Mutations in the Gabrb1 gene promote alcohol consumption through increased tonic inhibition

Quentin M. Anstee1,2,3,*, Susanne Knapp2,3,*, Edward P. Maguire4, Alastair M. Hosie5,*, Philip Thomas5, Martin Mortensen5, Rohan Bhome5, Alonso Martinez3,†, Sophie E. Walker6, Claire I. Dixon6, Kush Ruparelia7, Sara Montagnese7,†, Yu-Ting Kuo8,†, Amy Herlihy8, Jimmy D. Bell8, Iain Robinson9, Irene Guerrini10, Andrew McQuillin10, Elizabeth M.C. Fisher11, Mark A. Ungless8, Hugh M.D. Gurling10, Marsha Y. Morgan7, Steve D.M. Brown2, David N. Stephens6, Delia Belelli4, Jeremy J. Lambert4, Trevor G. Smart5 & Howard C. Thomas2,3

Alcohol dependence is a common, complex and debilitating disorder with genetic and environmental influences. Here we show that alcohol consumption increases following mutations to the γ-aminobutyric acidA receptor (GABAAR) β1 subunit gene (Gabrb1). Using N-ethyl-N-nitrosourea mutagenesis on an alcohol-averse background (F1 BALB/cAnN x C3H/HeH), we develop a mouse model exhibiting strong heritable preference for ethanol resulting from a dominant mutation (L285R) in Gabrb1. The mutation causes spontaneous GABA ion channel opening and increases GABA sensitivity of recombinant GABAARs, coupled to increased tonic currents in the nucleus accumbens, a region long-associated with alcohol reward. Mutant mice work harder to obtain ethanol, and are more sensitive to alcohol intoxication. Another spontaneous mutation (P228H) in Gabrb1 also causes high ethanol consumption accompanied by spontaneous GABA ion channel opening and increased accumbal tonic current. Our results provide a new and important link between GABAAR function and increased alcohol consumption that could underlie some forms of alcohol abuse.
Our understanding of the genetic and molecular basis of alcohol dependence is incomplete. Alcohol abuse has long been associated with facilitation of neurotransmission mediated by the brain’s major inhibitory transmitter, GABA, acting via GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs). Recently, a locus within human chromosome 4, containing GABA<sub>A</sub>R subunit genes encoding α2, α4, β1 and γ1 subunits has been associated with alcohol dependence in humans<sup>1</sup>-<sup>7</sup>. In particular, haplotypic variations in the GABRA2 gene encoding the α2 subunit have been repeatedly linked with alcohol dependence<sup>2</sup>-<sup>8</sup>,<sup>10</sup>-<sup>16</sup>. However, the neurobiological basis by which genetic variation translates into alcohol abuse is largely unknown.

Ionotropic GABA<sub>A</sub>Rs are pentameric ligand-gated ion channels, drawn from a family of 19 proteins, which underpins the expression of ~20–30 neuronal GABA<sub>A</sub>R isoforms<sup>11</sup>. These receptors have distinct physiological and pharmacological properties, are heterogeneously expressed in the mammalian CNS and as a consequence can differentially influence behavioural phenotypes<sup>12</sup>,<sup>13</sup>. Synaptic GABA<sub>A</sub>Rs mediate phasic inhibition, whereas extrasynaptic GABA<sub>A</sub>Rs are activated by ambient concentrations of GABA and mediate a tonic form of inhibition. Recent evidence has suggested roles for both forms of GABAergic transmission in the neurobiology of addiction<sup>14</sup>–<sup>17</sup>.

With regard to ethanol, both consumption and preference are reduced following disruption of GABA-mediated tonic inhibition in δ subunit knock-out (δ<sup>−/−</sup>) mice<sup>18</sup> and a similar impact on ethanol drinking was achieved by RNAi-induced suppression of either α4 (a subunit partner of the δ subunit) or δ subunit expression in the rodent nucleus accumbens (NAc)<sup>17</sup>,<sup>18</sup>. The reduced ethanol self-administration appeared to be a consequence of the altered reinforcing properties of the drug<sup>17</sup>,<sup>18</sup>. These actions on ethanol drinking and self-administration appear to be specific to the activity of α4βδ receptors and not a general effect on manipulating GABAergic activity in the NAc, as no differences in ethanol self-administration have been found in α2<sup>−/−</sup> or α5<sup>−/−</sup> mice<sup>19</sup>,<sup>20</sup>, when compared with wild-type (WT) counterparts.

It has been suggested that ethanol may exert a direct action on δ-GABA<sub>A</sub>Rs to enhance their function, which might account for the rodent self-administration data. However, whether ethanol exerts such direct effects is controversial<sup>21</sup>–<sup>24</sup>. An alternative interpretation of the behavioural data posits that the activity of NAc extrasynaptic GABA<sub>A</sub>Rs influences the activity of neural circuits underlying certain addictive behaviours such as the desire for alcohol. We were able to explore this possibility by exploiting the availability of two novel mutant mouse lines in which single point mutations in GABA<sub>A</sub>R β1 subunits have occurred. The first line was identified through a phenotype-driven N-ethyl-N-nitrosourea (ENU) mutagenesis screen<sup>25</sup>,<sup>26</sup> for alcohol-preferring mice, whereas the second was a spontaneous mutation identified using a genotype-driven approach<sup>27</sup>. Both mutant mouse lines display increased alcohol consumption and self-administration, and are characterized by GABA channels that can open spontaneously, promoting an increased tonic inhibition in NAc medium spiny neurons (MSNs). These findings strongly suggest that the large tonic conductance of MSNs contributes to the increased preference and intake of ethanol by Gabbr<sub>1</sub><sup>−/−</sup>L285R and Gabbr<sub>1</sub><sup>−/−</sup>P228H mice. These data reveal a novel link between GABA<sub>A</sub>R function and increased alcohol consumption that could lead to a better understanding of some forms of alcohol abuse.

### Results

**Alcohol-preferring mouse strains.** We identified two dominant mutations in Gabbr<sub>1</sub> that induced a phenotypic switch from alcohol aversion to a sustained, strongly heritable alcohol preference. One mutation was generated through phenotype-driven random ENU mutagenesis<sup>23</sup>,<sup>26</sup> (Gabbr<sub>1</sub>L285R), whereas the other was a spontaneous mutation (Gabbr<sub>1</sub>P228H<sup>11</sup>) identified through gene-driven screening of a DNA/sperm archive<sup>27</sup>. Both lines showed highly penetrant ethanol preference throughout over eight and five backcross generations, respectively.

