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Genome-wide Association Study Points to Novel Locus for Gilles de la Tourette Syndrome

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ABSTRACT

BACKGROUND: Tourette syndrome (TS) is a childhood-onset neurodevelopmental disorder of complex genetic architecture and is characterized by multiple motor tics and at least one vocal tic persisting for more than 1 year.

METHODS: We performed a genome-wide meta-analysis integrating a novel TS cohort with previously published data, resulting in a sample size of 6133 individuals with TS and 13,565 ancestry-matched control participants.

RESULTS: We identified a genome-wide significant locus on chromosome 5q15. Integration of expression quantitative trait locus, Hi-C (high-throughput chromosome conformation capture), and genome-wide association study data implicated the *NR2F1* gene and associated long noncoding RNAs within the 5q15 locus. Heritability partitioning identified statistically significant enrichment in brain tissue histone marks, while polygenic risk scoring of brain volume data identified statistically significant associations with right and left thalamus volumes and right putamen volume.

CONCLUSIONS: Our work presents novel insights into the neurobiology of TS, thereby opening up new directions for future studies.

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Tourette syndrome (TS) is a childhood-onset neurodevelopmental disorder characterized by multiple motor tics and at least one vocal tic persisting for more than 1 year (1). The prevalence of TS is estimated to be in the range of 0.6% to 1% in school-aged children (2,3). It is a highly heritable disorder (4) with a population-based heritability estimated at 0.7 (5,6) and single nucleotide polymorphism–based (SNP) heritability (h^2_{SNP}) estimates ranging from 0.21 (7) to 0.58 (4) of the total heritability. TS exhibits high polygenicity, and its genetic background is influenced by both common and rare variants of small effect spread throughout the genome (4,8,9). The results of two previously conducted genome-wide association studies (GWASs) (7,10) indicated enrichment of TS genetic susceptibility variants in tissues within the corticostriatal and corticocerebellar circuits and, in particular, the dorsolateral prefrontal cortex (7,10). Furthermore, gene set analyses of GWAS data have implicated ligand-gated ion channel signaling, lymphocytic, and cell adhesion and trans-synaptic signaling processes as potential biological underpinnings in the pathogenesis of TS (11). Polygenic risk scores derived from the second TS GWAS study predicted tic presence and severity at a statistically significant level (7,12). In addition, the hypothalamic-pituitary-adrenal axis was implicated in a recent cross-disorder GWAS analysis for TS, attention-deficit/hyperactivity disorder, and autism spectrum disorder (13).

Here, we present a genome-wide meta-analysis for TS that integrates novel and previously published data, resulting in a combined sample size of 6133 individuals with TS and 13,565

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ancestry-matched control subjects. We identify a novel genome-wide significant locus in the novel TS-EUROTRAIN (a training network for TS) GWAS and in the TS GWAS meta-analysis. Our results provide further insight into the genetic basis of TS.

METHODS AND MATERIALS

Datasets

The TS-EUROTRAIN dataset brings together 3 major TS cohorts, including 632 participants from the EMTICS (European Multicenter Tics in Children Study) (14), 763 participants from the TS-EUROTRAIN study (15), 238 participants from the TSGeneSEE (Tourette Syndrome Genetics: Southern and Eastern Europe Initiative) study (16), and 52 participants from Sweden. These studies included participants from multiple European sites who were diagnosed using DSM-IV-TR or DSM-5 criteria for TS, consistent with previously published TS studies. In total, we collected samples from 1685 individuals with TS (Table S1). In addition, 4454 population-matched control individuals were recruited. Ancestry-matched control subjects from the following public datasets were also used following appropriate approvals: British WTCCC2 1958 Birth Cohort samples (Study accession code: EGAS00000000028), German control samples obtained from the POPGEN biobank (17), and French control subjects from the Three City Study (18), leading to a total of 8558 general population control subjects (Table S1). Written informed consent was obtained from all participants as approved by the ethics committees of all participating institutions.

