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A population–specific *HTR2B* stop codon predisposes to severe impulsivity

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Impulsivity, describing action without foresight, is an important feature of several psychiatric diseases, suicidality and violent behaviour. The complex origins of impulsivity hinder identification of the genes influencing it and the diseases with which it is associated. Here we perform exon-focused sequencing of impulsive individuals in a founder population, targeting fourteen genes belonging to the serotonin and dopamine domain. A stop codon in *HTR2B* was identified that is common (minor allele frequency > 1%) but exclusive to Finnish people. Expression of the gene in the human brain was assessed, as well as the molecular functionality of the stop codon, which was associated with psychiatric diseases marked by impulsivity in both population and family-based analyses. Knockout of *Htr2b* increased impulsive behaviours in mice, indicative of predictive validity. Our study shows the potential for identifying and tracing effects of rare alleles in complex behavioural phenotypes using founder populations, and indicates a role for *HTR2B* in impulsivity.

Impulsivity is a broad term describing behaviour characterized by action without foresight, decreased inhibitory control and a lack of consideration of consequences[1]. Cognitive function, attention and responses to reward are factors that are thought to contribute to the trait of impulsivity. Although impulsivity can be an adaptive dimension of personality, intolerance for delay, disinhibition and the inappropriate weighting of contingencies are maladaptive[2]. The behavioural manifestations of impulsivity include suicide, addictions, attention deficit hyperactivity disorder (ADHD) and violent criminality[3], as well as antisocial personality disorder (ASPD), borderline personality disorder (BPD) and intermittent explosive disorder (IED). These behaviours and diagnoses, including impulsivity itself, are moderately heritable[4,5], indicating that it should be feasible to identify genes influencing them. Gene identification would also validate the idea that it is possible to deconstruct the multi-process origins of impulsivity. Still, studies demonstrating that genetic variation predicts impulsivity have been relatively sparse[6–11]. The fact that few genes influencing impulsivity have been discovered could reflect the complexity of the phenotype, the nature of the samples or the methodologies used.

To detect novel alleles that influence impulsivity, we studied severely impulsive Finnish criminal offenders and matched controls. This study had six components (as charted in Supplementary Fig. 1): resequencing and identification of putatively functional variants in severe impulsive probands from a founder population; association and linkage with impulsive behaviour; population genetics; evaluation of cognitive effects of the identified variant; gene expression and functionality; and animal studies.

Exon-centric sequencing was performed on fourteen genes involved in dopamine or serotonin function (the genes are listed in Supplementary Methods). Dysregulated activity of the monoamine neurotransmitters has been implicated in impulsivity both on a neuropharmacological basis and a genetic basis via gene knockouts and/or association studies with common functional variants. In rats, serotonin and dopamine interact in the control of impulsive choice, with differential actions in regions of the prefrontal cortex involved[12]. The spontaneous impulsivity of rats correlates with lower levels of dopamine D2 receptors in the nucleus accumbens, predicting liability to compulsive drug seeking and addiction[13]; also, in humans a reduction in D2 receptors, as well as a decrease in dopamine release, has been described in the ventral tegmental area of cocaine abusers[14]. The serotonin system has long been implicated in impulsivity[15,16] and, in particular, impulsive aggression and suicide. *Maoa* knockout mice have higher levels of monoamines and increased aggressive behaviour[17], and a functional variable number tandem repeat (VNTR) in the *MAOA* regulatory region (*MAOA-LPR*) moderates the effect of maltreatment on vulnerability to develop antisocial behaviour in humans[8,18]. It has been shown that a stop codon variant that produces complete deficiency of MAOA activity co-segregates with severe impulsivity[6]. Stress-modified associations with suicidality have been reported also for a polymorphism in the serotonin transporter (degenerate repeat polymorphic region *5-HTTLPR* in *SLC6A4*)[19,20].

Deep sequencing was recently successfully applied to gene identification in rare Mendelian disorders[21]. In the domain of complex disorders, sequencing revealed putatively functional alleles at a gene previously implicated by genome-wide association studies of type I diabetes[22]. Here we attempted to use sequencing to identify novel loci contributing to a non-Mendelian phenotype.

Sequencing Finnish impulsive subjects

Founder populations can increase power to detect effects of rare alleles. At autosomal loci, Finns are equally as diverse as other Europeans, yet a

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restricted number of founders and isolation have moulded the Finnish gene pool[23]. Many disease alleles are more abundant or unique to Finland and conversely some disease alleles common in other European populations are rare or nonexistent[23]. From the standpoint of identifying rare or uncommon alleles with roles in complex phenotypes, it is perhaps most important that Finnish ancestry seems to have reduced the genetic heterogeneity of various diseases. For seventeen Finnish disease alleles, 70% of disease chromosomes (and as many as 98% for some diseases) were attributable to a single allele[23].

Sequencing was conducted in 96 unrelated Finnish males with impulsive behaviour and an equal number of unrelated Finnish males free of psychiatric diagnoses (Supplementary Table 1 and Methods). Probands had ASPD, BPD or IED and were all violent offenders and arsonists who, because of the extreme nature of their crimes, underwent inpatient forensic psychiatric examination at the University of Helsinki at the time of their initial incarceration. ASPD and BPD share genetic risk for impulsive aggression[4], which is a central characteristic of both of these personality disorders. Impulsivity is also key to IED, described in the Diagnostic and Statistical Manual of Mental Disorders III-R (DSM-III-R) as a failure to resist aggressive impulses.