An ENU-induced Gabbr<sub>1</sub> mutation confers alcohol preference: Alcohol-avere male BALB/CaNN mice were exposed to ENU<sup>25</sup> and crossed to WT C3H/HeH females. G1 progeny (n = 1,047) were screened in a two-bottle choice test for preference for 10% (v/v) ethanol with mice showing ethanol preference backcrossed to C3H/HeH to test heritability. The ENU-induced mutation was localized to a region on mouse chromosome 5 (71.45–73.05 Mb), syntenic with a region on human chromosome 4, containing 11 genes including Gabbr<sub>4</sub> and Gabbr<sub>1</sub> (Supplementary Fig. S1). Sequencing identified just one mutation in the β1 subunit (Gabbr<sub>1</sub> exon 8), a leucine-to-arginine exchange (L285R) in the highly conserved third transmembrane domain (M3), near the M2–M3 linker (Supplementary Fig. S2A), an important area for GABA receptor activation and ion channel gating. The mutation was absent in both parental strains.

A spontaneous Gabbr<sub>1</sub> mutation confers alcohol preference: To determine whether other Gabbr<sub>1</sub> mutations modified alcohol drinking, we screened a DNA library containing ~10,000 unique samples from ENU mutagenized male mice (F1 C57BL/6jxC3H/HeH) and identified one sample carrying a non-synonymous proline-to-histidine mutation (P228H) within M1 of the β1 subunit. This proline is highly conserved in GABA<sub>A</sub>Rs from various species (Supplementary Fig. S2B). While absent in both background strains, the mutation occurred in the non-ENU mutagenized C3H DNA strand and therefore had arisen spontaneously. Significantly, as with Gabbr<sub>1</sub>L285R, Gabbr<sub>1</sub><sup>−/−</sup>P228H mutants displayed a heritable ethanol preference (Supplementary Fig. S3).

Gabbr<sub>1</sub>L285R mutant mice were significantly smaller than WT (P < 0.0001; Supplementary Fig. S4). A similar but less pronounced size difference was also observed in Gabbr<sub>1</sub><sup>−/−</sup>P228H mutant mice. Both mutant lines exhibited complete female infertility characterized by underdeveloped corpus lutei. Consequently, we could only study heterozygotes (Gabbr<sub>1</sub>L285R<sup>+/−</sup> and Gabbr<sub>1</sub>P228H<sup>+/−</sup>) and their corresponding WT mice. Reduced body size and impaired fertility most likely reflect hypothalamic–pituitary–endocrine deficiency and so analysis of anterior pituitary hormone content was performed. This demonstrated that carriage of either mutant Gabbr<sub>1</sub> allele (Gabbr<sub>1</sub>L285R<sup>+/−</sup> or Gabbr<sub>1</sub>P228H<sup>+/−</sup>) was associated with significant hypothalamic–pituitary axis dysfunction (Supplementary Tables S1 and S2). Mutant mice displayed a significant preference for ethanol (Gabbr<sub>1</sub>L285R<sup>+/−</sup>; Fig. 1a,b; Gabbr<sub>1</sub>P228H<sup>+/−</sup>; Supplementary Fig. S3), but not for similarly presented sucrose, saccharin or quinine solutions, suggesting taste or caloric requirements were not driving alcohol preference (Supplementary Table S3). Furthermore, all mice consumed similar daily fluid volumes, despite Gabbr<sub>1</sub>L285R<sup>+/−</sup> and, to a lesser extent, Gabbr<sub>1</sub>P228H<sup>+/−</sup> mutant mice possessing lower body weights (Supplementary Fig. S4). The co-segregation of both alcohol preference and body weight traits were observed throughout all eight (for Gabbr<sub>1</sub>L285R<sup>+/−</sup>) and five (Gabbr<sub>1</sub>P228H<sup>+/−</sup>) generations studied, being present in 123/130 (93%) of Gabbr<sub>1</sub>L285R<sup>−/−</sup> mice, effectively excluding the possibility of separate mutations causing these components.
an increased sensitivity of Gabrb1 ethanol administration (Supplementary Fig. S6), these data suggest differed significantly between mutant and WT mice following neither the peak, nor time course of blood or brain alcohol levels over 30 min by WT and mutant mice on a fixed ratio 4 (FR4) schedule for sucrose–ethanol reinforcers. Note increased number of lever presses for mutants trend towards a more rapid recovery in WT mice (806 ± 448 s (mean ± s.e.m.); \( P < 0.05 \) (n = 8 per group, ANOVA plus Bonferroni post hoc test)).

Figure 1 | Alcohol consumption and behavioural phenotype of Gabrb1L285R. (a) Ethanol (10% v/v) preference of male Gabrb1+/-L285R (mean = 68.37%, 95% CI: 65.61–71.13, n = 46) and WT littermates (24.67%, 22.20–27.13, n = 44; t-test \( P < 0.0001 \)). (b) Daily ethanol consumption (g per kg body weight): male Gabrb1+/-L285R (10.16, 95% CI: 9.08–11.25, n = 46); WT littermates (12.4, 0.71–1.75; t-test \( P < 0.0001 \), n = 44). (c) Amounts of ethanol earned in operant tests (g per kg body weight ± s.e.m.). *\( P < 0.05 \) (n = 8 per group, ANOVA plus Bonferroni post hoc test). (d) Numbers of lever presses ± s.e.m. over 30 min by WT and mutant mice on a fixed ratio 4 (FR4) schedule for sucrose–ethanol reinforcers. Note increased number of lever presses for mutants at reinforcer mixtures of 7% sucrose/5% ethanol and 10% sucrose/10% ethanol (n = 8/group). *\( P < 0.05 \), ANOVA plus Bonferroni post hoc test.

