Supplementary Information for

Modelling the polygenicity and clinical heterogeneity of human depression in mice to identify biomarkers predictive of treatment response

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SUPPLEMENTARY MATERIAL AND METHODS

Animals

Mice were selectively bred in animal facilities (Faculté de Médecine et de Pharmacie, Lyon 1 University, France) for high or low spontaneous mobility in the FST. The mice used as founders had a broad genetic background resulting from the eight-way cross of A/J, ABP/Le, BALB/cJ, C57BL/6J, C3H/HeJ, CBA/H, DBA/2J, and SWXL-4 mouse strains. These mice were kept on a 7 a.m.-7 p.m. light cycle with food and water ad libitum. If not indicated otherwise, behavioral assays were conducted on 10 to 14-week-old mice from generations eight to twenty. Testing was performed between 9 a.m. and 5 p.m..

Selection and breeding

The bidirectional selective breeding was initiated with a starting population of 992 animals (456 males and 436 females) born of 36 breeding pairs. These mice were then selected for helplessness in the TST and the FST. The selection of H-TST and NH-TST mice has previously been described [1]. Helplessness was estimated by counting bouts of immobility in a cylinder containing water for FST lines (see below). As for the TST line, we selected the two extreme 10% of all tested mice in a single FST. For the first generation, H-FST were then defined with an immobility time higher than 308 s for males and 310 s for females. Conversely, mice were included in the NH-FST group when their immobility time was lower than 196 s for males and 213 s for females. For the twenty following generations, males and females with the 10% most extreme phenotypes at each generation were then mated together for H-FST on one side, and NH-FST on the other side. Pups were weaned at 21±2 days, and animals were tested in the FST at age 42-50 days.

Behavioral studies

Forced Swim Test

Mice between 7 and 8 weeks of age were plunged individually into a vertical Plexiglas cylinder (25 cm high; 10 cm in diameter) filled with 9 cm deep water (21-23°C) for six minutes. Immobility (i.e., making only minimal movements to keep the head above water or floating) was measured by an observer blind to the condition of the mouse.

Tail Suspension Test

The TST was performed with a computerized device. Mice were suspended by the tail with adhesive tape to a hook connected to a strain gauge. This gauge transmitted movements to a computer calculating total duration of immobility during a 6-min test. Mice that climbed up their tail during the test session were excluded from the analysis.

Automated locomotor activity test

Exploratory motor activity was monitored in a 50 x 50 cm open-field arena for 45 minutes. Mice, aged 12 weeks, were placed for the test in a dimly lit, silent room. A video camera was placed in the ceiling overlooking the testing area and monitoring four open field cages. The test was performed using a video image processor (Videotrack, View Point, Lyon, France). The total distance travelled by the mice was recorded.

Sucrose preference test

Hedonic behavior was assessed using the two-bottle sucrose preference test [2]. The mice were 14 to 16 weeks old. Consumption of a 2% sucrose solution in one bottle and of water in a second bottle was measured (over a 3-day period) after a 6-day period of habituation with

switches between water and sucrose solution as only available fluid. Results are expressed as the percentage of sucrose solution consumed out of the total fluid drunk.

Light/dark box

Anxiety was first assessed using the light/dark box test [2]. This test is based on the innate aversion of rodents to brightly illuminated areas. The light/dark box have two compartments (20 cm large x 20 cm long x 30 cm high) connected by a 6 cm wide by 6 cm height opening located centrally at floor level. The time spent in the brightly 200 lux-lit white (anxiogenic) compartment was recorded during the 5-min testing session using the video- tracking device (Viewpoint).

Elevated Plus Maze

Anxiety was also assessed in the elevated plus maze. This maze is a plastic Greek cross placed 50 cm above the floor. The arms were 40 cm long and 6 cm wide. Two opposite arms are surrounded by walls (15 cm high, closed arms), while the two other arms are devoid of such walls (open arms). The mice, aged 13 weeks, were placed for the test in a dimly lit and quiet room. The time spent in the open (anxiogenic) arms over a 5-min period was recorded by the video-tracking device (Viewpoint).