The 96 cases were selected for resequencing from a larger cohort of Finnish violent offenders comprising 228 cases on the basis that they had the highest Brown–Goodwin Lifetime Aggression scores: 23.7 (standard deviation (s.d.) $\pm$ 4.9) as compared to 8.1 (s.d. $\pm$ 4.9) in controls. Their higher scores were indicators of a life history of aggressive, violent and impulsive behaviour as behavioural manifestations of impulsive temperament. The 96 male controls were free of DSM-III-R Axis I and II diagnoses and matched for age, and were selected for sequencing for single nucleotide polymorphism (SNP) discovery from a larger control cohort comprising 295 individuals. As compared to controls, cases also had significantly higher impulsivity (action on the spur of the moment) scores on the Karolinska Scales of Personality ($P < 0.0001$)[24]. However, analysis was conducted on a behaviourally based phenotype, rather than a measure of temperament, because behaviour has repeatedly shown the strongest relationship to biological predictors, including genes. Genetic loci previously implicated in impulsivity include the *MAOA* stop codon linked to impulsive behaviour in one Dutch family[6], *5-HTTLPR* at the serotonin transporter, which predicts suicidality[19,20], and the dopamine transporter VNTR, which has been associated with ADHD[11]. Impulsive behaviour also can be predicted by neurotransmitters and endocrine factors, as illustrated by associations with brain serotonin turnover[25], testosterone levels and a gene–testosterone interaction[9]. Animal behavioural pharmacology, gene knockout and strain-difference studies all primarily rely on measured behaviour. By selecting the most phenotypically extreme probands for sequencing, we increased the probability that we would detect functional variants altering impulsivity. Clinical and criminal records, including evaluation of premeditation and spontaneity of crimes, were available for all cases.

Exonic and promoter regions (comprising 82 kb) were amplified in pools of 12 genomic DNAs and sequenced simultaneously at $80\times$ coverage on an Illumina Genome Analyser, as described in Methods. Sequencing allowed us to identify and accurately estimate frequencies of alleles (Supplementary Fig. 8 compares frequencies determined by sequencing and genotyping; correlation coefficient $r = 0.94$). Of 360 SNPs identified, 44% were known (National Center for Biotechnology Information (NCBI) Build128). Frequencies of novel SNPs ranged as high as 0.2. Within 37 kb of protein-coding DNA, 25 synonymous SNPs, of which 9 were novel, and 26 nonsynonymous SNPs (nsSNPs), were detected. Of a total of 22 nsSNPs confirmed by Sanger sequencing, 10 were novel.

Association of putatively functional SNPs

Four nsSNPs were predicted to be functional according to both SIFT (sorting intolerant from tolerant) and PolyPhen (polymorphism phenotyping): *TPH2* Pro206Ser (rs17110563), *DRD1* Ser259Tyr, *HTR2B*

Arg388Trp and *HTR2B* Q20*, a stop codon (Supplementary Table 5). These four nsSNPs were genotyped in male Finnish cases and controls. In a global test of association with an aggregate of potential susceptibility variants, these four putatively functional variants were twice as common in cases (13.0%) compared to controls (6.5%, $\chi^2 = 6.76$, $P = 0.009$; Supplementary Table 6). However, this global association was driven by *HTR2B* Q20*. Seventeen out of two-hundred and twenty-eight cases were heterozygous for *HTR2B* Q20* compared to 7/295 controls ($\chi^2 = 7.26$, $P = 0.007$; Supplementary Table 6), with an allele frequency in controls of 0.012. Eighty-nine pedigrees comprising family members of the violent offenders were collected and all were genotyped without pre-selection for phenotype or genotype, identifying eight *HTR2B* Q20* carrier families (Fig. 1 and Methods). Affected status was defined as presence of ASPD, BPD, or IED. The transmission disequilibrium test detected over-transmission of Q20* to affected offspring (McNemar $\chi^2 = 5.0$, $P = 0.025$). Similarly, among affected individuals, 6/7 had Q20* transmitted, and among unaffected individuals 10/14 did not have Q20* transmitted (Supplementary Table 7). From the cumulative binomial distribution, previously proposed for linkage of functional loci in families[26], the likelihood of 16/21 or more linked outcomes was 0.013.

The *HTR2B* gene is on 2q36.3-q37.1, a location implicated in early-onset obsessive compulsive disorder[27] and illicit substance abuse[28]. However, resequencing of *HTR2B* in these two studies yielded no functional variants[27,28]. 5-HT2B receptor function in the brain is mainly unknown; however, it has been shown that 3,4-methylene-dioxymethamphetamine (MDMA, commonly known as ecstasy) selectively binds and activates 5-HT2B receptors, inducing serotonin release in mouse raphe nuclei, leading to dopamine release in the nucleus accumbens and ventral tegmentum[29], and 5-HT2B agonists increase serotonin transporter phosphorylation[30].

HTR2B Q20* in humans

We assessed molecular functionality of *HTR2B* Q20* by using RNA and proteins extracted from lymphoblastoid cell lines, and in addition *HTR2B* expression was measured in multiple brain regions, including the frontal cortex, by quantitative polymerase chain reaction (qPCR; Methods). Q20* led to variable nonsense-mediated RNA decay and blocked expression of the 5-HT2B receptor protein (Fig. 2 and Methods). *HTR2B* is widely expressed in the adult human brain, and the frontal lobe is one of the regions where it is most highly expressed (Methods and Supplementary Fig. 13).

