

Supplementary Appendix

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L-Histidine Decarboxylase and Tourette Syndrome

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Detailed Clinical Findings. All subjects meet both the Tourette Syndrome Classification Study Group (TSCSG) criteria for TS and the DSM-IV-TR criteria for Tourette disorder.

(1) is a male aged 47 years. His birth was the result of a full-term pregnancy to non-consanguineous parents. He was delivered vaginally in vertex position with a birth weight of 10 lbs. There was no reported maternal diabetes or medication use during pregnancy. His surgical history is significant for inguinal hernia repair at 2 years of age, removal of a cyst over the eye as a child and cholecystectomy in 2002. He has a history of sleep apnea, seasonal allergies, and eczema. (1) has history of motor tics including shoulder shrugs and neck stretching. Vocal tics included persistent clearing of the throat. He was diagnosed with Tourette syndrome and OCD at the age of 25 as part of a research study at another university. He is currently treated with Citalopram for OCD.

(2) is a female aged 45. She has no history of tics, OCD, trichotillomania (TTM) or ADHD. She reports no history of tics, TS, OCD, TTM or ADHD in her parents or relatives. Her medical history is significant for symptomatic chiari I malformation requiring surgery for posterior fossa decompression and release of tethered cord and craniocervical fusion. (2) has been evaluated by genetics for a connective tissue disorder of unknown etiology. She does not have any history of allergies or asthma. (2) and her husband, (1), have eight children (one set of fraternal twins) and have had seven first trimester miscarriages. With regard to the miscarriages: the first was a twin conception with very early loss of the co-twin; the 2nd, 3rd, 5th, and 6th losses were at 11 weeks gestation; the 4th miscarriage was at 6 weeks gestation. The 7th miscarriage was also at 11 weeks gestation and showed a 45,X mosaic Turner syndrome karyotype. High resolution karyotyping performed as a result of

multiple miscarriages revealed no abnormalities. As noted below, all eight children suffer from vocal and motor tics.

(3) is a female aged 23 years old. She was the first born of 8 children to (1) and (2). (3) is the result of a spontaneous dizygotic twin conception to non-consanguineous parents. At 11 weeks gestation twin demise was noted on ultrasound. Her mother was on bed rest for 4 months due to toxemia. Delivery was vaginal in the vertex position at 37 weeks gestation. (3) had a normal newborn course except for mild jaundice requiring only sun exposure. Her developmental history was unremarkable: she smiled at 5 weeks, crawled at 6.5 months, sat without support at 8.5 months, spoke her first words at 9 months and walked on her own at 14 months.

(3)'s medical history includes history of severe eczema on her legs as an infant. She currently has eczema limited to her hands. She has moderate seasonal allergies as well as myopia.

(3) is described as having had TS symptoms beginning at 14 months. Vocal tics have consisted of short screams and, more recently, coprolalia. Motor tics include repetitive scrunching of her face, eye blinking, lifting and pulling down of her shoulders, eyebrow lifting, hair pulling, tensing of arm, pursing of lips, and rolling of her neck. She was diagnosed with Tourette syndrome at the age of 12 by a neurologist. She also has a history of OCD which was most severe at presentation when she was 15 years of age. At that time, she could not touch outlet plugs and would wash her hands and wipe counters clean more

than fifty times per day. She has previously been treated with lorazepam and clonazepam. She is currently treated with Citalopram for OCD and TTM.

At age 21, (3) was diagnosed with Asperger syndrome. She was administered the *Weschler Adult Intelligence Test-3rd Edition (WAIS—III)* and scored in the superior range for the General Ability Index, verbal comprehension and perceptual organization and in the above average range for working memory and processing speed. She attended college. She stopped college courses after a particularly severe episode of hair pulling. She is currently employed and lives independently.

(4) is a male aged 22 years old. No complications in the prenatal period were noted except for maternal tachycardia. He was delivered vaginally after induction at 40 weeks gestation weighing 11 lbs. Developmental history as follows: he smiled at 1 month, rolled over at 4 months, sat without support at 6 months, crawled at 5 months, walked alone at 10 months, spoke his first words at 7.5 months. (4) stuttered between the ages of two to four. His medical history is significant for type I diabetes, strabismus, severe myopia, symptomatic chiari I malformation and tethered spinal cord. He has no significant history of allergies or sleep disturbance. He currently has osteoarthritis requiring physical therapy and leg braces. Previously, (4) has been evaluated by genetics for a possible connective tissue disorder. Echocardiogram revealed trace regurgitation of the tricuspid and mitral valves, but no dilation of the aorta. It was concluded at the time that while (4) does have joint hypermobility, long arm span, myopia, and a high, narrow palate, he did not fit the current criteria for Marfan syndrome, Ehler-Danlos syndrome or any other known connective

tissue syndrome. He has since been diagnosed with a variant of EDS. Tic onset was at age 8 with worst ever symptoms reported between 9-12 years of age. Motor tics included eye rolling, blinking, eye widening and arm tightening. Vocal tics consisted of throat clearing. There is no reported history of OCD, ADHD or learning difficulties.

(5) is a male aged 19 years old. His prenatal course was significant for maternal tachycardia and preterm labor at 6 months requiring turbutaline and bed rest. He was born vaginally in a posterior position at 36 weeks. His birth weight was 9 lbs 3oz and he was 20 inches in length. Normal Apgar scores were reported. No complications in the newborn period were reported other than mild clubbed feet requiring corrective shoes for treatment. His developmental milestones were unremarkable except for the onset of speech: he said his first words at 8 months but then did not speak again until 2 years of age when he began using phrased speech. He smiled at 6 weeks, rolled over at 4.5 months, crawled at 7 months, sat without support at 7.5 months and walked on his own at 14 months.

His medical history is significant for myopia, bicuspid aortic valve and aortic root dilation. (5) also had supraventricular tachycardia requiring cardiac ablation. Genetics exam revealed mild pectus excavatum and mild joint hypermobility. TGFBR 1 and 2 mutation testing was negative. (5) had spinal de-tethering in September 2008. He has symptomatic chiari I malformation but has not yet required surgery. He was also seen by an allergist due to symptoms but skin testing was negative. (5) was diagnosed with central and obstructive sleep apnea following a sleep study due to excessive sleepiness as a teenager. Tic onset

began at age 10. (5) was diagnosed with Tourette syndrome at age 15 by a neurologist. His tics included eye blinking, jaw snapping, echolalia and exhaling tics. (5) has also been diagnosed with OCD for which he currently is treated with fluoxetine. He has had reading comprehension difficulties. He is currently attending college.

(6) is a male aged 17. He was born via vaginal delivery at 39 weeks gestation with birth weight 8 lbs 15 oz and he was 21 ½ inches in length. Developmental milestones were as follows: he rolled over at 3.5 months, sat without support at 7.5 months, crawled at 8 months, walked alone at 13 months, said his first words at 12 months and his first sentences at 19 months.

(6)'s surgical history is significant for testicular torsion and removal of sebaceous nevus on his scalp at 12 months of age. He has mild aortic root dilation. He has been seen by genetics due to the cardiac involvement, pectus excavatum and high arched palate. At that time he did not meet the criteria for any known connective tissue disorder. (6) had allergies as an infant and toddler. He has eczema behind his ears and eyebrows.

(6) was diagnosed with Tourette syndrome by a neurologist. Tics developed as a toddler with eye rolling, nose twitching, facial grimaces, shoulder and neck stretches and noises consisting of sniffing. He also stutters. (6)'s tics had decreased with Clonidine. He does not have a reported history of OCD, ADHD or learning disabilities.

(7) is a female aged 14 years old. No complications were reported in the prenatal period. Full-term vaginal delivery at 38 weeks was in the vertex position. Birth weight was 9 lbs 13 oz and she was 21 ½ inches in length. Developmental milestones were within normal limits as follows: she smiled at 3 weeks, rolled over 2.5 months, sat alone at 6.5 months, crawled at 7 months, walked at 9 months, said her first word at 12 months and her first sentence at 17 months. The medical history is significant for 2-3 periods of pneumonia as a child, type 1 diabetes, autonomic dysfunction (tachycardia and narrowed vision), severe joint pain, and symptomatic chiari I malformation. Of note, seizures developed at age 5 after an episode of waking up unable to move the left side of her body. Past surgeries include spinal cord detethering. Currently she is awaiting spinal decompression and cranio-cervical fusion. She has been diagnosed with early onset osteo-arthritis and a variant of EDS. (7) does not have history of environmental allergies but had developed serum sickness after reaction to sulfa medication.

(7) developed vocal and motor tics at the age of 5 which included throat clearing, jaw tightening, jaw jutting, neck craning, eye rolling and mouth opening. Tics are exacerbated by stress. She does not carry a diagnosis of OCD, ADHD or learning disabilities.

(8) is a male aged 13 years. His prenatal history was uncomplicated except for maternal tachycardia. Vaginal delivery at 37 weeks gestation was in the vertex position. He had a nuchal cord and mild cyanosis requiring blow-by oxygen. Normal Apgar scores were reported. Birth weight was 8 lbs 8 oz and he was 19 ½ inches in length. The newborn nursery course was uneventful. Developmental milestones were as follows: he smiled at 6

weeks, rolled over at 9 weeks, sat alone at 7 months, crawled at 4 months, and walked at 12 months. His speech was delayed until 24 months. Medical history is significant for several hospitalizations related to a ketone utilization disorder. (8) has been evaluated for probable connective tissue disorder with pectus excavatum and hypermobile joints but no syndrome has been diagnosed. He does not have a history of allergies or eczema but does have history of sleep disturbance. A sleep study was ordered in May 2004 due to excessive snoring and breathing pauses. Results revealed some reduction in REM sleep. He has also been seen by cardiology after a bradycardic episode at age 8. EKG and echocardiogram were within normal. Brain MRI revealed chiari I malformation and hydromyelia syrinx. Surgical history is significant for spinal cord detethering.

(8) developed tics at the age of 4. Vocal tics include humming, rhythmic breathing, and snorting, motor tics include eye rolling and leg movements. He is currently taking Clonidine for tic control. He does not have a history of OCD, ADHD or a learning disability.

(9) is a male aged 9. He is the product of a spontaneous dizygotic pregnancy. Prenatal history is significant for maternal gestational diabetes treated with insulin and bed rest due to preterm labor at 20 weeks and 28 weeks. Delivery was via c-section at 35 weeks due to breech presentation. (9) was delivered second to twin (10), had apnea and was intubated and treated in the NICU for 24 hours. Birth weight was 5 lbs 13 oz and 18 ½ inches in length. He had jaundice and was treated with phototherapy for 3 days. He was discharged from the hospital at 1 week of age. Developmental milestones were as follows: he smiled at 6 weeks, rolled over at 5 months, sat without support at 6.5 months, crawled at 6 months,

and walked alone at 14 months. Speech was delayed with first words at 30 months. He had a normal audiogram at 4 years of age.