Sleep and Wakefulness Analysis

Electrodes for polygraphy sleep monitoring were implanted under general anesthesia as described in detail elsewhere [1, 3]. After a one-week recovery period, female mice (10 to 12-week-old) were placed in a Plexiglas barrel (20 cm in diameter) containing wood chips, food and water ad libitum, in a recording chamber for seven days. They were connected to a cable plugged in a rotating connector (Bilaney, Plastics One, Germany) to allow free movements.

Unipolar Electroencephalography (EEG) and bipolar electromyography (EMG) signals were amplified (MCP+, Alpha-Omega Engineering, Israel), digitized at 250 Hz, collected with an interface combining the Cambridge Electronic Design (CED) 1401 unit and Spike2 software to analyze off-line using custom scripts (Cambridge Electronic Design Limited, Cambridge, UK). Vigilance states were scored using a 5 s window frame according to standard criteria. After three days of habituation to their cable and new environment, polysomnographic recordings were performed for four days. The analyses were performed using the average of the data obtained after scoring the last two consecutive 24-hour periods.

Drug administration

Solutions were prepared daily. Doses always refer to the free bases. Acute treatments were performed on adult mice (10 to 14-week-old). They received an acute intraperitoneal injection with saline, 20 mg/kg fluoxetine HCl (Interchim, Montluçon, France) or 5 mg/kg escitalopram (sigma, France) or 3 mg/kg LY341495 (Bio-Techne, Mineapolis, MN, U.S.A.). All mice were tested in the TST 30 minutes after injection. Chronic treatments were performed in three-month-old mice treated daily, at 10 a.m., with fluoxetine HCl (10 mg/kg i.p.) or saline for three weeks. On day 22, H-FST or H-TST mice were tested (between 1 and 4 p.m.) in the TST.

RNA extraction and gene expression quantification

PFC samples were defined as sagittal brain sections of 2 mm of thickness in the frontal cortical region directly posterior to the olfactory bulb. Both left and right sides of the PFC have been collected. FastPrep® homogenizers pulverized PFC through simultaneous beating of specialized Lysing Matrix beads (type D). RNA was isolated on silica-based columns, DNase I digested and eluted with water using RNeasy Mini Kit following the manufacturer's instructions (Qiagen, Hilden, Germany). RNA qualities were assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, U.S.A.). Total RNA was quantified using DO 260 nm

on a NanoDrop 1000 spectrophotometer (ThermoScientific, Baltimore, MA, USA). Total RNA (50 ng) was amplified and labeled with Cyanine-3 in a round of *in vitro* transcription with Low Input Quick Amp Labeling Kit (Agilent Technologies) following the manufacturer's protocol. Following Agilent's protocol, arrays were hybridized 17 h at 65°C with rotation at 10 rpm and scanned on Agilent C microarray Scanner. The raw data were extracted by Feature Extraction Software v11.5.1 (Agilent Technologies).

Protein extraction, immunodetection and relative quantification

Mouse prefrontal cortices were mechanically grinded in 50 µL of lysis buffer, containing 50 mM Tris-HCl, 100 mM NaCl, 1% Triton X100, 5 mM Ethylene-Diamine-Tetra-Acetic acid (EDTA), 1 mM Phenylmethylsulfonyl fluoride (PMSF), and 1 mini-tablet of proteases and phosphatases inhibitors cocktail (Ref. A32961, Thermo Fisher Scientific, Waltham, MA, U.S.A.). Samples were then centrifuged for 10 minutes at 10 000 x g at +4 °C. Supernatant S1 proteins were collected and dosed using Bradford assay.

For the vesicular glutamate and the gamma-amino butyric acid (GABA) transporters (VGLUT1 and VGAT, respectively) immunodetections, 20 µg of non-heated S1 protein samples were resolved in NuPAGE™ Bis-Tris 10% polyacrylamide gels (Thermo Fisher Scientific) with 1X NuPAGE™ MOPS running buffer (Thermo Fisher Scientific). Proteins were transferred onto a low fluorescence polyvinylidene fluoride membrane overnight at +4 °C in 1X tris-glycine added with 10% methanol. For protein level normalization, total proteins were detected with No-Stain™ labelling kit (Thermo Fisher Scientific) as described by the manufacturer. Protein immunodetection was performed following the Azure fluorescent Western Blot workflow (Azure Biosystem, Dublin, CA, U.S.A.). As primary antibodies, we used a monoclonal mouse antibody against VGLUT1, clone 68B7 (Ref. 135 011, Synaptic Systems GmbH, Göttingen, Germany) and a polyclonal rabbit antibody against VGAT (Ref. 14471-1-AP, Proteintech, Rosemond, IL, U.S.A.) at a 1:5 000 dilution in 1X Azure fluorescent blot blocking buffer (Ref. AC2190, Azure