HTR2B Q20* is apparently exclusive to Finns. In >3,100 individuals representative of worldwide diversity, including the Human Genome Diversity Panel (Supplementary Table 8), one additional Q20* carrier was observed: a female with a Finnish surname and with alcoholism. Indicative of a common origin and founder population effect, Q20* was found on a single haplotype background (Supplementary Fig. 9), and in Finns who were likely to be non-admixed (Supplementary Fig. 2). Genetic subisolates have been identified within Finland, including families in Eastern Finland. Also, the Finnish population apparently was founded by two waves of migration: Eastern Uralic founders arrived 4,000 years ago, followed by Indo-European speakers 2,000 years later[23]. However, it is unlikely that the Q20* association is an occult admixture artefact because Q20* carriers are common across Finland (in Middle, Western and Eastern regions) (Supplementary Fig. 3), and cases and controls did not differ in Finnish ancestry (Supplementary Fig. 4 and Methods).

In the 17 violent offenders (from the case–control study) who carried Q20*, impulsivity had a strong role in their crimes. Although convicted for a variety of offences including homicide, attempted homicide, arson, battery and assault, 94% of their crimes were committed under the influence of alcohol. The crimes of the Q20* carrier probands occurred as disproportionate reactions to minor irritations and were unpremeditated, without potential for financial gain and recurrent. From court records up to an average age of 43, Q20* carriers had
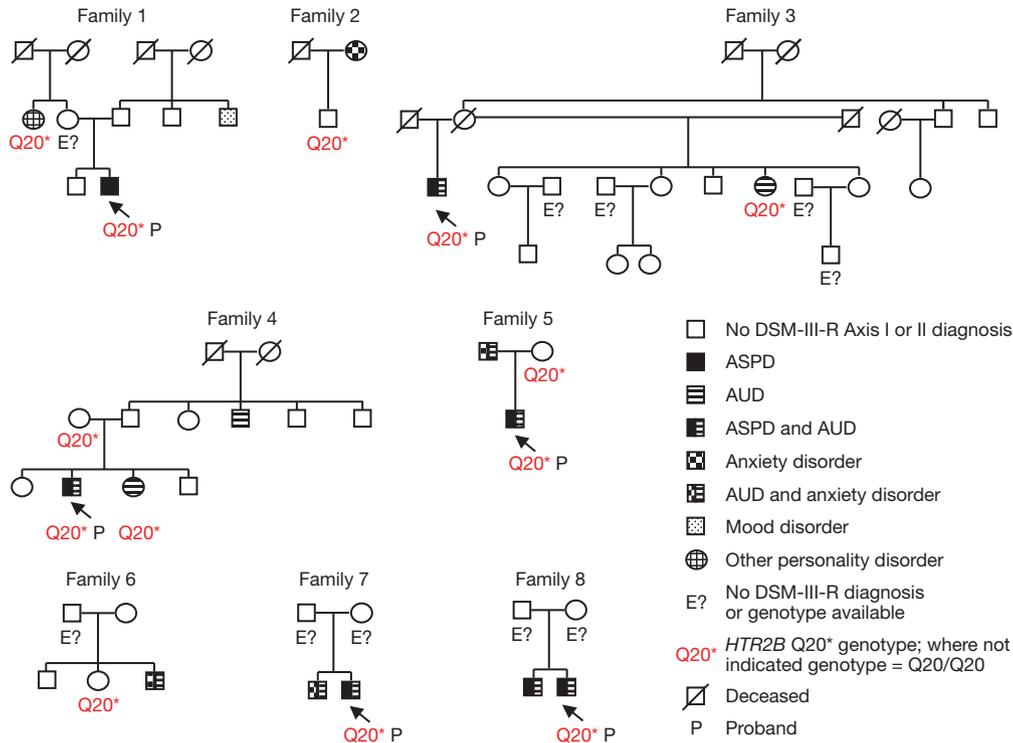
Figure 1 | *HTR2B* Q20* co-segregates with impulsivity. Co-segregation of *HTR2B* Q20* with ASPD and alcohol use disorder (AUD) in eight informative families.

committed an average of 5 violent crimes (range 2–12). The Q20* cases tended to fulfil the criteria for ASPD (82%) and IED (57% meeting 3 out of 4 IED criteria), except that alcoholism, ASPD and BPD are exclusory for IED. Overall, Q20* carriers were cognitively normal (mean IQ, 98; s.d., 14.9; range 75–124; two with IQ <87, Wechsler Adult Intelligence Scale).

