the
21 families with controls (Mann-Whitney U test, $U=76$, $p=0.06$) or the general population
22 of individuals with BD (Mann-Whitney U test, $U=626$, $p=0.69$; Figure 6).

23

24 **Discussion**

1 In the present study, we accumulated evidence that genetic variants in *CADPS* may
2 increase the vulnerability to BD. We first reported that common polymorphisms in
3 *CADPS* were more frequent in individuals with BD than in control population and
4 demonstrated that the difference was even greater when only patients with an early
5 age at onset were considered. This association was strengthened by difference in
6 allele frequencies observed in large populations of individuals with BD and controls
7 from the PGC.⁹ It was also consistent with previous genetic studies, which reported a
8 genetic linkage between BD and the 3p14 region, mainly with early-onset BD.³⁻⁵ Early-
9 onset BD has long been demonstrated to correspond to a homogeneous subgroup of
10 patients with a higher genetic component than later forms.⁴⁵⁻⁵⁰ Early-onset BD shares
11 a similar aetiology with late-onset BD with a small effect for common polymorphisms
12 and larger effects for very rare variants.⁵¹ By screening for rare variants in individuals
13 with early-onset BD, we observed that the missense variant frequency was 3 times
14 higher than in control populations, in contrast to synonymous variants for which no
15 significant difference was observed. This difference between synonymous and non-
16 synonymous substitution rates in patients and controls suggest that most of the rare
17 missense variants may have functional consequences subjected to purifying selection.
18 All missense variants identified in individuals with BD were predicted to alter protein
19 function (Supplementary Table 1). In addition, we have identified the deletion of a full
20 exon in one patient with BD with a strong impact on the protein sequence. Due to the
21 limited sensitivity of DNA chips, we may have overlooked the copy number variant
22 detection in affected and unaffected individuals. However, such a variation looks rare
23 since no similar deletion has been reported in public databases. Further experiments
24 confirmed for all but two variants (p.R959L and p.N205K) functional alteration of
25 *CADPS*. Although *in vitro* experiments showed that the *CADPS* mutations identified in

1 individuals with BD did not appear to affect the protein activity, cellular studies showed
2 that these mutations may affect either the amount of the protein (p.R195L and
3 p.S399L), the Ca²⁺-dependent exocytosis (p.I148_E185del and p.N1017I) or the ability
4 of CADPS to promote monoamine uptake (p.R195L and p.L482I). Results on
5 monoamine uptake and cellular secretion assays suggest that different domains of
6 CADPS may regulate distinct functions of the protein, as previously shown for both
7 CADPS and CADPS2.^{26, 36-38} Interestingly, the exon 2 deletion (p.I148_E185del)
8 showed a higher vesicular release probability and a higher mEPSC frequency than
9 wild type CADPS. This result is consistent with multiple studies on human induced
10 pluripotent stem cells-derived neurons, which showed that BD neurons are more
11 spontaneously active than control neurons.^{15, 52, 53} In addition, this mutation showed a
12 stronger depression of EPSC responses when trains of action potentials were applied,
13 suggesting that this mutation may result in an alteration of synaptic plasticity, which
14 has widely been reported to play a key role in BD pathophysiology and therapeutics.⁵⁴
15 More generally, our results suggest that abnormalities in Ca²⁺-dependent exocytosis
16 may increase the risk of developing an early-onset form of BD and thereby
17 corroborates the previous report of an association between *SNAP25*, a direct binding
18 partner of CADPS,²⁷ and early-onset BD.¹⁴ Numerous genetic studies have reported
19 calcium signalling as the most associated pathway with BD,^{9, 12, 16} which may have an
20 impact on the age at onset of the disease.⁵⁵ In addition, calcium signalling is among
21 the most affected pathways in cellular models of BD.⁵⁶
22 CADPS has been shown to trigger synaptic and large dense-core vesicle exocytosis.^{19,}
23 ^{21, 43, 57} All affected individuals with missense variants in our study were heterozygous
24 for *CADPS* mutations, which was not surprising since homozygous mutant mice for
25 *Cadps* die at birth.²¹ Interestingly, two mutations showed a significant lower protein

1 level in transfected cells. Adult heterozygous mutant mice for *Cadps* showed a
2 significant reduction of CADPS and have a reduction of the readily releasable pool and
3 a reduction of catecholamine secretion in neuroendocrine cells.²¹ Variations in
4 catecholamine and monoamines have been widely documented in individuals with BD
5 and are the target of many antidepressant and antipsychotic treatments.⁵⁸ This mouse
6 model showed both behaviours and biomarkers that bear resemblance to BD. In
7 addition, lithium reversed their manic-like behaviours. Interestingly, mutant mice for
8 CADPS resulted in over-reactivity to acute stress when animals experienced chronic
9 mild stress. This model perfectly matches with a two-hits model that has been
10 proposed for psychoses,⁵⁹ in which an early stress interacts with genetic factors to
11 increase the vulnerability to BD. Then further stressors in adolescence or early
12 adulthood trigger the disease in vulnerable subjects. This hypothesis is supported by
13 the observation that individuals carrying mutation in *CADPS* reported more childhood
14 trauma than other individuals with BD. Interestingly, the scores observed for mutated
15 individuals in these families were higher than those observed for unmutated ones.
16 Although, there is no doubt that childhood traumas are more frequent in families with
17 *CADPS* mutations, this difference suggests either that mutated individuals
18 experienced more childhood trauma than unmutated ones or that they were more
19 sensitive to trauma and thus scored higher to the questionnaire. Therefore, a functional
20 mutation in *CADPS* may affect the HPA axis, increasing the reactivity to stressful
21 events. This gene-environment interaction may thus result in changes in more central
22 physiological processes, as reflected by changes in BDNF level in our mouse model,
23 increasing the sensitivity to a later stressor that would trigger the disease. Although a
24 gender effect has been reported to interact with childhood trauma,^{60, 61} our sample was

1 too small to consider this parameter in our study and such analyses would need further
2 investigations in larger sample.

3

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

8

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19

20 **Author contributions**

21 S.J. designed the study, with the help of J.R. and D.N. for electrophysiological
22 experiments, C.K. and M.N.B. for mouse behavioural analyses and T.F.J.M for CADPS

1 *in vitro* analysis; S.J., J.S., C.K., A.N., A.H and E.C. generated genetic data and
2 conducted genetic analyses; J.S., N.P., A.H., V.L., R.T., S.M. and T.F.J.M. performed
3 biochemical experiments; C.K., G.G. and M.N.B. conducted behavioural analyses in
4 mice; D.N. and J.R. performed electrophysiological experiments on hippocampal
5 mouse neurons; C.H., B.E. and M.L. designed, collected and analysed clinical data,
6 with the help of C.B. and P.L.C. who collected biological samples in patients and
7 controls; S.J., J.S., D.N., C.K., B.E., M.N.B and T.F.J.M. wrote the article.

8

9 **Conflict of interest**

10 The authors declare no competing interests.

11

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16 554.
- 17
18

1 **Figure legends**

2 **Figure 1. Genetic exploration of *CADPS* in patients with BD.** (a) Manhattan plot
3 showing results of association studies conducted with 176 ht-SNPs spanning *CADPS*
4 on 456 patients with BD (blue) or 203 patients with early-onset BD (red) and 1,636
5 control individuals. Diamonds represent the most significantly associated SNP. The x
6 axis is the gene position according to NM_003716.3 and the y axis is the $-\log_{10}P$ -
7 value. The missense variants identified in individuals with an early-onset BD,
8 unaffected controls or both are shown in red, blue and purple, respectively (b) Family
9 pedigrees of early-onset BD patients with missense variants in *CADPS*. Affected
10 individuals are shown in black, with filled symbol for BD, half-filled symbol for unipolar
11 depression and quarter-filled symbol for hyperthymia. Individuals for whom DNA was
12 available are shown with a red border. (c) Comparison of disease probability in
13 mutated and unmutated individuals in the 6 families in which rare variants were
14 identified. See also Figure S1.

