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Generation and initial characterization of mice lacking full-length BAI3 (ADGRB3) expression

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Abstract

Brain-specific angiogenesis inhibitor 3 (ADGRB3/BAI3) belongs to the family of adhesion G protein-coupled receptors. It is most highly expressed in the brain where it plays a role in synaptogenesis and synapse maintenance. Genome-wide association studies have implicated ADGRB3 in disorders such as schizophrenia and epilepsy. Somatic mutations in *ADGRB3* have also been identified in cancer. To better understand the in vivo physiological role of *ADGRB3*, we used CRISPR/Cas9 editing to generate a mouse line with a 7-base pair deletion in *Adgrb3* exon 10. Western blot analysis confirmed that homozygous mutants ($Adgrb3^{\Delta 7/\Delta 7}$) lack full-length ADGRB3 expression. The mutant mice were viable and reproduced in Mendelian ratios but demonstrated reduced brain and body weights and deficits in social interaction. Measurements of locomotor function, olfaction, anxiety levels and prepulse inhibition were comparable between heterozygous and homozygous mutants and wild-type littermates. Since ADGRB3 is also expressed in organs such as lung and pancreas, this new mouse model will facilitate elucidation of ADGRB3's role in non-central nervous system-related functions. Finally, since somatic mutations

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CONFLICT OF INTEREST STATEMENT

All the authors declare no competing interests.

in *ADGRB3* were identified in patients with several cancer types, these mice can be used to determine whether loss of ADGRB3 function contributes to tumour development.

Keywords

ADGRB3; AGPCR; BAI3; Schizophrenia; seizure

1 | INTRODUCTION AND BACKGROUND

Brain-specific angiogenesis inhibitor 3 (BAI3/ADGRB3) is a member of the adhesion G protein-coupled receptor (ADGR) family, which is comprised of 33 receptors in humans.[1,2] ADGRB3 has a typical 7-transmembrane structure and a large extracellular N-terminus containing four thrombospondin type 1 repeats and a GPCR-autoproteolysis-inducing domain.[2-4] In humans, *ADGRB3* is mainly expressed in neurons and glial cells of the brain, with lower expression in skeletal muscle, lung, pancreatic β-cells and testes.[5] ADGRB3 was initially characterized as a putative anti-angiogenic factor[6,7] and subsequently shown to promote myoblast fusion.[8,9] The high level of *ADGRB3* expression in the brain prompted our characterization of its function in the central nervous system.[4] In the mouse brain, *Adgrb3* expression peaks at postnatal day 0 (P0), but it is stably expressed throughout adulthood.[6,10] *Adgrb3* is highly expressed in cerebellar Purkinje cells and excitatory neurons in the hippocampus and cortex of adult mice.[10] It is also highly expressed at postsynaptic elements[10-12] and has been demonstrated to interact with the reticulon 4 (RTN4) receptor,[13] neuronal pentraxins,[14] and complement-like proteins C1QL1–4.[15,16] In humans, single nucleotide polymorphisms (SNPs) and/or copy number variants (CNVs) in *ADGRB3* have been associated with schizophrenia,[17] addiction,[18] anxiety disorder,[19] and epilepsy.[20,21] Postmortem brain tissue from male patients with schizophrenia show reduced *ADGRB3* mRNA expression levels in the hippocampus.[22] Moreover, somatic mutations in *ADGRB3* have been identified in patients with several types of cancer including breast, lung and prostate, although whether these are driver or passenger mutations are unknown.[23] *ADGRB3* expression was also lower in human brain glioma specimens.[6]

Although SNPs and CNVs within *ADGRB3* have been associated with several neurological and psychiatric disorders, the in vivo role of ADGRB3 has only been examined within specific neuronal circuits and brain regions of mice. For example, deletion of *Adgrb3* in cerebellar Purkinje cells resulted in a deficit in motor learning as revealed by the horizontal optokinetic response assay, a cerebellum-dependent adaptive learning task.[16] In addition, while the deletion of *Adgrb3* in the olfactory bulb did not alter olfaction, appetite, or locomotion, it did prevent social olfactory learning.[24] To more broadly understand the role of ADGRB3 in the brain, we generated a mouse line with a 7-base pair (bp) deletion in *Adgrb3* exon 10 and examined heterozygous ($Adgrb3^{+/\Delta 7}$) and homozygous ($Adgrb3^{\Delta 7/\Delta 7}$) mutants for potential alterations in behaviour and seizure susceptibility.