In temperament—as measured by the Tridimensional Personality Questionnaire—Q20* carriers, like others with ASPD, score more highly in 'novelty seeking' and 'harm avoidance', but are otherwise more socially attached, empathic and dependent than the other violent offenders within the study group (Supplementary Data). Extrapolating from the Q20* frequency of 0.012 (and with 174 Q20* carriers directly
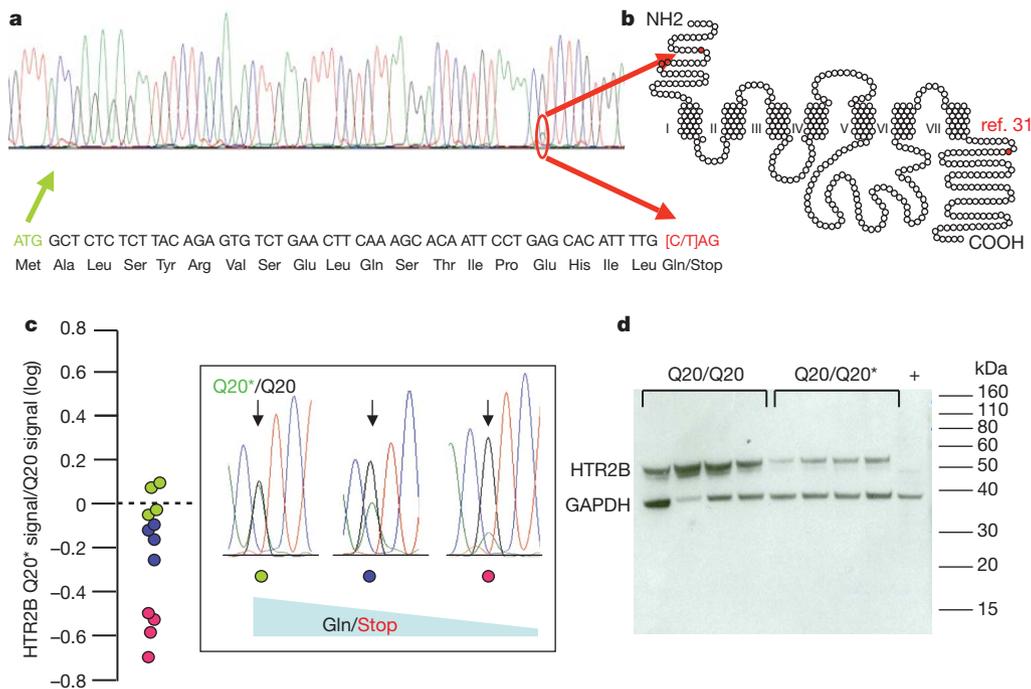

Figure 2 | *HTR2B* Q20* blocks protein expression. **a**, **b**, cDNA (**a**) and protein locations (**b**) of *HTR2B* Q20*. **b**, Labels I, II, III, IV, V, VI and VII refer to the seven transmembrane domains of the 5-HT2B protein and ref. 31 indicates the position in the 5-HT2B protein of a known, previously identified, *HTR2B* stop codon. **c**, Variable stop-codon-mediated RNA decay determined by cDNA sequencing of 12 Q20* heterozygotes. **d**, Q20*-mediated blockade of 5-HT2B protein expression in western blots (validated with three anti-5-HT2B antibodies; described in Methods). The 5-HT2B protein ratio was 1.93:1 in 14 Q20/Q20 homozygotes (mean, 1.78; s.d., 2.24) compared to 14 Q20/Q20* heterozygotes (mean, 0.92; s.d., 1.14) ($P = 0.03$) (Methods).

genotyped), 53,000 Finnish males (and as many females) are hetero-zygous. However, although few Q20* carriers are criminals, violent criminals with Q20* seem to represent some of the most impulsive individuals within our violent offender cohort. Among 100–155 homi-cides annually in the Finnish population of 5.3 million, there are few instances of multiple homicide. In our sample, only three individuals were convicted of multiple homicide, and all three carried the Q20* allele.

In our sample, the influence of Q20* was not due to interaction with MAOA or serotonin transporter genotypes (data not shown). However, it was not possible to rule out other gene interactions, or a modifying role of stress. Cerebrospinal fluid monoamine metabolite levels, another potential confounding factor, did not differ in Q20* carriers (Sup-plementary Data). Therefore, it is unlikely that their impulsivity was due to low turnover of serotonin, dopamine or norepinephrine or that Q20* substantially affects monoamine metabolism, as does the MAOA stop codon[6].

Risk conferred by Q20* seems to be modulated by sex and alcohol. Worldwide, suicide accounts for 1.5% of deaths, and Finland has a very high suicide rate, especially among men[32]. In our study, 70% of the Q20* male cases showed impulsive suicidal behaviour (for example, slashings, hanging attempts, drug overdoses) usually while intoxicated, for an average of 3.2 suicide attempts. At age 33.5 (s.d. ± 11), 66% had at least one life-threatening suicide attempt. It is unknown if suicide risk conferred by Q20* extends to the general population, whose mem-bers are at lower risk. Males are more likely to commit suicide[32] and to have ASPD and aggression, with a tenfold higher preponderance for the early-onset life-course-persistent variant of ASPD[33]. Moreover, alcohol-related violence is known to be higher among males, and the serotonin system is thought to contribute to individual differences in alcohol-facilitated impulsive aggression[34].

In the violent offender cohort, Q20* carriers were cognitively normal and in almost every instance acted out on their impulsivity only when inebriated. Having found the association of Q20* with impulsivity in a phenotypically extreme sample, it was important to define Q20* fre-quency and relationship to behaviour in the wider population, even though the only possible follow-up was in Finland. In >6,000 Finns ascertained epidemiologically (rather than from the criminal popu-lation), the Q20* allele frequency was 0.012 (the same as the frequency in controls) (Supplementary Table 9). We identified one Q20* homo-zygote, a young male adult with no major medical illness but with a history of violent behaviour while under the influence of alcohol (Supplementary Methods).

We followed up the cognitive effects of Q20* in 933 individuals in the FinnTwin12 and FinnTwin16 studies (22 with the stop codon) (Sup-plementary Methods). Overall, Q20* carriers were again cognitively normal. However, male (but not female) Q20* carriers had significantly lower Digit Span Forward ($P = 0.002$) and Backward ($P < 0.001$) scores, possibly indicating selective impairment in working memory (Supplementary Fig. 12), a specific measure of frontal cortical function.

