A single gene defect causing claustrophobia

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Claustrophobia, the well-known fear of being trapped in narrow/closed spaces, is often considered a conditioned response to traumatic experience. Surprisingly, we found that mutations affecting a single gene, encoding a stress-regulated neuronal protein, can cause claustrophobia. Gpm6a-deficient mice develop normally and lack obvious behavioral abnormalities. However, when mildly stressed by single-housing, these mice develop a striking claustrophobia-like phenotype, which is not inducible in wild-type controls, even by severe stress. The human GPM6A gene is located on chromosome 4q32-q34, a region linked to panic disorder. Sequence analysis of 115 claustrophobic and non-claustrophobic subjects identified nine variants in the noncoding region of the gene that are more frequent in affected individuals (P = 0.028). One variant in the 3’ untranslated region was linked to claustrophobia in two small pedigrees. This mutant mRNA is functional but cannot be silenced by neuronal miR124 derived itself from a stress-regulated transcript. We suggest that loose dynamic regulation of neuronal GPM6A expression poses a genetic risk for claustrophobia.

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Introduction

The neuronal tetraspan membrane glycoprotein Gpm6a has been implicated in neurite outgrowth and dendritic spine formation,1–3 but the lack of a mouse mutant has prevented any in vivo analysis of Gpm6a function. Specifically, the observation that Gpm6a expression in rodent brain is downregulated by cortisol or following physical restraint stress4 has been puzzling. As stress is a key factor for triggering mental disorders,5 we investigated the behavioral consequences of resident-intruder stress in mice lacking the Gpm6a gene. We report here the unexpected finding that the neuronal gene Gpm6a constitutes a genetic cause of a highly unusual ‘claustrophobia-like’ phenotype in null mutant mice, which otherwise develop completely normally. In fact, only Gpm6a mouse mutants that have experienced a mild ‘social stress’ exhibit this ‘claustrophobia-like’ behavior. Moreover, we translate this finding to human individuals, where we find rare sequence variants in the GPM6A gene associated with claustrophobia. Mechanistic insight is provided by the demonstration of a human variant-specific loss of GPM6A regulability. We conclude that regulability of the GPM6A gene under stress is required to avoid claustrophobia, which emerges as an unusual stress response.

Materials and methods

Generation and characterization of Gpm6a null mutant mice. All experiments were approved by the local Animal Care and Use Committee in accordance with the German Animal Protection Law. Mice with a targeted inactivation of the Gpm6a gene were generated. First a gene-targeting vector (Figure 1a) was constructed. From the cloned mouse (129SV) Gpm6a gene, a 6.5-kb fragment of intron 2 became the long homologous arm. A 1.5-kb fragment that included the 3’-part of intron 1 and 6 bp at the 5’-end of exon 2 became the short homologous arm. It was cloned with tailored PCR primers introducing HindIII (5’) and BamHI (3’) restriction sites. For negative selection, a neomycin-resistance gene (neo) under control of the herpes simplex virus (HSV) thymidine kinase (tk) promoter (kindly provided by R Sprengel, MPI Heidelberg) was utilized. The neomycin cassette was subcloned with tailored PCR primers introducing at both the 5’- and the 3’-end BamHI restriction sites and translation termination codons in all reading frames. For positive selection, a ClaI fragment of the HSV-tk under control of the HSV-tk promoter was subcloned into the vector. The construct was verified by molecular sequencing, and the vector backbone (pKS+ bluestrip, Stratagene Heidelberg, Germany) was linearized with NotI. Using

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standard procedures,6 R1 mouse embryonic stem cells (R1-ES, provided by A Nagy, Toronto, Canada), suspended in phosphate-buffered saline (PBS) with 40 mg linearized targeting vector, were electroporated using a Bio-Rad GenePulser (240 V and 500 μF, Bio-Rad, Munich, Germany). Transfected embryonic stem cells (2 × 10⁶) were cultured on gelatinized 10-cm dishes (Falcon, Heidelberg, Germany) for 1 day and then selected with 300 mg/l G418 and 2 μg/ml Gancyclovir. On day 10 after electroporation, 386 resistant clones were picked and one with homologous recombination was identified by semi-nested PCR. Amplification was (1) with forward primer (5’-GGGCTGACTTTTGGATTGG-3’) and reverse primer (5’-GCTCTCCACCCAAGCGGCG GAGAACCTGCGTGC-3’) and (2) on the first PCR product with alternative reverse primer (5’-GCAATCCATCTTGATTGAGAACCTGCGTGC-3’). Embryonic stem cells were microinjected into C57Bl6/6J blastocysts that were transferred to pseudo-pregnant foster mothers. Highly chimeric males (N = 4) were obtained that were bred to C57Bl6/6J females. We interbred heterozygous offspring to obtain homozygous mutant mice, which were born at the expected Mendelian frequency. Gpm6a null mutant mice are viable and fertile. For genotyping (Figure 1b), genomic DNA was isolated from tail biopsies using the DNeasy96 kit (Qiagen, Hilden, Germany) according