2 | MATERIALS AND METHODS

2.1 | Animals

Recombinant Cas9 protein from *Streptococcus pyogenes* and a synthetic 19-mer (5′-AUAGUGUCUUGGUGACCU-3′) targeting a region of exon 10 (5′-CCCAGGTCACCAAGA-CACTATT-3′) of the mouse *Adgrb3* gene were microinjected into individual fertilized eggs from C57BL/6NCrl mice (Charles River, Strain: 027), and the resulting embryos were implanted into recipient pseudo-pregnant females. Genomic DNA of probable founder pups was sequenced in order to identify mice with mutations in exon 10. A founder pup with a heterozygous 7-bp deletion in exon 10, predicted to result in a frame-shift and stop codon (Figure 1A), was identified and selected for generation of the mutant line. To verify germline transmission, the founder mouse (F0 generation) was crossed with C57BL/6NCrl mice (Charles River, Strain: 027) to generate F1 heterozygous mutants ($Adgrb3^{+/\Delta 7}$). $Adgrb3^{+/\Delta 7}$ mutants were backcrossed for four generations to reduce the likelihood of off-target substitutions. $Adgrb3^{+/\Delta 7}$ mutants were then intercrossed to generate wild-type (WT), heterozygous ($Adgrb3^{+/\Delta 7)}$ and homozygous ($Adgrb3^{\Delta 7/\Delta 7}$) offspring. $Adgrb3^{\Delta 7/\Delta 7}$ mice were genotyped as previously described.[25] Mice were housed in groups of three to five on a 12-h light/dark cycle with standard laboratory rodent chow (Lab Diet, #5001) and water available ad libitum. All experiments were performed by investigators who were blinded to genotype and in accordance with the Emory University Institutional Animal Care and Use Committee (IACUC) guidelines and the *Basic & Clinical Pharmacology & Toxicology* policy for experimental and clinical studies.[26]

2.2. | Western blot

Mouse brain tissue was homogenized in lysis buffer (50 mM Tris–HCl [pH 7.4], 150 mM NaCl, 1% [v/v] NP-40, 1 mM EDTA and 10% [v/v] glycerol). Lysates were then centrifuged for 10 min at 13500 × g. The supernatant was collected and protein concentration was determined using a BCA assay (Thermo Scientific, 23225). Laemmli sample buffer (4X) (500 mM Tris–HCl [pH 6.8], 44.4% [v/v] glycerol, 4.4% sodium dodecyl sulfate, 0.02% bromophenol blue) containing β-mercaptoethanol (10 mM) was added into the supernatant. Protein lysates (10 μg) from each mouse were subjected to SDS-PAGE electrophoresis without pre-boiling using an 8% polyacrylamide gel, followed by transfer to PVDF membranes (Bio-Rad, 1620177). Blots were blocked with tris-buffered saline with 0.1% Tween 20 and 5% non-fat dry milk, followed by incubation with a custom-made (GenScript), antigen-purified polyclonal rabbit anti-mouse ADGRB3 C-terminal antibody (epitope: GRREVQDAFRCRLRNCQDPINADSSSSFPNGHAQIMTDFEKDVDIACRSVLHKDIGP CRAATITGTLSRISLNDDEEEKGTNPEGLSYSTLPGNVISKVIIQQPTGLHMPSMNELS NPCLKKENTELRRTVYLCTDDNLRGADMDIVHPQERMMESDYIVMPRSSVSTQPSM KEESKMNIGMETLPHERLLHYKVNPE, 1:3000) or a mouse anti-β-actin antibody (Santa Cruz Biotechnology, sc-69879, 1:3000) overnight. The membrane was subsequently probed with a rabbit HRP tagged secondary antibody (Thermo Scientific, 31460, 1:5000) for ADGRB3 or a mouse HRP tagged secondary antibody (Thermo Scientific, 31430, 1:5000) for β-actin and processed for chemiluminescence detection using ECL (Thermo Scientific, 34580) (n = 3 mice/genotype).

2.3. | Brain and body weight assessment

Male *Adgrb3*$^{\Delta 7/\Delta 7}$, *Adgrb3*$^{+/\Delta 7}$ and WT littermates (2-3 months old) were weighed and anaesthetised with isoflurane. Brains were harvested and immediately weighed (n = 4–9 mice/genotype).

2.4. | Behaviour assessments

Behavioural experiments were conducted on male *Adgrb3*$^{\Delta 7/\Delta 7}$, *Adgrb3*$^{+/\Delta 7}$ and WT littermates (3 to 5 months old). Behavioural experiments were performed with two separate cohorts of mice. Cohort 1 was tested for open field and novel object recognition, novel cage and three-chamber social interaction. Cohort 2 underwent prepulse inhibition and the buried food test. All behavioural experiments were video recorded, and videos were analysed with ANY-Maze Video Tracking System (Stoelting Co.).