(9)'s medical history is significant for severe allergies to grass, pollen, trees, cats and dogs from ages 1 -5. He had a history of sleep apnea and breathing pauses while awake. MRI of the spine revealed mild convex left cervical scoliosis centered at C6/C7. Surgical history is significant for adenoidectomy at 4.5 years of age and spinal cord detethering. (9) was examined by genetics for possible connective tissue disorder at 6 years of age. Physical exam was remarkable for high/arched palate, bilateral dislocation of hips, and moderate joint laxity. He did not meet the criteria for any specific connective tissue disorder at the time. He has since been diagnosed with an EDS variant. Echocardiogram previously performed at 4 years of age was normal. He also has symptomatic chiari I malformation which has not yet required surgical intervention.

(9) has been diagnosed with Tourette syndrome by a neurologist. He has history of tics since the age of 18 months. These include eye rolling, growling, echolalia, and hitting his forehead. He has been also diagnosed with OCD. Compulsions include touching items from forehead to nose, tapping objects, sniffing hands and lining up objects. Currently he takes Clonidine for tics, but no medications for OCD.

(10) is a male aged 9 years. He is the dizygotic twin to (9). He also had difficulty breathing immediately after birth but recovered after suctioning. He was discharged with his twin at one week of age. Developmental milestones were as follows: he smiled at 6 weeks, rolled

over at 4.5 months, sat without support at 9 months, crawled at 6 months, and walked alone at 12 months. He had speech delay with first words at 30 months. He has not had a formal hearing evaluation.

(10) had bilateral strabismus with surgical correction at age 2. He has history of mild allergies to wheat, dairy, beef and peanut butter and intermittent asthma diagnosed at age 2. Past medications included Flovent, Albuterol, Zyrtec and Flonase. He also has history of eczema on his lower legs. He has not been diagnosed with a connective tissue disorder but is described as having thin skin, easy bruisability, and joint laxity and dislocation. He has been diagnosed with a tethered spinal cord but has not required surgery.

(10) has been diagnosed with Tourette syndrome by a neurologist. Tic onset at age 4 included eye rolling, throat clearing, eyebrow raising and jutting arm out. He has not been diagnosed with OCD, ADHD or a learning disability.

DNA Extraction for the index family. DNA was extracted from whole-blood using the PureGene DNA isolation kit (Gentra Systems, MN) according to the manufacturer's instructions. The quantity and quality of DNA was determined on a NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and DNA was stored at -20°C.

Genotyping. DNA samples were genotyped for 115,561 SNPs using the Affymetrix GeneChip® Human Mapping 100K Array Set which comprises Mapping50K_Hind240 and

Mapping50K_Xba240 Arrays according to the manufacturer's instructions. We analyzed cell intensity data using Affymetrix GeneChip® Genotyping Analysis Software (GTYPE) 4.0, and 95 % of all SNPs successfully passed the quality control criteria.

Prior to linkage analysis, all genotyped markers were checked for Mendelian inconsistencies using the Mega2 program. All SNPs showing inconsistency in transmission were removed from further analyses. We then generated a marker set by LD-based SNP pruning using the criteria that $r^2 < 0.08$ within 1Mb, the proportion of missing genotypes was less than 5%, the MAF $> 5\%$ (the relative minor allele frequency), and the Hardy-Weinberg equilibrium was greater than 0.05 with Affymetrix GeneChip Human Mapping 100K data representing Caucasian (CEU) populations.

Linkage analysis and loci identification. Corrected and LD pruned data were used to conduct parametric multipoint linkage analysis on affected individuals with Allegro 1.2 software program (DeCode genetics: Iceland) under a model of autosomal dominant inheritance with penetrances specified between 70 % and 99 %, a phenocopy rate of 0.01 and a disease allele frequency of 0.001. Allele frequencies of all genotyped SNPs were derived from the Affymetrix website based on Caucasian individuals previously genotyped. Map order and genetic inter-SNPs distances were taken from the Affymetrix website.

Confirmation of Linkage Using Microsatellite Short Tandem Repeat Markers.

Subsequently, chromosome 15 linkage region in all members of the family, both affected and unaffected, was fine mapped using a total of 16 microsatellite markers selected from Genome browser at an average heterozygosity of 75%. All forward primers were tagged on the 5' end with a fluorescent label (6-FAM, and TET) (**Supplementary Table 5**). The PCR

for genotyping the microsatellites started at 95 °C for 3 min followed by a 16 cycles in a Touchdown (TD) program (95°C for 30 s, 60°C for 30 s and 72°C for 30 s, followed by a 0.5°C decrease of the annealing temperature every cycle). After completion of the TD program, 16 cycles were subsequently performed (95°C for 30 s, 53°C for 30 s and 72°C s for 30 s) incubating at 4 °C. All PCR reactions were performed in 20 µl reactions using 1X PCR PreMixD buffer (Epicentre Biotech, Madison, WI), 525 nmol of each primer, 0.2µl TaqDNA polymerase (5 U/µl; Roche, Mannheim, Germany), and 15 ng of template DNA. Products were sequenced by the Keck Sequencing Facility (Yale University, New Haven, CT, USA) by using the sequencer 3730xl DNA Analyzer (Applied Biosystems). Genotyping was done using GeneMapper Software v3.7 (ABI Prism). The results were analyzed using the SimWalk2 program¹ with penetrances specified between 70% and 99%.

Copy Number Variants (CNVs). For Copy Number Changes, we evaluated (1) the father and one affected offspring and the unaffected using The Human *1M-Duo chip* (Illumina Inc), and (2) a total of 10,571 individuals available in our laboratory using CNV370-Duo, HumanHap300-v1, and HumanHap550v3 chips (Illumina Inc) (**Supplementary Table 6**). The scan data for each individual was visualized using the BeadStudio software package version 3.2.23 (Illumina, Inc.) with the Human Genome Build 18 as reference. The mean genotype success rate was 98.55% (range 98.16–99.07%) for samples considered as passing an initial quality check. For the affected family, CNV prediction was performed using visual inspection as well as three copy number detection algorithms, including PennCNV, QuantiSNP and a tool developed within our laboratory, GNOSIS. For evaluation of loss of function CNVs in the broader pool of apparently unaffected individuals, we extracted a total of 311,398 common SNPs from these three platforms to predict copy number

variants on chromosome 15. Log R ratio and B allele frequency of each sample were evaluated with PennCNV, QuantiSNP, and GNOSIS analytic tools. The Database of Genomic Variants (<http://projects.tcag.ca/variation/>) was reviewed to determine if the identified CNVs had been previously described.

Detection of Family Ethnicity and Genome-Wide Identity. We used Human *1M-Duo chip* (Illumina Inc) to identify the ethnicity of the pedigree. Illumina data were compared with HapMap samples representing Caucasian (CEU), Asian (CHB/JPT) and African (YRI) populations using PLINK.² A total of 37,808 independent high quality SNPs were used to perform multi-dimensional scaling (MDS) analysis on the matrix of pair-wise genome-wide IBS distances in 4 dimensions. MDS output was visualized in R. We used the PLINK program to perform advanced error checking (**Supplementary Fig. 1**).

Pairwise IBD was calculated using PLINK²; **Supplementary Table 7a**, LD pruned high quality SNPs from Affymetrix GeneChip® Human Mapping 100K Array Set for all family members was imported into PLINK (--genome function) to calculate the pi-hat value, which is the proportion of the genome showing inheritance by descent (IBD) estimates for parents and siblings.

Pairwise IBD was also calculated for the confirmation of unrelateness between father and mother by using 99 unrelated Caucasian samples with LD pruned high quality SNPs from Human *1M-Duo chip* data (**Supplementary Table 7b**).

Sequence Analysis. The candidate genes in the linkage interval were initially analyzed in the father, one affected offspring and the unaffected mother of the family by direct genomic

DNA sequencing of PCR amplicons. All primers were designed to span the exons and splice site junctions of candidate genes using the Primer3 program³ (primer sequences available upon request). The forward and reverse primers were chosen to produce amplicons of less than 500 base pairs. Larger exons were divided over several amplicons, each with a size of less than 500 base pairs. A total of 750 amplicons of 51 genes were analyzed with an average size of approximately 400 bp. All samples were sequenced on one strand at Agencourt Bioscience (Beverly, MA). The sequence results were analyzed using Sequencher software v4.8 (Gene Codes Corp. Ann Arbor, MI).

Genomic sequence for the coding region of all candidate genes and all coding and 5'UTR-3'UTR' regions of *HDC* gene were obtained from the UCSC Genome Browser. All PCR reactions were carried out in 96/384-well plates containing 1X PCR PreMixD buffer (Epicentre Biotech, Madison, WI), 1 μ L 10 μ mol of each of the primers, 2 μ L of extracted DNA (10ng/ μ L), 0.03 μ L cloned Pfu DNA polymerase, 0.6 μ L Taq DNA polymerase, and sterile water in a total reaction volume of 25 μ L. The PCR protocol consisted of a denaturation step at 95°C for 5 min, followed by 35 cycles of a 95°C denaturation for 30 sec, an annealing step at an optimized temperature for 30 sec, and a 1 min extension step at 72°C. The reaction was completed with a 10 min extension step at 72°C.

***HDC* Stop-Codon Confirmation.** The PCR product was re-amplified from DNA stocks in all nine affected and one unaffected family members. PCR reactions and protocols were described above. All PCR products were purified using QIAquick kits from Qiagen (Valencia,

CA) and then directly sequenced on both strands at the W. M. Keck Facility (Yale University, New Haven, CT).

***HDC* screening in family members, independent cases and control groups.** All 12 coding exons and 30bp of the flanking intron-exon boundaries of *HDC*, 5' UTR/3'UTR and promoter region which includes 595 bp upstream of 5'UTR were amplified for all family members (**Supplementary Table 8**). PCR products were screened to detect any possible *HDC* mutations by direct sequencing at Agencourt Bioscience (Beverly, MA).

Independent cases used for sequencing of *HDC* in this study consisted of 720 Caucasian, Hispanic, and Asian subjects diagnosed with TS and in some cases one or more co-morbid disease such as ADHD, OCD, OCB, anxiety, depression. Of these, 71% were male and 29% were female. DNA samples were derived from whole blood..

We used randomly selected individuals from a population of Caucasians controls for mutation screening via Sanger Sequencing and SNP genotyping. Males and females accounted for 65.2% and 34.8 % of the samples, respectively. The self-identified racial distribution was 82.9% Caucasian, 13.6% African-American, and 3.5% other races. These individuals were not screened for TS syndrome or comorbid disorders.