15

16 **Figure 2. Characterization of missense variants identified in *CADPS*.** (a) Protein
17 location of missense variants identified in individuals with an early-onset BD (red),
18 unaffected controls (blue), or both (purple). (DBD) dynactin binding domain; (C2) C2
19 domain; (PH) pleckstrin homology domain; (MHD) munc homology domain; (DCVB)
20 dense core vesicle binding domain. (b) Protein expression level of wild type and
21 mutant isoforms of *CADPS*. Protein level has been evaluated by Western-blot assay
22 in transiently transfected COS cells using antibody directed against *CADPS* and
23 TUBA. (c) Two under-expressed mutant isoforms of *CADPS* containing either the
24 p.R195L or the p.S399L mutations were evaluated for protein stability. Transfected
25 COS cells were treated with 100 μ g/ml cycloheximide (CHX) over a 24 hours time

1 period. Cell lysates were subjected to Western blot analysis with anti-CADPS and
2 quantified before to be plotted on graph. The value of untreated cells was set as 1.
3 The p.S399L mutation significantly decreases the half-life of the protein by a factor 3.
4 All data are presented as mean \pm s.e.m. ** $p < 0.01$; **** $p < 0.0001$. WT, wild type. See
5 also Figure S2.

6
7 **Figure 3. Mutations in CADPS decreased the FFN511 release and monoamine**
8 **uptake.** PC12 cells were electroporated with vector containing CADPS mutations. In
9 the evoked-exocytosis Krebs-Ringer buffer containing 90mM KCl, release of
10 FFN511 after 1min is higher for cells containing CADPS than the mock, as seen in
11 cells by microscopy (a) or by FACS (b). (c) Release of FFN511 in PC12 cells has
12 been compared wild type and mutated CADPS. Exocytosis of FFN511 was
13 significantly decreased for p.I148_E185del, p.N1017I and the double mutant
14 p.L482I_N1017I, indicating that these mutations altered the released of vesicular
15 monoamines. (d) VMAT1 expressing CHO cells were transfected either with wild type
16 or mutant CADPS and serotonin transport with VMAT1 was compared. Serotonin
17 uptake occurred for 10min a 37°C using a solution of potassium-glutamate-ATP
18 containing 40nM[H3]serotonin and 100 μ M of no-labeled serotonin. Serotonin uptake
19 was significantly reduced for p.R195L and p.L482I mutants, suggesting these
20 mutations altered the CADPS ability to promote monoamine uptake. All data are
21 presented as mean \pm s.e.m. ** $p < 0.01$; *** $p < 0.001$. See also Figure S3 and
22 supplementary Table 1.

23
24 **Figure 4. Cultured neurons expressing CADPS_{p.I148_E185del} or CADPS_{p.S399L}**
25 **exhibit altered short-term plasticity characteristics. (a, j) Sample traces of action**

1 potential (AP)-evoked excitatory postsynaptic currents (EPSCs) (i) spontaneously
2 occurring miniature EPSCs (mEPSCs) (ii) and sucrose-evoked EPSCs (iii) recorded
3 in cultured CADPS-CADPS2 double knock-out hippocampal neurons (DKO), which
4 expressed indicated CADPS cDNA. **(b, k)** Lentiviral expression of the
5 p.I148_E185del (blue) and the p.S399L mutation (red) rescued the AP-triggered
6 EPSCs amplitude. No difference has been observed neither in the readily releasable
7 pool (RRP) charge measured in presence of 0.5M sucrose solution **(c, l)**, nor in the
8 median amplitude of spontaneously occurring mEPSCs **(e, n)** for none of the
9 mutants. An increased vesicular release probability **(d)** and an increased frequency
10 of spontaneously occurring mEPSCs **(f)** has been observed for p.I148_E185del but
11 not for p.S399L **(m, o)**. **(g, p)** Averaged EPSC responses during a 40 Hz AP train. **(h,**
12 **i, q, r)** Paired-pulse ratio during trains of APs at indicated frequencies was decreased
13 in p.I148_E185del **(h)** but increased in p.S399L **(q)**. No difference was observed for
14 steady-state EPSC responses for none of the mutants **(i, r)**. Bars in plots depict
15 median and 5-95 percentile. N = 6 cultures for each of the two comparisons. *
16 $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. See also Figure S4.

17

18 **Figure 5. Heterozygous mutant mice for CADPS display manic-like behaviours**

19 **and a higher sensitivity to stress.** *Cadps*^{+/-} mice showed an increased locomotor
20 activity in the open field **(a)**, in home cage **(b)**, and during the forced swim test **(c)**,
21 and a tendency has been observed in the tail suspension test **(d)**. *Cadps*^{+/-} mice
22 showed a lower corticosterone level in basal condition **(e)**, but are hypersensitive to
23 chronic **(f)** and acute stress **(g)**. Animals unexposed (C) and exposed (S) to
24 unpredictable chronic mild stress are indicated in black and red, respectively.
25 Unstressed *Cadps*^{+/-} mice had a lower hippocampal BDNF level **(h)**. Unpredictable

1 chronic mild stress (red) increased the BDNF level only in *Cadps*^{+/-} mice (i), for which
2 the hippocampal BDNF level was correlated with the plasma level of corticosterone
3 (j). (k) Basal locomotor hyperactivity of *Cadps*^{+/-} mice was rescued by lithium (Li)
4 when compared with non-treated *Cadps*^{+/-} animals (Con.). Data in (a) are presented
5 as mean ± s.e.m. Bars represent median (b-k) * p<0.05; ** p<0.01; *** p<0.001. WT,
6 wild type. See also Figure S5.

7

8 **Figure 6. Assessment of childhood trauma in families with mutations in**
9 **CADPS, 355 independent patients with BD and 86 unaffected controls.** Patients
10 with BD experienced more childhood trauma than unaffected controls. Patients with
11 mutation in *CADPS* experienced more childhood trauma than both unaffected
12 controls and the general population of patients with BD. In families with *CADPS*
13 mutations, mutated subjects have a higher score than unmutated subjects. Data are
14 presented as median ± interquartile range. ** p<0.01; **** p<0.0001.