Genotyping, Merging, and Imputation

Samples from the TS-EUROTRAIN dataset were genotyped on the Illumina HumanOmniExpress BeadChip at deCODE genetics. The control samples obtained from collaborators and public repositories were genotyped on multiple Illumina arrays, and full details are provided in the respective references and Table S1. We applied standard GWAS quality control procedures to our data before and after the imputation, as described in a previous GWAS performed by the PGC (Psychiatric Genomics Consortium) (7). Quality control procedures included the removal of samples that fit any of the following criteria: call rate < 0.98, absolute value of inbreeding coefficient < 0.2, genomic sex discrepancy with reported sex, and formation of pairs with relatedness > 0.1875. We applied variant quality control, excluding markers with call rate < 0.98, differential missingness between cases and controls > 0.02, Hardy-Weinberg equilibrium p value $< 10^{-6}$ in controls and $< 10^{-10}$ in cases.

The quality control steps were applied on each dataset separately. Imputation was performed on the Sanger Imputation Server using a reference panel of 64,940 European ancestry haplotypes (version 1.1) from the HRC (Haplotype Reference Consortium) (19,20). We performed batch effect tests in samples of same status (case/control) between different sources, as they are shown in Table S1, excluding markers that achieved a p value $< 10^{-5}$. X chromosome data were excluded from the final analysis. To avoid ancestry bias, we matched the ancestry of the control subjects to individuals with TS at a 3:1 ratio, using the first 5 principal components (PCs) as the basis for a final dataset of 1438 individuals with TS and 4356 control subjects on 2,949,675 markers.

Genome-wide Association Study

We conducted a GWAS using an additive logistic regression model on the best-guess genotypes produced by imputation. We incorporated the PCs identified by Tracy-Widom statistics, as calculated by EIGENSOFT (21,22), as well as sex and imputation batch. We excluded SNPs when their minor allele frequency (MAF) was <1% and minor allele count was <10 in either cases or controls. We set the level of genome-wide significance at $p = 5 \times 10^{-8}$. We estimated confounding bias in our results by performing linkage disequilibrium score regression (LDSC) with ldsc (23) and using the attenuation ratio as well as the p value of the intercept to evaluate our results. Results were plotted using matplotlib in Python and the package region-plot (24).

Meta-analysis

We conducted a meta-analysis with the results of the second TS GWAS study conducted by the TS Working Group of the PGC (TSGWAS2) (7). Sample overlap was verified through genotypic identity-by-descent analysis, so the TS-EUROTRAIN GWAS was reanalyzed after the overlapping samples were excluded (124 cases and 279 controls), leading to a non-overlapping sample of 1314 cases and 4077 ancestry-matched control subjects. The summary statistics were used as input to METASOFT (25). METASOFT implements an array of methods for meta-analysis, especially in the case of heterogeneity in the results. In our study, we employed Han and Eskin's random effects model, which separates hypothesis testing from effect-size estimation and has been demonstrated to increase statistical power under heterogeneity compared with the conventional random effects model (26,27). We also used METASOFT to produce m values, which are estimates of the posterior probability that an effect exists, with small values indicating the absence of an effect, large values indicating the presence of an effect, and intermediate values indicating ambiguity (25).

Heritability and Heritability Partitioning

SNP-based heritability was estimated through LDSC (23). We further investigated heritability by partitioning into functional categories using stratified LDSC (28). TS heritability was partitioned into 53 functional categories as well as into 220 cell type–specific and 10 cell-type-group–specific annotations produced on the data derived from the Roadmap Epigenomics Project (29). The significance threshold for the heritability enrichments was defined at a Benjamini-Hochberg false discovery rate (FDR) of $p < .05$.