2.5 | Open field and novel object recognition

Locomotor activity and anxiety levels were assessed by open field. Learning and memory was assessed by novel object recognition. The tasks were performed over a 4-day period as previously described.[27,28] The apparatus is comprised of opaque Plexiglas walls (60 cm × 60 cm × 60 cm). On day 1, each mouse was allowed to explore the empty apparatus for 10 min; the total distance travelled and the time spent in the centre were scored as a measure of locomotor activity and anxiety, respectively. On days 2 and 3, two identical objects (cube or sphere) were placed in the centre of the apparatus, and the experimental mouse was allowed 10 min to explore. On day 4, one of the objects from days 2 and 3 was replaced with a novel object (e.g., a sphere was replaced with a cube or vice versa). The objects and the location of the novel *versus* familiar object were counterbalanced. A discrimination ratio was calculated for each mouse (time exploring the novel object/(time exploring the novel object + time exploring the familiar object) (n = 8–9 mice/genotype).

2.6 | Three-chamber social interaction

Sociability and social discrimination were assessed using the three-chamber social interaction paradigm as previously described.[27,28] The experiment consisted of three 10-min trials. In Trial 1, the experimental mouse was placed in the centre chamber, with an empty cylindrical wire cage in the left and right chambers; the mouse was allowed to freely explore. In Trial 2, an age-matched male C57BL/6J mouse (stranger) was placed under one of the wire cages. Sociability was defined as the time spent exploring the stranger mouse *versus* the empty cage. In Trial 3, a second age- and sex-matched C57BL/6J mouse (novel mouse) was placed under the previously empty wire cage. As a metric of social discrimination, the time spent exploring the familiar *versus* novel mouse was calculated (n = 8 mice/genotype).

2.7 | Novel cage

Novel cage experiment was performed as previously described.[27,28] Each mouse was placed into a novel standard mouse cage (33 cm × 18 cm × 15 cm), and the time spent grooming, digging, rearing and circling were recorded over a 10-min period (n = 7–10 mice/genotype).

2.8 | Prepulse inhibition (PPI)

PPI is a measure of sensorimotor gating, the process by which the brain filters out irrelevant sensory stimulation. It is assessed by measuring the inhibition of the reflex response to a startle stimulus that occurs when a weaker stimulus (prepulse) precedes the startle stimulus. Each mouse was placed in a sound-attenuated chamber (San Diego Instruments). For the startle response, the mouse was exposed to auditory-evoked startle stimuli (120 dB, 20 ms), and its response was measured by a piezoelectric accelerometer. The mouse was then subjected to 12 startle trials (120 dB, 20 ms), 12 prepulse trials (20 ms white noise at 74, 78, 82 or 86 dB at 100-ms intervals) and 12 prepulse/startle trials (20 ms white noise at 74, 78, 82 or 86 dB at 100-ms intervals and 20 ms 120-dB startle stimulus). The startle and prepulse trials were randomized. The percent PPI was calculated [100 — (startle amplitude on prepulse–pulse trials/startle amplitude on pulse alone trials) × 100] for each experimental mouse (n = 11–13 mice/genotype).

2.9 | Buried food test

Olfactory function was examined using the buried food test as described previously.[27] Two days before test day, chocolate-flavoured pellets (Bio-Serv F05472-1) were introduced into the home cage of the experimental mice to allow for habituation to the novel and palatable stimulus. Mice were food-deprived 1 day before the test day. On the test day, chocolate pellets were buried in a random corner of a clean mouse cage. The latency for the experimental mouse to find and eat (grasp and bite) the chocolate pellets was recorded. A maximum feeding latency score of 900 s was assigned if the mouse did not feed within 15 min (n = 7–10 mice/genotype).[29]

2.10 | Seizure induction

Susceptibility to induced seizures was tested in 3- to 5-month-old male and female *Adgrb3*$^{\Delta 7/\Delta 7}$, *Adgrb3*$^{+/\Delta 7}$ and WT littermates.

2.11 | 6 Hz seizure induction

6 Hz psychomotor seizures were induced as previously described.[30-33] A topical analgesic was applied to the cornea (0.5% tetracaine hydrochloride) prior to seizure induction. Experimental mice were subjected to corneal electrical stimulation (3 s at 17 mA for males and 13 mA for females) using a constant current device (ECT Unit 57 800; Ugo Basile, Comerio, Italy). Behavioural seizures were scored on a modified Racine scale: RS0 = no abnormal behaviour; RS1 = immobile ≥ 3 s, RS2 = forelimb clonus, paw waving, RS3 = rearing and falling (n = 8–14 mice/sex/genotype).

2.12 | Flurothyl-induced seizures

Flurothyl seizure induction was performed as previously described.[28,33-35] Latencies to the first myoclonic jerk (MJ) and generalized tonic–clonic seizure (GTCS) were recorded (n = 7–9 mice/sex/genotype).

