Supplementary methods and materials

pVAAST analysis

The variants in the VCF files were converted to Genome Variation Format (GVF) with the vaast_converter. Genotypes with quality scores (GQ) of less than 30 were converted to no calls. The variants were annotated using the Variant Annotation Tool[1]. Then, annotated variants for the entire pedigree were combined into one condensed file using Variant Selection Tool (VST). Lastly, the pVAAST analysis was conducted under both dominant and recessive modes of inheritance. Single nucleotide variants and short insertion/deletions overlapping coding and splice sites were included in the analysis, which was performed allowing up to 1% prevalence of a variant in the background sample. The background sample consists of 427 genomes from the 1000 Genomes project that were sequenced using the Complete Genomics platform [2]. It includes a wide variety of ethnicities, including 80 Africans, 96 Europeans, 93 Chinese and several other populations. Candidate genes were ranked based on their disease-causing probability and evidence of their segregation with the phenotype in the pedigree. The candidate gene list and detailed commands for pVAAST runs can be found in Table S4.

Code availability

Please see supplementary Table 4 for commands calling the candidate genes.

Generation and maintenance of induced pluripotent stem cells (iPSCs)

CD4+ T cells from peripheral blood draws were isolated using the CD4+ T cells isolation kit (Miltenyi Biotec, CA) and subsequently stimulated with Dynabeads Human T-Activator CD3/CD28 (ThermoFisher Scientific, MA) for 4 days in T cell growth medium (RPMI 1640 medium + 15%FBS + 1% glutamine + 1% Pen/Strep). To reprogram T cells into iPSCs, stimulated CD4+ T cells were transduced with Sendai virus vectors carrying transgenes: Oct4, Sox2, Klf4 and c-Myc (CytoTune-iPS reprogramming kit). Two days after transduction, CD4+ T cells were plated on X-ray-inactivated mouse embryonic fibroblast feeder cells in T cell growth medium. Two days later, the medium was replaced with the KOSR medium (20% Knockout serum replacement, 20ng/mL bFGF, 1% GlutaMax and 1% NEAA in Knockout DMEM/F12) and iPSC colonies appeared around 9 days post-transduction. iPSC colonies were then manually picked and seeded onto plates coated with Matrigel (BD Biosciences, CA) and cultured in mTeSR medium (Stemcell Technologies, Canada) for expansion. iPSCs were passaged weekly using Dispase (Stemcell Technologies, Canada).

Generation and maintenance of neural progenitor cells (NPCs) and neurons

At day 0, iPSCs were split 5:1 (passage 1) onto Matrigel-coated plates using Dispase and were cultured in 1:1 mTeSR/NBM medium (NBM: Neurobasal Medium supplemented with 1% N2, 2% B27 without vitamin A, 1% Insulin Transferrin Selenium and 2mM L-Glutamine) with 500ng/ml Noggin (PeproTech, NJ) for 5 days during which medium was refreshed every 2 days. At day 5, culture medium was changed to NBM with 500ng/ml Noggin and was refreshed every two days until the cells become 90% confluent. At approximately day 10 to 12, cells were scraped off the plates manually with cell scrapers and were plated (passage 2) onto 20ug/ml laminin-coated 10cm

dishes and were subsequently cultured in NBM medium without Noggin. NBM medium was changed every 2 days and was replaced by NPM medium (50% DMEM/F12 with GlutaMAX, 50% Neurobasal Medium, 0.5% N2, 0.5% B27 without vitamin A and 20ng/ml bFGF) when the cells became 60%-70% confluent. At around day 24, the culture dishes became 90% confluent and cells from one 10cm dish were passaged (passage 3) onto 3 T75 flasks coated with Matrigel using Accutase (Stemcell Technologies, Canada). After 3-4 days, when the T75 flasks became confluent, the monolayer cells were plated onto 10 cm dishes for RNA and protein extractions, or plated onto glass coverslips for immunocytochemistry, or cryopreserved in mFreSR (Stemcell Technologies, Canada) for storage.

To generate neurons, the neural progenitor cells were plated on 10cm culture dishes as a monolayer in NPM. NPM was then replaced with neural differentiation medium (NDM: Neurobasal Medium with 1x B27 Supplement (ThermoFisher Scientific, MA) and 10ng/ml human recombinant BDNF (Peprotech, NJ). NDM was changed every 2 days until cells were collected for functional assays.

Karyotyping

One or two confluent wells of iPSCs from a 6 well plate were re-plated onto a Matrigel-coated T25 flask. Cells were sent to Cell Line Genetics for karyotyping.