TaqMan Genotyping. The nonsense mutation (W317X), a missense mutation (V186M) found in a second affected family, two missense mutations (R8G and P303S) identified in controls and the putative (-/C) insertion reported as rs35490626 were genotyped using Custom TaqMan SNP Genotyping Assays (Applied Biosystems) in an additional control

sample of 6413 unrelated subjects, including 5427 individuals not evaluated for psychiatric disorders and 986 Caucasian neurologically normal controls from the NINDS repository (Supplementary Table 6). A custom TaqMan SNP Genotyping Assays consist of a mix of unlabeled polymerase chain reaction (PCR) primers and the TaqMan minor groove binding group (MGB) probe (FAM™ and VIC® dye-labeled) (Applied Biosystems). Primers and probe design used in the TaqMan assays are given in **Supplementary Table 9**.

PCR was performed using 2.5 µl of TaqMan® Universal Master Mix, 10 ng of DNA, 0.125 µl of Custom TaqMan® SNP Genotyping Assay mix (40-fold mix) and water to bring the final reaction volume to 5 µl. The PCR thermal cycling was as follows: initial denaturing at 95 °C for 10 min; 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Thermal cycling was performed and read using a on ABI Prism 7900 high-throughput sequence detection system.

Each 384-well plate contained 376 samples of an unknown genotype and 4 reaction mixtures containing reagents but no DNA (no-template control) and 4 reaction mixtures containing positive controls. Post-PCR data from all the plates were evaluated using SDS 2.2.1 software package and fluorescence from the two reporters was plotted.

Amino acid Alignment. Conservation of rare non-synonymous variant was investigated in *HDC* proteins from different organisms. BLAST program (<http://www.ncbi.nlm.nih.gov/blast/>) was used to search the sequence database to identify similar proteins in the species. Then the amino acid sequences were aligned with the use of the ClustalW2 program (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>) with the following sequences: Human (*Homo Sapiens*; NP_002103), Chimpanzees (*Pan troglodytes*; XP_510396), Rhesus monkeys (*Macaca mulatta*; XP_001114161), dog (*Canis*

lupus familiaris; XP_544676), cow (*Bos taurus*; NP_001019722), horse (*Equus caballus*; XP_001499698), mouse (*Mus musculus*; NP_032256), brown rat (*Rattus norvegicus*; NP_058712), The Gray Short-tailed Opossum (*Monodelphis domestica*; XP_001380521), chicken (*Gallus gallus*; XP_413833), Wild boar (*Sus scrofa*; XP_001925377) The California sea slug (*Aplysia californica*; AAP34326), jewel wasp (*Nasonia vitripennis*; XP_001603214), honey bee (*Apis mellifera*; XP_392129), fruit fly (*Drosophila melanogaster*, NP_523679) **(Supplementary Fig. 3).**

HDC Expression. mRNA was isolated from the Epstein-Barr virus immortalized lymphoblastoid cell line for each member of family using RNeasy kit (Qiagen). Also, RNA was isolated from fresh blood obtained from only father using PAXgene blood RNA Kit. Blood was collected in PAXgene. DNase (Turbo DNase, Ambion) treated mRNA was reverse transcribed into cDNA according to the manufacturer's instructions (Omniscript RT kit). cDNA products were used for PCR reaction containing 3 µl cDNA, 1 X Power SYBR Green PCR master mix (Applied Biosystems), 200 nM of the forward 5'-TTCCTGTGCCCCGAGTTC-3' and reverse primers 5'-GGATCTGCCAGTGCATGAAGT-3' and nuclease-free water to a final volume of 20 µl. Primer pair was designed with an amplicon size of 214 using Primer Express Software (Applied Biosystems) on the exon-exon junction and tested for specificity using NCBI's BLAST software. The PCR conditions were an initial incubation at 50°C for 2 min followed by a 95°C incubation for 10 min to activate the HotstarTaq DNA Polymerase, then 50 cycles at 94°C (30 s), 58°C (1 min), 72°C (30 sec).

To detect any cross contamination of reagents and surfaces, we used primers that crossed exon-exon boundaries and a "No Template Control" (NTC) which has all of the RT-PCR reagents except the cDNA template. Also, to detect any DNA contamination, we used minus-reverse transcriptase control ("No Amplification Control" or NAC; containing all the RT-PCR reagents, except the reverse transcriptase) in RT-PCR.

Before sequencing, PCR products were first separated by agarose gel electrophoresis and gel purified using the Gel Extraction Kit (QIAGEN). Gel purified products were sequenced on both complementary strands of cDNA.

Immunohistochemistry

Human brain specimen. Post-mortem human brain specimens were collected following guidelines on the research use of human brain tissue from the Croatian Institute for Brain Research, University of Zagreb Medical School. Tissues from the Zagreb Neuroembryological Collection were obtained with approval from the Medical Ethical Committee at the University of Zagreb Medical School. For each tissue donation appropriate written informed consent and approval were obtained. The study was approved by the Human Investigation Committee at Yale University School of Medicine.

Immunohistochemistry of mouse brain. Experiments were carried out in accordance with protocols approved by the Institutional Animal Care and Use Committee at Yale University School of Medicine. An adult CD1 mouse (older than 3 months) was purchased from Charles River Laboratories (Wilmington, MA) was anesthetized and intracardially

perfused with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS). The brain was then cryoprotected in 30% sucrose in PBS, sectioned at 40 μm using a sledge microtome on the coronal plane and processed free-floating. For diaminobenzidine (DAB) staining, sections were treated with 1% H_2O_2 , washed in PBS, preincubated in blocking solution (BS) containing 5% normal donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA), 1% bovine serum albumin, 0.1% glycine, 0.1% L-lysine, and 0.03% Triton-X-100 for one hour. The sections were then incubated with goat anti-Histidine H3 Receptor antibody (Santa Cruz Biotechnology, Santa Cruz, CA; catalog # sc-17921; 1:25 dilution) for 36 hrs at 4°C. After subsequent washes with PBS, sections were incubated with donkey anti-goat Biotin-SP (Jackson ImmunoResearch Laboratories, 1:250 dilution) in BS for 2 hours at room temperature. More washes were performed followed by two hour incubation with Vectastain ABC Elite solution (Vector Laboratories, Burlingame, CA). Sections were developed using 0.05% DAB (pH 7.4), 0.2% glucose, 0.01% nickel ammonium sulfate, 0.04% ammonium chloride and 8 $\mu\text{g}/\text{ml}$ glucose oxidase, and then rinsed, mounted onto glass slides, allowed to dry, dehydrated and cover-slipped.

For immunofluorescence, sections were placed straight into BS solution without H_2O_2 treatment followed by the same incubation with anti-Histidine H3 Receptor. After washes with PBS, the sections were incubated with secondary antibody [donkey anti-goat cyanine 3 (Jackson ImmunoResearch Laboratories, 1:250 dilution)] for 2 hours at room temperature. The sections were mounted, cover-slipped with DAPI-containing Vectashield (Vector Laboratories) and analyzed using a confocal microscope (Zeiss).

Immunohistochemistry of human brain. The collected human brains were fixed in 4% PFA, cryoprotected in 30 % sucrose, frozen by immersion in 2-methylbutane and stored at -80°C . Blocks were sectioned frozen at $60\ \mu\text{m}$ using a cryostat and processed free-floating. Target retrieval was first performed following the manufacturer's protocol. Briefly the sections were incubated with Dako Target Retrieval Solution (Dako North America, Inc., Carpinteria, CA) at 97°C for 30 minutes and then allowed to cool to room temperature. DAB staining was then performed as described as with the mouse brain sections.

HDC constructs. The full-length cDNA clone of HDC (accession number BC130527) was purchased from Open Biosystems, Huntsville, AL. The clone contained an amino acid variant that did not correspond to the patient and was fixed using QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) with the sense and antisense primer: 5'-GGAGAGACGTGTGACGCCAGACGTGCAGCCTGGC-3'. The coding region was then amplified with the primers: 5'-GAATTCATGATGGAGCCTGAGG-3' and 5'-CTCGAGCTAAACCATGGCCTGC-3' containing the restriction sites EcoRI and XhoI respectively. The resulting product was digested and inserted into the pGEX-6P-1 vector (GEHealthcare). Additionally the coding region was inserted into the pcDNA3.1(-) vector (Invitrogen, Carlsbad, CA) which includes a T7 promoter by using the primers: 5'-CTCGAGTTATGATGGAGCCTGAGG-3' and 5'-GAATTCCTAAACCATGGCCTGC-3' containing the sites XhoI and EcoRI respectively.

The W317Stop mutation was engineered for both HDC constructs with the QuikChange kit using the sense and antisense primer: 5'-GACTGTAAGTGGTTCTGAGTCAAGGACAAGTAC-3'.

Similarly the 54-kDa HDC was constructed by creating an early stop codon using the sense and antisense primer 5'-TCTCATCCTGAGTCAGTAATGTACTTCCCAACCCAG-3. All clones were fully sequenced.

Recombinant protein expression and refolding. *E. coli* BL21DE3 cells were transformed with pGEX-HDC-54 encoding the wild-type 54 kDa form, and the truncated pGEX-HDC W317Stop, in which W317 is replaced with a stop codon. The cultures were grown at 37°C until reaching an OD₆₀₀ of 0.6-0.8, and then induced with 0.10 mM isopropylthiogalactoside for 4 hrs at 25°C. Following induction, the cells were harvested and resuspended in 10 ml of 20 mM Tris (pH 7). Cells were lysed by sonication, pellets were prepared, and the inclusion bodies comprised of the expressed GST-fusion proteins were dissolved in 7 M guanidine hydrochloride containing 2 mM EDTA and 10 mM DTT. The dissolved inclusion body preparations were then ultracentrifugated at 125,000 g for 30 min in a Beckman Ti70 rotor, and the supernatant was refolded using the Profoldin protein folding kit (SCF06) according to the manufacturer's instruction (Westborough, MA). This folding kit was chosen after initial optimization of protein recovery and enzymatic activity for the wild-type 54 kDa HDC using a screening kit obtained from the same manufacturer. Refolded proteins were then dialyzed overnight against 10 mM ammonium acetate buffer prepared from a 100 mM stock and adjusted to pH 6, analyzed by SDS-PAGE and Coomassie staining to assess purity, and used for the HDC enzymatic assay. Protein amounts were quantified using the Pierce protein estimation kit (BCA method).