2.13 | Statistical analysis

Prism v 9.3.1 software (GraphPad, San Diego, California) was used for statistical analyses. A two-way ANOVA followed by Sidak's multiple comparisons test was used to compare sociability, social discrimination and PPI data between each genotype. For novel object recognition, a one-sample parametric t-test was used to compare the time spent with the novel object *versus* 50% chance. A one-way ANOVA followed by Tukey's multiple comparisons test was used to analyse brain/body weight measurements, the total distance travelled and total time spent in the centre of the open field, the latency to the MJ and GTCS during flurothyl seizure induction, the startle response and feeding latency. An unpaired nonparametric Mann–Whitney *U* test was used to compare Racine scores between each genotype after 6 HZ seizure induction. A chi-square test was used to compare the observed number of offspring of each genotype to the expected Mendelian ratio and sex ratio. All results are presented as mean ± SEM, and a p value < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Generation and characterization of *Adgrb3*$^{\Delta 7/\Delta 7}$ mice

To generate *Adgrb3*$^{\Delta 7/\Delta 7}$ mice, we used CRISPR/Cas9 to target exon 10. A founder mouse with a 7-bp deletion (CCCAGGT) in exon 10 (Figure 1A), predicted to result in a frameshift and premature stop codon, was identified and used to generate the mouse line by backcrossing to C57BL/6NCrl for four generations (N4 generation). Male and female *Adgrb3*$^{+/\Delta 7}$ mice at the N4 generation were intercrossed to generate heterozygous and homozygous mutants and WT littermates that were used for all experiments. Western blot analyses showed full-length ADGRB3 protein expression (~172 kDa) was absent in *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants (Figure 1B). Four additional proteins at ~100, 72, 48 and 27 KDa were also detected but were present in both *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants and WT littermates. Whether these represent non-specific bands or result from alternative transcripts requires further study. Nissl staining of brain sections did not reveal any gross structural abnormalities between WT littermates and *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants (Figure 1C). From a total of 31 litters (217 mice), offspring of each genotype was born in the expected 1:2:1 Mendelian ratio (Figure 1D), and an equal ratio of male and female pups was observed (Figure 1E). All genotypes were viable and fertile.

Adgrb3$^{\Delta 7/\Delta 7}$ mutants, followed for over 600 days, displayed no evidence of spontaneous cancer formation, suggesting that gene loss is not sufficient to initiate cancer (data not shown). Aged mice had no obvious visible defects except for the development of severe dermatitis starting at ~250 days in ~40% of the female *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants, which mandated their termination per IACUC guidelines. No age and sex-matched *Adgrb3*$^{+/\Delta 7}$ mutants or WT littermates developed the same severity of dermatitis as female *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants.

3.2 | *Adgrb3*$^{\Delta 7/\Delta 7}$ mice exhibit reduced brain and body weight

We compared brain and body weights of 2- to 3-month-old male mice of each genotype. The average brain weight of *Adgrb3*$^{+/\Delta 7}$ and *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants was significantly less

than WT littermates (*Adgrb3*$^{+/\Delta 7}$, $p < 0.05$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $p < 0.001$), and the average brain weight of *Adgrb3*$^{\Delta 7/\Delta 7}$ mice was significantly lower than *Adgrb3*$^{+/\Delta 7}$ ($p < 0.05$) (Figure 2A). The body weight of *Adgrb3*$^{\Delta 7/\Delta 7}$ mice was also significantly less than WT littermates ($p < 0.05$) (Figure 2B). However, brain/body weight ratios were similar across genotypes (Figure 2C).

3.3 | *Adgrb3*$^{\Delta 7/\Delta 7}$ mice exhibit deficits in social discrimination

We observed no statistically significant differences between *Adgrb3*$^{+/\Delta 7}$ and *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants and WT littermates in measurements of locomotor activity (Figure 3A), anxiety levels (Figure 3B,D), repetitive behaviours (Figure 3E), startle response and percent PPI (Figure 3F,G). All genotypes spent significantly more time exploring the novel object compared to 50% chance in the novel object recognition task (WT, $p < 0.001$; *Adgrb3*$^{+/\Delta 7}$, $p < 0.0001$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $p < 0.01$), suggesting intact long-term recognition memory (Figure 3C).

In the three-chamber social interaction task, all genotypes spent significantly more time exploring the stranger mouse than the empty cage in the “sociability” trial, demonstrating normal sociability (WT, $p < 0.05$; *Adgrb3*$^{+/\Delta 7}$, $p < 0.01$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $p < 0.001$) (Figure 4A). During the “social discrimination” trial, WT littermates and *Adgrb3*$^{+/\Delta 7}$ showed a significant preference for the novel mouse compared to the familiar mouse, demonstrating normal social discrimination (WT, $p < 0.05$; *Adgrb3*$^{+/A7}$, $p < 0.001$). In contrast, *Adgrb3*$^{\Delta 7/\Delta 7}$ mice did not show a statistically significant preference for the novel mouse, suggesting a deficit in social discrimination (Figure 4B). In the buried food test, the latency to find buried food was comparable across all genotypes (Figure 4C), demonstrating that the observed deficit in social discrimination in the *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants is unlikely to be due to olfactory dysfunction.