Htr2b$^{-/-}$ mice

Although severe developmental consequences have been observed in Htr2b knockout mice, approximately 50% of the mice that survive the first postnatal week are apparently normal as adults[35]. These mice were reported to be impulsive in an open field novelty test[29]. We assessed Htr2b knockout mice for five separate measures of impulsivity and novelty seeking: delay discounting, activity in a novel environ-ment, exposure to a novel object, motor activity after a dopamine D1 receptor agonist, and decreased latency to eat in the novelty suppressed feeding test (hyponeophagia). The Htr2b$^{-/-}$ mice were more impulsive and more responsive to novelty in all of these tests (Fig. 3). In rats, both impulsivity and response to novelty are predictors for the development of addiction-like behaviours[36]. In addition to their differences in beha-viour, Htr2b$^{-/-}$ males had a threefold elevation in plasma testosterone (Fig. 3 and Supplementary Methods). Testosterone (measured in the

cerebrospinal fluid of nine heterozygous violent offenders) also seemed to be higher in human males carrying Q20* (Supplementary Fig. 11). This raises the possibility of an interaction between Q20* and testoster-one contributing to impulsive behaviours, as was reported between MAOA and testosterone in the same population of Finns that we studied here[9].

Discussion

The aim of this study was to identify genetic variation associated with impulsivity, an intermediate phenotype thought to contribute to several psychiatric disorders including addictions[12]. The goal is to track shared genetic factors in these diseases and to contribute to their reconceptua-lization on a neurobiological basis. Another purpose of identifying genes influencing impulsivity is to determine which of the potential aetiologies and types of impulsivity, for example novelty seeking versus executive dysfunction[36], are important in human populations. The dis-covery of genes influencing impulsive behaviour would validate the idea that it is possible to deconstruct the multi-process origins of impulsive behaviour.

HTR2B Q20* is associated and co-segregates with disorders char-acterized by impulsivity, reflected in severe crimes committed on the spur of the moment—as documented by criminal and clinical records—and under alcohol intoxication, a condition where impulse control is impaired. Thus, the Q20* allele can be regarded as one determinant of behavioural variation. However, the presence of Q20* was not in itself sufficient: male sex, testosterone level, the decision to drink alcohol, and probably other factors such as stress exposure, all have important roles. Although relatively common in Finland, HTR2B Q20* is unlikely to explain a large fraction of the overall variance in impulsive behaviours. There are likely to be many pathways to impulsivity in its various manifestations, and the genetic association may be present only in the most phenotypically extreme.

It is unsurprising that a stop codon variant discovered by sequen-cing within a founder population is common only in it, and even restricted to it. However, this observation is also in line with the significance of Q20* as a complete loss of function variant, and with the behavioural consequences in some heterozygous carriers. The relatively high frequency of Q20* in Finns would thus reflect its status as a founder mutation, in contrast with MAOA, COMT and SLC6A4 (previously known as HTT) alleles that are common worldwide, more modestly affect molecular function, and may have counterbalancing selective advantages. However, it is highly unlikely that Finns are unique in possessing a severe genetic variation leading to impulsivity. There is the previous example of the MAOA stop codon found in one Dutch family. On average, ten or more heterozygous stop codons reside in the genomes of each individual of European ancestry[21], but perhaps because the source populations from which the probands were sequenced did not have founder characteristics, no common stop codon had yet been reported for a neurotransmitter gene. Although rare variants identified in founder populations are more likely to be confined to those populations, analyses of the relationship between gene variation and phenotype can be conducted within the founder population, identifying new candidate genes and pathways influencing behaviour or other aetiologically complex phenotypes.

As has often been illustrated, the availability of mouse genetic models, including gene knockouts, offers an opportunity to test the predictive validity of genetic discoveries and to define effects in contexts where genetic background and environment are better controlled. The Htr2b mouse knockout reveals more general effects of 5-HT2B deficiency on behaviour, including effects on novelty seeking. This could be explained by pleiotropic actions of the serotonin 2B receptor. On the other hand, the effect of the Htr2b knockout on delay discounting seems to validate the effect of the Q20* stop codon on impulsivity in people. In people, we observed a significant association between the HTR2B Q20* variant and impairment in working memory, a neurocognitive process contributing or predictive of executive cognitive function. The ability to store and
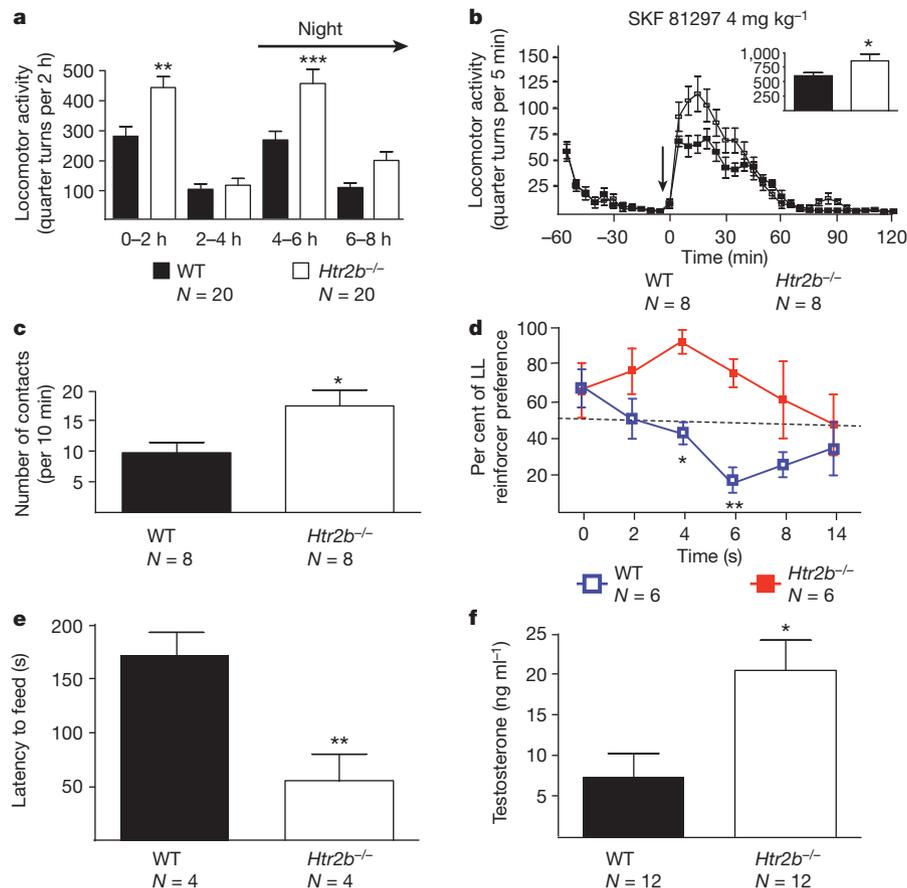
Figure 3 | Increased impulsivity and novelty seeking in *Htr2b*$^{-/-}$ mice.
a, b, Increased locomotor response of *Htr2b*$^{-/-}$ mice to environmental novelty (**a**) and to a dopamine D1 receptor agonist (SKR 81297) (**b**). WT, wild type. **c**, Increased number of contacts of *Htr2b*$^{-/-}$ mice with a novel object. **d**, Increased delay discounting of *Htr2b*$^{-/-}$ mice. LL, large and late hole, nose pokes leading to delivery of a larger but later reward. **e**, Reduced hyponeophagia in 18-h starved Htr2b$^{-/-}$ mice. **f**, Male *Htr2b*$^{-/-}$ mice have threefold higher plasma testosterone levels as compared to control mice. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Error bars are data $\pm$ standard error.