Fluorescence-quenching assay. The enzymatic activity of histidine decarboxylase was assayed as described.⁴ Briefly, the assay is based on the self assembly of a complex between the fluorescent dye Dapoxyl and the water soluble macrocycle cucurbit[7]uril (CB7). This complex is highly fluorescent compared to uncomplexed Dapoxyl. CB7 has a high affinity for histamine, the enzymatic product of the histidine decarboxylase, but only a very low affinity for the substrate histidine. Upon enzymatic turnover of histidine to histamine, histamine therefore gradually displaces the Dapoxyl from its macromolecular complex with CB7. The gradual displacement of the Dapoxyl from the host guest complex is monitored spectroscopically as a decrease in fluorescence as a function of time. The reaction mixture contains 2.5 μM of Dapoxyl (Invitrogen, Carlsbad, CA), 10 μM CB7 (Sigma-Aldrich, St. Louis, MO), and 50 μM histidine (Sigma-Aldrich, St. Louis, MO) as described³. Fluorescence was measured using a QM-1 fluorescence spectrometer (Photon Technology International, Birmingham, NJ) by using $\lambda_{\text{excitation}} = 336\text{nm}$ and $\lambda_{\text{emission}} = 380\text{nm}$ for the CB7-Dapoxyl pair. Enzymatic reactions were carried on in a 0.5 ml quartz glass cuvette and temperature was kept constant at 25°C. Purified proteins were added at 4 $\mu\text{g}/\text{ml}$ at 500 seconds after mixing the reagents, which allowed the background fluorescence to become stable. The time point of enzyme addition was set as the start point of the fluorescence recordings analyzed here. Recombinant GroEL protein was used as negative control and was a kind gift of Drs. Art Horwich and Navneet Tyagi, Department of Genetics and HHMI, Yale University.

Assay for Dominant Negative Effect using Liquid Chromatography-Mass Spectrometry.

***In vitro* translation and histamine reaction.** The wt (54-kDa HDC), mut (W317Stop) and

empty vector (pcDNA3.1 with no insert) were digested with HindIII to linearize the plasmids and purified using a Qiagen PCR purification kit (Qiagen, Valencia, CA). The resulting amount of DNA was quantitated and used as templates for the following experiments using *in vitro* T7 transcription/translation (TNT T7 Coupled Kit, Promega, Madison, WI). Unless otherwise noted, 0.5 µg of DNA template was used in a 50 µl *in vitro* reaction. To ensure proper expression, Transcend tRNA (Promega), which is a biotinylated lysine, was added to an aliquot from each reaction. These were analyzed using SDS-PAGE, transferred to a PVDF membrane, and detected with Streptavidin-Horseradish Peroxidase. All three conditions, wt, mut and co-translation of wt and mut, ran at expected size. The intensity of the bands for the co-translation condition was similar to that of the wt and mut translated separately (see Figure 3D). For the enzymatic reaction, the HDC proteins were generated using the *in vitro* system and then added to 1X Reaction Buffer (consisting of 0.25 mM L-Histidine, 5 µM pyroxidal phosphate and 50 mM sodium phosphate, pH 6.8) for a final volume of 200 µl. The reaction was incubated for 60 minutes at RT. 10 µg of 1,3-diaminopropane, was then added to each reaction product to provide for normalization in the LC-MS experiments, and the entire mixture was brought to a volume of 600 µl using Reaction Buffer. The solution was applied to an Oasis MCX 1 cc (30 mg) extraction cartridge (Waters, Milford, MA) for solid-phase extraction (SPE). The procedure for SPE was performed as described elsewhere.⁵ The amount of histamine in the eluted solution was quantitated using liquid chromatography-mass spectrometry.

To determine the parameters for the assay, we compared wt and all mut proteins at a 1:1 ratio (0.5 µg of DNA template for each) derived from the co-translation experiments versus

the same ratio derived from a mixture of wt and mut proteins that were translated independently. The resulting amount of histamine was similar in both cases. We elected to use co-translated products given the additional internal controls provided for loss of product during sample preparation. A dosage curve was established using a fixed amount of wt (0.5 μg) and adding increasing amounts of mut template (0 - 1 μg) to the *in vitro* translation reaction.⁶ Empty vector was added so that the total amount of DNA for each reaction was 1.5 μg . Each condition was performed in duplicate and measured in triplicate using liquid chromatography-mass spectrometry (Fig. 3E-F and Table S10).

Histamine quantitation using liquid chromatography-mass spectrometry.

Liquid chromatography-mass spectrometry was performed on an Applied Biosystems 4000 QTRAP triple quadrupole mass spectrometer interfaced with a Waters nanoAcquity UPLC system running Analyst 1.5 software. 4 μl of the SPE sample, which was run in triplicate, was loaded onto a Symmetry C18 nanoAcquity trapping column (180 μm x 20mm 5 μm) with 1% acetonitrile (ACN)/0.1% formic acid (FA) at 15 μL per minute for 3 minutes. After trapping, the compounds were resolved on an Altanis C18 nanoAcquity column (75 μm x150mm 1.7 μm) with a 15 minute, 2-22.5% ACN/0.1% FA linear gradient (0.280 $\mu\text{L}/\text{min}$ flow rate). Positive ion electrospray was performed using a NanoSpray II XYZ source containing a 15 μm i.d. SilicaTip (New Objective, Cambridge, MA, USA). Multiple Reaction Monitoring scanning was carried out with two transitions (75.1/58.1 – 1,3-diaminopropane and 112.0/95.0 – histamine) and with a 50 millisecond dwell time per transition at unit resolution. Linear concentration curves and sample quantitation were calculated with the program Multiquant 1.1 (Applied Biosystems), using an external

calibration. The external calibration was generated from a standard curve of the molecule, histamine, run immediately prior to the samples. The linear histamine concentration curve was calculated from triplicate MRM assays on histamine at the following concentrations (pmol/ μ L): 0, 0.024, 0.097, 0.39, 1.56, 6.25, 25, and 100, with a constant concentration of 6.25 pmol/ μ L of 1,3 diaminopropane. Data was then exported from Multiquant and uploaded into the Yale Protein Expression Database (YPED)⁷ for viewing, along with a PowerPoint file of images of the spectra.

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Table S1. Phenotypic Information for Pedigree

Age/Sex	DSM-IV Criteria*	Symptom Onset	Symptoms	Co-Morbid Psychiatric Dx	Medications
47M	TD	Childhood	nose twitching, shoulder shrugging, neck stretching, throat clearing, sniffing, elbow flapping	OCD	Citalopram
45F	None	NA	None	None	None
23F	TD	14 months	eye blinking, shoulder shrugging, eyebrow lifting, arm, neck, thigh, abdominal and hip tensing, lip pursing, hair pulling, swearing, jaw jutting, thumb flexing, arm shaking, humming, sucking in,	OCD/Asperger's syndrome	Citalopram, guanfacine
22M	TD	8 years	eye rolling, blinking, eye widening, arm tensing, nose twitching, throat clearing	None	none
19M	TD	10 years	eye blinking, jaw snapping, echolalia, exhaling	OCD/Reading difficulties	Clonidine, Fluoxetine
17M	TD	Toddler	eye rolling, nose twitching, facial grimaces, shoulder and neck stretches, sniffing, hard blinking, throat clicking	None	Clonidine
14F	TD	5 years	eye rolling, throat clearing, jaw tightening, jaw jutting, neck craning, and mouth opening, arm tightening, neck tensing	None	none
13M	TD	4 years	eye rolling, humming, rhythmic breathing, snorting, and leg movements, pounding chest, crossing eyes, head shaking and forceful head nodding	None	Clonidine
9(B)M	TD	18 months	eye rolling, growling, echolalia, hitting forehead, tapping forehead on floor, yelping, touching others, throat clearing,	None	Clonidine
9(A)M	TD	4 years	eye rolling, throat clearing, sniffing, arm jutting	None	none

*DSM-IV nomenclature uses the term Tourette disorder (TD) instead of Tourette syndrome (TS). These are interchangeable and the diagnostic criteria used throughout the text is DSM-IV as noted.

Table S2. Total of 51 genes are predicted within the LOD-2 interval of chromosome 15q

Gene Name	Description
<i>SHC4</i>	Rai-like proteine
<i>KIAA0256</i>	Hypothetical protein LOC9728
<i>COPS2</i>	COP9 constitutive photomorphogenic homolog
<i>GALK2</i>	Galactokinase 2 isoform 2
<i>C15orf33</i>	Hypothetical protein LOC196951
<i>FGF7</i>	Fibroblast growth factor 7 precursor
<i>DTWD1</i>	DTW domain containing 1
<i>ATP8B4</i>	Adenosine triphosphatase involved in phospholipid transport within the cell membrane
<i>KIAA1939</i>	Homo sapiens mRNA for KIAA1939 protein
<i>SLC27A2</i>	Solute carrier family 27 (fatty acid
<i>GABPB1</i>	GA binding protein transcription factor, beta
<i>USP8</i>	Ubiquitin specific peptidase 8
<i>USP50</i>	Ubiquitin specific protease 50
<i>TRPM7</i>	Transient receptor potential cation channel
<i>HDC</i>	Histidine decarboxylase
<i>CHAK1</i>	Homo sapiens channel-kinase 1
<i>SPPL2A</i>	Signal peptide peptidase-like 2A
<i>TNFAIP8L3</i>	Tumor necrosis factor, alpha-induced protein
<i>DMXL2</i>	Abundant expression of Dmxl2 in brain only, and subcellular distribution analysis revealed enrichment in the synaptic vesicle fraction
<i>LYSM2</i>	LysM, putative peptidoglycan-binding, domain
<i>AP4E1</i>	Adaptor-related protein complex 4, epsilon 1
<i>CYP19A1</i>	Cytochrome P450, family 19
<i>GLDN</i>	Gliomedin: has a role in the formation of the nodes of Ranvier along myelinated axons.
<i>SCG3</i>	Secretogranin III
<i>LEO1</i>	Leo1, Paf1/RNA polymerase II complex component
<i>TMOD2</i>	Tropomodulin (TMOD) is the actin-capping protein for the slow-growing end of filamentous actin. Expression in neuronal tissues
<i>TMOD3</i>	Tropomodulin 3 (ubiquitous)
<i>MAPK6</i>	Mitogen-activated protein kinase 6
<i>GNB5</i>	Guanine nucleotide-binding protein, beta-5
<i>BCL2L10</i>	BCL2-like 10 (apoptosis facilitator)
<i>MYO5C</i>	Myosin VC
<i>MYO5A</i>	Myosin VA
<i>MYR12</i>	Myosin V (Fragment).
<i>ARPP-19</i>	Cyclic AMP phosphoprotein, 19 kD
<i>KIAA1370</i>	Hypothetical protein LOC56204
<i>ONECUT1</i>	One cut homeobox 1
<i>WDR72</i>	WD repeat domain 72
<i>UNC13C</i>	Involved in neurotransmitter release.
<i>C15orf15</i>	Ribosomal protein L24-like
<i>RAB27A</i>	Ras-related protein Rab-27A
<i>PIGB</i>	Phosphatidylinositol glycan, class B
<i>CCPG1</i>	Cell cycle progression 1 isoform 2
<i>DYX1C1</i>	Dyslexia susceptibility 1 candidate 1 isoform c
<i>PYG01</i>	Pygopus homolog 1
<i>PRTG</i>	Protogenin
<i>NEDD4</i>	Neural precursor cell expressed, developmentally
<i>RFX7</i>	Regulatory factor X domain containing 2
<i>TEX9</i>	Testis expressed 9
<i>ZNF280D</i>	Suppressor of hairy wing homolog 4 isoform 1
<i>TCF12</i>	Transcription factor 12 isoform a
<i>MNS1</i>	Meiosis-specific nuclear structural 1