3.4 | Male *Adgrb3*$^{\Delta 7/\Delta 7}$ mice show a modest increase in susceptibility to flurothyl-induced seizures

In the 6 Hz paradigm, acute psychomotor seizures were induced via corneal stimulation,[36] and seizure severity was scored using a modified Racine scale as we previously described.[27] Racine scores were found to be comparable between all genotypes regardless of sex (Figure 5A,D), indicating there were no statistically significant differences in the severity of these behavioural seizures between genotypes. In the flurothyl seizure induction paradigm, acute seizures were induced via inhalation of flurothyl, a $GABA_A$ antagonist.[37] Male *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants displayed shorter average latencies to the first myoclonic jerk (MJ) ($P < 0.05$) (Figure 5B); however, the average latency to the first generalized tonic–clonic seizure (GTCS) was comparable between genotypes (Figure 5C). Regardless of genotype, there were no statistically significant differences in average latencies to the MJ or GTCS among female mice (Figure 5E,F).

4 | DISCUSSION

Multiple SNPs and CNVs in *ADGRB3* have been implicated in schizophrenia and epilepsy[17,20,21,38-40]; however, it remains unclear whether ADGRB3 plays a causal role

in these disorders. To date, functional analyses of identified SNPs have not been conducted and most CNVs include several adjacent genes. Similarly, somatic mutations were identified in *ADGRB3* in several cancers, including lung and ovarian,[23] but their significance as disease drivers have not been investigated. A recent study reported a consanguineous family in which two siblings harbored a homozygous intragenic duplication of *ADGRB3* introns 3–17. The probands had intellectual disability, cerebellar atrophy, psychosis, epilepsy and anxiety disorder.[41] The duplication was predicted to alter the reading frame and reduce *ADGRB3* expression via nonsense-mediated decay of the mRNA transcript. However, the authors were unable to directly examine mRNA levels since *ADGRB3* is almost exclusively expressed in the brain.[41] Thus, to better understand the clinical relevance of reduced *ADGRB3* expression, we generated a mouse line that constitutively lacks full-length *Adgrb3* gene expression. Western blotting with a C-terminal antibody confirmed that the homozygous mutants lack full-length ADGRB3 protein.

The deletion of *Adgrb3* had a modest impact on seizure thresholds, with the only observed statistically significant alteration being a reduction in the latency to the first flurothyl-induced MJ in male $Adgrb3^{\Delta 7/\Delta 7}$ mutants (Figure 5B). In addition to ADGRB3, the ADGRB subfamily also includes ADGRB1 (BAI1) and ADGRB2 (BAI2).[3,4] We previously demonstrated that male and female mice lacking full-length *Adgrb1* ($Adgrb1^{-/-}$) exhibit significantly increased susceptibility to flurothyl-and 6 Hz-induced seizures.[27] Seizure susceptibility has not yet been examined in $Adgrb2^{-/-}$ mutants.

With the exception of a deficit in social discrimination, we found that the $Adgrb3^{\Delta 7/\Delta 7}$mutants exhibited fairly normal behaviour. We previously reported that $Adgrb1^{-/-}$ mutants show deficits in both social discrimination and sociability.[27] While the novel object recognition task revealed deficits in recognition memory in $Adgrb1^{-/-}$ mutants, the performance of $Adgrb3^{\Delta 7/\Delta 7}$ mutants in this task was comparable to their WT littermates. When examined using the Morris water maze test, $Adgrb1^{-/-}$ mutants exhibited deficits in spatial learning and memory,[42] while normal performance was observed in $Adgrb2^{-/-}$ mutants.[43] $Adgrb2^{-/-}$ mutants also displayed a higher level of hippocampal neurogenesis and greater antidepressant-like behaviour as assessed by the forced swim test.[43]

The behaviour alterations observed with loss of each member of the ADGRB subfamily might reflect, in part, the impact on different associated G-protein independent signalling pathways. ADGRB1 is a phosphatidylserine mediated phagocytic receptor,[44,45] and the loss of ADGRB1 expression leads to reduced phagocytic uptake by glial cells and increased MDM2-mediated p53 degradation, both of which could contribute to the observed abnormalities in social behaviour and memory performance, and increased seizure susceptibility.[46-50] ADGRB2 inhibits vascular endothelial growth factor (VEGF),[51] which is known to stimulate adult hippocampal neurogenesis and promote antidepressant effects.[52] Therefore, the lack of ADGRB2 can lead to the activation of VEGF, which might be the underlying cause of the antidepressant-like behaviour in $Adgrb2^{-/-}$ mice.[43]

With the exception of the identified deficit in social discrimination, the performance of $Adgrb3^{\Delta 7/\Delta 7}$ mice was similar to WT littermates in the selected behavioural assays.