Figure 1 Generation of Gpm6a null mutant mice and discovery of behavioral consequences following stress. (a) Strategy to inactivate the mouse Gpm6a gene. A neomycin resistance cassette flanked by translation stop codons in all reading frames was fused into exon 2, which is the first exon present in all Gpm6a transcripts. (b) PCR genotyping to identify wild-type (WT) and mutant Gpm6a alleles. (c) Immunoblot analysis of cortex homogenates using antibodies directed against Gpm6a or tubulin, with or without prior deglycosylation using PNGaseF. Gpm6a was undetectable in Gpm6a null mutants. Note that the abundance of Gpm6a was considerably reduced in heterozygous mice. (d) Immunohistochemistry of brain sections with antibodies directed against Gpm6a or the related proteolipid protein (PLP). Note that Gpm6a was not detected in Gpm6a null mutant mice that showed unchanged PLP expression. (e) Elevated plus maze (EPM) behavior of psychosocially stressed (resident-intruder paradigm) as well as of sham-stressed Gpm6a null mutants (KO) reveals a prominent claustrophobia-like phenotype (N = 17–19 per group). (f) Sample track recording of EPM performance, illustrating a Gpm6a mutant spending much less time in closed arms compared with its WT littermate. (g) Single housing (a prerequisite of performing the resident-intruder stress) is sufficient to induce a claustrophobia-like phenotype in Gpm6a mutants (N = 7–9 per group), which is absent upon group housing, and (f) to reveal a clear genotype difference in the expression of a stress-regulated gene, Fkbp5, in mouse amygdala (N = 7–9 per group). Mean ± s.e.m. presented. DAPI, 4’-6-diamidino-2-phenylindole; HSK-tk, herpes simplex virus-thymidine kinase.
to manufacturer’s directions. In a PCR co-amplification reaction, the presence of the Gpm6a wild-type (WT) allele was shown using forward primer #1 (5’-TGCTCTTCTAC AGGGTGCT-3’) and reverse primer #2 (5’-CCTCCA TCCTGTCATTCC-3’), which yielded a 560-bp fragment. We identified the targeted allele with forward primer #1 and reverse primer #3 (5’-GCAATCCATCTGTTCAATGGC-3’), yielding a 310-bp fragment. For protein analysis (Figure 1c), we prepared total cortex lysates from WT, heterozygous and homozygous mice and determined the protein concentration according to Bradford, and boiled the samples (5 min) before loading. For immunoblot, we separated 40 µg of total cortical protein and transferred the samples on poly(vinylidene fluoride) membranes (Hybond-P, Amersham Biosciences, Glattbrugg, Switzerland). We blocked the membrane in 5% milk powder in PBS with 0.1% Tween (30 min at 37 °C). Following wash, membrane was incubated with horseradish peroxidase-conjugated secondary antibody (Dianova, Hamburg, Germany, 1:5000 in blocking buffer). Immunoreactive bands were visualized by enhanced chemiluminescence (Pierce, Bonn, Germany). For immunohistochemistry (Figure 1d), WT and Gpm6a null mutant mice were anesthetized with Avertin (250 mg/kg body weight; Sigma), perfused with Hank’s balanced salt solution, and the isolated brains were post-fixed for 1 h. Vibratome sections (thickness 12 µm, Leica VT 1000S, Leica Biosystems, Wetzlar, Germany) were permeabilized with 0.4% Triton X-100 in PBS (30 min, room temperature), blocked in 4% horse serum in PBS (30 min, room temperature) and incubated with antibodies against Gpm6a (M6, rat monoclonal, 1:25; kind gift by Carl Lagenaur, Pittsburgh, USA) or proteolipid protein (A431, rabbit polyclonal, 1:500) at 4 °C for 24 h. After wash, sections were incubated with appropriate fluorochrome-coupled secondary antibodies (Dianova, Hamburg, Germany; 2 h, room temperature) and washed three times. Sections were imaged with Leica DMRXA and OpenLab 2.0 software (Improvison, Tübingen, Germany).

Behavioral testing. For behavioral testing, mice were housed in groups of three to five in standard plastic cages, food and water ad libitum. The temperature in the colony room was maintained at 20–22 °C, with a 12-h light/dark cycle (light on at 0700 hours). Behavioral experiments were conducted by an investigator, blinded to the genotype, during the light phase of the day (between 0800 hours and 1700 hours). For behavioral experiments, eight different cohorts of mice were used. The order of testing in the first cohort was as follows: elevated plus maze (EPM), open field, hole board, rotarod, pre-pulse inhibition, fear-conditioning, and visual cliff. In further cohorts, EPM release in closed arms, EPM in the dark, mouse light/dark box test, mouse wide/narrow box test, EPM retesting (‘exposure treatment’) and hearing were performed. For electroretinogram, olfaction testing and corticosterone determination upon metabolic cage exposure, separate cohorts were used. Age of mice at the beginning of testing was 19 weeks. Inter-test interval varied depending on the degree of ‘test invasiveness’ but was at least 1 day. During all tests, the investigator was ‘blinded’, that is, unaware of mouse genotypes. For comprehensive test description of basic tests, that is, EPM, open field, hole board, rotarod, visual cliff test (vision), buried food finding test (olfaction), sucrose preference test (motivation), pre-pulse inhibition, cued and contextual fear-conditioning, and ultrasound vocalization analysis, please see El-Kordi et al.9 Described in the following are additional, modified or specifically designed tests.

EPM with release in closed arms. In this modified version, mice were placed in the closed arms in the same plus-maze described above. This test was done to address potential motor factors influencing the time spent in arms. The test was otherwise conducted in the same manner as the classical EPM.

EPM in darkness. This test was again performed like the classical EPM, just in full darkness to address potential visual/perceptual factors affecting behavior in open/closed space. The behavior of mice was monitored via infrared camera.

Hot plate test. The hot plate test is used as a measure of pain sensitivity. Mice were placed on a metal plate (Ugo Basile, Comerio, Italy), preheated up to 55 °C. The latency of hind paw licking or jumping was recorded. Mice were removed from the platform immediately after showing the response. A 40-s cutoff time was supposed to prevent wounds, although none of the tested mice reached it.

Assessment of hearing by the acoustic startle response. Individual mice were placed in small metal cages (90 × 40 × 40 mm3) to restrict major movements and exploratory behavior. The cages were equipped with a movable platform floor attached to a sensor that records vertical movements of the floor. The cages were placed in four sound-attenuating isolation cabinets (TSE GmbH, Bad Homburg, Germany). Startle reflexes were evoked by acoustic stimuli delivered from a loudspeaker that was suspended above the cage and connected to an acoustic generator. The startle reaction to an acoustic stimulus (pulse), which evokes a movement of the platform and a transient force resulting from this movement of the platform, was recorded with a computer during a recording window of 100 ms and stored for further evaluation. The recording window was defined from the onset of the acoustic stimulus. An experimental session consisted of a 2-min habituation to 65 dB background white noise (continuous throughout the session), followed by a baseline recording for 1 min at background noise. After baseline recording, stimuli of different intensity and fixed 40 ms duration were presented. Stimulus intensity was varied between 65 and 120 dB, such that 19 intensities from this range were used with 3 dB step. Stimuli of the each intensity were presented 10 times in a pseudorandom order with an interval ranging from 8 to 22 s. The amplitude of the startle response (expressed in arbitrary units) was defined as the difference between the maximum force detected during a recording window and the force measured immediately before the stimulus onset. Amplitudes of responses for each
The behavior of Gpm6a knockout (KO) and wild-type (WT) littermates in classical elevated plus maze (EPM); (b) in EPM performed in the darkness; and (c) in EPM upon release in closed arms. (d) Weekly exposure to EPM (over 3 weeks) led to reduction of closed arm aversion seen in Gpm6a KO. This adjustment also explains the weaker closed-arm avoidance seen in mice of b and c, which had had one previous exposure to EPM. (e) In a newly developed wide/narrow box test, WT mice spent more time in the narrow area (left graph), whereas Gpm6a KO did not show narrow space preference. (f) In the light/dark box, Gpm6a KO mice entered the light area faster; (g) explored less holes (requiring nose pokes in narrow holes); (h) spent less time in the center of the open field, and (i) exerted higher baseline freezing in the fear-conditioning chamber. (j) Exposed to narrow metabolic cages for 3 h, Gpm6a KO excreted higher levels of corticosterone via urine. (a-c): N = 17–18; (d): N = 8; (e): N = 32–35; (g-h): N = 17–18; (i, j): N = 12. Mean ± s.e.m. presented.