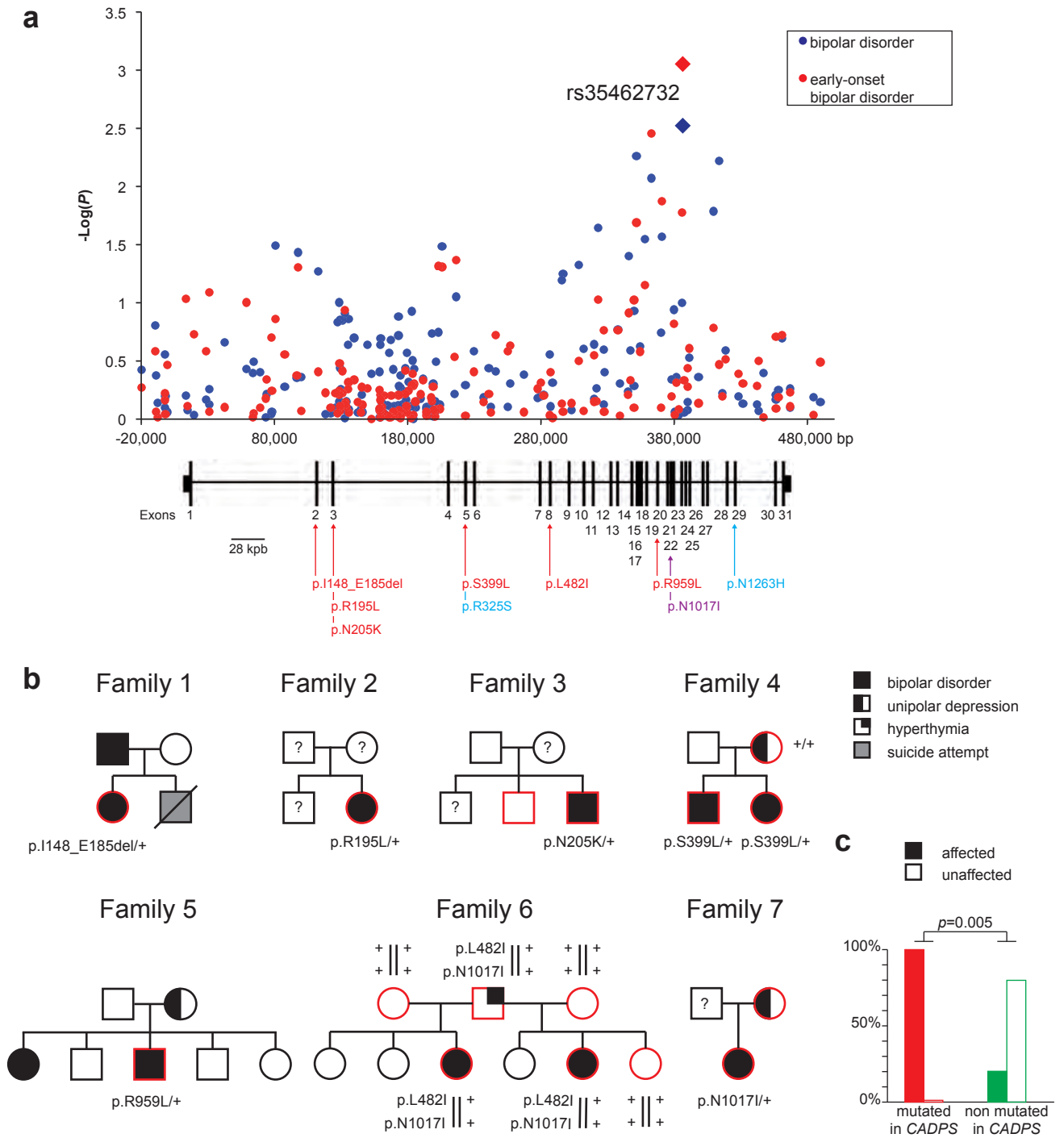


Figure 1.

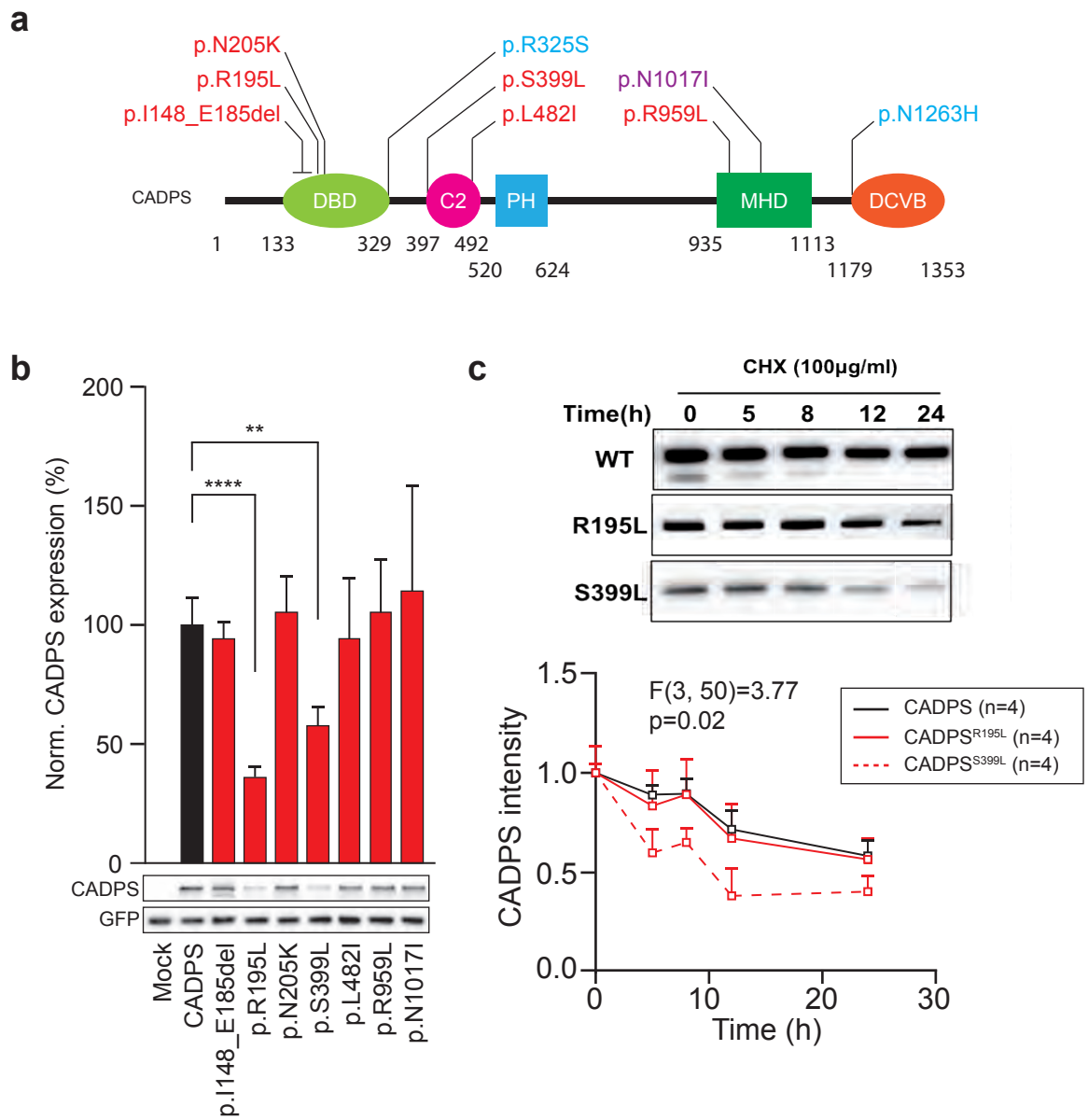


Figure 2.