This result was surprising as ADGRB3 is associated with a wide range of psychiatric and neurological disorders and several cancer types.[4,6,14,17,20,22,23,41] It is possible that *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants might exhibit abnormalities in behaviours that were not examined in the current study. For example, mice lacking the ADGRB3 ligand, C1QL3,[15] display altered circadian activity,[53] and mice lacking C1QL1[15] exhibit impaired cerebellar motor learning[16] and reduced auditory brainstem response during audiometric testing.[54] Furthermore, we cannot exclude the possibility that the residual bands detected by Western blotting in *Adgrb3*$^{\Delta 7/\Delta 7}$ mice might represent functional ADGRB3 protein isoforms. Shorter N-terminal truncated forms of ADGRB1 were recently shown to arise from usage of an alternative promoter in intron 17 of *ADGRB1*.[55] However, these ADGRB1 isoforms did not rescue the neurological deficits found in mice lacking full-length ADGRB1.[42] Finally, it should be noted that a mild increase in seizure susceptibility was only observed in male *Adgrb3*$^{\Delta 7/\Delta 7}$ mice, suggesting possible sex effects. Additional studies using both sexes should be conducted to further explore the role for ADGRB3 in neurological and psychiatric disorders and to examine whether the loss of ADGRB3 might accelerate tumour formation in the context of other genetic alterations.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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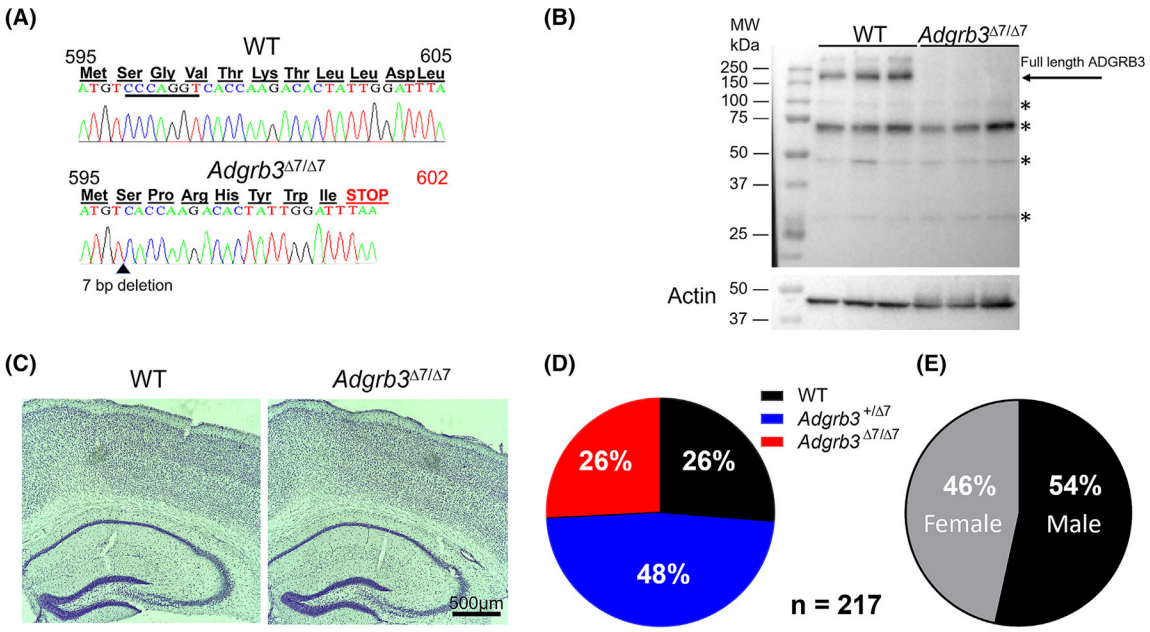

FIGURE 1.

Generation and characterization of *Adgrb3*$^{\Delta 7/\Delta 7}$ mice. (A) Exon 10 was targeted by CRISPR/Cas9. A 7-bp deletion in exon 10 was confirmed by Sanger sequencing. (B) Western blot shows the absence of full-length ADGRB3 protein (~172 kDa) in *Adgrb3*$^{\Delta 7/\Delta 7}$ mice. *Smaller ~100, 72, 48 and 27 kDa bands are observed in *Adgrb3*$^{\Delta 7/\Delta 7}$ mice and WT littermates. Whether these are non-specific or represent shorter isoforms of ADGRB3 remain to be determined. (C) Nissl staining of coronal brain sections show no obvious structural defects in *Adgrb3*$^{\Delta 7/\Delta 7}$ mice. (D) Matings between *Adgrb3*$^{+/\Delta 7}$ mice yielded offspring of each genotype in the expected Mendelian ratio and a similar ratio of males and females (E).