**Mouse light/dark box test.** The apparatus (36 × 20.5 × 19 cm³) consisted of two equal acrylic compartments, one roofed, dark and one white, with a 300 lx light intensity in the white compartment and separated by a divider with an opening (size: 5.7 × 5 cm³) connecting both compartments. Each mouse was tested by placing it in the black/dark area, facing the white one, and was allowed to explore the novel environment for 5 min. The roof of the dark compartment was closed after releasing the mouse. The number of transfers from one compartment to the other and the time spent in the illuminated side were measured. This test exploited the natural conflict between the animal’s drive to explore a new environment and its tendency to rather stay in a closed, dark and protected environment and to avoid bright light.

**Mouse wide/narrow box test.** This inhouse-made box (test arena: length 60 cm, width 60 cm and height 30 cm) consisted of two equal (each 30 cm length) gray plastic compartments. One compartment was wide and open, the other one narrow (consisting of 30 × 5 × 30 cm³ corridor). Mice were placed in the wide compartment, facing the narrow corridor. Light intensity in the wide compartment was 300 lx, in the corridor 150 lx. Time to enter the corridor was recorded by a stopwatch. The behavior was recorded throughout the 10 min testing period by a PC-linked overhead video camera. ‘Viewer 2’ software was used to calculate velocity, distance travelled, number of visits of and time spent in both compartments.

**Electroretinogram.** Before the experiments, animals were dark adapted for at least 12 h and all preparations were carried out under dim red light.10 Mice were anaesthetized by intraperitoneal injection of ketamine (0.125 mg g⁻¹) and xylazine (2.5 μg g⁻¹). Supplemental doses of 1/4 the initial dose were administered when changes in the constantly monitored electrocardiogram or movements indicated that the animals were waking up. Mice were placed on a heated mat (Hugo Sachs Elektronik–Harvard Apparatus, March, Germany) that kept the body temperature constant at 37 °C under the control of a rectal thermometer. The head of the mouse was placed inside a custom-designed Ganzfeld bowl illuminated by a ring of 20 white light-emitting diode. The pupil of the left eye was dilated with 1% atropine sulfate and a silver wire ring electrode was coupled to the corneal surface using electrode gel. The eye and electrode were kept moist by a drop of 0.9% saline applied every 30 min. Subcutaneous needle electrodes were inserted between the eyes (reference) and near the tail (ground). Electrical potentials were amplified 1000 times, filtered between 0.1 and 8 kHz and notch-filtered at 50 Hz using custom-designed hardware. The Tucker Davis System III hardware and BioSig software (Tucker-Davis Technology, Alachua, FL, USA) were used for
stimulus control and recordings. Scotopic responses to 10 white light flashes were averaged for each stimulus condition. Interstimulus intervals were 5 s for light intensities below 1 cd s m$^{-2}$ and 17 s for light intensities above 1 cd s m$^{-2}$. The amplitude duration functions and latencies of the A-waves, B-waves and oscillatory potentials in response to 0.1, 1 and 5 ms long-light flashes ranging between 0.0003 and 10 cd s m$^{-2}$ was analyzed using custom-written matlab (Mathworks, Natick, MA, USA) software.

Corticosterone excretion. Urine samples were collected using inhouse-made metabolic cages. Mice were placed in small, narrow metal cages ($90 \times 40 \times 40$ mm$^3$) to restrict major movements and exploratory behavior, thus resulting in stress-induced corticosterone release. These cages had a wire-mesh floor enabling urine collection via a funnel. The funnel was fixed on top of a collecting flask. Mice (12 per genotype) were placed in the metabolic cages at 2200 hours for 3 h each. Urine was collected at 0100 hours. Concentrations of corticosterone were measured using a commercially available EIA kit (BIO TREND, Cologne, Germany) according to the manufacturer’s protocol. Urine creatinine was determined photometrically (Jaffé method). Sample analysis of WT and knockout (KO) animals was performed blinded and in random order. Values were expressed as nmol per day per g body weight.$^{11}$

Resident-intruder (psychosocial stress) test. The procedure is described in detail elsewhere.$^{12,13}$ Briefly, male mice of both genotypes (28 days old) were randomly assigned to either the 'stress' or 'sham stress' group. As intruders, they were subjected for 21 days (1 h daily, from 0900–1000 hours) to resident male mice (male FVB, 2–3 months old, habituated to resident cages for $\geq10$ days). To prevent injuries, direct interaction was immediately terminated at the first attack (usually occurring after a few seconds) by putting a grid cage ($140 \times 75 \times 60$ mm$^3$) over the intruder. Afterwards, intruder mice were placed back in their home cage. Mice were confronted with a different resident every day. Sham stress consisted of placing the intruder mouse in an empty novel cage for 1 h.

Restraint stress paradigm. Mice were kept undisturbed for at least 1 week until a single 6-h restraint stress was performed in a separate room (with mice left in their home cages and put in wire mesh restrainers, secured at the head and tail ends with clips) during the light period of the circadian cycle as described.$^{14}$ Control animals were left undisturbed.

Amygdala dissection. Mice were anaesthetized (intraperitoneal sodium pentobarbital 50 mg kg$^{-1}$) and perfused transcardially (ice-cold PBS). Amygdalae were dissected from a coronal slice – 0.58 to –2.3 mm relative to Bregma and stored in RNA later (Qiagen) at 4°C until processed.$^{14}$

Quantitative reverse transcription-PCR from amygdala. Amygdala tissue was homogenized in Quizaol (Qiagen, Hilden, Germany). Total RNA was isolated by using the miRNasy Mini Kit (Qiagen). First strand cDNA was generated from total RNA using N9 random and Oligo(dT) 18 primers. The relative concentrations of mRNAs of interest in different cDNA samples were measured out of three replicates using the threshold cycle method (deltaCt) for each dilution and were normalized to the normalization factor of Hprt1 and H2afz genes calculated by the geNorm analysis software. Reactions were performed using SYBR green PCR master mix (ABgene, Foster City, CA, USA) according to the protocol of the manufacturer. Cycling was done for 2 min at 50°C, followed by denaturation at 95°C for 10 min. The amplification was carried out by 45 cycles of 95°C for 15 s and 60°C for 60 s. The specificity of each primer pair was controlled with a melting curve analysis. For quantitative PCR, we used the following primers:

- mFkbp5_forward: 5′-ATTTGATTGCGAGATGTG-3′
- mFkbp5_reverse: 5′-TCTTCAACCGGCTTTGTC-3′
- mNpy5r_forward: 5′-CCCGGAGAGCTTATGGAGA-3′
- mNpy5r_reverse: 5′-TCT GTAGTCTCCAGGGC-3′
- mHprt1_forward: 5′-GCTTTGCTGGTGAAGGACCTC TCGAA-3′
- mHprt1_reverse: 5′-CCCTGGAGACTATTAGTCA AGGGCAT-3′
- mH2afz_forward: 5′-ACACGCGACCCACCTCCTGGAGTA-3′
- mH2afz_reverse: 5′-CTCGCAGAATGTATGCTAGAGGGCAT-3′

miR124. First strand cDNA synthesis and reactions were generated from total RNA using the TaqMan MicroRNA RT Kit, TaqMan MicroRNA Assay for hsa-miR124, TaqMan MicroRNA Assay for snoRNA142 as a housekeeper and TaqMan MicroRNA Assay for snoRNA142 as a housekeeper and TaqMan 2 X Universal PCR Master Mix (ABgene) according to the manufacturer’s protocol. Cycling was done with 10 min denaturation at 95°C and amplification for 40 cycles at 95°C for 15 s and 60°C for 60 s.

Human sample

Claustrophobic subjects. The present study was approved by the Ethics Committee of the Georg-August-University. A total of 47 subjects with clinical diagnosis of claustrophobia according to Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV)$^{15}$ were included (Table 1). Healthy subjects (N = 13) were recruited by e-mail announcements in the Max Planck Institutes of Experimental Medicine (MPIEM) and Biophysical Chemistry (MPBPC). Patients suffering from psychiatric conditions other than psychotic disorders (N = 16; that is, N = 7 affective disorder, N = 5 anxiety disorder, N = 2 substance use disorder, N = 2 others) were recruited from the psychiatric hospital of the Georg-August-University Göttingen. In addition, N = 18 schizophrenic individuals with a claustrophobic phenotype were selected from the GRAS data collection.$^{16}$ Claustrophobic subjects were invited to the outpatient unit of the MPIEM for examination. In the case of GRAS patients, extensive telephone interviews were performed instead. Subjects underwent detailed claustrophobia relevant phenotyping, after validation of diagnoses using DSM-IV criteria$^{15}$ by a trained psychologist/psychiatrist. The subsequent examination procedure comprised a short questionnaire regarding sociodemographic information, history of physical and psychiatric diseases, specifically for this project.
Table 1. Comparison of sociodemographic, general clinical and anxiety/claustrophobia relevant parameters in claustrophobic and non-claustrophobic subjects independent of mutation status

| Sociodemographics | Total sample (N = 115) Mean ± s.d. | Claustrophobic subjects (N = 47) Mean ± s.d. | Non-claustrophobic subjects (N = 68) Mean ± s.d. | Statistics | P<sup>a</sup> |
|-------------------|-----------------------------------|-------------------------------------------|-----------------------------------------------|------------|---------|
| Age in years      | 43.56 ± 13.22                     | 43.87 ± 12.11                             | 43.35 ± 14.02                                 | 0.733      |         |
| Education in years| 14.43 ± 3.55                      | 14.31 ± 3.85                              | 14.52 ± 3.35                                  | 0.830      |         |
| Gender            |                                    |                                           |                                               |            |         |
| Female            | 81                                 | 70.4                                      | 13                                             | 27.7       | 21      | 30.9 | 0.139 | 0.710 |
| Male              | 34                                 | 29.6                                      | 34                                             | 72.3       | 47      | 69.1 |         |        |
| Ethnicity         |                                    |                                           |                                               |            |         |
| Caucasian         | 112                                | 97.4                                      | 47                                             | 100.0      | 65      | 95.6 | 2.129 | 0.546 |
| African           | 1                                  | 0.87                                      | —                                             | —          | 1       | 1.47 |         |        |
| Other             | 2                                  | 1.73                                      | —                                             | —          | 2       | 8.94 |         |        |
| Marital status    |                                    |                                           |                                               |            |         |
| Single            | 57                                 | 49.6                                      | 24                                             | 51.1       | 33      | 48.5 | 3.545 | 0.471 |
| Married           | 33                                 | 24.7                                      | 12                                             | 25.5       | 21      | 30.9 |         |        |
| Divorced          | 19                                 | 26.0                                      | 11                                             | 23.4       | 11      | 16.2 |         |        |
| Widowed           | 3                                  | 2.6                                       | —                                             | —          | 3       | 4.4  |         |        |
| Main diagnoses according to DSM-IV |                                    |                                           |                                               |            |         |
| No clinical diagnosis | 27                          | 23.5                                      | 13                                             | 27.7       | 14      | 20.6 | 1.135 | 0.567 |
| Schizophrenia     | 43                                 | 37.4                                      | 18                                             | 38.3       | 25      | 36.8 |         |        |
| Other clinical diagnoses | 45                          | 39.0                                      | 16                                             | 34.0       | 29      | 43   |         |        |

Prevalence of anxiety disorders

| Comorbid anxiety disorder<sup>c</sup> | 68 | 59.1 | 29 | 61.7 | 39 | 57.4 | 0.702 |
| Panic disorder                  | 29 | 25.2 | 15 | 31.9 | 14 | 20.6 | 0.194 |
| Agoraphobia<sup>d</sup>         | 56 | 48.7 | 47 | 100.0 | 9 | 13.2 | <0.0001 |
| Social phobia                   | 18 | 15.7 | 9  | 19.1 | 9  | 13.2 | 0.440 |
| Specific phobia                 | 38 | 33.0 | 20 | 42.6 | 18 | 26.5 | 0.106 |
| Generalized anxiety disorder    | 13 | 11.3 | 7  | 14.9 | 6  | 8.8  | 0.375 |
| Obsessive compulsive disorder   | 18 | 15.7 | 9  | 19.1 | 9  | 13.2 | 0.440 |