Self-administration of ethanol over 1 h caused ataxia, consistent with alcohol intoxication (Supplementary Fig. S5A). To determine whether the features of intoxication observed in mutant mice during the 1 h operant sessions were due to increased intake or a heightened sensitivity to alcohol, WT and mutant mice were given a standard body-weight-adjusted dose of ethanol (3 g per kg) and loss of righting reflex (ethanol 3.5 g per kg; Supplementary Fig. S5). Although study groups were relatively small (n = 7–8), limiting statistical power, there was a strong tendency for the mutant mice to be more impaired than the WT mice (two-way ANOVA, main effect of genotype: \( F(1,13) = 4.46; \ P = 0.05 \), and to recover more slowly (two-way ANOVA, time point*genotype interaction: \( F(1,13) = 1.76; \ P = 0.08 \)). Genotype did not affect the rate of loss of righting reflex, but there was a trend towards a more rapid recovery in WT mice (806 ± 140 versus 1466 ± 448 s (mean ± s.e.m.); t-test, \( t = 1.41; \ P < 0.1 \)). As neither the peak nor time course of blood or brain alcohol levels differed significantly between mutant and WT mice following ethanol administration (Supplementary Fig. S6), these data suggest an increased sensitivity of Gabrb1+/-L285R mice to the ataxic effects of ethanol (Supplementary Figs S5B,C and S6).

To ensure ataxia did not affect performance, we analysed only the first 30 min of each operant self-administration session. Although rates of lever pressing declined with lower sucrose and higher ethanol concentrations (\( P < 0.001 \), two-way ANOVA n = 8 per group), these were still higher with 7/5% and 10/10% v/v sucrose/ethanol (Fig. 1c,d) in Gabrb1+/-L285R mice compared with WT (\( P < 0.01 \), two-way ANOVA n = 8 per group). Above 5% v/v ethanol, mutant mice worked harder to obtain ethanol, irrespective of the sucrose concentration, tending towards lower lever-pressing rates for unadulterated sucrose (\( P < 0.1 \), two-way ANOVA n = 8 per group). Thus, the Gabrb1L285R mutation is specific in affecting the motivation to consume alcohol.

Consistent with higher response rates, inter-response times (IRTs) were significantly shorter in the mutant mice (Supplementary Fig. S7). In order to obtain information on patterns of responding within a session, we analysed the pattern of IRTs within sessions. Interestingly, WT mice showed increases in IRTs as the session progressed, consistent with them satiating on alcohol. In contrast, the Gabrb1+/-L285R mice maintained their shorter IRTs (Supplementary Fig. S7). This pattern suggests that, in comparison with the WTs, their desire for alcohol decreased more slowly as they consumed alcohol.

Following the tests of alcohol self-administration, the mice were tested over two sessions in extinction (that is, lever pressing no longer resulted in fluid presentation). Their response rates declined when alcohol was no longer delivered following lever presses. The higher response rates maintained by Gabrb1+/-L285R mice during extinction sessions (Supplementary Fig. S8) were not significant, suggesting that there were no major differences in motivation to obtain ethanol under deprivation conditions. However, mice could not consume ethanol during extinction sessions and so any differences in rate of satiation to ethanol would not influence responding.

**Mutant GABA\(_A\)R β1 subunits increase NAc tonic inhibition.** The NAc is an important brain region for understanding the neurobiology of reward and addiction. Implicating GABA\(_A\)Rs, specific suppression of either the GABA\(_A\)R α4 or δ subunits in the NAc decreases both ethanol consumption and preference in rats\(^{16,17}\). We therefore determined how the β1 mutations affected GABA\(_A\)R function in NAc slices. Whole-cell voltage-clamp
Indeed, picrotoxin (100 μM) reduced GABAAR-mediated inward current induced by the agonist THIP, at a concentration sufficient to shut spontaneously open mutant Gabrb1<sup>+/−L285R</sup> MSNs, implying that the large tonic current was not caused by increased δ-GABA<sub>R</sub> expression (Table 1). In contrast to bicuculline (a partial negative allosteric modulator<sup>29–31</sup>) and gabazine, picrotoxin (100 μM) and gabazine, shuts spontaneously open mutant δ-GABA<sub>R</sub> channels<sup>29,32</sup>. Indeed, picrotoxin (100 μM), which is a non-competitive antagonist of GABA<sub>A</sub>R-gated chloride channels, when co-applied with bicuculline, produced an additional outward current selectively in Gabrb1<sup>+/−L285R</sup> neurons (Table 1; Fig. 2c), whereas gabazine (20 μM), which shares a common binding site with bicuculline, prevented the outward current induced by bicuculline (30 μM), but not by picrotoxin (100 μM; Table 1, Fig. 2d,e).

β<sub>1</sub>subunit mutations influence NAc phasic inhibition. The β<sub>1</sub> subunit mutations also affected GABA<sub>A</sub>-mediated phasic inhibition. For Gabrb1<sup>+/−L285R</sup> and Gabrb1<sup>+/−P228H</sup> MSNs the frequency of mIPSCs was reduced and their decay times prolonged. Additionally, the mIPSC amplitude was increased for Gabrb1<sup>+/−L285R</sup> relative to WT MSNs (Fig. 2h,i; Table 2), which was associated with an increased population of large amplitude mIPSCs, exhibiting slow decays. A scatter plot revealed a cluster of large amplitude (peak amplitude >105 pA), slowly decaying (T70 > 19 ms) events prevalent in Gabrb1<sup>+/−L285R</sup> MSNs constituting only 0.7% of the total number of events for WT MSNs, but 8.8% (>10-fold increase) of the Gabrb1<sup>+/−L285R</sup> MSNs (Supplementary Fig. S9). Conversely, the proportion of events with a peak amplitude < 105 pA and T70 < 19 ms decreased from 81.7% for WT to 54.2% for the Gabrb1<sup>+/−L285R</sup> MSNs. This loss is accounted for not only by the greater percentage of events with a peak amplitude >105 pA and T70 > 19 ms but, additionally, by a higher proportion of events with an amplitude >105 pA (T70 ≤ 19 ms) and of those with a T70 > 19 ms (but pA ≤ 105 pA) (Supplementary Fig. S9). Such events may originate from a population of mutant post-synaptic β<sub>1</sub>-GABA<sub>A</sub>Rs, with increased open probabilities compared with WT β<sub>1</sub>-GABA<sub>A</sub>Rs.