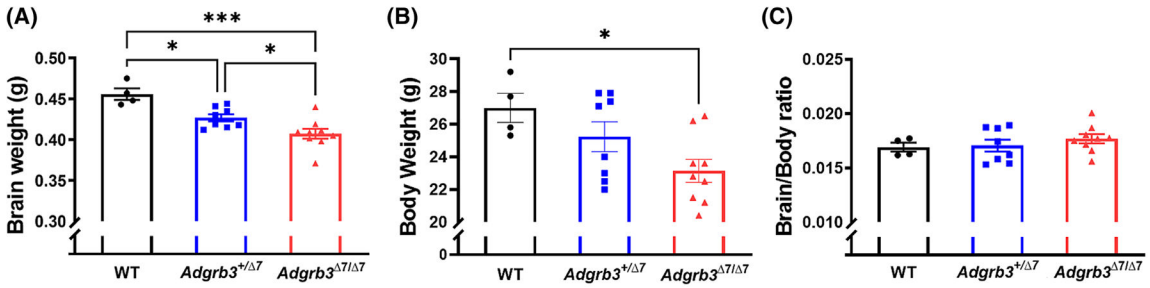

FIGURE 2.

Male Adgrb3$^{\Delta 7/\Delta 7}$ mice (2- to 3-month-old) exhibit lower brain and body weight. *Adgrb3*$^{\Delta 7/\Delta 7}$ and *Adgrb3*$^{+/\Delta 7}$ mice had lower brain (A) and body weight (B) compared to WT littermates. (C) Brain/body weight ratio was comparable across all three genotypes.WT, $n = 4$; *Adgrb3*$^{+/\Delta 7}$, $n = 8$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 9$. Mean ± SEM. *$p < 0.05$, ***$p < 0.001$.

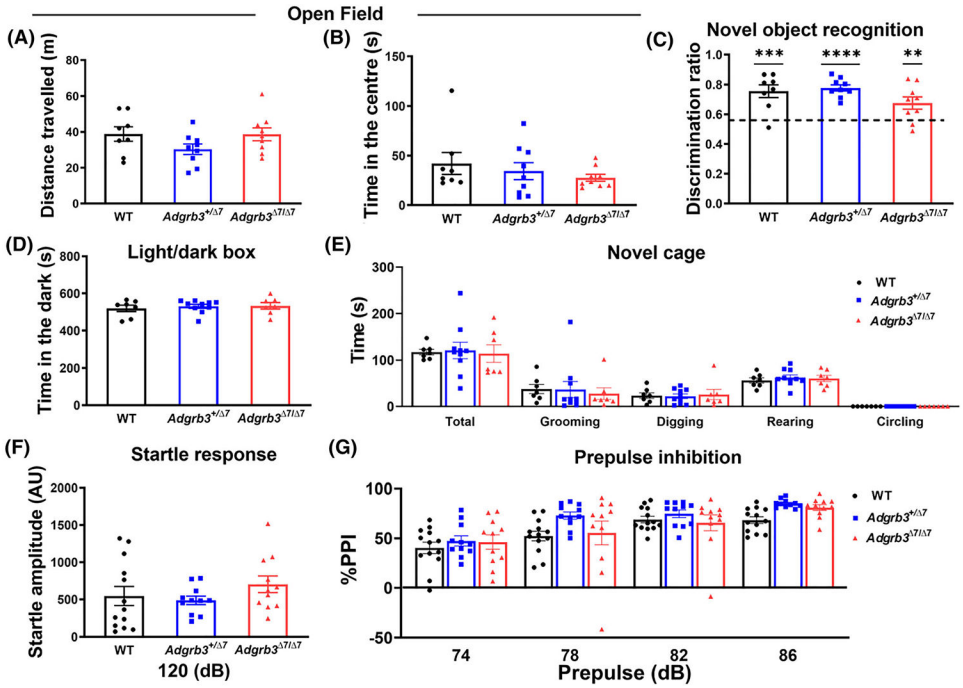

FIGURE 3.