Genetic Correlations

Bivariate LDSC (23) was conducted to identify genetic correlations between the TS-EUROTRAIN GWAS, the TS-EUROTRAIN/GWAS2 meta-analysis, and TSGWAS2 (7). We then examined each of these studies' cross-disorder correlations with multiple psychiatric and neurological disorders for which data were accessible and are listed in Table S2. To avoid

confounding due to sample size, we selected summary statistics from studies with more than 5000 samples and all studies, except anxiety (h^2_{SNP} z score = 2.5), have a heritability z score $>$ 4. For the correlation analysis, we used the European LD scores and merged alleles based on the HapMap3 reference panel for each trait, excluding markers residing in the major histocompatibility complex region on chromosome 6. The significance threshold was defined by Benjamini-Hochberg FDR as $p < .05$.

Polygenic Risk Scoring

We used PRSice-2 (30) for our polygenic risk score (PRS) analysis. We performed a unilateral PRS analysis between the TS-EUROTRAIN cohort and the TSGWAS2 (7) cohort, using the TSGWAS2 summary statistics as discovery and the TS-EUROTRAIN GWAS, after excluding the overlapping samples, as the target dataset. The TSGWAS2 summary statistics were clumped on the LD information of the TS-EUROTRAIN GWAS, using a window of 250 kb and an r^2 threshold of 0.1. PRSice performed the scoring on subsets of the dataset based on 9 thresholds of p-value leniency (5×10^{-8}, 10^{-4}, 10^{-3}, .01, .05, .1, .2, .5, and 1). The resulting PRS was tested for association with TS using logistic regression with the previously mentioned ancestry components, sex, and imputation batch as covariates. The model fit for best p-value threshold was run using 10,000 permutations. Liability scale was calculated on the variance explained by the PRS (R^2) using a TS population prevalence of 1%.

Biological Annotation of Results

We used FUMA (31) to perform gene-based and gene set analyses on the results from the TS-EUROTRAIN GWAS and the subsequent TS-EUROTRAIN/GWAS2 meta-analysis. The genetic variants were assigned to protein-coding genes based on their GRCh37 build genomic position using a ±20-kb window size. After quality control, 18,089 genes contained at least 1 variant and as such were used for the gene-based analysis, thus setting the Bonferroni threshold at $p < 2.764 \times 10^{-6}$. The gene-based association results were subsequently used for gene set analysis under a competitive model. Tissue expression analysis was conducted on the GTEx (Genotype-Tissue Expression) version 8 expression data (32,33). We investigated chromatin contact points through Capture Hi-C (high-throughput chromosome conformation capture) data available from the 3D Genome Browser (34) using promoter-centered, long-range chromatin interaction data derived from human dorsolateral prefrontal cortex tissues (35).

We performed a set-based association analysis using PLINK (36,37) on the gene sets that had previously been identified as significantly associated with TS (11,38). We used logistic regression as the association model on the genotypes and PCs that were identified by Tracy-Widom statistics in the GWAS. Another repetition of this step was performed with the χ^2 association test to test for this method's robustness to population structure. We proceeded to run the analysis on all samples using a 10-kb genomic window size and a million permutations. Because the permutations were performed on the phenotypic status of the samples, and only served as a method of association of the trait with the gene sets, we also corrected the results by defining the significance threshold through Bonferroni correction.

Investigating Genetic Relationships With Subcortical Brain Volumes

Using the TS-EUROTRAIN/GWAS2 meta-analysis summary statistics as a base, we computed TS PRS (PRS_{TS}) for individuals in the UK Biobank (UKBB) (39) using PRScs (40) and subsequently evaluated the association between risk scores and subjects' 14 subcortical volumes (FIRST) that were available in the UKBB (category 1102). Because we had access to individual-level UKBB data, it was possible to calculate the association between PRS_{TS} and different brain volumes instead of just evaluating their genetic correlation with LDSC. After quality controls (see Supplemental Methods), 29,798 samples with brain magnetic resonance imaging phenotypes available were included in the analysis. To calculate the PRS for these individuals, we applied the continuous shrinkage method implemented in PRScs to obtain the updated effect sizes for all SNPs in TS-EUROTRAIN/GWAS2 meta-analysis summary statistics using European 1000 Genomes data as the reference for LD structure. We then used these updated effect sizes to calculate the PRS using the score function in PLINK (37). Regressions between PRS_{TS} and brain volume measurements were performed using the PHESANT tool (41) while using age, sex, genotyping batch, and the first 10 UKBB PCs as covariates. The significance threshold was defined by Benjamini-Hochberg FDR as $p < .05$. In addition, to leverage results from larger datasets for which individual-level data were not accessible, we used LDSC to test the genetic correlation of the TS-EUROTRAIN/GWAS2 meta-analysis with 7 subcortical brain volume GWAS summary statistics from the ENIGMA (Enhancing Neuro Imaging Genetics through Meta Analysis) consortium (42).