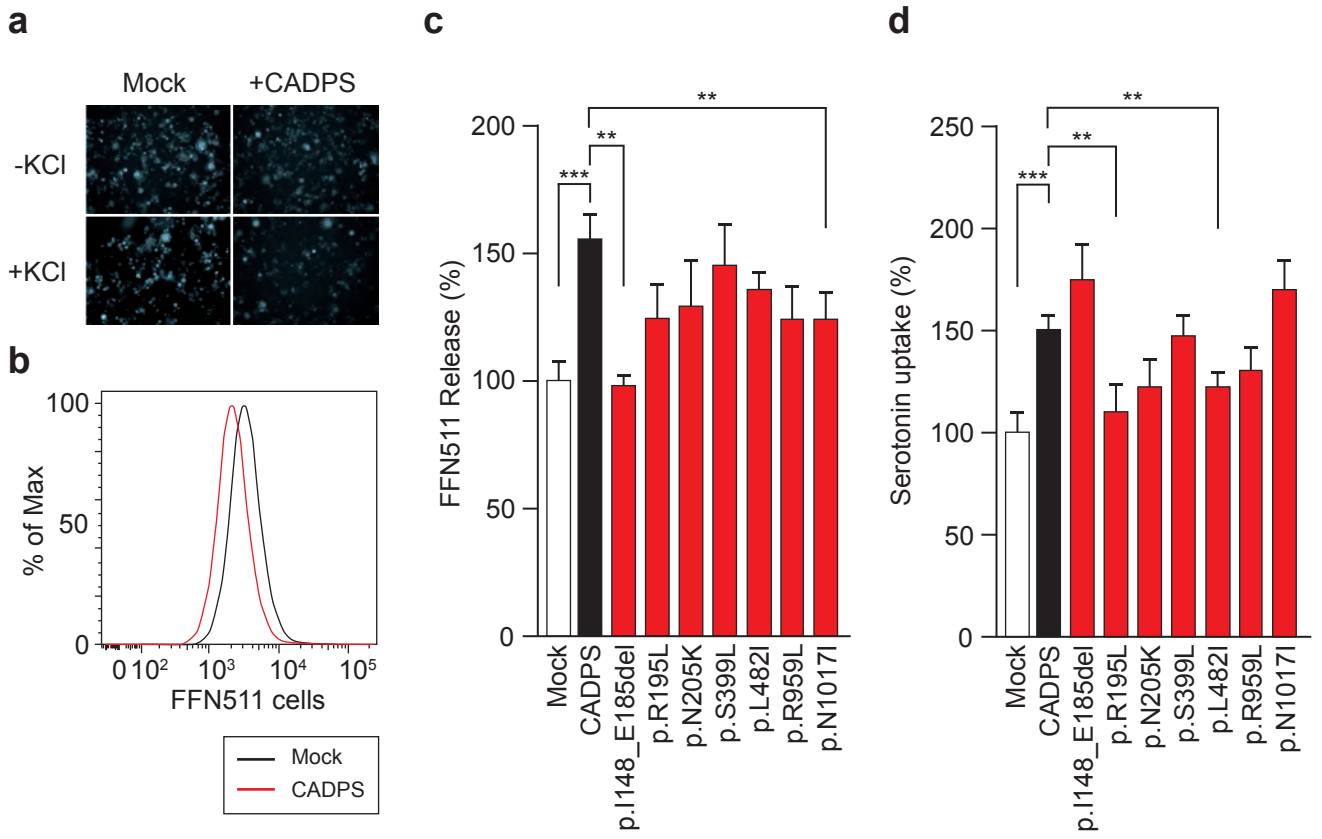


Figure 3.

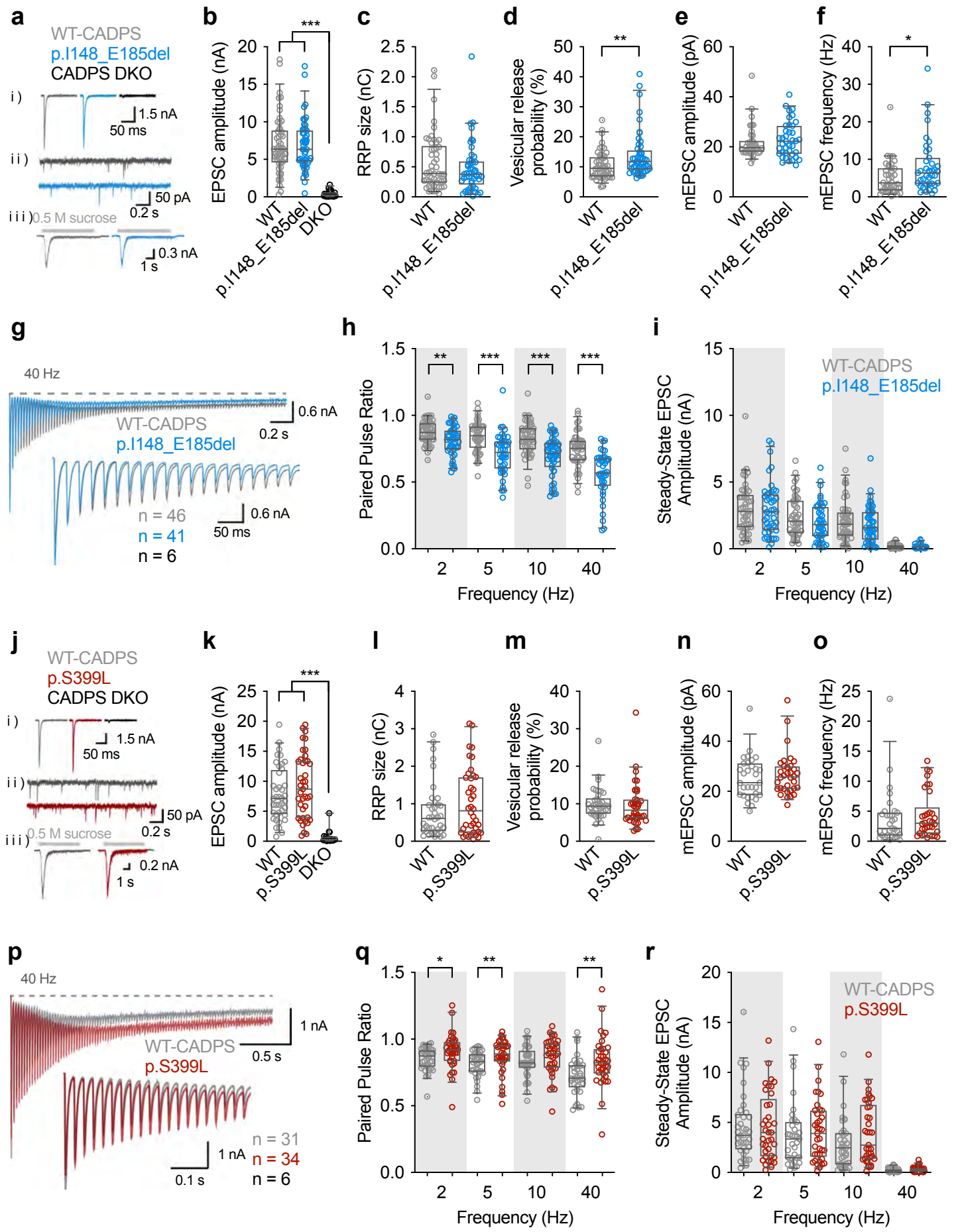


Figure 4.