Table S3. Non-synonymous mutations found in genes within chromosome 15 linkage interval

Gene Name	Amino Acid Substitution	Nucleotide Variation			rs number	Mutation Type	Allele frequency from hapmap CEU population
		Father	Mother	Child			
<i>MAPK6</i>	L290V	C/G	C/C	C/G	rs35697691	Missense	G: 0.1±0.05
<i>DMXL2</i>	T497K	A/A	C/C	A/C	rs17524906	Missense	A: 0.258±0.08
<i>ZNF280D</i>	V568I	A/A	G/G	G/A	rs28620278	Missense	A: 0.381±0.09
<i>HDC</i>	W317X	G/A	G/G	G/A	novel	Nonsense	

Table S4. Missense variants detected in HDC in the TS probands and control samples

Exon	Nucleotide Variation	Amino Acid Substitution	Carriers	Mutant Allele Frequency ^a	Complete Conservation ^b	Polyphen	Sift	Notes
TS Probands (N=720)								
4	G/A	V186M	1	0.0000779	No	Benign	Affect protein function	Mother (OCD and depression) has same mutation. Father is wild type
Unscreened^c Controls (N=360)								
1	A/G	R8G	1	0.0000779	No	Damaging	Affect protein function	
7	C/T	P303S	1	0.0000779	Yes	Damaging	Affect protein function	

^a The allele frequencies for mutations were estimated to be 1/12,826 after genotyping in additional control samples (Table S6).

^b Conservation was evaluated in an alignment of 11 vertebrate and 4 invertebrate species (**Supplementary Fig. 3**). A residue was considered completely conserved if it was invariant across all species.

^c These control samples were not evaluated for TS, OCD or other neuropsychiatric disorders

Table S5. Microsatellite Short Tandem Repeat Markers used to confirm Linkage Analysis

STR name	Other name	Start	End	Chr band	cM	Max het.	Left Primer	Right Primer	Distance bps	Label
AFM205YE1	D15S123	45851812	45852003	15q21.1	45.62	0.8	AGCTGAACCCAATGGACT	TTTCATGCCACCAACAAA	191-207	FAM
AFMA345XH9	D15S992	46627034	46627372	15q21.1	45.62	0.8	AGCTGAGAAATGCCTTCTATAAAT	GAGGGCCACCTTGATAGT	228-274	FAM
AFM218YF12	D15S126	47089559	47089840	15q21.1	45.62	0.85	GTGAGCCAAGATGGCACTAC	GCCAGCAATAATGGGAAGTT	188-218	FAM
AFM150XF4	D15S119	47280418	47280662	15q21.1	45.62	0.726	AACAGAAAATCCGTAACATAACATA	ACTTTTGTGCCATTTAGAGATT	185-197	FAM
AFMB330XD5	D15S1017	49768798	49768956	15q21.2	45.62	0.63	TCAAGTAAGGCNATTATTATACAGA	CCACAAGCTGGACTGAGAAT	123-149	FAM
AFM326VD9	D15S1032	50484442	50484761	15q21.2	45.62	0.7133	AGCTTTAACTTCCATGAGTTTC	CTAATCTCTGGTGCATAGTGA	137-167	TET
AFMB324YH9	D15S1016	51320121	51320470	15q21.3	47.29	0.88	GATCCGTCACATAATGGC	ACACCTCAGCTTTCCTGG	239-267	FAM
AFMB076WC9	D15S1003	51698599	51698915	15q21.3	47.85	0.788	TGGTAGTACCCCTGGATACCTG	AATCTTTGGATATGGCTCTGCT	200-218	TET
AFMA085WG1	D15S1049	53542517	53542821	15q21.3	47.85	0.74	CACTCCAGCCTAAGGAACAC	TGTCAAAGATGGCTTTTATTACC	168-186	FAM
AFM296WG5	D15S1029	53750335	53750737	15q21.3	47.85	0.73	AAGAGTAAAACCTCCGTCACAAACAC	AGATTTGAGTCTCTGCACAGTAAG	273-285	FAM
AFM189YC1	D15S121	53840822	53841179	15q21.3	47.85	0.67	TTGTATCAGGGATTGGTTA	TGTTGTCGCTTCAGTACATA	258-264	TET
AFMA106XG1	D15S962	54362316	54362671	15q21.3	47.85	0.68	AATTCTGCTCATTGGGG	GGATATTTTGGAACTGCACT	282-294	FAM
CHLC.ATA20E12	D15S648	55165564	55165873	15q21.3	47.85	0.774	GGCATCATTCTGAGGATGTC	ATGCGTTCACTTCACAGTT	242-260	FAM
GATA153F11	CHLC.GATA153F11	55221723	55222047	15q21.3	48.98	0.704	AATGAATCCTGTGGTGTTGG	TCCAGAATAACAAGATGCTGC	209-229	TET
AFM098YG1	D15S117	56266839	56267255	15q22.1	51.21	0.82	GCACCAACAACCTATCCCAA	CCCTAAGGGGTCTCTGAAGA	132-150	FAM
AFM333YB5	D15S1033	56548483	56548838	15q22.1	52.33	0.73	GGATTACTTGGGCCAGG	TAAACGGCTTCAGGGAAC	157-169	FAM

Table S6. Screened Samples for CNV analysis and rs35490626 Taqman genotyping

Samples used for CNV analysis		
DNA's from	# of individuals	Ethnicity
Tourette group	840	Caucasian
Autism group	2,195	Caucasian, Latin America, Asian
Finland	7,014	Caucasian
Non-Ashkenazi Jewish	522	Caucasian
Total	10,571	
Samples used for TaqMan SNP genotyping		
Poland	375	Caucasian
New Zealand	360	Caucasian
Finland	730	Caucasian
Holland	715	Caucasian
Germany	715	Caucasian
Australian	700	Caucasian
Japanese	752	Japanese
^a NINDS	986	Caucasian
Other control subjects	1,080	Caucasian, Hispanic, African-American, and other races
Total	6,413	

^aNINDS (National Institute of Neurological Disorders and Stroke) samples are neurologically normal control samples

Table S7a. IBD estimates for parents and siblings.

IID1 ^a	IID2 ^b	EZ ^c	Z0 ^d	Z1 ^e	Z2 ^f	PI_HAT ^g	PHE ^h	DST ⁱ	PPC ^j	RATIO ^k
Father	Mother	0	0.8747	0.1173	0.008002	0.06665	0	0.756798	0.9958	2.281
Father	Child#1	0.5	0	0.9711	0.02895	0.5145	1	0.847203	1	NA
Father	Child#2	0.5	0	0.953	0.04704	0.5235	1	0.85005	1	NA
Father	Child#3	0.5	0	0.9591	0.04093	0.5205	1	0.849088	1	NA
Father	Child#4	0.5	0	0.9561	0.04392	0.522	1	0.849559	1	NA
Father	Child#5	0.5	0	0.9483	0.05169	0.5258	1	0.850781	1	NA
Father	Child#6	0.5	0	0.9424	0.05759	0.5288	1	0.851711	1	NA
Father	Child#7	0.5	0	0.9528	0.04723	0.5236	1	0.85008	1	NA
Father	Child#8	0.5	0	0.9018	0.09819	0.5491	1	0.858098	1	NA
Mother	Child#1	0.5	0	0.9482	0.05183	0.5259	0	0.850803	1	NA
Mother	Child#2	0.5	0	0.8893	0.1107	0.5554	0	0.860071	1	NA
Mother	Child#3	0.5	0	0.9325	0.06754	0.5338	0	0.853275	1	NA
Mother	Child#4	0.5	0	0.9425	0.05752	0.5288	0	0.851699	1	NA
Mother	Child#5	0.5	0	0.9515	0.04847	0.5242	0	0.850275	1	NA
Mother	Child#6	0.5	0	0.9392	0.06082	0.5304	0	0.852218	1	NA
Mother	Child#7	0.5	0	0.926	0.07397	0.537	0	0.854288	1	NA
Mother	Child#8	0.5	0	0.886	0.114	0.557	0	0.860582	1	NA
Child#1	Child#2	0.5	0.2661	0.3173	0.4166	0.5752	1	0.881701	1	10.77
Child#1	Child#3	0.5	0.254	0.5222	0.2238	0.4849	1	0.852566	1	10.15
Child#1	Child#4	0.5	0.1401	0.5466	0.3133	0.5866	1	0.877992	1	21.45
Child#1	Child#5	0.5	0.2367	0.4654	0.2979	0.5306	1	0.86596	1	12.48
Child#1	Child#6	0.5	0.2601	0.4996	0.2403	0.4901	1	0.85456	1	11.08
Child#1	Child#7	0.5	0.1727	0.5296	0.2977	0.5625	1	0.872295	1	17.01
Child#1	Child#8	0.5	0.2574	0.4811	0.2615	0.502	1	0.858155	1	8.889
Child#2	Child#3	0.5	0.2349	0.4619	0.3032	0.5342	1	0.866969	1	11.06
Child#2	Child#4	0.5	0.2476	0.4517	0.3007	0.5266	1	0.865314	1	11.01
Child#2	Child#5	0.5	0.2329	0.4297	0.3374	0.5523	1	0.872554	1	11.68
Child#2	Child#6	0.5	0.2525	0.5098	0.2377	0.4926	1	0.854911	1	10.24
Child#2	Child#7	0.5	0.2105	0.4482	0.3414	0.5655	1	0.875403	1	12.3
Child#2	Child#8	0.5	0.2773	0.3768	0.3459	0.5343	1	0.869464	1	7.784
Child#3	Child#4	0.5	0.1963	0.5265	0.2772	0.5405	1	0.866719	1	13.98
Child#3	Child#5	0.5	0.1712	0.5472	0.2817	0.5552	1	0.869922	1	16.09
Child#3	Child#6	0.5	0.2796	0.4237	0.2967	0.5085	1	0.861485	1	10.04
Child#3	Child#7	0.5	0.2081	0.5068	0.2852	0.5385	1	0.866796	1	12.83
Child#3	Child#8	0.5	0.2845	0.4039	0.3116	0.5135	1	0.863346	1	7.87
Child#4	Child#5	0.5	0.2498	0.5315	0.2187	0.4844	1	0.852185	1	10.41
Child#4	Child#6	0.5	0.2287	0.5061	0.2652	0.5182	1	0.861598	1	11.51
Child#4	Child#7	0.5	0.2011	0.4882	0.3108	0.5549	1	0.871529	1	14.92
Child#4	Child#8	0.5	0.2364	0.4098	0.3538	0.5587	1	0.874774	1	9.629
Child#5	Child#6	0.5	0.1681	0.4627	0.3692	0.6005	1	0.883996	1	19.15
Child#5	Child#7	0.5	0.3366	0.4432	0.2202	0.4418	1	0.843766	1	7.87
Child#5	Child#8	0.5	0.2422	0.5165	0.2413	0.4995	1	0.856496	1	9.531
Child#6	Child#7	0.5	0.2103	0.4282	0.3615	0.5756	1	0.878576	1	14.92
Child#6	Child#8	0.5	0.1706	0.4849	0.3445	0.587	1	0.879875	1	13.04
Child#7	Child#8	0.5	0.2493	0.4152	0.3355	0.5431	1	0.870624	1	9.84