RESULTS

Mega-analysis of TS-EUROTRAIN GWAS

GWAS analysis was performed as a mega-analysis on the combined genetic data of all TS-EUROTRAIN samples (1438 TS cases and 4356 controls) using a logistic regression model on the best-guess genotypes (genotype probability $>$ 0.9) with INFO score $>$ 0.9 and MAF $>$ 0.01. As covariates, we included the ancestry components 1, 2, 4, and 5 to account for population stratification as identified by analysis of variance statistics (Table S3), sex, and imputation batch.

The TS-EUROTRAIN GWAS identified 3 highly correlated ($r^2 > 0.8$) genome-wide significant SNPs located near the *NR2F1* antisense RNA 1 long noncoding (lnc) RNA (*NR2F1-AS1*) locus (Figures S1 and S2A). The strongest signal was found for rs2453763 (chr5:92376460:T/A, odds ratio = 0.7512, $p = 2.62 \times 10^{-8}$, MAF = 0.3581), a variant 350 kb upstream of *NR2F1-AS1* and associated with decreased risk for TS. The imputation statistics for this SNP indicate high imputation quality (MAF = 0.3581, INFO = 0.99). The proximal SNPs were rs2009416 (chr5:92415111:T/C, odds ratio = 0.7532, $p = 3.31 \times 10^{-8}$, MAF = 0.3562) and rs1496337 (chr5:92411293:T/C, odds ratio = 0.7534, $p = 3.33 \times 10^{-8}$,

MAF = 0.3563). Conditional analysis using the lead SNP as covariate showed no secondary signals in the region. The top ($p < 10^{-5}$) loci detected in the novel GWAS are reported in Table 1. LDSC analysis of the summary statistics did not provide evidence for genomic inflation (λ_{GC} = 1.07, intercept = 1.0061, intercept p value = .28, attenuation ratio = 0.0887), while using the full GWAS SNPs, the λ_{GC} was 1.05 (Figure S1B).

TS-EUROTRAIN Meta-analysis With TSGWAS2

The TS-EUROTRAIN GWAS was then meta-analyzed with summary statistic results from what had previously been the largest meta-analysis of TS conducted to date (TSGWAS2) (7) using Han and Eskin's random effects model (25,26). Because there was small but known sample overlap between the 2 studies, the TS-EUROTRAIN GWAS was reanalyzed after excluding the overlapping samples (124 cases and 279 controls), resulting in a dataset of 1314 cases and 4077 ancestry-matched controls. The results in the reduced sample were very similar to those found using the full dataset TS-EUROTRAIN GWAS (Figure S3). Only variants overlapping in both studies were included, leading to a total of 6133 cases, 13,565 controls and 1,955,677 variants in the final meta-analysis.