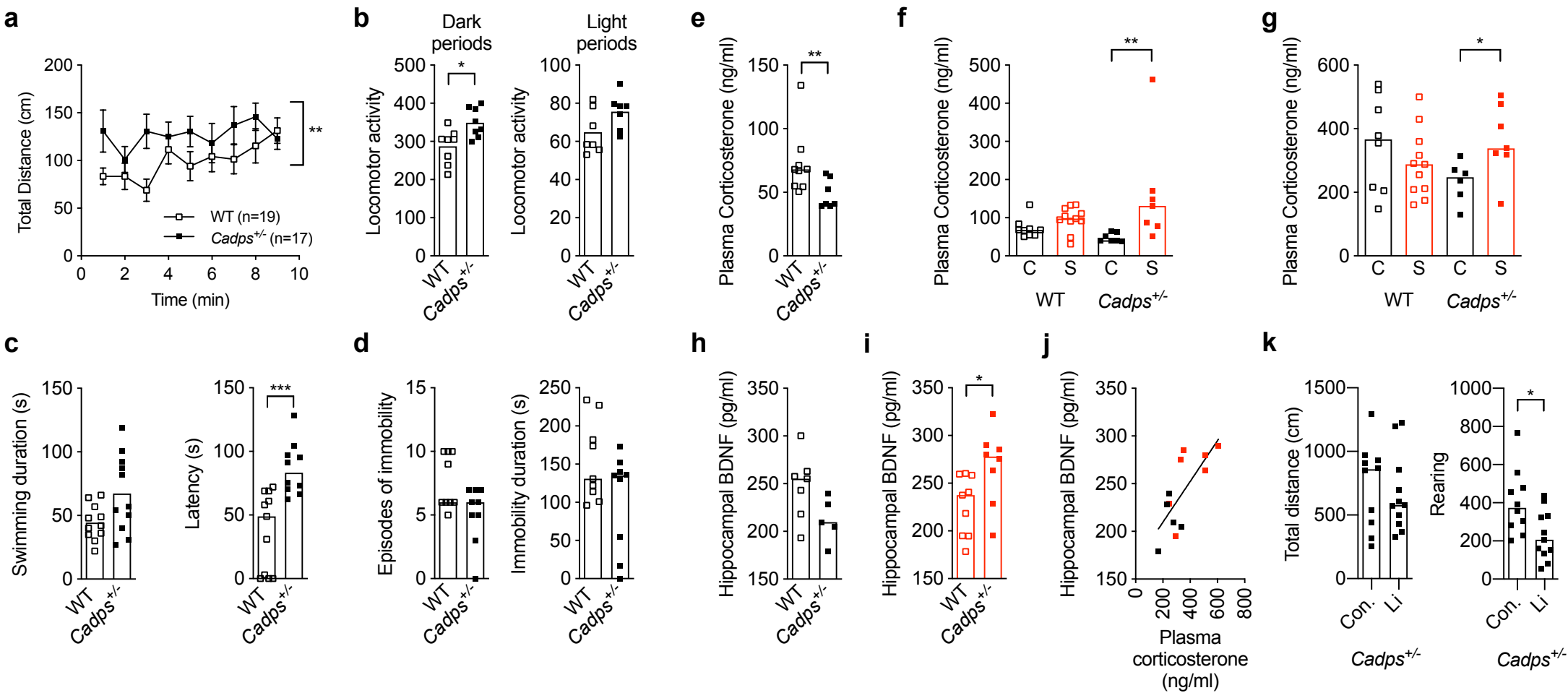


Figure 5.

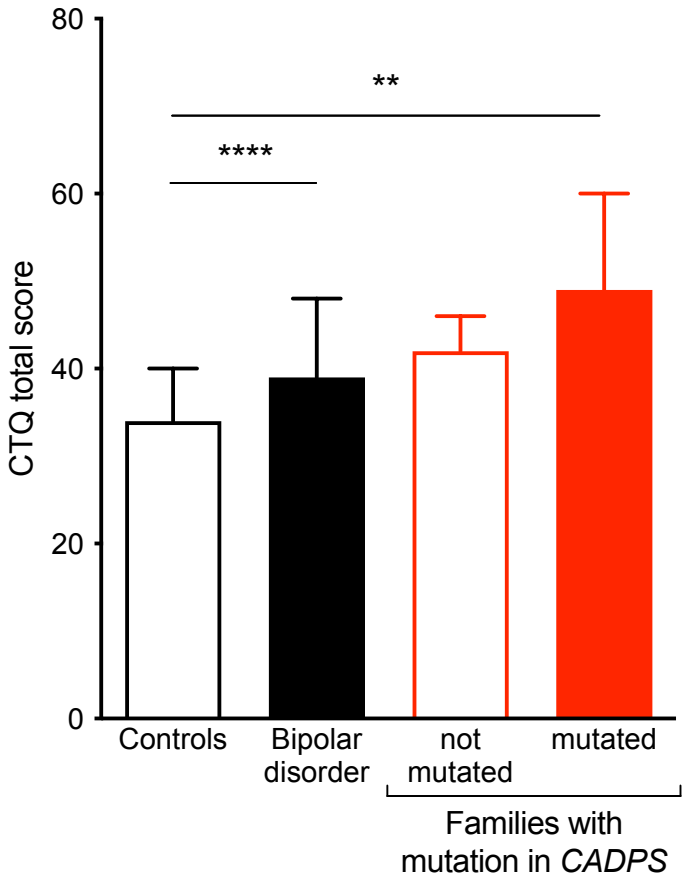


Figure 6.

Supplementary information for

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

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Supplementary Materials and Methods

Supplementary Figures 1 to 5

Supplementary Materials and Methods

Subjects

For sequencing analyses, 132 individuals (56 males and 76 females) with early-onset BD (mean age 37) and 184 individuals (105 males and 79 females) with no personal history of psychiatric disorder or suicidal behaviour (mean age 43) were recruited into two French university-affiliated psychiatry departments (Paris-Créteil and Bordeaux) and in the Inserm Centre d'Investigation Clinique 1430 (Créteil, France), respectively. Patients were interviewed by a trained psychiatrist using the *diagnostic interview for genetic studies* (DIGS)¹ and included according to the DSM-IV² criteria for BD type I or II with an age at onset lower than 22. The age at onset was defined as the age of first thymic episodes (depressive, manic or hypomanic) and determined by reviewing medical case notes and information from semi-structured interviews. The threshold for early-onset BD was defined as 22, based on previous admixture analyses.³ The childhood trauma questionnaire (CTQ) was fulfilled by 355 (147 males and 208 females) affected and 86 unaffected individuals (40 males and 46 females) of whom 257 patients and 48 controls had available DNA and were included in genetic analyses.

Human genotyping and copy-number variation detection

DNA was isolated from lymphocytes either directly from venous blood sample or after transformation by Epstein Barr virus as previously described.⁴ DNA from individuals with BD was genotyped using HumanHap550 BeadArrays (Illumina, San Diego, CA, U.S.A.) at the *Centre National de Génotypage* (CEA, Evry, France) and imputed according to the CEU population of the HapMap project.⁵ Case/control association analyses were performed using the PLINK software v1.07.⁶ Copy number variations were detected by comparison of the fluorescence level over the whole chromosome

and by loss of heterozygosity using the SniPeep software (R. Toro, Institut Pasteur, France). The exon 2 deletion of *CADPS* was confirmed by quantitative polymerase chain reaction (PCR) analysis using one internal and two external couples of primers and Mesa Green qPCR master mix plus (Eurogentec, Liege, Belgium). Each real-time PCR was performed in triplicate and assessed by comparing C_T at a determined threshold between the three amplicons and the three individuals.

Cloning and in vitro mutagenesis

Human cDNA of *CADPS* isoform 1 (NM_003716.3) has been obtained from clone hh10147 (ORK04421) provided by the Kasuza DNA Research Institute (KDRI, Chiba, Japan) and subcloned into pcDNA3.1 plasmid (Life Technologies, Carlsbad, CA, U.S.A.) using the KpnI restriction enzyme. The internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV) and the gene encoding the enhanced green fluorescent protein (eGFP) were added to the vector downstream to *CADPS* using the EcoRI restriction enzyme. We then used QuickChange Site-Directed Mutagenesis kit (Agilent Technologies, Santa Clara, CA, U.S.A.) in order to introduce mutations identified in patients with BD by *in vitro* mutagenesis.

CADPS-mKate2-His₆ and *CADPS*-twinStrep constructs were generated by PCR amplifying *CADPS* from the constructs described above with forward and reverse primers, and subcloned into the pcDNA3.1 mKate2-His₆ vector⁷ or the *CADPS*-twin-Strep plasmid. All constructs were confirmed by Sanger sequencing.

Antibodies

Antibodies against *CADPS* (sc-136402, Santa Cruz Biotechnology, Dallas, TX, USA), the green fluorescent protein (GFP, A6455, Life Technologies) and against the α -

tubulin (TUBA, T5168, Sigma Aldrich) were used for protein quantification by western blot analyses.