^aIID1; Individual ID for first individual, ^bIID2; Individual ID for second individual, ^cEZ; Expected IBD sharing given PED file, ^dZ0; P(IBD=0), ^eZ1; P(IBD=1), ^fZ2; P(IBD=2), ^gPI_HAT; P(IBD=2)+0.5*P(IBD=1) (proportion IBD), ^hPHE; Pairwise phenotypic code (1,0,-1 = AA, AU and UU pairs), ⁱDST; IBS (pairwise identity-by-state (IBS)) distance (IBS2 + 0.5*IBS1)/(N SNP pairs), ^jPPC; IBS binomial test Ratio of Hethet; ^k RATIO; IBS 0 SNPs (expected value is 2)

Table S7b. Pairwise IBD was calculated using 99 independent Caucasians along with parents and one offspring genotyping data from Human 1M-Duo chip data

IID1 ^a	IID2 ^b	EZ ^c	Z0 ^d	Z1 ^e	Z2 ^f	PI_HAT ^g	PHE ^h	DST ⁱ	PPC ^j	RATIO ^k
Father	Mother	0	0.9658	0.0342	0.0000	0.0171	0	0.72368	0.7578	2.0451
Father	Child	0.5	0.0035	0.9935	0.0031	0.4998	1	0.82756	1	593.85
Mother	Child	0.5	0.0029	0.9941	0.003	0.5	0	0.82761	1	600.14

^aIID1; Individual ID for first individual, ^bIID2; Individual ID for second individual, ^cEZ; Expected IBD sharing given PED file, ^dZ0; P(IBD=0), ^eZ1; P(IBD=1), ^fZ2; P(IBD=2), ^gPI_HAT; P(IBD=2)+0.5*P(IBD=1) (proportion IBD), ^hPHE; Pairwise phenotypic code (1,0,-1 = AA, AU and UU pairs), ⁱDST; IBS (pairwise identity-by-state (IBS)) distance (IBS2 + 0.5*IBS1)/(N SNP pairs), ^jPPC; IBS binomial test Ratio of Hethet; ^k RATIO; IBS 0 SNPs (expected value is 2)

Table S8. HDC primer sequences and associated PCR amplification conditions

Amplicon	Primers (Forward/Reverse)	Annealing PCR Temperature (°C)
5'UTR-CDS-1	GGAGCTAAGGTCAAAGAAAGAACC TTCTAATGCTCCCATCAAATGTC	60
CDS-2	TCTTTACCAAGTCTCTTGGCCC TGACCCAAAGCAGGTCTTTCTC	60
CDS-3	GAGATGGTCTCTGGAGAAAGAAAG CCTAGGATACCACTCCCTGCTAC	60
CDS-4	AAATGTTCCCATTGACACCATC GTTAGGGTTTGGCTTGTCTTTC	60
CDS-5	TTTGGCAAGAGAATACAGAAAGG TCAAGGACCAAGATTCCAGAAG	60
CDS-6	ATATTTGGTTCCCGAGTCTTAGG CAAATTCCTTCCTCACATCC	60
CDS-7	TCTGTGGTGAAATCTTTCAGG TAATCCCATAGAGCTGGTTTCAG	60
ZXY-5UTR.1	AGTAGGAGAGCCTTCGGTTTTC CCTTTTTCTTCCACTGGGTAAAG	60
ZXY-5UTR.2	CCTTCCCTCTAACTTTTGTATTCTG ATAAGCAGCATCGATGTGGAG	60
CDS-8-9	TCATTTAGCCAAAGCACATCATC GGTATGCATTGGCAGTATTATGAG	60
CDS-10	AACAGATAATGCCAGAAATGC TGGTTAACAAAGAGAATCAAGTCC	60
CDS-11	CATTTAGCAGTCTGAAAGTTTATGC TAAAGGAAGTGAAGTCTCCATCG	60
CDS-12.1	TCTGTGAATGATGTCTTGTGGTG ATTGTGAGGGGTTTACAGAGTC	60
CDS-12.2	TGTCCTCCTTCCTGTTTACAGTTAC ATACAATTGTGAGGGGTTTACAG	58.4
3'UTR1	AATGCAGCTCTCAATGTGGAC CCCAGACTGGTTAATCAATGTTTC	60
<i>HDC-beu</i> -3UTR	GTGCCCCGTCTTTGTAAGTCAG ACCTAGGGGAAAATTAAGGCAAG	60
<i>HDC-bet</i> -3UTR	AGTATCTTCCCTCTGTGGCTAGG CTACCCTTTTCTTTTCTGAAGG	60
<i>HDC</i> -Promoter	CCCCTTCTACTTCTTTAAGTGC CCTTCTCACAGATGGACACG	60

Table S9. Oligonucleotides and Taqman fluorogenic probes

Name of Probe	Primer/Probe Name	Primer and probe sequences (5'-3')
<i>HDC-W317X</i>	Forward	AGCCACTAATGCCCATTTGGA
	Reverse	CTGCTGCAGCTTGTACTTGTC
	FAM	AACCCACAGAGTCAAG
	VIC	ACCCACAGGGTCAAG
<i>HDC-V186L</i>	Forward	TGCTGATGAGTCCTGCCTAAATG
	Reverse	GGCAACTCACCTGGTCAGA
	FAM	CCCGACTCATGGCCTA
	VIC	CCGACTCGTGGCCTA
HDC-R8G	Forward	AGCCCAAGGGAGATGATGGA
	Reverse	CAGGGATGCCCGTTGCT
	FAM	CTCTCTCTCCGTACTCC
	VIC	CCTCTCTCTCTGTACTCC
HDC-P303S	Forward	TTCCTGTGCCCCGAGTTC
	Reverse	CAGTACAGTCAAATGCACCATCAT
	FAM	CTTGGAAGAATTAAG
	VIC	ACTTGGAAGGATTAAG
HDC-rs35490626	Forward	AGATTCCAGTCTCTCAGGATGTCA
	Reverse	GCCACTATCCAGGACAAGTTAATCA
	FAM	TGACATCCCCAGTTTAC
	VIC	TGACATCCCCAGTTTAC

Table S10. Results from the Dominant Negative Effect Assay of W317X using Liquid Chromatography-Mass Spectrometry.

sample	wt (ug)	mut (ug)	ratio of mut/wt	area ratio	corrected area	quality	IS quality	signal noise	histamine conc.	average conc.	stdev
1a	0.5	0	0	4.78	1946405.47	1.00	1.00	7533.63	34731.60	34108.86	539.31
				4.65	2717477.42	1.00	1.00	8786.48	33798.07		
				4.65	1772930.31	1.00	1.00	7164.72	33796.91		
1b	0.5	0	0	3.35	1622946.30	1.00	1.00	8947.79	24345.82	23250.56	959.70
				3.15	1474733.61	1.00	1.00	6635.11	22849.01		
				3.11	1758013.20	1.00	1.00	8018.72	22556.86		
2a	0.5	0.125	0.25	2.28	1336647.87	1.00	1.00	5111.68	16531.95	17358.87	716.16
				2.45	1251900.11	1.00	1.00	5644.21	17766.23		
				2.45	1350637.27	1.00	1.00	6601.78	17778.42		
2b	0.5	0.125	0.25	2.45	1568680.78	1.00	1.00	7693.35	17772.19	17670.75	502.62
				2.49	1342285.73	1.00	1.00	6638.26	18114.92		
				2.36	1368649.25	1.00	1.00	5637.98	17125.16		
3a	0.5	0.25	0.5	1.60	901614.26	1.00	1.00	4039.69	11629.00	11327.32	506.25
				1.48	1121477.14	1.00	1.00	4949.93	10742.85		
				1.60	822413.51	1.00	1.00	3504.77	11610.10		
3b	0.5	0.25	0.5	1.97	1120862.99	1.00	1.00	3777.36	14342.34	15084.97	656.48
				2.15	989625.66	1.00	1.00	4990.83	15587.98		
				2.11	1147614.49	1.00	0.86	5300.99	15324.60		
4a*	0.5	0.5	1	0.30	131061.61	1.00	1.00	852.03	2202.07	2159.27	37.14
				0.29	147907.59	1.00	0.93	758.62	2140.20		
				0.29	211926.33	1.00	0.99	1122.14	2135.55		
4b	0.5	0.5	1	1.46	632370.12	1.00	1.00	2017.62	10585.02	10940.27	442.76
				1.49	522949.74	1.00	1.00	2055.14	10799.48		
				1.57	691751.23	1.00	1.00	3542.95	11436.30		
5a	0.5	1	2	0.86	382993.23	1.00	1.00	2072.70	6233.67	5941.26	253.44
				0.80	338758.94	1.00	0.94	1263.30	5805.15		
				0.80	344364.96	1.00	0.98	1337.12	5784.94		
5b	0.5	1	2	1.19	689738.83	1.00	1.00	3123.48	8635.51	9365.65	650.03
				1.32	661705.22	1.00	1.00	3600.26	9580.01		
				1.36	617952.85	1.00	1.00	2967.51	9881.42		
6a	0	0.5		0.05	30202.79	1.00	1.00	185.86	371.71	381.36	10.66
				0.05	30712.16	1.00	1.00	201.00	392.81		
				0.05	36978.33	1.00	1.00	218.17	379.55		
6b	0	0.5		0.12	46535.80	1.00	1.00	390.62	846.75	832.78	34.31
				0.11	41523.25	1.00	1.00	251.18	793.69		
				0.12	62442.36	1.00	1.00	443.94	857.91		

Each condition was performed in replicate, designated (a) and (b), and then measured in triplicate.