Both the L285R and the P228H mutations were associated with a reduced mIPSC frequency compared with WT counterparts (Supplementary Table S2). Activation by THIP of presynaptic δ-GABA<sub>A</sub>Rs, located either on accumbal interneurons (the main source of MSN somatic input) or on neighbouring MSNs, reduces GABA release onto MSNs (mIPSC frequency, control: 1.9 ± 0.2 Hz; +THIP 1 μM: 1.1 ± 0.2 Hz, data presented as mean ± s.e.m., n = 5; P < 0.05 paired t-test). Therefore, it is conceivable that pre-synaptically located spontaneously open

### Table 1 | NAc tonic conductance.

| Conditions | WT | Gabrb1<sup>+/−L285R</sup> | WT | Gabrb1<sup>+/−P228H</sup> |
|------------|----|----------------|----|----------------|
| I<sub>hold</sub> (pA) | 212 ± 24 | 284 ± 14 || 156 ± 12 | 296 ± 20 |
| n = 12 | n = 19 | n = 15 | n = 12 |
| RMS (pA) (control) | 4.3 ± 0.2 | 7.9 ± 0.2 | 3.9 ± 0.1 | 7.0 ± 0.2 |
| n = 12 | n = 19 | n = 15 | n = 12 |
| RMS (pA) (+ Bic) | 2.7 ± 0.2 | 4.4 ± 0.2 | 2.6 ± 0.1 | 3.5 ± 0.1 |
| n = 6 | n = 9 | n = 11 | n = 8 |
| Δ RMS (pA) (+ Bic) | 1.1 ± 0.2 | 3.7 ± 0.3 | 1.1 ± 0.1 | 3.7 ± 0.2 |
| n = 6 | n = 9 | n = 11 | n = 8 |
| I<sub>Bic</sub> (pA) | 22 ± 3 | 120 ± 8 | 22 ± 3 | 115 ± 9 |
| n = 6 | n = 11 | n = 11 | n = 8 |
| I<sub>Gabz</sub> (pA) | 17 ± 4 | 19 ± 3 | 16 ± 5 | 46 ± 10 |
| n = 5 | n = 4 | n = 4 | n = 3 |
| I (pA) Bic + Ptx | 27 ± 5 | 171 ± 8 | 20 ± 10 | 137 ± 26 |
| n = 4 | n = 4 | n = 3 | n = 3 |
| I (pA) Ptx after Bic | 4 ± 2 | 41 ± 8 | -5 ± 3 | 19 ± 6 |
| n = 4 | n = 4 | n = 3 | n = 3 |
| RMS (pA) (Bic + Ptx) | 2.9 ± 0.2 | 2.9 ± 0.2 | 2.6 ± 0.1 | 2.8 ± 0.1 |
| n = 4 | n = 4 | n = 3 | n = 3 |
| I<sub>THIP</sub> (pA) | 60 ± 15 | 41 ± 9 | ND | ND |
| n = 7 | n = 4 | ND | ND |

Bic, bicuculline; Gabz, gabazine; ND, not determined; Ptx, picrotoxin
Statistical significance of mutant relative to its WT: *P<0.05; **P<0.01; ***P<0.001.
Statistical significance of I<sub>hold</sub> relative to I<sub>Bic</sub>: *P<0.05; **P<0.001.
Statistical significance of Gabrb1<sup>+/−P228H</sup> relative to Gabrb1<sup>+/−L285R</sup>: P<0.05. (unpaired Student’s t-test used in all cases)
Data presented as mean ± s.e.m.
Figure 2 | β1 mutations influence tonic and phasic inhibition of MSNs. Whole-cell recordings from accumbal MSNs of Gabrb1<sup>+/L285R</sup> (a), Gabrb1<sup>+/P228H</sup> (b) and WT littermates. Note for both Gabrb1<sup>+/L285R</sup> and Gabrb1<sup>+/P228H</sup> MSNs the larger outward currents produced by bicuculline (30 μM). Scale bars apply to both a and b. (c) Outward currents induced by bicuculline (30 μM), gabazine (20 μM) and picrotoxin (PTX, 100 μM) from WT, Gabrb1<sup>+/L285R</sup> and Gabrb1<sup>+/P228H</sup> MSNs (means ± s.e.m.; n = 3–12). (d) Whole-cell recording from a Gabrb1<sup>+/L285R</sup> MSN. Gabazine (20 μM) abolishes the mIPSCs, but produces only a relatively small outward current. However, gabazine prevents the further outward current usually produced by bicuculline for this mutant, but not that produced by picrotoxin. (e) Mean outward current induced by GABA<sub>A</sub>R antagonists for Gabrb1<sup>+/L285R</sup> MSNs (means ± s.e.m.; n = 4). Whole-cell recordings from a Gabrb1<sup>+/P228H</sup> (f) and a WT MSN (g). Gabazine (20 μM) abolishes mIPSCs, but additionally produces only a relatively small outward current for both the Gabrb1<sup>+/P228H</sup> and WT MSNs. However, for the Gabrb1<sup>+/P228H</sup> MSN gabazine prevents a further outward current by subsequent bicuculline, but this antagonist does not prevent the outward current produced by picrotoxin. By contrast, for WT MSNs after gabazine both bicuculline and picrotoxin are inert. Superimposed averaged mIPSCs are shown for WT (black) and Gabrb1<sup>+/L285R</sup> (h), or Gabrb1<sup>+/P228H</sup> (i) MSNs (grey). Right-hand traces of each pair are normalized to the mean peak amplitude of the appropriate WT mIPSC. Note the prolonged mIPSCs for Gabrb1<sup>+/L285R</sup> and Gabrb1<sup>+/P228H</sup> over WT. Scale bars apply to both h and i. *P<0.05, **P<0.001 (unpaired Student’s t-test for mutants relative to WTs).
βL-GABAA Rs either on interneurons or neighbouring MSNs would similarly influence GABA release, thereby reducing MSN mIPSC frequency.

**Mutant β1 subunit expression and spontaneous channel opening.** To explore how the β1_1L285R and β1_2P228H mutations affected GABAA R function, we used heterologous expression of WT and mutant recombinant GABAA Rs in HEK293 cells. Immunocytochemistry was used to assess the expression levels of myc epitope-tagged WT and mutant β1 subunits. The expression levels of β1_1L285R along with γ2 and γ2L subunits, including enhanced green fluorescent protein (eGFP), revealed no differences either for cell surface or total overal fluorescence (Supplementary Fig. S10). Similarly, the cell surface membrane and intracellular expression levels for β1_2P228H were also unaltered compared with WT β1 subunits (Supplementary Fig. S11).

To examine whether the β1 subunit mutations (L1285R and P228H) altered GABAA R receptor physiology and pharmacology, whole-cell recording was performed on α2β1_1L285R,2 and α2β1_2P228H,2 receptors expressed in HEK293 cells. Relative to WT, both mutations reduced the maximum current density induced by saturating GABA concentrations (1 mM; Fig. 3a).