Biosystem) and incubated for 1.5 hours at room temperature. As secondary antibodies, we used a goat anti-mouse IR800 (Ref. AC2135, Azure Biosystem) and a goat anti-rabbit IR700 (Ref. AC2128, Azure Biosystem) at a 1:10 000 dilution in 1X Azure fluorescent blot blocking buffer for 1 hour at room temperature.

References

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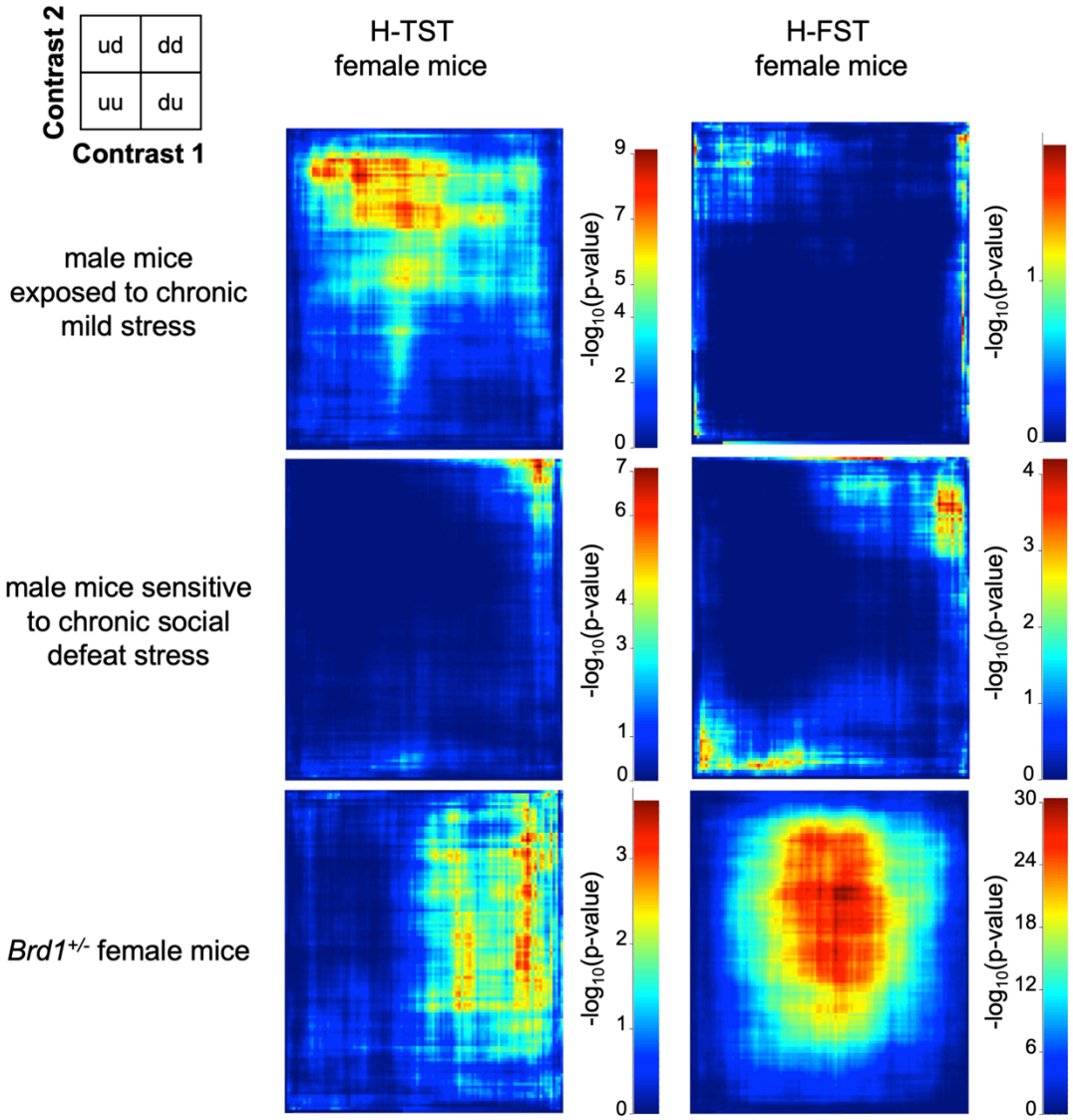
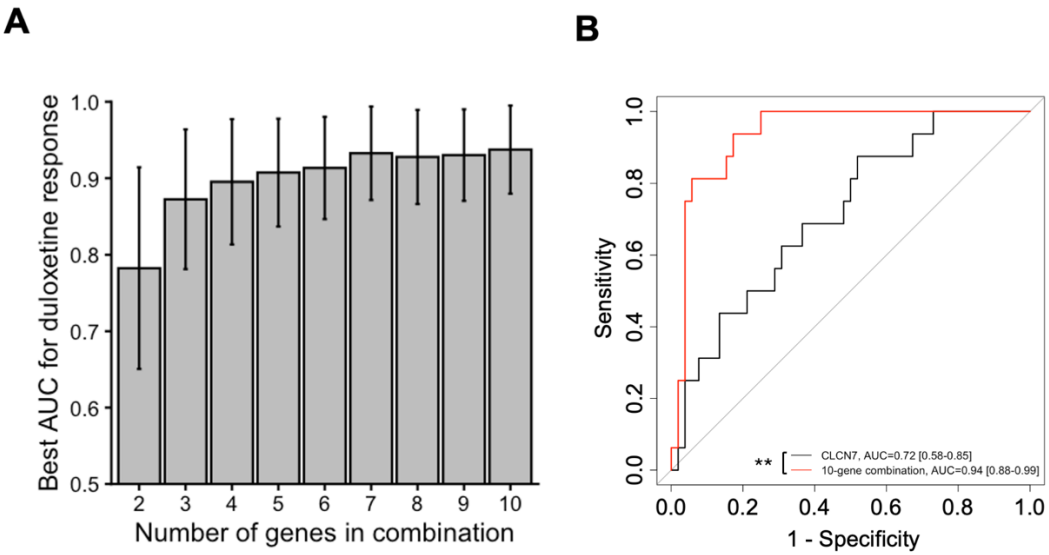
2. El Yacoubi M, Rappeneau V, Champion E, Malleret G, Vaugeois J-M. The H/Rouen mouse model displays depression-like and anxiety-like behaviors. Behav Brain Res. 2013;256:43–50.
3. Arthaud S, Varin C, Gay N, Libourel P-A, Chauveau F, Fort P, et al. Paradoxical (REM) sleep deprivation in mice using the small-platforms-over-water method: polysomnographic analyses and melanin-concentrating hormone and hypocretin/orexin neuronal activation before, during and after deprivation. J Sleep Res. 2015;24:309–319.



Supplementary Figure 1. Rank-rank hypergeometric overlap heatmaps of H-TST and H-FST prefontal cortex gene expression patterns with other mouse models with anxiodepressive-like phenotypes. H-TST and H-FST mice were compared to NH-TST and NH-FST, respectively. The other models were compared non exposed animals to environmental stressors or wild-type mice for the *Brd1* model. d, downregulated gene expression; u, upregulated gene expression.



Supplementary Figure 2. Predictive probabilty of gene combinations on treatment response to duloxetine. A. Best area under the curve (AUC) with 95% confidence interval for predicting response to duloxetine, obtained via receiver operating characteristic (ROC) analysis for multiple gene combination. **B.** ROC curves comparison between the best unique gene predictor (*CLCN7*) and the best 10-gene combination (*ATF3*+*GNPTAB*+*FBLN1*+*IGFBP3* +*TLE1*+*SLC38A9*+*TES*+*CD200*+*BCAT2*+*KBTBD7*). **p<0.01.