CADPS protein purification

HEK293FT cells were cultured in DMEM supplemented with 10% FBS and Geneticin. CADPS-mKate2-His₆ and CADPS-twinStrep wild type and mutant plasmids were transfected using a standard calcium phosphate protocol. At 2d post-transfection, cells were lysed into 20 mM HEPES, 300 mM NaCl, 5 mM DTT, 1% Triton X-100 and protease inhibitors. His-tag lysis buffer contained 5 mM imidazole. Cell debris was pelleted and CADPS-mKate2-His₆ supernatants were added to equilibrated Ni NTA beads (Qiagen, Hilden, Germany), washed, and eluted with 20 mM HEPES, 300 mM NaCl, 200 mM imidazole. CADPS-twinStrep supernatants were added to equilibrated Strep-actin resin, washed, and eluted with elution buffer (IBA GmbH). Eluates were buffer exchanged into 20 mM HEPES, 150 mM NaCl using Zeba Spin Desalting Columns (Thermo Fisher Scientific). Purified CADPS and mutant proteins were quantified by SDS-PAGE with BSA as standards. Gels were stained with SYPRO Ruby (Invitrogen), imaged by GE ImageQuant and quantified with FIJI (ImageJ; NIH, Bethesda, MD). Purified wild type CADPS proteins with either of two tags exhibited similar activity in the permeable cell assay in 60s incubations at 2.5-20nM concentrations (Supplementary Figure S2a).

Permeable cell secretion assay

PC12 cells (clone 2-6) were cultured as previously described.⁸ The cells were incubated overnight with 1.5 mM norepinephrine and 0.5 mM ascorbate, permeabilized by single pass through a ball homogenizer, washed extensively, and primed for

regulated secretion as previously described.⁹ Purified CADPS proteins were added to an electrochemical chamber with permeabilized cells in potassium glutamate buffer (0.02 M HEPES [pH 7.2], 0.12 M K glutamate, 0.02 M K acetate, 0.002 M EGTA). A rotating disk electrode was lowered into the chamber and CaCl₂ (to 10 μM Ca²⁺ free) was injected to stimulate norepinephrine secretion.⁹ CADPS mutants were paired with wild type controls.

Behavioural studies in mice

Except for circadian rhythms, experiments were conducted during the light phase of a 12h light/dark schedule (lights on at 7:30 a.m.) in a sound-attenuated room under controlled illumination. Baseline behavioural studies were performed on adult naïve *Cadps*^{+/-} heterozygous male mice and their wild type littermates. Sample size has been determined in order to have more than 80% chance to detect an effect size higher than 1.5 with an alpha error of 0.05. Observers were blinded to group allocation during data collection and analyses.

Motor activity and exploratory behaviour in an open field

The exploratory behaviour was individually recorded for 9 min in an open field (55 × 55 × 30 cm white polyvinyl chloride box) under homogeneous illumination (100 lux) and automatically scored (videotrack ViewPoint, France).

Circadian activity and food and water intake

Spontaneous locomotor activity and rears were measured using individual boxes equipped with infra-red captors. The quantity of water and food consumed during the

test period was measured using automated pellet feeder and lickometer (Imetronic, Pessac, France).

Mice were first tested under light/dark cycle (12/12h: light on at 7:00) for 1 week (188 h) including 8h habituation and 7 complete light/dark circadian cycles. Afterwards, they are returned to their home cages and placed in a constant darkness for 21 days. They were then tested again in constant darkness for one week (188 h).

Forced swimming test

The forced swimming test was adapted from Porsolt *et al.*¹⁰ Animals were forced to swim individually for 6 min in a vertical glass cylinder (height: 28 cm; diameter: 15 cm) containing 23.5 cm of water maintained at $25\pm 1^{\circ}\text{C}$. The latency before the first episode of immobility, the number of immobility episodes, total immobility duration and total activity duration were recorded by an observer blinded to the genotype. An animal was judged to be immobile whenever it remained floating in the water in a slightly hunched but upright posture, the head just above the water.

Tail suspension test

Mice were suspended by the tail with tape to a vertical aluminium bar for 6 min at 30 cm from the floor. The latency before the first episode of immobility and total immobility duration were recorded for each individual by an observer blinded to the genotype.

Acoustic startle reflex reactivity and pre-pulse inhibition

Acoustic startle reactivity and pre-pulse inhibition (PPI) of startle were assessed in a single session using standard startle chambers (SR-Lab Startle Response System, San Diego Instruments, USA). Ten different trial types were used: acoustic startle

pulse alone (110-dB), eight prepulse trials in which either 70, 75, 85 or 90-dB stimuli were presented alone or preceded the pulse, and finally one trial (NOSTIM) in which only the background noise (65 dB) was presented to measure the baseline movement in the Plexiglas cylinder. In the startle pulse or prepulse alone trials, the startle reactivity was analyzed and in the prepulse plus startle trials the amount of PPI was measured and expressed as percentage of the basal startle response.

Sucrose preference

On the first day, mice were habituated overnight to sucrose in their home cages, replacing water with 0.8 % sucrose solution. From the second to the 4th days, mice were individually transferred to the testing cages 2h before the light off with food available *ad libitum*. One hour later, two bottles of water and 0.8 % sucrose were provided for 15h. All the 3 days measurements were used for evaluation of sucrose preference.

Unpredictable Chronic Mild Stress

To test the effect of chronic stress on *Cadps*^{+/-} heterozygous mice, behavioural studies were performed on 6 months old *Cadps*^{+/-} heterozygous males and their wild type littermates placed under unpredictable chronic mild stress protocol (UCMS) for 4 months. The stressful environments included social isolation, overcrowded cage, tilted cage, empty cage, damp sawdust cage, addition of rat feces, restraint space, circadian disturbance (reversed light/dark cycle), period of continuous overnight illumination, overnight period of difficult access to food (without a reduction in the daily food ration). To be unpredictable to them mice, these conditions were selected randomly every day and applied for 1 to 12h in duration with 1 to 12h interval.

Acute stress and corticosterone level quantification

In order to quantify corticosterone secretion before and after acute stress, three blood samples were collected from mouse tails between 9:00 and 11:00 a.m. using a microcuvette capillary tube. Twenty-four hours before the experiments, *Cadps*^{+/-} and wild type males submitted or not to unpredictable chronic mild stress (UCMS) were moved from the vivarium to a temperature and light controlled behavioural testing room for acclimation. Before acute stress, tail clipping was used for basal blood collection in freely walking mice to minimize stressful environment in less than 2 min to avoid corticosterone secretion due to stress. For acute stress blood collection, mice were then immediately restrained in a 50-ml plastic tube with sufficient ventilation for 30 min before a second blood sample collection. Plasmas were isolated by centrifugation and stored at -80°C. Plasma corticosterone concentrations were determined in triplicate using a 1:100 dilution and a Corticosterone ELISA kit (Abnova, Taipei City, Taiwan) according to manufacturer's instructions.

Hippocampal BDNF level

After acute stress, brains were rapidly extracted from mice. Bilateral hippocampi were dissected, immediately frozen on dry ice and stored at -80°C. At the time of analysis, 400µl of lysis buffer (100mM PIPES, 500mM NaCl, 0.2% Triton X-100, 2%BSA, and 2mM EDTA) containing freshly prepared protease inhibitors (200µM PMSF, 0.3µM aprotinin, and 10µM leupeptin) were added to each sample.¹¹ After sonication, homogenates were centrifuged at 16,000g for 30 min at 4°C and supernatants were removed. Twofold dilutions were used to determine BDNF concentrations in duplicate

using a BDNF E_{max}® ImmunoAssay system (Promega, Madison, WI, USA), according to manufacturer's instructions.