Immunocytochemistry

iPSCs were plated on Matrigel coated plastic chamber slides (Nunc Lab-Tek), NPCs were plated on Matrigel coated glass coverslips and neurons were plated on poly-L-ornithine (15ug/ml)/laminin (1ug/ml)/fibronectin (2ug/ml) mix (Sigma-Aldrich, MO)-

coated glass coverslips. Cells were fixed with 4% paraformaldehyde for 10 min and were permeabilized with 1x PBS solution containing 0.5% Triton X-100 and 1% bovine serum albumin (BSA) for 5 min. Cells were blocked in 1x PBS solution containing 1% BSA . Primary and secondary antibodies were both diluted in 1x PBS solution containing 0.2% Triton X-100 and 1% BSA. Cells were mounted with SlowFade Gold Antifade Mountant (Thermo Fisher Scientific, MA) and images were captured using Zeiss LSM 510 META confocal microscope. Information on antibodies is shown in Table S2

Flow Cytometry

iPSC colonies were detached from the wells as single cells using Accutase. After being washed 2 times with PBS, cells were fixed with 4% PFA for 15 min at RT and permeabilized, blocked with Perm/Wash Buffer (BD Biosciences, CA) for 15 min at RT. Proper amount of primary antibodies anti-OCT4 (1:2000) and anti-TRA -1-60 (1:1000) (EMD Millipore, CA) were added into Perm/Wash Buffer and incubated with cells overnight at 4°C. After washing the cells 3 times with Perm/Wash Buffer, secondary antibodies goat anti-Mouse IgG1, PE and goat anti-Mouse IgM Heavy Chain Cross-Adsorbed, Alexa Fluor 488 (Thermo Fisher Scientific, MA) were both diluted at 1:500 in Perm/Wash Buffer and incubated with the cells in the dark for 30-45min. Cells were washed with Perm/Wash Buffer 2 times and resuspended in PBS for flow cytometry analysis.

To stain for NESTIN and SOX2 in NPCs, we used eBioscience™ Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher Scientific, MA) and “one-step protocol:

intracellular protein (nuclear) protein". Anti-Human Nestin Alexa Fluro488 and Anti-Sox2 eFluro 660 antibodies (Thermo Fisher Scientific, MA) were used.

Calcium imaging

Fluorescent calcium indicator dye Fluo4 AM and cell viability dye calcein red-orange AM (Thermo Fisher Scientific, MA) were used for imaging. The coverslip was placed in the Quick Release Imaging Chamber (Warner Instruments, CT) and medium was replaced with pre-warmed HBSS. 100mM KCl Tyrode's solution was perfused to depolarize the neuronal cultures. Images were captured using a Zyla sCMOS camera (Andor Technology, UK) and a 20X 0.75 NA objective at 0.25 sec/0.5sec intervals for 1 min and intensity was quantified using NIH ImageJ software.

.**Cycloheximide (CHX) treatment**

For each differentiation process, four lines of neurons at 29 days (D29) of differentiation were untreated, treated with DMSO or treated with 100ug/ml CHX for ~15 hrs. D30 neurons were then collected for RNA extraction. RNAs were converted to cDNA as described in the methods section. *PNKD* (L) transcript specific primers were used to measure *PNKD* (L) transcript level by qPCR. D30 neurons from three independent differentiations were included for analysis.

Statistics

Two-sided student *t*-test was used when comparing the *PNKD* (L) transcript expression in unaffected and TD affected cells. Error bar represents the standard error. Sample size is 3 for each sample under each condition.

Supplementary clinical evaluations of the TD multiplex family

Of the five siblings, four (including the proband) met DSM-IV-TR [3] criteria for TD and one sibling met criteria for a chronic tic disorder not specified in the DSM, namely chronic tic disorder-combined subtype [4], defined as a history of a single motor (rather than multiple motor) and at least one vocal tic, with onset prior to age 18 and persistence for at least a year. The sibship consists of an older sister (5001), a set of MZ twin females (5002 and 5003) which includes the proband, and a younger set of DZ twins- one male (5005) and one female (5004). The proband's MZ twin was not sequenced, but zygosity was confirmed. The father (4001) was also diagnosed with chronic tic disorder- combined subtype. The paternal grandmother (3002) was diagnosed with a chronic motor tic disorder and paternal grandfather (3001) was diagnosed with a tic disorder, not otherwise specified (onset age 66), consisting of a recent-onset recurrent motor tic of less than a year's duration. Both paternal grandparents and the three oldest siblings were diagnosed with OCD. The father was diagnosed with subclinical OCD (consisting of obsessions and compulsions that were non-impairing and only mildly distressing) while the mother (4002) was diagnosed with OC symptoms (defined as non-interfering, non-distressing obsessions and compulsions)[5]. The youngest sibling was also diagnosed with subclinical OCD and probable autism spectrum disorder.

Reference

1. Hu H, Roach JC, Coon H, Guthery SL, Voelkerding KV, Margraf RL *et al.* A unified test of linkage analysis and rare-variant association for analysis of pedigree sequence data. *Nat Biotechnol* 2014; **32**(7)**:** 663-669.

2. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO *et al.* A global reference for human genetic variation. *Nature* 2015; **526**(7571)**:** 68-74.

3. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th, Text Revision edn: Washington, DC, , 2000.

4. Dietrich A, Fernandez TV, King RA, State MW, Tischfield JA, Hoekstra PJ *et al.* The Tourette International Collaborative Genetics (TIC Genetics) study, finding the genes causing Tourette syndrome: objectives and methods. *Eur Child Adolesc Psychiatry* 2015; **24**(2)**:** 141-151.

5. McMahon WM, Carter AS, Fredine N, Pauls DL. Children at familial risk for Tourette's disorder: Child and parent diagnoses. *Am J Med Genet B* 2003; **121B**(1)**:** 105-111.