integrate knowledge about possible choices with the current context enables the individual to select appropriate cognitive strategies and generate optimal reactions. This is coherent with the impulsivity observed in *HTR2B* Q20* cases, who seemed deficient in the ability to weigh the consequences of their acts.

The use of deep sequencing to detect a stop codon associated with impulsivity in a founder population reveals a role for the *HTR2B* gene in behaviour. It also indicates that this approach may be applicable to other complex behavioural traits, including those diseases for which impulsivity is itself an intermediate phenotype.

METHODS SUMMARY

Fourteen serotonergic and dopaminergic genes were resequenced (Solexa GA2) in 96 Finnish Caucasian male violent offenders and 96 matched controls free of psychiatric diagnoses. Exon-centric sequencing was performed by amplifying 108 regions, for a total of 82 kb, in pools of 12 subjects. *HTR2B* Q20* was genotyped in a Finnish sample of 228 cases and 295 controls, in 89 Finnish families, and in 5,684 individuals belonging to either a Finnish family data set ($N = 1,885$), the Older Finnish Twin cohort ($N = 2,388$) or the FinnTwin16 and FinnTwin12 studies ($N = 1,411$), as described in detail in Supplementary Methods, and in >3,100 samples representing worldwide diversity. Genotyping was performed with a custom 5′ exonuclease assay (Applied Biosystems 7900) using these primers and probes: forward primer, 5′-AGAGTGTCTGAACTTCAAAGCACAA-3′; reverse primer, 5′- TCCAGACCAGTTAGAAGAGATAACGT-3′; probe 1, 5′-AGGTGCTCTGCAAAAT-3′; probe 2, 5′-AGGTGCTCTACAAAAT-3′.

One-hundred and eight-six ancestry informative markers were genotyped on 1536-SNP arrays (Illumina). qPCR for *HTR2B* expression in 13 human brain regions was determined by ABI Taqman gene expression assays (Hs01118766 and Hs00168362). β-actin was the internal control. Total protein and total RNA

were extracted from lymphoblastoid cell lines using the TRIzol LS reagent protocol (Invitrogen). Nonsense-mediated RNA decay was detected by sequencing on a 3700ABI capillary sequencer complementary DNA from *HTR2B* Q20/Q20* heterozygotes. HT2B protein was measured in 12 Finnish Q20/Q20 homozygotes and 14 Finnish Q20/Q20* heterozygotes. Blots were probed with antisera raised against the amino-terminal (mouse monoclonal antibody; Novus Biologicals), internal (goat polyclonal antibody; Santa Cruz Biotechnology), or carboxy-terminal (rabbit polyclonal antibody; Santa Cruz Biotechnology) regions of the HT2B receptor, and GAPDH antibody (Millipore). Densitometry was performed using National Institutes of Health (NIH) ImageJ. *Htr2b*$^{-/-}$ knockout mice were made in a pure 129Sv/PAS background and compared to 129/SvPAS control mice (8–10 weeks old) for four measures of response to novelty and for delay discounting.

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Author Contributions L.B. and D.G. drafted and revised the manuscript, conceptualized the study, and performed molecular, clinical and statistical analyses. L.M., S. Doly, S. Diaz and A.B. performed behavioural analyses in mice and statistical analyses. J.K. performed clinical and statistical analyses. Q.Y. performed statistical analyses. R.T., M.V. and J.S. performed clinical analyses. T.P. directed molecular analyses. J.W. performed molecular analyses. C.A.H and Z.Z. helped direct molecular analyses. L.P. helped direct clinical analyses. C.A.H., Z.Z., J.K., T.P., J.S., M.V. and E.C. revised the manuscript. D.G., L.M., J.K., C.A.H., L.P., L.D., E.C., R.J.R. and M.V. also helped with organization and support.