Claustrophobia Relevant Items (Short CLQ-G)

| Subscale ‘restriction’ | Total sample Mean ± s.d. | Claustrophobic subjects Mean ± s.d. | Non-claustrophobic subjects Mean ± s.d. | P<sup>a</sup> |
|------------------------|--------------------------|------------------------------------|----------------------------------------|--------------|
| Dark room              | 2.18 ± 1.66              | 3.25 ± 1.22                        | 1.44 ± 1.51                           | <0.000001    |
| Well-lit room          | 1.51 ± 1.46              | 2.53 ± 1.27                        | 0.78 ± 1.12                           | <0.000001    |
| Sleeping bag           | 1.04 ± 1.45              | 1.87 ± 1.58                        | 0.47 ± 1.01                           | <0.000001    |
| Trunk                  | 2.18 ± 1.67              | 3.53 ± 1.04                        | 1.25 ± 1.36                           | <0.000001    |
| MRI scanner            | 1.65 ± 1.67              | 3.17 ± 1.05                        | 0.60 ± 1.11                           | <0.000001    |
| Mean of subscale       | 1.71 ± 1.36              | 2.67 ± 0.87                        | 0.91 ± 1.00                           | <0.000001    |

| Subscale ‘suffocation’ | Total sample Mean ± s.d. | Claustrophobic subjects Mean ± s.d. | Non-claustrophobic subjects Mean ± s.d. | P<sup>a</sup> |
|------------------------|--------------------------|------------------------------------|----------------------------------------|--------------|
| Elevator               | 1.07 ± 1.39              | 2.13 ± 1.36                        | 0.34 ± 0.84                           | <0.000001    |
| Breathe                | 0.83 ± 1.09              | 1.26 ± 1.24                        | 0.54 ± 0.87                           | <0.001       |
| Crowded room           | 1.82 ± 1.57              | 3.04 ± 1.12                        | 0.97 ± 1.25                           | <0.000001    |
| Under a car            | 1.23 ± 1.44              | 2.17 ± 1.51                        | 0.59 ± 0.97                           | <0.000001    |
| Sauna                  | 1.00 ± 1.44              | 2.04 ± 1.56                        | 0.28 ± 0.75                           | <0.000001    |
| Mean of subscale       | 1.19 ± 1.08              | 2.13 ± 0.83                        | 0.54 ± 0.70                           | <0.000001    |
| Mean of questionnaire  | 1.45 ± 1.17              | 2.50 ± 0.74                        | 0.73 ± 0.82                           | <0.000001    |

Abbreviation: MRI, magnetic resonance imaging.
<sup>a</sup>Mann-Whitney U-test.
<sup>b</sup>Fisher’s exact test/χ²-square test.
<sup>c</sup>Anxiety disorders other than agoraphobia.
<sup>d</sup>Agoraphobia includes claustrophobia.

devolved abbreviated German version of the Claustrophobia Questionnaire (CLO)<sup>17</sup> (Short CLO-G) and the screening questions of the Structured Clinical Interview of Diseases<sup>18</sup> for anxiety disorders.

Non-claustrophobic subjects. A total of 68 subjects, who did not suffer from claustrophobia, were matched to the claustrophobic subjects regarding age, gender and clinical diagnosis where applicable (Table 1). Again, healthy subjects (N = 14) were recruited by e-mail announcements in the MPIEM and MPBPC. Patients suffering from psychiatric conditions other than psychotic disorders (N = 29; that is, N = 18 affective disorder, N = 4 general anxiety disorder, N = 4 substance use disorder, N = 3 others) were recruited.
from the psychiatric clinic of the Georg-August-University Göttingen. Furthermore, 25 schizophrenic non-claustrophobic individuals were selected from the GRAS data collection.16 The examination procedure comprised the same battery of questionnaires as for the claustrophobic subjects (above).

Pedigrees. To explore whether particular variations in GPM6A are transmitted in families together with claustrophobia, we tried to contact all available family members of the three claustrophobic individuals carrying the genetic variation at locus c.*1834T>C. Only for two of the subjects, SIWO and THKA (Figure 3b), it was possible to contact a sufficient number of relatives. Claustrophobia diagnosis according to DSM-IV criteria was confirmed by a telephone interview carried out by a trained psychologist. Swabs for genetic analysis and a short sociodemographic questionnaire, also containing items regarding the history of physical and psychiatric diseases, the Short CLQ-G and the screening questions of the Structured Clinical Interview of Diseases for anxiety disorders,18 were communicated via mail.

Abbreviated German version of the CLQ (Short CLQ-G). To quantitatively assess the severity of claustrophobic anxiety, nine items of the CLQ17,19 were selected and translated into German language (Supplementary Table 1). One item measuring fear experienced during magnetic resonance imaging was added to the restriction subscale because this situation may induce claustrophobia.20,21 The CLQ is the most commonly used questionnaire for the psychological assessment of claustrophobia and has excellent psychometric properties (Cronbach’s \( \alpha \): 0.95; test–retest reliability: 0.89).17 It is composed of two subscales measuring two distinct but related fears: fear of restriction and fear of suffocation. Anxiety severity is measured on a 5-point Likert scale. To cover both subscales, five items from the suffocation and four items from the restriction subscale with high ecological validity were selected for construction of the Short CLQ-G. Given the substantial reduction in item number (\( \approx 60\% \)), the Short CLQ-G still achieves high internal consistency (total scale: 0.932, restriction: 0.909, suffocation: 0.835) and split-half reliability (0.952, splits matched for mean item difficulty) for the whole subject sample (\( N = 115 \); \( N = 47 \) claustrophobic subjects; \( N = 68 \) non-claustrophobic subjects; Supplementary Table 1).

GPM6A sequencing. DNA from all subjects participating in this study (\( N = 115 \)) was isolated from blood with the
Claustrophobia in Gpm6a null mutant mice

A El-Kordi et al.

JET Quick Kit (Genomed, Loehne, Germany). For analysis of pedigree members (swabs), DNA was isolated with the Isohelix DNA Swab Kit (Biolab Products, Goedendorf, Germany). PCR reaction: All exons, the putative promoter region of Ex2b and the 3' untranslated region (3'UTR) of GPM6A were PCR-amplified from peripheral blood mononuclear cells (PBMCs) of claustrophobic patients with the mutation in the 3'UTR of GPM6A were PCR-amplified from peripheral samples. Primers are listed below. Sequencing: The PCR amplicons were purified from unincorporated primers and deoxyribonucleotide triphosphates by digesting with 1 U Shrimp Alkaline Phosphatase and 5 U Exonuclease I (Exo) according to the manufacturer's instructions (USB Europe GmbH, Staufen, Germany). Sequencing was carried out using the dideoxy chain termination method with the BigDye Terminator v3.1 Cycle Sequencing Kit on a 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Raw data were processed with Sequencing Analysis 5.2 (Applied Biosystems) and with different modules of the software package Lasergene 7.0 (DNASTAR, Madison, WI, USA).