As native GABAA Rs contain two β subunits and because our in vivo studies necessarily used heterozygous mice, a proportion of native GABAA Rs could comprise a mixture of WT and mutant β1 subunits. To examine the amplitudes of GABA-evoked currents under these conditions, we recreated a binomial mixture of GABAA Rs in HEK293 cells by co-expressing α2 and β1 with either β1_1L285R or β1_2P228H and γ2 subunits in an equimolar ratio, thereby reproducing native receptor isoforms likely to be present in heteroygotes. Assuming receptor subunit assembly proceeds according to binomial probabilities, we would expect a mixture of pantemeric receptors (2α:2β:1γ) to include: WT, full mutant (both β1 subunits are mutated) and two forms of partial mutant receptors containing only one copy of the mutant β1 subunit. The maximum current densities induced by saturating concentrations of GABA (up to 1 mM) were larger for receptors containing only one copy of the mutant β1 subunit than those observed with full mutant receptors, and this approached that of WT receptors (Supplementary Fig. S12).

GABA concentration–response curves revealed that GABA potency was increased (~2–3-fold) by β1_1L285R and β1_2P228H (Fig. 3b,d; Supplementary Table S4). Significantly, the holding current (at − 40 mV) was greater for many mutant receptor-expressing cells with WT (Supplementary Table S4), indicative of spontaneous GABA channel activity. Indeed, picrotoxin (100 µM) induced outward currents in the absence of GABA for cells expressing β1_1L285R, and to a lesser extent for β1_2P228H mutant receptors, reflecting spontaneous channel activity (Fig. 3c). The spontaneous current revealed by picrotoxin (I_pTX) accounted for ~3–15% of the total current ( = I_GABA,max + I_pTX) for these mutant receptors.

We also examined the level of spontaneous current for α4β1γ2 receptors, with WT or mutant β1 subunits, as an alternative isoform that may populate synaptic and/or extrasynaptic sites. Maximal current densities and holding currents exhibited greater variability for the β1 mutants compared with WT (Supplementary Table S4), and α4β1_1L285R,2 exhibited a spontaneous current revealed by picrotoxin (Fig. 3c).

To examine the gating of GABA ion channels underlying the spontaneous current, we used outside-out patches from HEK293 cells expressing α2β1γ2, α2β1_1L285R,2 or α2β1_2P228H,2 receptors (Fig. 3e). Spontaneous channel activity was evident with mutant receptors, but absent in WT, and abolished by the GABA antagonist, bicuculline (50 µM), acting as a negative allosteric modulator29 in the absence of GABA. Activating WT α2β1γ2 GABA channels with 10 µM GABA induced single-channel currents that were indistinguishable from the spontaneous openings observed with the mutant receptors (Fig. 3e) and with only minor differences in open and shut time durations (Supplementary Table S5). Overall, β1_2P228H, but to a lesser extent, β1_1L285R caused spontaneous channel opening and increased receptor sensitivity to GABA when co-assembled with either α2 or α4 and γ2 subunits.

Given the importance of α and δ subunits for tonic current in NAc MSNs16,17,28 we explored whether β1 mutants also conferred spontaneous channel activity on δ-GABAA Rs. Using HEK293 cells, β1 subunits were expressed with either α2 (co-localized on same chromosome as β1, and expressed in accumbal synapses20), or α4 (co-expressed in the accumbens with δ forming extrasynaptic receptors)28. The β1_1L285R mutation increased the maximum current density to saturating GABA (300 µM) for both α2β1_1L285R,4 and α4β1_1L285R,4 receptors, compared with WT equivalents (α2/4β1δ), whereas β1_2P228H did not (Supplementary Fig. S13). Both β1 mutants increased GABA sensitivity by 3–5-fold at α2β1δ-GABA ARs (Fig. 4a, Supplementary Table S4), similar to the increased sensitivity for mutant α2β1γ2 GABA ARs (Fig. 3b), but only β1_1L285R affected α4β1δ receptor sensitivity to GABA (Fig. 4b).

In the absence of GABA, picrotoxin (100 µM) induced outward currents only for α2/4β1_1L285R,4δ GABA ARs (Fig. 4c,d), with α2/4β1_2P228H,4δ showing similar levels of activity to WT. Given that β1_2P228H conferred substantial spontaneous activity on δ-GABAA Rs, compared with β1_1L285R, the increased tonic currents recorded in MSNs of the NAc and caused by the β1 mutants are most likely due to the presence of α2/4β1_1L285R,4, α2/4β1_2P228H,4δ isoforms.

**Alcohol and GABAA Rs.** Although alcohol may directly modulate some GABAA Rs, this remains controversial21,22,55,36. Here ethanol (30–200 mM) co-applied with low concentrations (EC10) of GABA did not modulate α2/4β1γ2 GABA ARs containing either WT or mutant β1 subunits in HEK cells (Supplementary Table S4). Alcohol intoxication is associated with increased production of the endogenous neurosteroid

---

**Table 2 | Brain slice electrophysiology: synaptic transmission.**

|            | WT       | WT       | Gabbr1_1L285R | Gabbr1_2P228H |
|------------|----------|----------|---------------|--------------|
| Peak Amp. (pA) | 62 ± 4   | 88 ± 6** | 53 ± 2        | 64 ± 5       |
| Rise time (ms) | 0.5 ± 0.02 | 0.5 ± 0.02 | 0.6 ± 0.02 | 0.5 ± 0.02   |
| t_w (ms)     | 9.5 ± 0.6 | 14.4 ± 1.3** | 7.8 ± 0.5 | 10.9 ± 0.9*  |
| Frequency (Hz) | 2.1 ± 0.5 | 0.6 ± 0.1** | 2.9 ± 0.5 | 0.8 ± 0.02*** |

Statistical significance of mutant relative to the appropriate WT: *P<0.05; **P<0.01; ***P<0.001 (unpaired Student’s t-test).