The TS-EUROTRAIN/GWAS2 meta-analysis (Figure 1; Table S4) pointed again to the 3 genome-wide significant SNPs of the TS-EUROTRAIN GWAS, which remained genome-wide significant in the meta-analysis, with rs2453763 again being the most strongly associated (chr5:92376460:T/A, random effects $p = 4.05 \times 10^{-8}$). SNP rs10209244 (chr2:161561898:A/G, random effects $p = 6.16 \times 10^{-8}$, MAF = 0.01) that resides 200 kb downstream of *RBMS1* (Figure S2B) was the next hit and did not manage to achieve genome-wide significance. The top loci detected from the meta-analysis are reported in Table 2. LDSC analysis of the summary statistics did not provide evidence for genomic inflation (λ_{GC} = 1.16, intercept = 1.016, intercept p value = .11, attenuation ratio = 0.0869).

Genetic Relationship Between the TS-EUROTRAIN GWAS and TSGWAS2

The SNP with the strongest signal in the previously published TSGWAS2 study was absent from the TS-EUROTRAIN dataset due to differences in the reference panels used (1000 Genomes for TSGWAS2 and HRC for the novel study) and stringent batch effect quality control performed on the novel dataset. However, we observed no genome-wide significant heterogeneity (Cochran's Q test p value $< 5 \times 10^{-8}$) in the meta-analysis.

To explore the relationship between the TS-EUROTRAIN GWAS and TSGWAS2, we used LDSC (23) to compute their genetic correlation after excluding the overlapping samples. LDSC identified a strong genetic correlation between the 2 studies (rg = 0.95, $p = 6 \times 10^{-8}$) and provided evidence of consistency across them (Figure 2). Investigation of the gene sets previously found to be associated with TS (11,38) also resulted in successful replication of the associations for the lymphocytic, the ligand-gated ion channel signaling, the cell adhesion and trans-synaptic signaling, and the astrocyte-neuron metabolic coupling gene sets (Table S5).

Table 1. Top Regions ($p < 10^{-5}$) in the TS-EUROTRAIN GWAS

SNP ID	Chr	p Value	A1	OR	SE	N	MAF Cases	MAF Controls	Location	kb	Nearest Genes
rs2453763	5	2.623×10^{-8}	T	0.751	0.051	25	0.314	0.373	chr5:92322427..92559372	236.946	*NR2F1-AS1*
rs2197383	3	4.681×10^{-7}	A	0.595	0.103	165	0.050	0.077	chr3:79889497..80380401	490.905	*ROBO1*
rs3773057	3	1.388×10^{-6}	T	0.198	0.335	23	0.003	0.019	chr3:29563164..29627731	64.568	*RBMS3, RBMS3-AS3*
rs9382365	6	1.829×10^{-6}	G	0.677	0.081	77	0.087	0.112	chr6:54418351..54531232	112.882	*FAM83B, TINAG*
rs152061	5	2.085×10^{-6}	T	1.26	0.049	196	0.434	0.379	chr5:64778944..64989139	210.196	*ADAMTS6, CENPK, ERBB2IP, NLN, PPWD1, SGTB, TRAPPC13, TRIM23*
rs2278796	1	6.882×10^{-6}	T	1.26	0.052	9	0.330	0.279	chr1:204951209..204971553	20.345	*CNTN2, DSTYK, NFASC, RBBP5, TMCC2, TMEM81*
rs34940828	3	7.531×10^{-6}	C	2.10	0.16	64	0.027	0.015	chr3:123213895..123398466	184.572	*ADCY5, CCDC14, MYLK, MYLK-AS1, PTPLB, SEC22A*
rs2076218	1	9.746×10^{-6}	A	1.25	0.05	3	0.380	0.340	chr1:209745395..209768699	23.305	*C1orf74, CAMK1G, DIEXF, G0S2, HSD11B1, IRF6, LAMB3, MIR205, MIR205HG, MIR4260, TRAF3IP3*

The total sample size was 1438 cases and 4356 controls on 2,949,675 variants. One variant was identified as genome-wide significant ($p < 5 \times 10^{-8}$), Chromosome and region (based on hg19) are shown for index SNPs (LD $r^2 > 0.1$), as well as the number of LD-associated markers in proximity (N). A1 refers to the associated allele. The OR and standard error are shown for the association between A1 and TS. MAF indicates the allelic frequency of allele 1 in the dataset. The nearest reported genes were determined by genomic location (±500 kb). The analysis was restricted to variants with MAFs ≥ 0.01 and information quality (INFO) scores ≥ 0.9. Chromosome X was not analyzed because it was absent from a significant portion of the acquired datasets.