Primers for GPM6A sequencing approach

| Amplified region | Primer sequence (5'–3') | Size (bp) |
|------------------|-------------------------|-----------|
| Exon 1           | fw GAAGAAAGAGGAGATGACAAGG 653 |
|                  | rv GTCTGAGGCCGAGGACATT     |
| Promoter region  | fw GTGCTGCTGGTATTGGAAGTG 810 |
| Exon 2b          | rv CTAAACATTAGGCGACCCAAACACAG 755 |
|                  | fw GAGGGAGAAAAGGAAAAACACAG 788 |
| Exon 2b          | rv GAAACATCTATGCTCTGCTG 694 |
|                  | fw GAAGCTCTGGTGGGAGGAG 709 |
| Exon 3           | rv GATTGTGCTCCGACTTCTAATC 694 |
|                  | fw GGAGGATAAAGTAAGATG 640 |
| Exon 4           | rv GAGACGGAGAAGGAGAAG 904 |
|                  | fw GCACTTACATCAACAGTG 955 |
| Exon 5           | rv GGGAGGATAAAGTAAGATG 694 |
|                  | fw GCCAACGTATGGATTCCGACT 709 |
| Exon 6_7         | rv GGAACCTGCTTGGATTTGATTAG 955 |
|                  | fw GACCTACCTACCCATTTGTTCC 904 |
| Exon 8*          | rv GGAAGTACAGGGCTCATTAC 1543 |
| 3'UTR*           | fw ATGGTACTTTGGAAGAATTCACAC 1543 |

*For sequencing exon 8 and the associated 3'UTR additional primers were designed to cover the full sequence.

Exon8v2: 5' - GGGTCCCTTGAAGGTATTACCT-3' 3'UTRw2: 5' - GGCAATCGATATTGTTGACCC-3' 3'UTRw2: 5' - CACCTTACAGCATTGGTGAG-3'

Computational micro RNA (miRNA) search. To explore putative miRNA-binding sites in the GPM6A 3'UTR, several analyses were performed. TargetScan, version 6.2 (http://www.targetscan.org) was used to identify miRNA-binding sites. Screening and ΔAG prediction analysis for both alleles of GPM6A were carried out using established algorithms (http://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html).

Expression analysis after nucleofection. Peripheral blood mononuclear cells (PBMCs) of claustrophobic patients with the mutation in the 3'UTR (N = 2) and three matches per subject were freshly isolated using the standard Ficol-Paque Plus isolation procedure (GE Healthcare, Munich, Germany). Using the Amaxa Nucleofector II Device (T-020), 6 × 10⁶ cells were transfected with neg miRNA #2 or hsa-miR124 (Applied Biosystems) and cultured in RPMI supplemented with 10% fetal calf serum. After 24 h, cells were harvested and RNA extracted with the mirNeasy Mini Kit (Qiagen). cDNA was synthesized using 200 U SuperScriptIII (Invitrogen, Karlsruhe, Germany). For quantification with quantitative reverse transcription-PCR, the cDNA was used 1:10 diluted and four replicates per sample were performed; for 4 μl diluted cDNA, 5 μl Power SYBR mix (Applied Biosystems) and 1 pmol of each primer (see below) were added. Cycle threshold (CT) values for GPM6A were standardized to CT values of GAPDH.

hGPM6A_forward: 5'-TGAGATGCGAAGAATCGTG-3'  
hGPM6A_reverse: 5'-CTTCCACCACATCAGCAAAAT-3'  
hGAPDH_forward: 5'-CTGACTCTCAACAGCGACACC-3'  
hGAPDH_reverse: 5'-TGCTGTACGCAAATTTGTTG-3'

Statistical analyses. Data were analyzed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA; http://www.spss.com) (human data analyses) and Prism 4 for Windows version 4.03 (GraphPad Software, Inc., La Jolla, CA, USA) (mouse data analyses). Unless otherwise stated, the data given in figures and text are expressed as mean ± s.e.m., and were compared by two- or three-way analysis of variance with post-hoc planned comparisons or by analysis of variance for repeated measurements, Mann–Whitney U-test and χ² test, where appropriate.

Results

Gpm6a null mutant mice appear essentially normal in development and basic behavior. We have generated Gpm6a null mutant mice (KO) to explore the role of Gpm6a in the behavioral response to stress (Figures 1a–d). Homozygous KO mice were born at the expected Mendelian frequency and are long-lived. By western blot analysis, heterozygous mice expressed about 50% of the protein (Figure 1c), demonstrating that Gpm6a abundance can be regulated at the transcriptional level (Figure 1c), demonstrating that Gpm6a abundance can be regulated at the transcriptional level in vivo (see below). Gpm6a KO mice reproduce well and exhibit no obvious developmental defects (data not shown). Also, in a basic behavioral test battery, which included the analysis of motor and sensory functions, motivation and sensorimotor gating, we found no difference from WT littermate controls (Supplementary Figure 1).

Mild stress induces a claustrophobia-like phenotype in Gpm6a null mutant mice. Unexpectedly, when applying the resident-intruder paradigm in order to assess the response to experimental stress, we noticed that sham-stressed Gpm6a null mutant mice exhibit a prominent phenotype in the EPM, consisting of a specific avoidance of closed arms. To our knowledge, such a behavioral response, which we like to term ‘claustrophobia’ in mice, has not been reported before. This phenotype is specifically striking, because normal rodents rapidly seek closed and narrow spaces to hide, which is a protective trait. Interestingly, the claustrophobia-like phenotype was only marginally striking, because normal rodents rapidly seek closed and narrow spaces to hide, which is a protective trait. Interestingly, the claustrophobia-like phenotype was only marginally
mice), we asked whether the relatively mild stress of social withdrawal might have been sufficient to trigger the claustrophobia-like phenotype in Gpm6a mutants. Indeed, single-housed, but not group-housed, Gpm6a mutants showed claustrophobia (Figure 1g). In these experiments, 10 days of single housing were sufficient to cause downregulation of the stress-responsive gene Fkbp5 in the amygdala of WT mice. Importantly, this downregulation was absent in Gpm6a mutant mice, demonstrating a perturbation of the normal stress response even at the molecular level (Figure 1h). A comparable result was obtained for Npy5r as another marker of stress (data not shown).