Lithium treatment

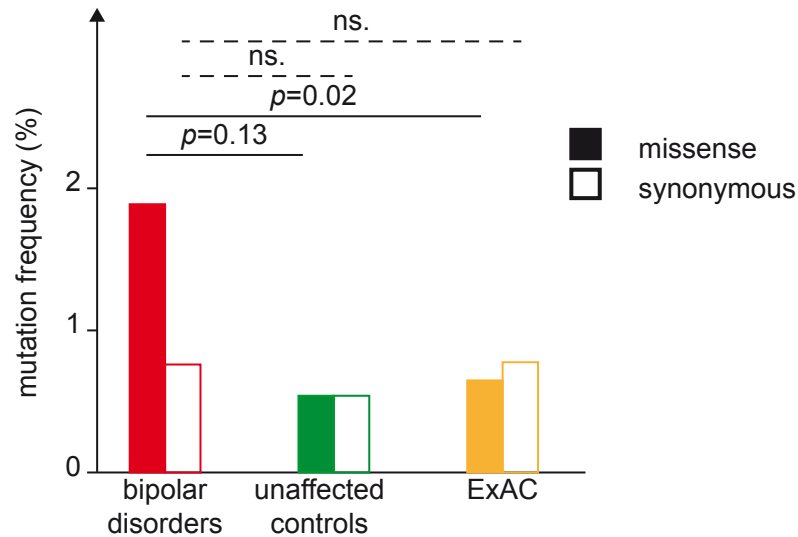
Mice were fed either with lithium carbonate-containing chow or control chow (Genobios, Laval, France) for three weeks. The lithium group was fed with 0.2% lithium carbonate chow for a week followed by two weeks with 0.4% lithium carbonate chow before starting the behavioural assays. A saline solution (9g/L NaCl in water) was provided in addition to normal water for both treated and untreated animals to counterbalance the lithium toxicity.

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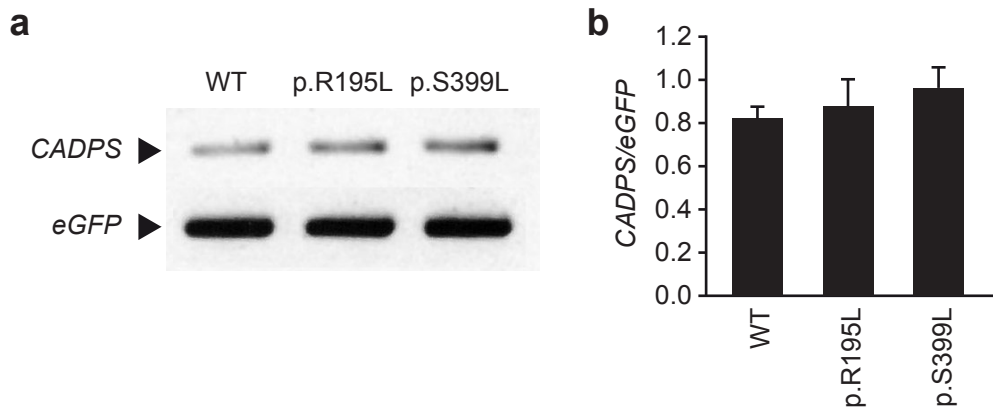
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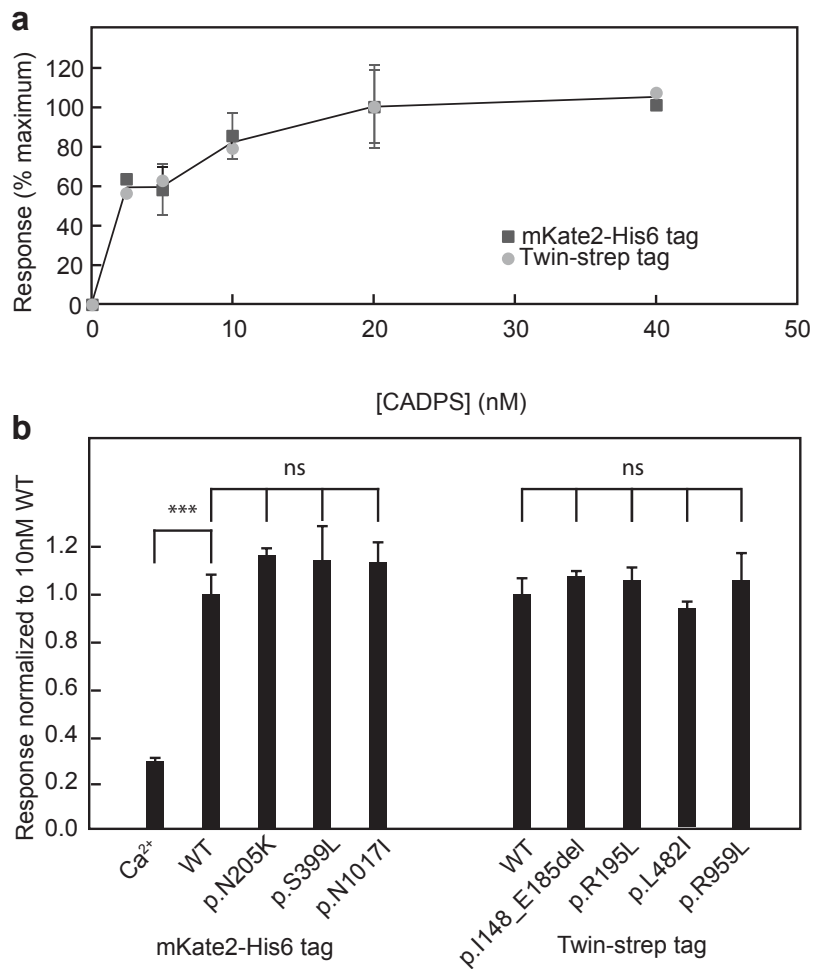
Supplementary Figures



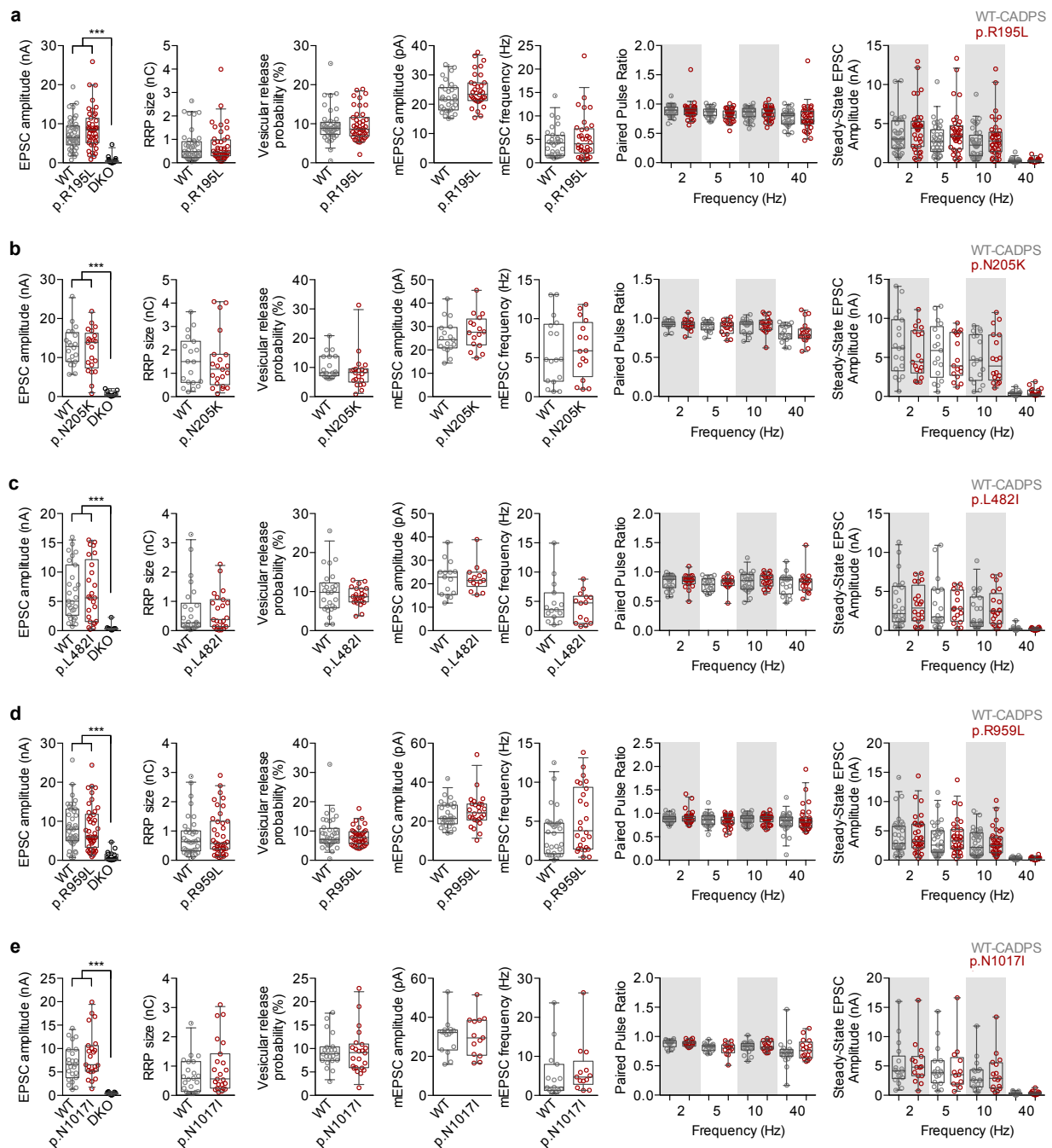
Supplementary Figure S1. Rare variant frequency in 132 individuals with early-onset BD (red), 184 unaffected controls (green) and 21,070 subjects from the Exome Aggregation Consortium (ExAC) (yellow) (<http://exac.broadinstitute.org>). Only rare variants with allele frequency lower than 1‰ in the ExAC database were considered. Missense variations (filled bars) were 3 times higher in patients with early-onset BD than in unaffected individuals (Fisher's exact test, OR=3.53, 95%CI[0.57;37.32], $p=0.13$) or when compared to the non-psychiatric and non-Finnish European population from ExAC (Fisher's exact test, OR=3.38, 95%CI[1.08;8.11], $p=0.02$). No significant difference was observed for synonymous substitutions (opened bars) between the three populations.