Chr, chromosome; GWAS, genome-wide association study; LD, linkage disequilibrium; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism; TS, Tourette syndrome.

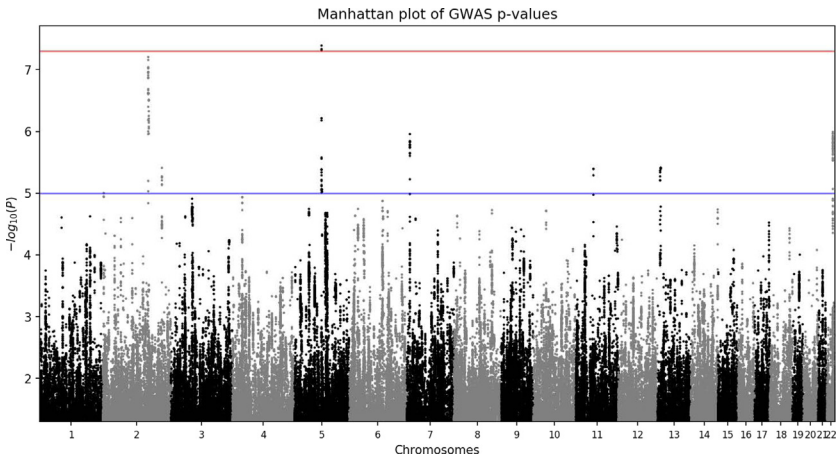

Figure 1. The Manhattan plot for the genome-wide association meta-analysis of TS with the TS-EUROTRAIN and the TSGWAS2 datasets (6133 TS cases and 13,565 controls of European descent on 1,955,677 variants) using Han and Eskin's random effects model (25). The $-\log_{10}(p)$ values for the association tests (two-tailed) are shown on the y-axis, and the chromosomes are ordered on the x-axis. One genetic locus on chromosome 5 surpassed the genome-wide significance threshold ($p < 5 \times 10^{-8}$, indicated by the red line). Gray and black differentiate adjacent chromosomes. GWAS, genome-wide association study; TS, Tourette syndrome.

PRS analysis displayed consistency between the 2 studies. PRSs were computed using the summary statistics of TSGWAS2 as a training dataset and the TS-EUROTRAIN raw genotypes as discovery in PRSice (43). The best fit p-value threshold was determined at $p = .1182$ (model fit $p = 1.26 \times 10^{-28}$) (Figure 3A, B). The maximum amount of variance explained by the best fit model was estimated by Nagelkerke's R^2 at 3.3%.

Cross-disorder Analysis

Pairwise genetic correlations were computed between 10 psychiatric and 6 neurological traits using LDSC (23), and results are shown in Figure 2. Benjamini-Hochberg FDR correction with an $\alpha = 0.05$ was used to correct for multiple testing. After correction, the TS-EUROTRAIN/GWAS2 meta-analysis was significantly correlated with obsessive-compulsive disorder ($rg = 0.39$, $p_{FDR} = 3.4 \times 10^{-3}$). We also observed genetic correlations significant at $p < .05$ for the meta-analysis with attention-deficit/hyperactivity disorder, schizophrenia, and migraine, which, however, did not remain significant after FDR correction.

Heritability Estimation and Partitioning

We used LDSC (23) to estimate h^2_{SNP} using the summary statistics of the novel GWAS and the meta-analysis. The summary statistics were merged with the HapMap3 marker panel provided by the authors. For the TS-EUROTRAIN GWAS, analysis yielded an h^2_{SNP} estimate of 0.4385 (SE = 0.1167) on the observed scale and 0.2736 (SE = 0.0728) assuming a TS prevalence of 0.01 on the liability scale, while the LDSC analysis intercept was computed at 1.0157 (SE = 0.013) ($p = .028$), and the ratio of stratification to polygenicity was estimated at 0.0863 (SE = 0.0711). For the meta-analysis, the h^2_{SNP} estimate was 0.3504 (SE = 0.0439) and 0.2184 (SE = 0.0269) on the liability scale.