* The histamine concentration for sample 5a at 0.5ug was significantly lower than its biological counterpart and appears to be an outlier when compared to the data set as a whole. This data point, if accurate, would support an exaggerated dominant negative effect at a 1:1 ratio compared to that illustrated in Fig 3.

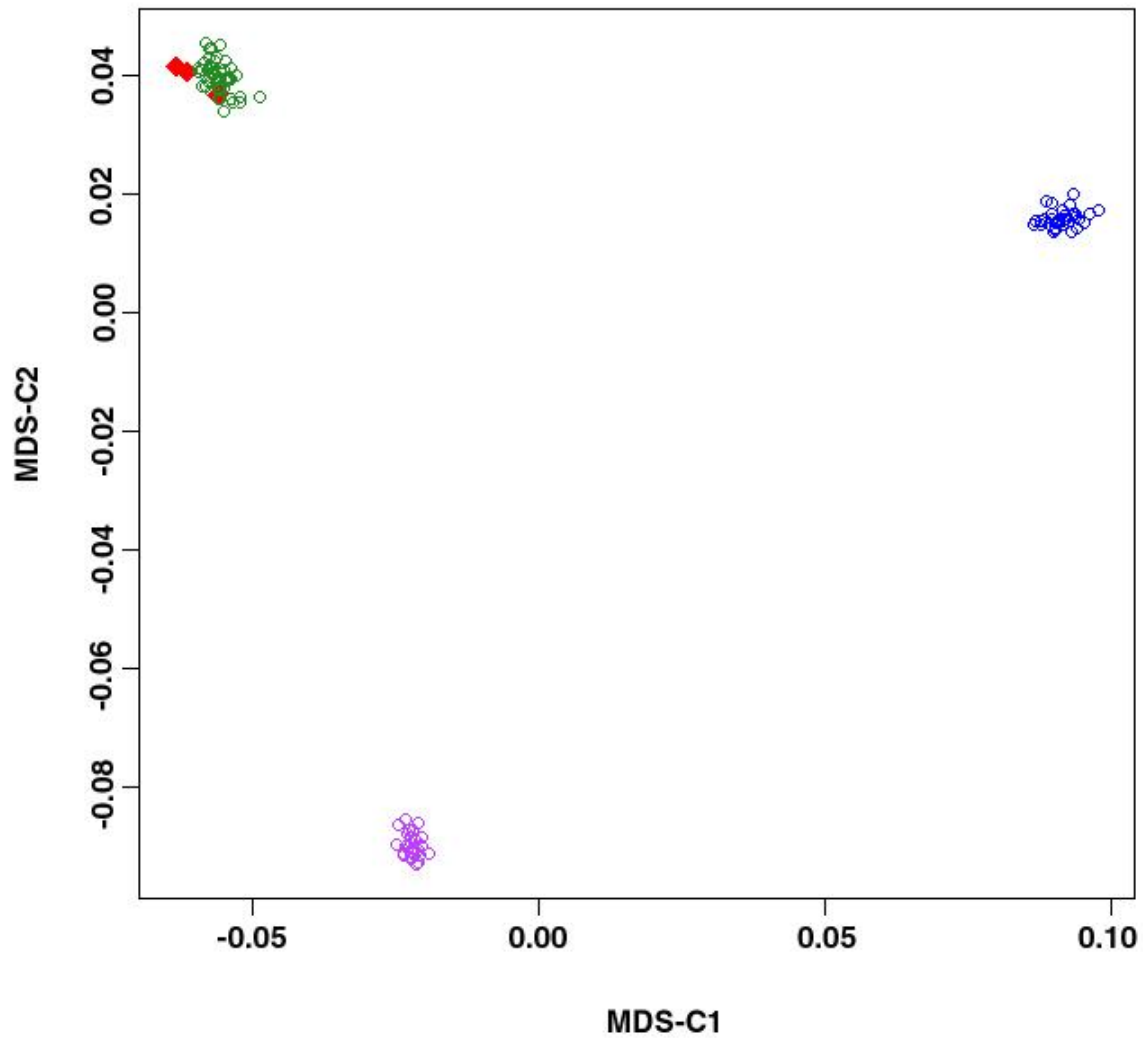


Figure S1: MDS (Multidimensional scaling) analysis tightly clustered Tourette family members (father, mother and a child) with Caucasian HAPMAP samples. The first (C1) and second (C2) dimensions were plotted. In the graph; Red diamonds indicate Tourette Family members, green circles; Caucasian (CEU), blue and purple circles; African (YRI), and Asian (JPT-CHB) HAPMAP samples, respectively.

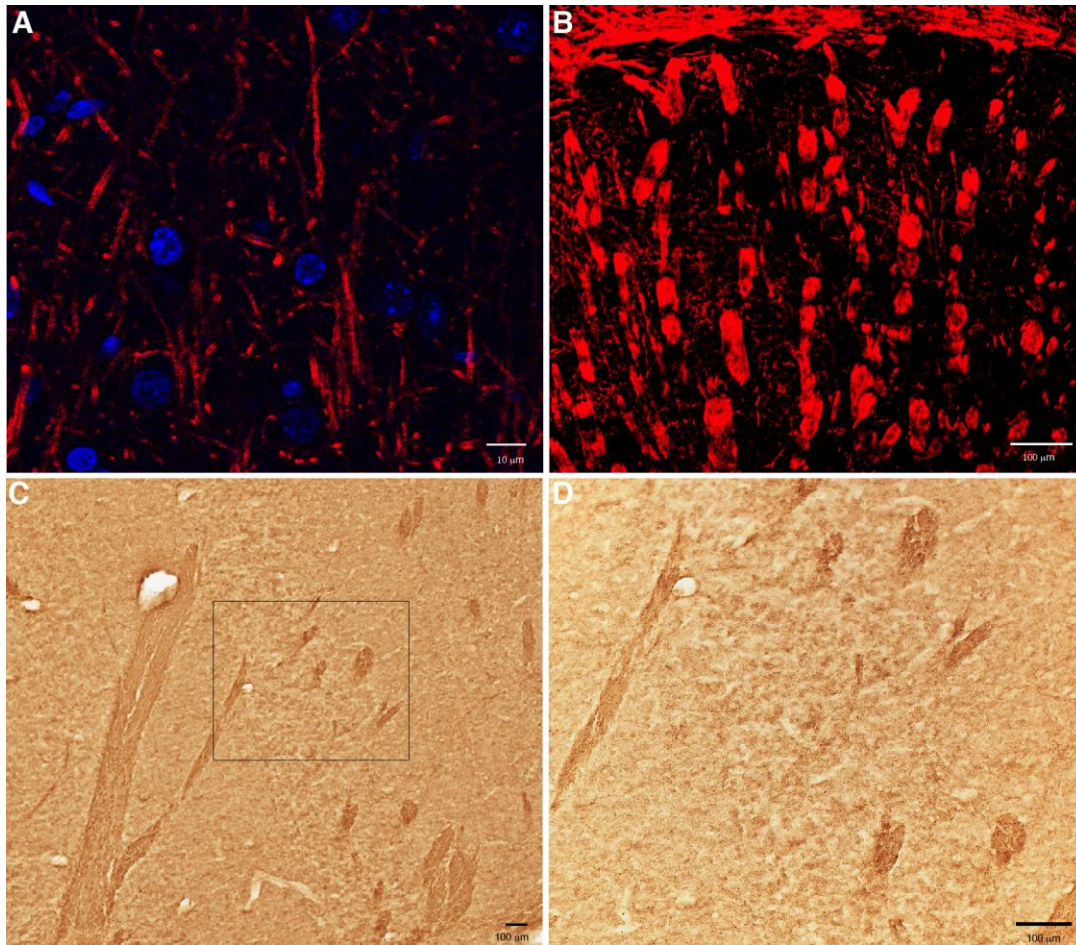


Figure S2: Histidine H3 Receptor (*H3R*) expression in mouse and human brain. (A) Confocal microscopy of *H3R* staining in adult mouse cortex, layer 5. *H3R* is highly expressed in the axons. *H3R* staining is in red with the cell nuclei shown in blue (DAPI). (B) Large bundles of axonal projections are seen in the adult mouse striatum. At the top, part of the corpus callosum is shown, also highly positive for *H3R* expression. (C) *H3R* staining in adult human striatum is depicted at low magnification and at high magnification (D), where the box represents the region under higher magnification. Similarly *H3R*-positive axonal bundles are seen in the striatum.

(A)

W317X

<i>Homo sapiens</i>	AAAYAGTAFLCPEFRGFLKGI EYADSFTFNPSKMMVHFDCTGFVVKDKYK	323
<i>Pan troglodytes</i>	AAAYAGTAFLCPEFRGFLKGI EYADSFTFNPSKMMVHFDCTGFVVKDKYK	323
<i>Macaca mulatta</i>	AAAYAGTAFLCPEFRGFLKGI EYADSFTFNPSKMMVHFDCTGFVVKDKYK	323
<i>Canis lupus familiaris</i>	AAAYAGTAFLCPEFRGFLKGI EYADSFTFNPSKMMVHFDCTGFVVKDKYK	342
<i>Bos taurus</i>	AAAYAGTAFLCPEFRGFLKGI EYADSFTFNPSKMMVHFDCTGFVVKDKYK	323
<i>Equus caballus</i>	AAAYAGTAFLCPEFRGFLKGI EYADSFTFNPSKMMVHFDCTGFVVKDKYK	323
<i>Mus musculus</i>	AAAYAGTAFLCPELRGFLEGI EYADSFTFNPSKMMVHFDCTGFVVKDKYK	330
<i>Rattus norvegicus</i>	AAAYAGTAFLCPELRGFLEGI EYADSFTFNPSKMMVHFDCTGFVVKDKYK	326
<i>Monodelphis domestica</i>	AAAYAGTAFLCPEFREFLKGI EYADSFTFNPSKMMVHFDCTGFVVKDKFK	322
<i>Gallus gallus</i>	AAAYAGTAFLCPEFRLFLDGI EYADSFANPSKMMVHFDCTGFVVKDKYK	322
<i>Sus scorfa</i>	AAAYAGTAFLCPEFRGFLKGI EYADSFTFNPSKMMVHFDCTGFVVKDKYK	339
<i>Aplysia californica</i>	AAAYAGSAFICPEFRSWMAGI EFSDFSANPSKWL MVHFDCSAMVVKDARA	342
<i>Apis mellifera</i>	AAAYAGSAFVCPFRGWLKGI EYADSI AFNPSKWL MVHFDCTAMVVKSSQA	322
<i>Nasonia vitripennis</i>	AAAYAGSAFVCPFRSWMKGI EYADSFANPSKWL MVHFDCTAMVVRNSQS	321
<i>Drosophila melanogaster</i>	AAAYAGSAFICPEFRTWLRGI ERADSI AFNPSKWL MVHFDATA L VVRDSTA	322