Supplementary Figure S2. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of the wild type (WT) and two mutant forms of CADPS containing p.R195L and p.S399L missense variants. (a) RT-PCR of WT and mutant isoforms of *CADPS*. For each reaction, total RNA was extracted from COS-7 cells transfected with pcDNA3.1 plasmid containing *eGFP* as well as either the WT or mutant isoforms of *CADPS*. After extraction, total RNA has been reverse-transcribed and amplified using specific primers flanking either *CADPS* or *eGFP*. **(b)** Relative quantification of WT and mutant *CADPS* isoforms using *eGFP* to control transfection and transcription efficacies. All data are presented as median \pm interquartile range (n = 2).

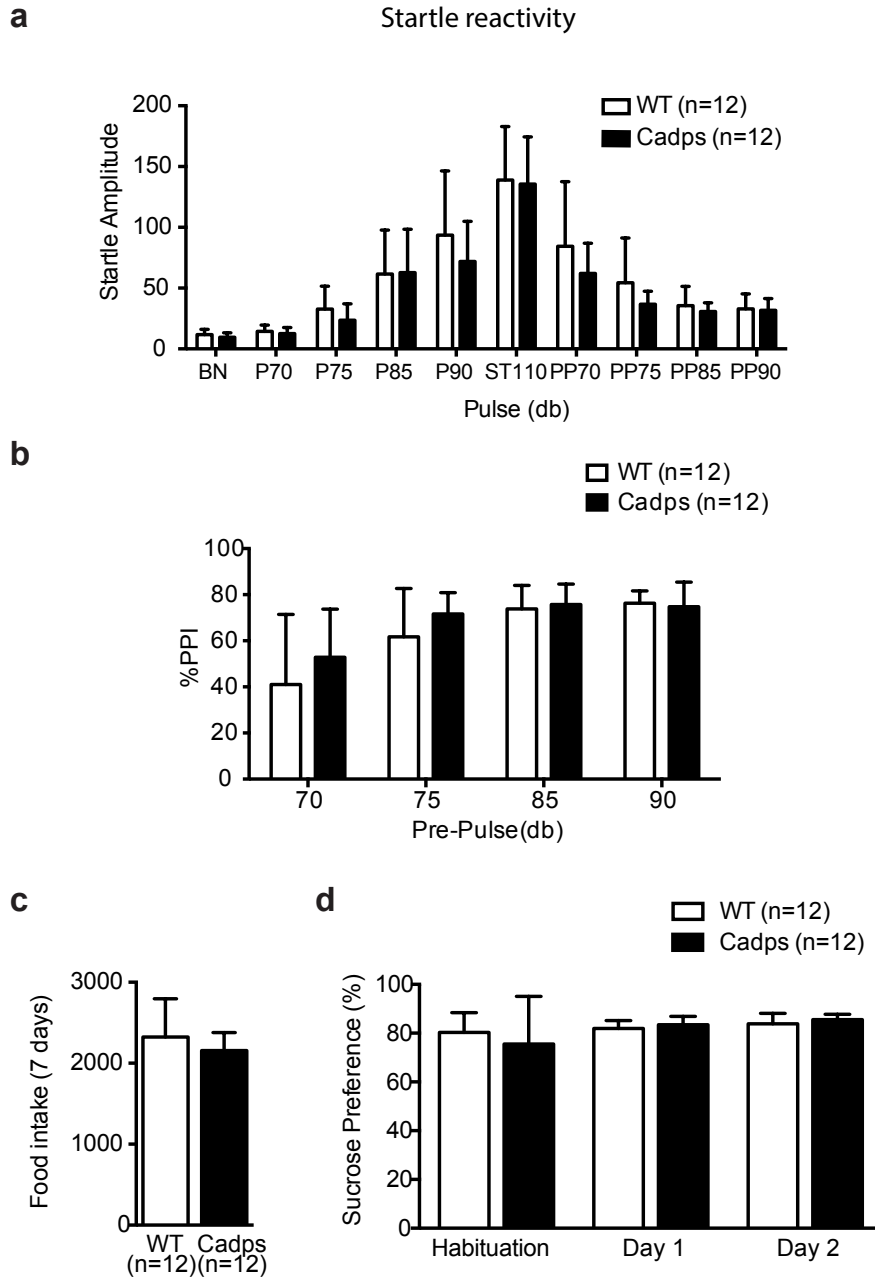


Supplementary Figure S3. Activity of wild type and variant CADPS proteins in permeable cell secretion. Indicated CADPS proteins were purified and tested in a permeable PC12 cell secretion assay. **(a)** Wild type tagged CADPS proteins were tested at the indicated final concentrations. **(b)** The indicated wild type (WT) and variant CADPS proteins were purified and tested at 10 nM. All data are presented as mean \pm s.e.m. ($n = 3-7$); ns = not significant; *** $p < 0.001$.



Supplementary Figure S4. Physiological exploration of five mutations in CADPS.

Lentiviral expression of the p.R195L (a), p.N205K (b), p.L482I (c), p.R959L (d) and p.N1017I (e) mutations rescue sucrose-evoked EPSCs amplitude in autaptic hippocampal CADPS DKO neurons, and do not impact neither the size of the readily releasable pool (RRP), the vesicular release probability, the mEPSC amplitude or frequency, the paired-pulse ratio nor the steady-state EPSC amplitude. All data are presented as mean \pm s.e.m.



Supplementary Figure S5. Behavioural analysis of *Cadps*^{+/-} mice. *Cadps*^{+/-} mice showed no difference for auditory startle reflex reactivity (a), pre-pulse inhibition (b), food intake (c) and sucrose preference (d), when compared with wild type littermates. All data are presented as mean ± s.e.m.