Data presented as mean ± s.e.m.
allopregnanolone, an allosteric potentiator of GABA$_A$Rs. However, WT and $\beta_1$ mutant GABA$_A$Rs remained equally sensitive to potentiation by allopregnanolone (1 $\mu$M) and benzodiazepines (0.5 $\mu$M diazepam), and to inhibition by Zn$^{2+}$ (100 $\mu$M) or bicuculline (50 $\mu$M) (Supplementary Figs S14 and S15). Similarly, neither WT nor mutant mice showed no differences in preference for saccharin or quinine solutions, discounting taste as a driver for high alcohol consumption in the mutants. That Gabrb1$^{+/+}$/L285R mice worked harder than WT to obtain alcohol compared with sucrose solutions indicates that motivation for alcohol was also not driven by caloric requirement or taste. Importantly, mutants undertook this extra work despite possessing lower body weights than their WT counterparts. The mutant mice also showed greater sensitivity to the sedative/ataxic effects of ethanol, which together with increased consumption, contributed to an increased incidence of intoxication.

**Discussion**

ENU mutagenesis of alcohol-averse BALB/cAnN mice generated a stable line carrying a non-synonymous dominant mutation in Gabrb1 (L285R) with a strong phenotypic preference for alcohol. Given that the probability of a second ENU-induced mutation within a 5-Mb region is estimated to be $P < 0.002$, adopting a Poisson model method, and a Markov chain Monte Carlo method, the probability of a second functional mutation within the 1.6-Mb genomic region of interest is extremely remote and would continue to halve with each of the subsequent eight backcross generations that have been studied. This possibility has been further excluded by the absence of other exonic mutations following comparative sequencing across the candidate region. Importantly, the identification of a second, independent line with a spontaneous, non-synonymous single base-pair mutation (P228H) in the same gene that also consistently exhibits ethanol preference and similar phenotypic traits over the five backcross generations studied, provides further confirmation that the phenotype is caused by mutating Gabrb1.

Mutant and WT mice showed no differences in preference for saccharin or quinine solutions, discounting taste as a driver for high alcohol consumption by the mutants. That Gabrb1$^{+/+}$/L285R mice worked harder than WT to obtain alcohol compared with sucrose solutions indicates that motivation for alcohol was also not driven by caloric requirement or taste. Importantly, mutants undertook this extra work despite possessing lower body weights than their WT counterparts. The mutant mice also showed greater sensitivity to the sedative/ataxic effects of ethanol, which together with increased consumption, contributed to an increased incidence of intoxication.
Previous studies in man demonstrated significant allelic association between the risk of alcohol dependence and GABRA2 and GABRB1 polymorphisms. Although deleting Gabra2 in mice did not alter ethanol self-administration, and human polymorphisms and mouse mutations may lack common effects on receptor function, our studies are supportive of the view that the GABA<sub>R</sub> β1 subunit is a modifier of alcohol consumption.

The NAc is associated with reward and has an important role in addiction. Our recordings from MSNs within the accumbal core revealed that the β1 subunit mutations greatly impact upon both synaptic and tonic inhibitory transmission. For Gabrb1<sup>+/L285R</sup> and Gabrb1<sup>+/P228H</sup>, mIPSCs were prolonged and less frequent. Our previous demonstration that MSNs express synaptic α2-GABA<sub>R</sub>s, the changed mIPSC kinetics suggest that β1 subunits may co-assemble with α2 and γ2 subunits at inhibitory synapses. The GABA<sub>R</sub>-mediated tonic conductance was greatly increased in the NAc of Gabrb1<sup>+/L285R</sup> and Gabrb1<sup>+/P228H</sup> mice. The differential effects of GABA<sub>R</sub> antagonists suggest that this perturbation results from spontaneous activity of mutant β1-GABA<sub>R</sub>s, though increased receptor sensitivity to GABA and, for Gabrb1<sup>+/P228H</sup>, an increase in ambient GABA may also contribute.

Our recombinant receptor studies indicate that mutant β1 subunits efficiently co-assemble with α and γ or δ subunits and are functionally expressed at the cell surface, with minimal effect on their pharmacological properties. Nevertheless, both β1<sup>P228H</sup> and β1<sup>L285R</sup> mutations increased receptor sensitivity to GABA and caused their channels to open spontaneously in the absence of GABA, particularly for L285R. Modelling the likely subunit combinations of synaptic α2- and extrasynaptic δ-containing GABA<sub>R</sub>s found in accumbal MSNs, we recreated recombinant equivalents of extrasynaptic (α2/β1δ) and synaptic (α2/β1γ2) type receptors. Mutating the β1 subunit initiated spontaneous GABA channel opening, with β1<sup>L285R</sup> exhibiting a greater degree of spontaneous activity, implying that both α2β1<sup>L285R</sup>γ and α2β1<sup>L285R</sup>δ receptors are the most significant contributors to the increased spontaneous (tonic) current in the NAc of the Gabrb1<sup>+/L285R</sup> mouse. In comparison, β1<sup>P228H</sup> GABA<sub>R</sub>s were less spontaneously active, but such activity was nevertheless clearly evident for α2β1<sup>P228H</sup>γ2.

Recombinant GABA<sub>R</sub>s (α2/α4β1γ2, α2/α4β1δ) incorporating either WT or mutant β1 subunits were consistently insensitive to ethanol. Similarly, neither tonic nor phasic inhibition of WT and β1 mutant MSNs was affected by ethanol. Although low ethanol concentrations (∼10–30 mM) have been reported to enhance neuronal and recombinant δ-GABA<sub>R</sub> function, other studies have failed to corroborate such effects.

Thus, increased accumulant tonic current is associated with β1 subunit mutations leading to increased ethanol consumption, but not as a consequence of direct effects of ethanol on GABA<sub>R</sub>s. Notably, both ethanol preference and consumption are reduced in δ<sup>−/−</sup> mice, a genetic manipulation that reduces accumulant tonic currents. The importance of the tonic current for ethanol reward is emphasized by RNAi knockdown of either α4 or δ subunits in the accumbens, which reduced ethanol consumption and preference without affecting water or sucrose intake. Collectively, this strongly suggests that the large tonic conductance of MSNs contributes to the increased preference and intake of alcohol by Gabrb1<sup>+/L285R</sup> and Gabrb1<sup>+/P228H</sup> mice.