METHODS

Human studies. Written informed consent was obtained from each participant. Protocols were approved by the Institutional Review Board (IRB) of the NIH and the National Institute of Mental Health (NIMH), by the Office for Protection from Research Risks (OPRR), Indiana University IRB, by the University of Helsinki Department of Psychiatry IRB, by the University of Helsinki Central Hospital IRB, the University of Turku Central Hospital IRB, and by the Ministry of Social Affairs and Health and the Ethics Committee of the National Public Health Institute of Finland.

Animal studies. Mice were housed under controlled environmental conditions. Behavioural tests and animal care were conducted in accordance with standard ethical guidelines (NIH's "Guide for the Care and Use of Laboratory animals", and the European Communities Council European Communities Directive 86/609 EEC). All experiments involving mice were approved by the Ile de France Regional Ethics Committee for Animal Experiments.

Finnish violent offenders' cohort and controls. Cases were 228 unrelated Finnish male violent offenders and arsonists (Supplementary Table 1) who, because of the extreme nature of their crimes, underwent forensic psychiatric examination at the time of their initial incarceration. They were studied as inpatients at the University of Helsinki[25,37]. These subjects were diagnosed with the Structural Clinical Interview for DSM (SCID) according to DSM-III-R criteria for ASPD, BPD and IED. Excluded were subjects with schizophrenia or a history of psychosis. Ninety-six cases were selected for resequencing from the larger Finnish case cohort, comprising 228 individuals with diagnoses of ASPD, BPD and IED, on the basis that they had the highest Brown–Goodwin Lifetime Aggression (BGLAS) scores[38], with scores of 23.7 (s.d. ± 4.9) out of a theoretical maximum of 36. Controls ($N = 295$) were unrelated, nonimpulsive Finnish volunteers recruited by advertisements in local newspapers, paid for their participation and psychiatrically interviewed by trained psychiatrists. Cases and controls were independently blind-rated from interview data by two research psychiatrists under the supervision of a senior research psychiatrist. Inter-rater reliability was high, and differences were resolved by the senior psychiatrist. Controls were free of ASPD, BPD, IED, psychosis or schizophrenia but some had mood or anxiety disorders or alcohol use disorder (Supplementary Table 1). Ninety-six male controls free of Axis I and II diagnoses and matched for age were selected for sequencing for SNP discovery from a cohort of 295 controls. Controls had a BGLAS score of 8.1 (s.d. ± 4.9).

A total of 89 pedigrees were collected. Family members were interviewed using the SCID and diagnosed using DSM-III-R criteria. DNA and data were available for 397 subjects in families. Genomic DNA was prepared from lymphoblastoid cell lines.

Resequencing. For the exon-centric targeting of 14 candidate genes, we custom-designed or used Applied Biosystem oligonucleotide primers to amplify 108 target regions that covered exons, flanking regions and ~800–1,000 bp of the upstream regions of 14 genes, for a total of 82 kb (Supplementary Table 2).

DNA samples were individually quantified in three replicates by RT–PCR, using TaqMan RNase P Detection Reagent kits (FAM) and Roche human DNA standards, and were normalized to $10 \, \text{ng} \, \mu\text{l}^{-1}$. Eight DNA pools (12 subjects per pool) were made with equal amounts of DNA from 96 Finnish cases and in parallel fashion eight pools were made from 96 Finnish controls. Average sequencing coverage per individual per nucleotide was 80×.

For DNA amplification, DNA pools were amplified in 108 separate PCR reactions (Supplementary Methods).

Before DNA sequencing, amplicon concentrations were normalized using SequalPrep Normalization Plate kits (Invitrogen). All amplicons from the same DNA pool were combined. The DNA was sheared by sonication and purified with QIAquick PCR purification kits (QIAGEN). Genomic DNA preparation kits and protocol (Illumina) were used to prepare sequencing libraries.

Analysis of sequence data was carried out by calling sequences from image files with the Illumina Genome Analyser Pipeline and aligning them to human reference sequences from NCBI build 36.3 using the Illumina Eland software. Each 36-base read was uniquely mapped to the human reference genome. Sequence reads with more than two mismatches were excluded. Sequence reads with alternative alleles that did not exactly match the reference genome did uniquely map to the corresponding location in the reference sequence. Additional results are described in Supplementary Data.

Capillary electrophoresis sequencing. nsSNPs were validated by Sanger sequencing using the BigDye Terminator Sequencing Mix (Applied Biosystems) and analysed on the Applied Biosystems 3730 DNA Analyser. Of 26 nsSNPs, 22 were validated, and overall 30/34 SNPs tested in this way were validated.

Predicted functionality. Missense, nonsense and synonymous variants were predicted to be probably damaging or damaging for protein function via PolyPhen and SIFT amino acid substitution prediction methods. Four variants

(*DRD1* S259Y, *HTR2B* R388W, *HTR2B* Q20* and *TPH2* P206S—rs17110563) scored as damaging or intolerant by both methods were used in a global test of proportion of rare functional variants in cases (ASPD, BPD or IED) and controls. Genotypes of the four SNPs were collapsed so that an individual was coded as 1 if a rare allele was present and otherwise as 0. Frequencies of putatively functional variants were globally compared between cases and controls, with the null hypothesis being a lack of difference between cases and controls in the proportion carrying the putatively functional variants. A case–control association test was also performed for *HTR2B* Q20* alone. Pearson χ^2 test was used to test the null hypothesis. All analyses were conducted using JMP software v7.0 (SAS Institute). The criterion for statistical significance was set at 0.05.