We proceeded to partition the heritability of the meta-analysis GWAS by functional genomic categories using stratified LDSC (28) on the full baseline model and a model based on the Roadmap epigenomics data, as provided by the authors (28). The full baseline model included 24 main overlapping functional categories and identified statistically significant enrichment in 2 categories after Benjamini-Hochberg FDR correction at an $\alpha = 0.05$. The H3K4me1 sites category (enrichment value 1.61, $p = 9.5 \times 10^{-4}$) was the top significant signal in the analysis, with the conserved elements category (enrichment value 2.05, $p = 3.8 \times 10^{-3}$) being the secondmost significant signal. The Roadmap model includes epigenomic mapping data from 395 tissues (29), and when applied to our data for heritability partitioning, it yielded 13 statistically significant modifications after Benjamini-Hochberg FDR correction at an $\alpha = 0.05$. These 13 signals marked the enrichment of the histone marks H3K27ac, H3K4me1, and H3K9ac on 5 brain tissues. H3K27ac was identified on the angular gyrus, the cingulate gyrus, the dorsolateral prefrontal cortex, and the inferior temporal lobe; H3K4me1 on the angular gyrus, the cingulate gyrus, the dorsolateral prefrontal cortex, the inferior temporal lobe, and the substantia nigra; and H3K9ac on the angular gyrus, the anterior caudate, the dorsolateral prefrontal cortex, and the inferior temporal lobe (Tables S6 and S7).

Biological Annotation and Enrichment Analysis

Functional mapping, annotation, and gene set enrichment using the functional mapping and annotation pipeline did not produce significant results. The identified top signals from the TS-EUROTRAIN GWAS and the TS-EUROTRAIN/GWAS2 meta-analysis reside in large intergenic regions that exceed the distance limits set by the software and were thus excluded from the annotation step of the pipeline. The top signal of the TS-EUROTRAIN/GWAS2 meta-analysis gene-based analysis was *RANGAP1* ($p = 3.36 \times 10^{-6}$) on chromosome 22; it did not meet the genome-wide significance threshold ($p = 2.8 \times 10^{-6}$) (Table S8). MAGMA tissue expression analysis using functional mapping and annotation did not produce any statistically significant results for the TS-EUROTRAIN GWAS or the meta-analysis (Figures S4A, B and S5A, B). MAGMA tissue expression analysis of the meta-analysis, using the 53-tissue-sample set from GTEx, indicated stronger putative enrichment in various brain tissues, with the top signals in the cerebellar hemisphere, cerebellum, and frontal cortex (Brodmann area 9). Using the 30-tissue-sample set from GTEx, stronger evidence

Table 2. Top Regions ($p < 10^{-5}$) in the TS GWAS Meta-analysis (TS-EUROTRAIN and TSGWAS2)

SNP ID	Chr	*p* Value	OR	MAF	MAF EUR (1KG)	N	Location	kb	Nearest Genes
rs2453763	5	4.054×10^{-8}	0.83	0.358	0.347	25	chr5:92322427..92559372	236.946	*NR2F1-AS1*
rs10209244	2	6.156×10^{-8}	2.32	0.012	0.001	29	chr2:161422880..161676570	253.691	*MIR4785, RBMS1*
rs13401916	2	2.441×10^{-7}	2.08	0.014	0.003	13	chr2:161945103..162055548	110.446	*LOC100996579, LOC101929512, PSMD14, TANK, TBR1*
rs139469	22	9.997×10^{-7}	0.89	0.345	0.327	33	chr22:41451185..41627527	176.343	*ACO2, CHADL, DNAJB7, EP300, EP300-AS1, L3MBTL2, MIR1281, MIR4766, MIR6889, PHF5A, RANGAP1, RBX1, SLC25A17, ST13, TEF, TOB2, XPNPEP3, ZC3H7B*