Our study identifies GABA<sub>R</sub>s containing the β1 subunit as a key element in modulating alcohol consumption and suggests that spontaneous GABA channel opening and increased tonic inhibition in the accumbens are critically important factors in this debilitating behavioural phenotype that is so costly to individuals and society.
Methods

Mice. Mice were housed under standard conditions with a commercial diet (SDS, UK) and drinking water ad libitum. Using alcohol-averse background strains, 12-week-old male BALB/cAnN mice were exposed to ENU\(^2\) and crossed to WT C57BL/6J females. G1 progeny (n = 1,047) were screened in a two-choice test for preference for 10% (v/v) ethanol at age 7–12 weeks. Mice showing ethanol preference (>2.5 s.d. above a control non-mutagenized BALB/cAnN or C3H/HeH cohort) were backcrossed to 8–12-week-old C57Bl/6J females to test heritability. Our study was approved by the local ethical review panels of the MRC Mammalian Genetics Unit (MRC Harwell), the University of Dundee and the University of Sussex, and complies with the UK Animal (Scientific Procedures) Act 1986.

Phenotyping. Male and female 7–12-week-old adult mice were singly housed with free choice of water or ethanol (3 or 10% v/v) during two 10-day test periods. Consumption was determined by weighing the drinking bottles. The amount consumed was corrected for leakage and evaporation and expressed as grams ethanol consumed daily per kg body weight of the mice measured at the beginning of each period. Preference was calculated as the ratio of ethanol over total amount of liquid imbibed. Taste preference was determined similarly using 15 and 120 mM sucrose solution for 24 h and 60 mM sucrose (no calories) and 0.05 mM quinine. For self-administration of ethanol studies, animals (n = 8 group) were trained using a sucrose-fading procedure to self-administer up to 10% v/v ethanol\(^5,\)\(^6,\)\(^7\). Intoxication was scored as normal, mild ataxia or ambulatory impairment, upon removal of the animals from the operant boxes at the end of daily sessions.

Mapping of ENU Mutation. A genome scan was performed on 13 animals (2 G2, 11 G3) displaying high ethanol preference using 86 microsatellite markers distributed throughout the genome to differentiate DNA of BALB/cAnN or C3H/HeH origin. A further 169 animals were used to identify informative recombinants. Fine mapping with additional microsatellite markers in the candidate region was undertaken to narrow the location of the mutation.

Candidate gene sequencing. Exons and the exon/intron borders of twelve candidate genes were sequenced from both directions in mutant and WT mice. Oligonucleotides were designed using Ensembl v36 predictions (http://ensembl.org). Data were analysed with BioEdit software. Primer sequences can be found in Supplementary Table S6.

Electrophysiology and analysis of brain slice preparations. Coronal slices (300 μm) containing the NAc were prepared from male Gabra\(_2\)\(^{-}\)\(^{-}\)\(^{-}\)//Gabra\(_{2}\)\(^{-}\)\(^{-}\) (n = 12 mice) and WT littermates (age 2–5 months). As previously described\(^14\), slices were cut in oxygenated ice-cold maintenance solution containing (mM): 140 Kglutamate, 15 NaCl, 4 NaHCO\(_3\), 0.8 KPO\(_4\), 1.25 NaHPO\(_4\), 2 CaCl\(_2\), 10 glucose and 2 MgCl\(_2\). Solutions were equilibrated with 5% CO\(_2\) in 95% air for 1 h before experiments (pH 7.4). The slices were maintained at 32–33°C in a humidified chamber with warm 5% CO\(_2\) in 95% air (pH 7.2–7.3 with NaOH, 300–308 mOsm). Slices were transferred to the recording chamber (37°C) and equilibrated for 1 h. Whole-cell patch-clamp recordings from the dorsal striatum were obtained using an Axopatch 200B amplifier with patch pipettes (9–11 MΩ for Gabra\(_2\)\(^{-}\)\(^{-}\)\(^{-}\)//Gabra\(_{2}\)\(^{-}\)\(^{-}\) and 7–8 MΩ for WT) with 8-pole Bessel filter (36 dB per octave). Drugs and Krebs solution were applied using a modified U-tube. GABA, bicuculline, picrotoxin and ethanol were dissolved directly into Krebs solution (adjusted to pH 7.4). Allopregnanolone and diazepam were dissolved in dimethyl sulfoxide (DMSO). For whole-cell studies of ethanol potentiation, the GABA current evoked by a low GABA concentration (EC\(_{10}\)) was initially determined, then 30–200 mM ethanol was applied for 30–60 s prior to the co-application of EC\(_{10}\) GABA and ethanol. Allopregnanolone potentiation was determined by co-application with EC\(_{10}\) GABA and the neurosteroid. Dose–response relationship data were fitted with a non-linear least squares fitting routine using Origin 6.1 (Microcal). Data points represent the mean ± s.e.m. of at least three experiments. The relative proportion of spontaneous GABA\(_A\) receptor activation was assessed by dividing the amplitude of currents induced by saturating concentrations of PTX (100 μM) by the summed current amplitudes induced by saturating concentrations of PTOX and GABA (1× PTOX + 1× GABA + 2× I\(_{TOX}\)). Single-channel currents were recorded at room temperature from excised outside-out membrane patches maintained at –70 mV (sampling rate 50 kHz, low pass-filtered at 10 kHz during recording, and at 2–5 kHz during analysis). Patches showing multiple simultaneous channel openings (channel stacking) exceeding 5% of all detected openings were discarded.

Confocal microscopy and immunocytochemistry. Gabar\(_{2}\) receptor subunits were visualized. Confocal images of the GABA\(_A\) receptor expressed in HEK293 cells and incubated at room temperature with 9E10 antibody (to the extracellular myc epitope; Santa Cruz Biotechnology) followed by TRITC- or Cy5-conjugated secondary antibody. The myc epitope in the J2 subunit is electrophysiologically silent\(^4\). Transfected HEK293 cells were fixed in phosphate-buffered saline (PBS) containing 4% paraformaldehyde for 15 min before being quenched with 50 mM NH\(_4\)Cl in PBS for 10 min. After washing in PBS, cells were incubated for 45 min at room temperature with 9E10 antibody. Cells were washed and then mounted in glycerol before imaging using a Zeiss LSM510 Meta, confocal microscope. Data were collected and amplifier offset and gain were set at the same levels for all FITC, TRITC and Cy5 images of WT and mutant receptors to compare expression intensities. The scanning slice depth was set to 2.1 μm.

Statistical analysis. Analyses were performed using SPSS v14 and SigmaStat software (SPSS, USA). Data are presented as mean ± s.e.m. or 95% confidence intervals unless otherwise stated. Student’s t-tests were used for two group comparisons (paired or unpaired as appropriate). Multifactorial linear model analysis was used to investigate the effect of genotype on alcohol preference, correcting for generation, gender and weight. Two-way ANOVA, with the between-subject factor
References

1. Whiting, P. J., McKernan, R. M. & Wafford, K. A. Structure and pharmacology of vertebrate GABA receptor subtypes. Int. Rev. Neurobiol. 38, 95–138 (1995).