Genotyping. *HTR2B* Q20* was genotyped in 228 Finnish cases and 295 Finnish controls and in 89 pedigrees belonging to the Finnish cohort for a total of 352 subjects. Taking into account the fact that some families had affected probands, we genotyped a total of 872 Finnish DNAs. In addition to the Finnish case/control and family data set and over 3,100 samples representing worldwide diversity, we also genotyped a total of 5,684 individuals belonging to either a Finnish family data set ($N = 1,885$), or to the Older Finnish Twin cohort ($N = 2,388$) or the FinnTwin16 and FinnTwin12 studies ($N = 1,411$), as described in Supplementary Methods.

Genotyping of Q20* was performed with a custom 5′ exonuclease assay (Applied Biosystems 7900) using these primers and probes: forward primer, 5′-AGAGTGTCTGAACTTCAAAGCACAA-3′; reverse primer, 5′-TCCAGAC CAGTTAGAAGAGATAACGT-3′; probe 1, 5′-AGGTGCTCTGCAAAAT-3′; probe 2, 5′-AGGTGCTCTACAAAAT-3′.

Ancestry informative markers. A panel of 186 ancestry informative markers were genotyped on 1536-SNP arrays (Illumina)[39]. No difference was detected between cases (ASPD, BPD and IED) and controls in proportions of ancestries. The pattern of measured ancestry for seven ancestry factors derived separately for each subject was compared between controls ($N = 279$) and cases ($N = 220$) with reference to the Human Genome Diversity Panel (HGDP) (1,051 DNAs representing 51 populations worldwide).

Finnish ancestry was measured using 177 ancestry informative markers in 29 Q20* carriers, 580 other Finns, and 200 individuals representing 10 European populations in HGDP. Principal component analysis was performed with EIGENSTRAT.

For *HTR2B* RNA and protein expression studies, total protein and RNA were extracted from lymphoblastoid cell lines using the TRIzol LS reagent protocol (Invitrogen).

HTR2B cDNA sequencing for nonsense-mediated decay. Nonsense-mediated RNA decay was detected by sequencing cDNA from *HTR2B* Q20/Q20* heterozygotes on a 3700ABI capillary sequencer (Fig. 2 and Supplementary Methods). The sequences of the upstream and downstream oligonucleotides were as follows: 5′-gagtgtttggcatggttaca-3′ and 3′-accaggcaggacatagaaca-5′ (Supplementary Methods). *HTR2B* Q20 and Q20* transcripts were quantified by comparing the relative intensities of the Q20 and Q20* sequencing peaks within each heterozygous individual (Supplementary Methods).

Western blots. HT2B protein was measured in 12 Finnish Q20/Q20 homozygotes and 14 Finnish Q20/Q20* heterozygotes. Western blots were prepared using 50 μg of protein per lane on a 10% Bis-Tris gel (Invitrogen). Separated proteins were transferred to nitrocellulose using the iBlot transfer system (NuPage; Invitrogen). Blots were probed with antisera raised against the amino-terminal (mouse monoclonal antibody; Novus Biologicals), internal (goat polyclonal antibody; Santa Cruz Biotechnology) or carboxy-terminal (rabbit polyclonal antibody; Santa Cruz Biotechnology) regions of the HT2B receptor, and GAPDH antibody (Millipore).

Antibody binding was visualized on X-ray film (Kodak XAR) using chemiluminescence (ECL Plus, GE Healthcare). Densitometry was performed using NIH ImageJ. Ratios between the 5-HT2B receptor and the housekeeping protein GAPDH were calculated to normalize 5-HT2B protein quantity.

qPCR for HTR2B in human brain. qPCR for *HTR2B* expression in 13 human brain regions was determined by ABI Taqman gene expression assays (Hs01118766 and Hs00168362). β-actin was the internal control.

Neuropsychological assessment. Neuropsychological assessment was conducted on both the combined FinnTwin16 and FinnTwin12 cohorts (described in Supplementary Methods) for measures of verbal intellectual ability, working memory and executive function. Working memory was assessed with the Digit Span Forward and Backward subtests of the Wechsler Memory Scale-Revised (WMS-R). We analysed the combined FinnTwin16 and FinnTwin12 data sets. A linear regression model was constructed using performance on the working memory test as the dependent variable and sex and genotype as independent variables. Sex was a significant predictor, so the sample was stratified into male and female. Male heterozygotes performed significantly worse on the Digit Span

Backward and Forward tests, and combined score (Supplementary Table 12 and Supplementary Fig. 12). All statistical analyses were conducted using Stata (version 11, Stata Corp, College Station, Texas, USA). The criterion for statistical significance was set at 0.05. Bonferroni correction for multiple testing was applied, as presented in Supplementary Table 12.

Htr2b knockout mice. $Htr2b^{-/-}$ knockout mice (50% males and 50% females) were made in a pure 129Sv/PAS background. Wild-type 129/SvPAS mice (8–10 weeks old), bred in-house, were used as controls.

Novelty seeking and impulsive behaviour in $Htr2b^{-/-}$ knockout mice were investigated using five experimental measures: novelty-induced locomotion; locomotor reactivity in response to a dopamine D1 receptor agonist; exposure to a novel object; delay discounting; and novelty-suppressed feeding. Plasma testosterone levels were measured.

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