Adgrb3$^{\Delta 7/\Delta 7}$ mice and WT littermates show comparable performance in open field, novel object recognition, light/dark box, novel cage paradigm and PPI. In the open field task, there was no significant effect of genotype on (A) total distance travelled and (B) total time spent in the centre of the open field. (C) All three genotypes spent significantly more time exploring the novel object, indicating intact object recognition memory. WT, $n = 8$; *Adgrb3*$^{+/\Delta 7}$, $n = 9$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 9$. (D) Comparable anxiety levels were observed across all genotypes as assessed by the light/dark box paradigm. (E) In the novel cage paradigm, no significant differences were observed between genotypes in the time spent grooming, digging, rearing and circling. WT, $n = 7$; *Adgrb3*$^{+/\Delta 7}$, $n = 10$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 7$. Mice of all three genotypes exhibited comparable (F) startle response and (G) prepulse inhibition. WT, $n = 13$; *Adgrb3*$^{+/\Delta 7}$, $n = 11$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 11$. Mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

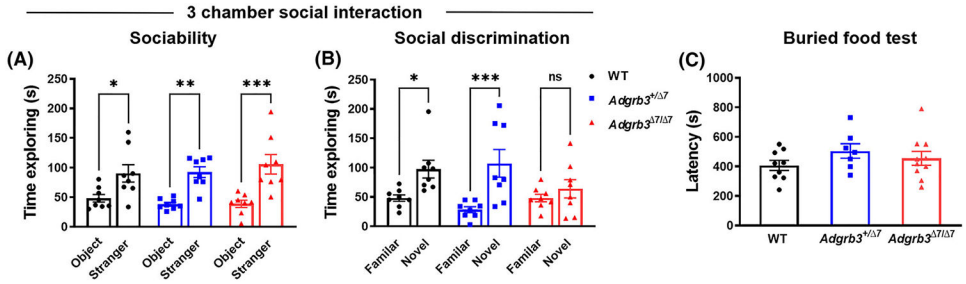

FIGURE 4.

Adgrb3$^{\Delta 7/\Delta 7}$mice exhibit deficits in social discrimination. (A) Mice of all genotypes spent more time exploring a stranger mouse than an empty cage (object), demonstrating intact sociability. (B) *Adgrb3*$^{\Delta 7/\Delta 7}$ mice failed to discriminate between a novel and a familiar mouse, indicating a deficit in social discrimination. WT, $n = 8$; *Adgrb3*$^{+/\Delta 7}$, $n = 8$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 8$. (C) The latency to find buried food was comparable across genotypes. WT, $n = 9$; *Adgrb3*$^{+/\Delta 7}$, $n = 7$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 10$. Mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

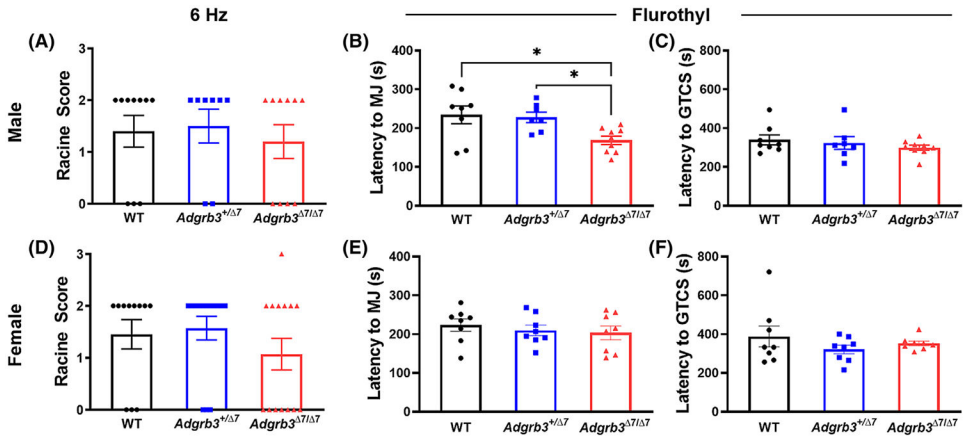

FIGURE 5.

A modest increase in susceptibility to flurothyl-induced seizures in male *Adgrb3*$^{\Delta 7/\Delta 7}$ mice. (A and D) Susceptibility to 6 Hz induced seizures was comparable between same-sex mice of all genotypes. Male: WT, $n = 10$; *Adgrb3*$^{+/\Delta 7}$, $n = 8$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 10$; female: WT, $n = 11$; *Adgrb3*$^{+/\Delta 7}$, $n = 14$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 14$. (B and E) Male, but not female *Adgrb3*$^{\Delta 7/\Delta 7}$ mutants exhibited shorter latencies to the first flurothyl-induced myoclonic jerk (MJ). (C and F) Male and female mice of all genotypes exhibited comparable latencies to the first generalized tonic–clonic seizure (GTCS). Male: WT, $n = 8$; *Adgrb3*$^{+/\Delta 7}$, $n = 7$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 9$; female: WT, $n = 8$; *Adgrb3*$^{+/\Delta 7}$, $n = 8$; *Adgrb3*$^{\Delta 7/\Delta 7}$, $n = 8$. Mean ± SEM. *$p < 0.05$.