**Extra behavioral tests underline the claustrophobia-like phenotype in Gpm6a−/− mice.** As claustrophobia-like behavior in mice has to our knowledge never been reported before, we performed a large number of extra behavioral tests in eight independent cohorts of male mice in order to substantiate this unusual phenotype. In fact, claustrophobia upon single housing was found in all cohorts of Gpm6a mutants and maintained when EPM was performed in darkness, using infrared cameras or when mice were released in closed arms (Figures 2a–c). This behavioral response did not rely on whisker functions or vocalizations, as confirmed by whisker cutting and ultrasound recording, respectively (data not shown). Similar to an ‘exposure therapy’ in humans, repeated EPM testings of mutants reduced and ultimately eliminated the claustrophobia-like behavior (Figure 2d; note also the weaker closed arm avoidance of mutants in Figures 1g and 2b; Supplementary Figure 2B, showing cohorts that already had one previous EPM test session). Also, other tests confirmed our diagnosis of ‘claustrophobia’, such as a specifically designed wide/narrow box, a light/dark box and the hole board test, in all of which mutant mice lacked preference for narrow and dark spaces (Figures 2e–g), that is, displayed a highly abnormal behavior, considering that rodents naturally prefer these spaces to hide and thereby protect themselves from predators.

Further tests demonstrated slightly increased general anxiety, again reminiscent of the known human claustrophobic phenotype. Mutants spent less time in the center of the open field and showed increased ‘baseline freezing’ in the fear-conditioning box (Figures 2h,i). The collection of urine from mutant mice that were kept for 3 h in narrow metabolic cages, revealed a significantly higher corticosterone excretion compared with their WT littermates (at similar urine creatinine values: WT 0.35 ± 0.08 versus KO 0.39 ± 0.06 mg per g body weight and day; N=12/group; P > 0.1), indicative of an increased stress level (Figure 2j). As phobias/panic disorders in humans are more prevalent in females than in males,25 we additionally examined female mutant mice and confirmed a very similar behavioral pattern as in male mice, that is, an unaltered basic behavior and the avoidance of closed arms in EPM (Supplementary Figure 2).

**First considerations on a functional compensation for loss of Gpm6a in null mutant mice.** Interpreting stress at the level of gene expression changes is difficult, because the encoded proteins can be ‘upstream’ or ‘downstream’ of stress perception, and either contribute to or protect from abnormal stress response. This complicates the prediction of cause and effect in a pathological situation. Gpm6a mRNA is downregulated by chronic social stress and also following prolonged cortisol treatment.26 As stimulation of the hypothalamus–pituitary–adrenal (stress) axis leads to cortisol release, it is likely that downregulated Gpm6a expression mediates adaptation of the brain to stress and is therefore a healthy response that serves a feedback function in neuronal circuits exposed to stressful signals. The loss of Gpm6a in null mutant mice is clearly tolerated, presumably by the functional compensation of structurally related membrane proteins that are co-expressed in development (but are likely not stress regulated). One candidate for functional compensation is the neuronal Gpm6b gene, which encodes a highly related protein27 with a similar (but not identical) spatio-temporal expression in brain.28,29 and which is, unlike Gpm6a, not among the identified stress-regulated genes.26,30 In fact, this gene is upregulated under basal conditions in the amygdalae of Gpm6a mutant mice (KO: 1.04 ± 0.06; WT: 0.86 ± 0.05, normed to Hprt1 and H2afz; P < 0.05). To further investigate compensatory functions between the two genes, we cross-bred Gpm6a mutant mice with a newly generated line of Gpm6b null mutant mice.31 The resulting double-mutant mice develop normally and reproduce well, but show 20% unexplained mortality at age 1 month. Further evidence that Gpm6a and Gpm6b have overlapping functions was found in cultured cortical neurons, in which the loss of both proteins reduced the collapse response of growth cones to soluble ephrin-B5, a repulsive signal.31 This significant but clearly limited evidence of compensation strongly suggests that several (but not all) Gpm6a functions are redundantly served by Gpm6b and presumably other neuronal proteins. If stress-induced down-regulation of Gpm6a expression in vivo were part of a neuroprotective stress response, it would be plausible that Gpm6a null mutant mice can develop normally but are selectively affected at the behavioral level, simply because Gpm6a compensating genes (such as Gpm6b) lack the necessary downregulation following stress exposure.

**Selected genomic sequencing of GPM6A reveals associations with claustrophobia.** As polymorphisms of human GPM6A, specifically in the noncoding region, could likewise interfere with dynamic gene regulation, we explored the association of this gene with a predisposition to human claustrophobia. A sample of 115 adult subjects (N = 47 self-reported claustrophobics and N = 68 non-claustrophobic controls) were recruited and interviewed with special emphasis on general anxiety and claustrophobia (Table 1). The sociodemographic description of the human sample revealed similar distributions between claustrophobic and non-claustrophobic individuals with regard to age, educational background, gender, ethnicity and marital status. Moreover, cases and controls were well matched for comorbid disease state. The prevalence of DSM-IV anxiety disorders other than claustrophobia (Table 1, included under agoraphobia) did not substantially diverge between claustrophobic cases and controls. More than half of the total sample (59%) reported to suffer from at least one (additional...
anxiety disorder. Expectedly, most individuals suffered from any kind of specific phobia (33%), followed by panic disorder (25%), social phobia and obsessive-compulsive disorder (both 16%). Generalized anxiety disorder was least frequent in our sample (11%). Claustrophobic subjects displayed higher severity ratings on all 10 items of an abbreviated German version of the CLQ17 (Short CLQ-G; essentially all \( P < 0.00001 \)). Despite a 60% reduction in item number, the Short CLQ-G showed still very good psychometric properties comparable to the original instrument (Supplementary Table 1).

On all 115 subjects, we performed genomic sequencing of GPM6A covering all exons and flanking noncoding regions. This identified nine single-base substitutions in GPM6A, all of which were rare (most of them previously unreported) variants in the noncoding regions. Interestingly, in claustrophobic individuals, the sequenced regions were significantly more polymorphic than in non-claustrophobic controls (\( P = 0.028 \); Figure 3a). To investigate whether particular variants of GPM6A are also genetically linked to claustrophobia, we examined two families that shared sequence abnormalities in the 3’UTR. This allowed us to include information on more than one family member (\( N = 10 \)) within two small pedigrees (Supplementary Table 2). Indeed, the sequence variants in the 3’UTR/noncoding region exon8 were consistently found in claustrophobic (but not in non-claustrophobic) individuals (Figure 3b). Unfortunately, the pedigrees were too small to assess significance. Interestingly, however, when comparing all mutation carriers in our sample of 115 individuals with all non-mutation carriers (independent of the claustrophobia diagnosis) significantly higher scores for most claustrophobia-relevant items were found associated with the mutation status (Supplementary Table 3).