2. Edenberg, H. J. et al. Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. Am. J. Hum. Genet. 74, 705–714 (2004).

3. Enoch, M. A. The role of GABA(A) receptors in the development of alcoholism. Pharmacol. Biochem. Behav. 90, 95–104 (2008).

4. Parsian, A. & Zhang, Z. H. Human chromosomes 11p15 and 4p12 and alcohol dependence: possible association with the GABRB1 gene. Am. J. Med. Genet. 88, 533–538 (1999).

5. Porjesz, B. et al. Linkage disequilibrium between the beta subfamily of the human EEG and a GABRA receptor gene locus. Proc. Natl Acad. Sci. USA 99, 3729–3733 (2002).

6. Beck, B. H., Mukhopadhyay, N., Tai, H. J. & Weeks, D. E. Analysis of alcohol dependence phenotype in the COGA families using covariates to detect linkage. BMC Genet. 6(Suppl 1): S143 (2005).

7. Agrawal, A. et al. Association of GABRA2 with drug dependence in the collaborative study of the genetics of alcoholism sample. Behav. Genet. 36, 640–659 (2006).

8. Bauer, O. L. et al. Variation in GABRA2 predicts drinking behavior in project MATCH subjects. Alcohol Clin. Exp. Res. 31, 1780–1787 (2007).

9. Covault, J., Gelernter, J., Hesselbrock, V., Nellissery, M. & Kranzler, H. R. Allergic and haplotypic association of GABRA2 with alcohol dependence. Am. J. Med. Genet. B Neuropsychiatr. Genet. 129B, 104–109 (2004).

10. Dick, D. M. et al. The role of GABRA2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. Behav. Genet. 36, 577–590 (2006).

11. Olsen, R. W. & Sieghart, W. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol. Rev. 60, 243–260 (2008).

12. Girke, T., Schwarzer, C., Wiesendaler, A., Sieghart, W. & Sperr, G. GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101, 815–850 (2000).

13. Rudolph, U. & Mohler, H. Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annu. Rev. Pharmacol. Toxicol. 44, 475–498 (2004).

14. Boehm, 2nd S. L. et al. gamma-Aminobutyric acid A receptor subunit mutant mice: new perspectives on alcohol actions. Biochem. Pharmacol. 68, 1581–1602 (2004).

15. Engin, E., Liu, J. & Rudolph, U. alpha2-containing GABA(A) receptor: a target for the development of novel treatment strategies for CNS disorders. Pharmacol. Ther. 136, 142–152 (2012).

16. Nie, H., Rewal, M., Giller, T. M., Ron, D. & Janak, P. H. Extrasynaptic delta-containing GABA receptor in the nucleus accumbens dorsomedial shell contributes to ethanol intake. Proc. Natl Acad. Sci. USA 108, 4459–4464 (2011).

17. Rewal, M. et al. alpha4-containing GABA receptor in the nucleus accumbens mediate moderate intake of alcohol. J. Neurosci. 29, 543–549 (2009).

18. Mihalek, R. M. et al. GABA(A)-receptor delta subunit knockout mice have multiple defects in behavioral responses to ethanol. Alcohol Clin. Exp. Res. 25, 1708–1718 (2001).

19. Dixon, C. I., Walker, S. E., King, S. L. & Stephens, D. N. Deletion of the gab2 gene results in hypersensitivity to the acute effects of ethanol but does not alter ethanol self administration. PLoS One 7, e47135 (2012).

20. Stephens, D. N., Pistoivakova, J., Worthing, L., Attack, J. R. & Dawson, G. R. Role of GABAA alpha5-containing receptors in ethanol reward: the effects of targeted gene deletion, and a selective inverse agonist. Eur. J. Pharmacol. 526, 240–246 (2005).

21. Wallner, M., Hanchar, H. J. & Olsen, R. W. Enthaloh enhances alpha 4 beta 3 delta and delta 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc. Natl Acad. Sci. USA 100, 15218–15223 (2003).

22. Borghese, C. M. et al. The delta subunit of gamma-aminobutyric acid type A receptors does not confer sensitivity to low concentrations of ethanol. J. Neurosci. Exp. Ther. 316, 1360–1368 (2006).

23. Santhakumar, V., Wallner, M. & Otis, T. S. Ethanol acts directly on extrasynaptic subtypes of GABA receptor to increase tonic inhibition. Alcohol 41, 211–221 (2007).
radioimmunoassay reagents, performed by D. Carmignac (NIMR) and I. Huhtaniemi’s laboratory.

**Author contributions**
H.C.T., S.D.M.B. and E.M.C.F. conceived the research. The ENU screen was conducted by A.M., S.K., Q.M.A. and H.C.T. *In vivo* phenotyping and preference testing was conducted and overseen by S.K. and Q.M.A. Endocrine studies were performed by I.R. *In vitro* electrophysiology studies were conducted by A.M.H., P.T., M.M., R.B. and T.G.S. *In vivo* operant conditioning, ethanol kinetics and ataxia studies were performed by S.E.W., C.I.D. and D.N.S. *Ex vivo* brain slice electrophysiology studies were conducted by E.P.M., D.B. and J.J.L. Additional phenotyping, data/sample acquisition and scientific support was performed by Y.-T.H., A.H., M.A.U., J.D.B., S.M., K.R., H.M.D.G., A.M. and I.G. Data analysis and interpretation was performed by Q.M.A., S.K., T.G.S., D.N.S., E.P.M., D.B., J.J.L. and H.C.T. The manuscript was written and revised by Q.M.A., S.K., D.B., J.J.L., D.N.S., T.G.S. and H.C.T. All authors reviewed and approved the submitted manuscript. D.N.S., D.B., J.J.L., T.G.S. and H.C.T. are joint senior authors.

**Additional information**
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

**Competing financial interests:** The authors declare no competing financial interests.

**Reprints and permission** information is available online at http://npg.nature.com/reprintsandpermissions/

**How to cite this article:** Anstee, Q. M. et al. Mutations in the *Gabrb1* gene promote alcohol consumption through increased tonic inhibition. *Nat. Commun.* 4:2816 doi: 10.1038/ncomms3816 (2013).