*****:**:***:* :: *** :***:*****:*****:..:***:.

(B)

V186M

<i>Homo sapiens</i>	PDAD E SCLNARLVAYASDQA HSSVEKAGLISLVKMKFLPVDDNFS LRGEA	223
<i>Pan troglodytes</i>	PDAD E SCLNARLVAYASDQA HSSVEKAGLISLVKMKFLPVDDNFS LRGEA	223
<i>Macaca mulatta</i>	PDAD E SCLNARLVAYASDQA HSSVEKAGLISLVKMKFLPVDDNFS LRGEA	223
<i>Canis lupus familiaris</i>	PGADESSLNARLVAYASDQA HSSVEKAGLISLVKMKFLPVDDNFS LRGEA	242
<i>Bos taurus</i>	PEADESFLNARLVAYASDQA HSSVEKAGLISLVKMKFLPVDDNFS LRGEA	223
<i>Equus caballus</i>	PGVDESSLNARLVAYASDQA HSSVEKAGLISLVKMKFLPVDDNFS LRGA V	223
<i>Mus musculus</i>	PDAN E SSLNARLVAYTSDQA HSSVEKAGLISLVKIRFLPVDDNFS LRGEA	230
<i>Rattus norvegicus</i>	PNADESSLNARLVAYASDQA HSSVEKAGLISLVKIKFLPVDDNFS LRGEA	226
<i>Monodelphis domestica</i>	PDVDESSLNSRLVAYASDQA HSSVEKAGLISLVKMKFLPVDKNFS LRGET	222
<i>Gallus gallus</i>	PGADESSLNARLVAYASDQA HSSVEKAGLISLVKMKFLPVDDNFS LRGET	222
<i>Sus scorfa</i>	PGADESSLNARLVAYASDQA HSSVEKAGLISLVKMKFLPVDDNFS LRGEA	239
<i>Aplysia californica</i>	PDKDEAWINGRLTGYCSDQA HSSVEKAGLIGLVKMRFLPSDENLS LRGST	242
<i>Apis mellifera</i>	PDRLPAEINSRLVAYCSDQA HSSVEKAGLIGLVRMKYIESDDELSMRGET	222
<i>Nasonia vitripennis</i>	PDLLPAEINSRLVAYCSDQA HSSVEKAGLIGLVRMRYIDSDDNLSMRGEK	221
<i>Drosophila melanogaster</i>	PGYQDAEINARLVAYCSDQA HSSVEKAALIGLVRMRYIEADDDLAMRGKL	222

* : :*.***:.* *****.***.***:..: * :..:***

(C)

R8G

<i>Homo sapiens</i>	-----MMEP-----E EYRERGREMVDYICQYLSTVRRERRVT	31
<i>Pan troglodytes</i>	-----MMEP-----E EYRERGREMVDYICQYLSTLRERRVT	31
<i>Macaca mulatta</i>	-----MMEP-----E EYRERGREMVDYICQYLSTVRRDRVT	31
<i>Canis lupus familiaris</i>	MVVASNFLLCDSFVSPSPLVSHRERE RER GKEMVDYICQYLSTVRRERRVT	50
<i>Bos taurus</i>	-----MMEP-----E EYRER GKEMVDYICQYLSTVRRERRVT	31
<i>Equus caballus</i>	-----MMEP-----E EYRER GKQMVDYICQYLSTVRRERRVT	31
<i>Mus musculus</i>	-----MMEPCEYREYREY RARGKEMVDYISQYLSTVRRERQVT	38
<i>Rattus norvegicus</i>	-----MMEPSEYREY---QARGKEMVDYICQYLSTLRERQVT	34
<i>Monodelphis domestica</i>	-----MDL-----E EYRER GKEMVDYIFQYLSTVRRERRVT	30
<i>Gallus gallus</i>	-----MEP-----E EYRRRGKEMVDYICQYLSNVRERRVT	30
<i>Sus scorfa</i>	-----MDLERDPHKRRRTLPLKFI SFTINLQGH SAGYQELVSLDLN	41
<i>Aplysia californica</i>	--MAEDLLSEQCKSSLPPGGMTVEEY RKR GKEMVDYIADYFLDIRSRRVF	48
<i>Apis mellifera</i>	-----MNLEEYRKHGKEMVDYIADYLENIRSRRVY	30
<i>Nasonia vitripennis</i>	-----MNLEEYRQH GKEMVDYIADYLENIRKRRVY	30
<i>Drosophila melanogaster</i>	-----MDFKEYRQR GKEMVDYIADYLENIRERRVY	30

:: : * : . :

(D)	P303S	
<i>Homo_sapiens</i>	AAAYAGTAFLCPEFRGFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKYK 323
<i>Pan_troglodytes</i>	AAAYAGTAFLCPEFRGFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKYK 323
<i>Macaca_mulatta</i>	AAAYAGTAFLCPEFRGFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKYK 323
<i>Canis_lupus_familiaris</i>	AAAYAGTAFLCPEFRGFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKYK 342
<i>Bos_taurus</i>	AAAYAGTAFLCPEFRGFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKYK 323
<i>Equus_caballus</i>	AAAYAGTAFLCPEFRGFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKYK 323
<i>Mus_musculus</i>	AAAYAGTAFLCPELRGFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKYK 330
<i>Rattus_norvegicus</i>	AAAYAGTAFLCPELRGFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKYK 326
<i>Monodelphis_domestica</i>	AAAYAGTAFLCPEFRFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKFK 322
<i>Gallus_gallus</i>	AAAYAGTAFLCPEFRFLKGYEYADSFVFN	PSKWMVVHFDCTGFVVKDKYK 322
<i>Sus_scorfa</i>	AAAYAGTAFLCPEFRGFLKGYEYADSFTFN	PSKWMVVHFDCTGFVVKDKYK 339
<i>Aplysia_californica</i>	AAAYAGSAFICPEFRSWMAGIEFSDFVFN	PSKWLVMVHFDCTAMVVKDARA 342
<i>Apis_mellifera</i>	AAAYAGSAFVCPEFRGFLKGYEYADSIVFN	PSKWLVMVHFDCTAMVVKSSQA 322
<i>Nasonia_vitripennis</i>	AAAYAGSAFVCPEFRSWMKGYEYADSFVFN	PSKWLVMVHFDCTAMVVRNSQS 321
<i>Drosophila_melanogaster</i>	AAAYAGSAFICPEFRVLRGIERADSIVFN	PSKWLVMVHFDCTALVVRDSTA 322
	*****:**:**:* :: *** :***:*****:*****.:**:	

Figure S3: The amino acid alignment of *HDC* across species using ClustalW2 program.

The *HDC* variants that resulted in an amino acid change are depicted in the alignment. Variants found in the cases are shaded with pink (**A** and **B**) and variants found in the controls are shaded with yellow (**C** and **D**). An asterisk (*) highlights identical residues, conserved substitutions are marked with a colon (:), and period (.) indicates semiconserved substitutions.

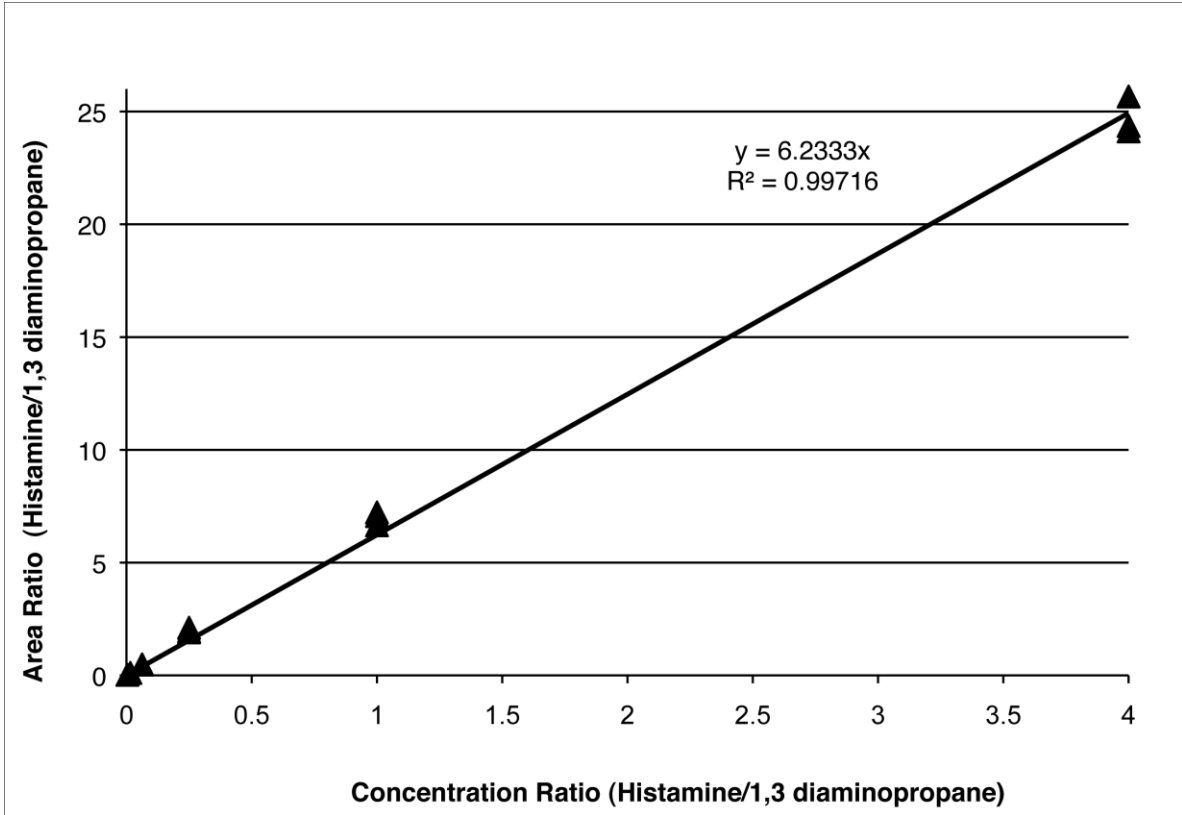


Figure S4: Histamine calibration curve generated from liquid chromatography-mass spectrometry. The linear histamine concentration curve was calculated from triplicate MRM assays on histamine at the following concentrations (pmol/ μ l): 0, 0.024, 0.097, 0.39, 1.56, 6.25, 25, and 100 with a constant concentration of 6.25 pmol/ μ L of 1,3 diaminopropane. This histamine calibration curve was then used to calculate the amount of histamine produced from the experimental *in vitro* translated wt and mut HDC proteins.