**A single-base substitution in the 3’UTR of GPM6A delivers first mechanistic insight.** To gain mechanistic insight into the possible role of GPM6A sequence variants in the noncoding region, we focused on the newly identified substitution T to C at position c.*1834 in the 3’UTR of exon8, consistently associated with claustrophobia in the two pedigrees. In vertebrates, the c.*1834-T allele is conserved from human to zebrafish (Figure 3c). Mechanistically, this position is of particular interest because it is located within the seed sequence of miR124. This miRNA is expressed in the adult brain, but has only been studied in neuronal development and for its role in neuroplasticity. We asked whether miR124 is also found in the amygdalae of mice and stress regulated. To this end, WT mice were exposed to restraint stress for 6 h, followed immediately by amygdala dissection. Indeed, we detected a significant upregulation of miR124 (Figure 3e) under stress.

**Discussion**

The behavioral analysis of Gpm6a mutant mice has led to the unexpected finding that a single neuronal gene can cause an isolated behavioral defect, best described as claustrophobia. Belonging to the category of agoraphobia/panic disorder, claustrophobia is often assumed to be a conditioned response, following a related traumatic experience. In our model, claustrophobia-like behavior was observed in mice with a strong genetic predisposition (that is, Gpm6a deficiency) when combined with rather mild chronic stress. Interestingly, there was no obvious relationship between the quality of stress (that is, single-housing) and the very specific avoidance behavior. This not only suggests that loss of Gpm6a expression is a key genetic determinant of claustrophobia, but also sufficient to turn an unrelated stressor into a trigger of a unique behavioral response. We note that Gpm6a itself is widely expressed in the CNS, including hippocampus and amygdala as known sites of fear conditioning. Thus, there are no reasons to believe that the encoded membrane protein has evolved in the context of specific behavioral functions. It is much more likely that membrane protein Gpm6a, similar to other proteolipids, is a cholesterol-associated tetraspan, which binds other neuronal membrane proteins, which provide functional specificity. It is thus intriguing that Gpm6a has been found to stimulate endocytosis of \( \mu \)-opioid receptors from the surface of neuronal cells. We note that opioids are well known to be involved in regulation of fear/anxiety and their extinction in mouse and man.

Virtually nothing was known about the cause of claustrophobia. Typically, anecdotal evidence suggested traumatic experiences, such as in individuals that became trapped alive, but these incidents cannot explain the high frequency of claustrophobia in otherwise normal people. The cause or trigger of some cases of claustrophobia may still be related to exposure to narrow spaces, traumatic brain injury and other traumatic experiences, such as surviving of mining accidents, but these are mostly poorly documented. Our report of a mutant mouse model for claustrophobia suggests that also human claustrophobia can have a familial predisposition. We could identify a genetic component of claustrophobia, involving GPM6A expression and its post-transcriptional regulation by the (stress-regulated) neuronal miR124. These data suggest that GPM6A may contribute to the normal stress response in mouse and human. Larger studies in human samples would be required to assess exactly to what extent variants of GPM6A act as a claustrophobia-susceptibility gene.

At first glance, the two findings in mouse and human appear contradictory, because the claustrophobic phenotype was associated with the murine Gpm6a null mutation and the human GPM6A c.*1834-C allele. The latter is predicted to encode a more stable mRNA, due to the loss of its miR124-binding site. However, both findings can be reconciled with the compensation of Gpm6a (in the null mutant) by related proteins, such as Gpm6b. These proteins substitute for
Gpm6a in neurons and allow mutant animals to develop and behave normally. However, when exposed to stress the expression of these genes is not downregulated (unlike Gpm6a), as evidenced by the gene expression profiling that had identified and later confirmed Gpm6a as the only stress-responsive proteolipid in the adult brain. Along these lines, we note that miR124, which acts as a stress-regulated mediator of GMP6A downregulation, as shown here, does not have comparable functional binding sites in GPM6B. Thus, loss of dynamic proteolipid expression in neurons (and the inability to downregulate these proteins) may predispose to abnormal stress response, rather than the loss of Gpm6a per se.

The detailed downstream mechanisms will have to be explored in other conditional mouse mutants in the future. Gpm6a drives the rate of endocytosis that downregulates the steady-state level of μ-opioid receptors at the surface of neuronal cells. Thus, our data are compatible with a hypothetical model, in which a stress-induced phobia/panic disorder might be caused (in part) by a reduced feedback regulation of endogenous opioid receptor signalling. Obviously, interactions with other proteins that also influence behavior may be functionally relevant, and we note that the human serotonin transporter has been reported to interact in cis with GPM6A and GMP6B (and Jana Haase, Dublin, Ireland, personal communication), whereas another study has implicated this serotonin transporter in human panic disorders. In turn, GMP6A may also be relevant as a modifier of other diseases, and it is intriguing that an association has been found between GMP6A and the severity of depression in patients with schizophrenia. The ramification of GMP6A downstream mechanisms are therefore likely complex and beyond the scope of this study. However, by placing the dynamic expression of GMP6A/Gmp6a both upstream and downstream of stress perception in the brain, we suggest a working model of GMP6A/Gmp6a as a neuronal ‘brake’ for maintaining a healthy stress response.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

MK and HW in the lab of K-AN generated the Gmp6a KO mice. Pdm-S and JP carried out the molecular biological characterization of the mice under supervision of HW and K-AN. All behavioral experiments of mice were designed, performed and analyzed by AEK. Electrophysiology measurements were done by NS, ultrasound vocalizations by KH. Mouse amygdala dissections and quantitative reverse transcription-PCR from amygdala tissue were performed by SS under supervision of RP. The corticosterone assay was conducted by SS. AK developed and evaluated the Short CLQ-G, was responsible for telephone interviews, recruiting and examination of claustrophobic patients, control subjects, family members as well as data analyses. She was assisted by MB and BS. SG designed the genetic study, performed GMP6A sequencing/data analysis, cell culture, nucleodetection and corresponding expression analysis. She was supported by CH. MB did the computational mRNA search. GF and RP gave input to data analysis, interpretation and literature citation. K-AN and HE initiated the project, designed the whole translational study and wrote the manuscript. HE and K-AN had full access to all the data of the study and take responsibility for data integrity and accuracy of data analysis.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)