The analysis was performed on 6133 cases and 13,565 controls on 1,955,677 variants using Han and Eskin's random effects model (25). One variant was identified as genome-wide significant ($p < 5 \times 10^{-8}$) while the second top hit reached $p < 10^{-7}$. Chromosome and region (based on hg19) are shown for index SNPs (LD $r^2 > 0.1$), as well as number of LD-associated markers in proximity (N). The nearest reported genes were determined by genomic location (±500 kb). MAF indicates the allelic frequency of the minor allele in the dataset. MAF EUR (1KG) indicates the frequency of the minor allele in the meta-analysis in the 1000 Genomes Project European samples.

Chr, chromosome; GWAS, genome-wide association study; LD, linkage disequilibrium; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism; TS, Tourette syndrome.

of potential enrichment could be identified in the brain, followed by the pituitary and the ovary (Figure S5A, B).

Regarding the top SNP in our GWAS, the GTEx portal (32) reports SNP rs2453763 to be significantly associated as a splicing quantitative trait locus for *CTD-2091N23.1* in the tibial nerve and as an expression quantitative trait locus for *NR2F1* and *NR2F1-AS* in the smooth muscles of the esophagus and for *CTD-2091N23.1* in cultured fibroblasts (Table 3). Capture Hi-C (34) showed strong evidence for the SNP being related to the regulation of *NR2F1* (Figure S6A).

Genetic Correlation to Subcortical Brain Volumes

The genetic correlation analysis for our TS-EUROTRAIN/GWAS2 meta-analysis and ENIGMA GWAS summary statistics for 7 subcortical brain volumes (42) did not reveal any significant correlation (Table S9). However, with individual-level data available within the UKBB, we were also able to test for an association between PRS_{TS} and specific brain phenotypes. Our results highlighted the previously described relationship (44) between genetic risk for TS and putamen volume. We observed that increase in the genetic risk of TS was associated with decrease in right putamen (beta = −0.0175, adjusted p = .0069) and left pallidum (beta = −0.0137, adjusted p = .043) volumes. Significant associations were also observed between PRS_{TS} and bilateral thalamic volume, indicating that increase in PRS_{TS} was associated with decrease in right thalamus volume (beta = −0.0138, adjusted p = .035) and left thalamus volume (beta = −0.0132, adjusted p = .037) (Table S10).

DISCUSSION

We present results from a novel TS GWAS and integrate them with a previous study to report the largest GWAS meta-analysis on TS at this time (6133 individuals with TS of European ancestry and 13,565 matched control subjects). We found one novel, independent genome-wide significant locus associated with TS on chromosome 5q15, upstream of a gene cluster that harbors *NR2F1-AS*, *NR2F1*, and *lnc-NR2F1*. The top associated SNP is located within *CTD-2091N23.1*, a gene encoding an lncRNA that has yet to be functionally characterized. Our study provides novel insights into the genetic cause and pathophysiology of TS. However, replication of our results in independent samples and even larger studies are needed to ultimately elucidate the background of this complex disorder and lead toward interventions that will be informed by genetic discoveries.

The 5q15 region that is highlighted by our study has previously been implicated in neurodevelopmental phenotypes (45–48). The 5q14-5q15 regions have been reported to contain fragile sites that are associated with genomic and epigenomic instability as well as being linked to schizophrenia and autism (49). The exact genes have not yet been identified, with recent evidence suggesting a role for *NR2F1*-related genes, and more intriguingly, the *lnc-NR2F1* gene. *lnc-NR2F1* is an lnc RNA locus discovered to be recurrently mutated in individuals with autism spectrum disorders and intellectual disability, with translocations in this locus reported to show patterns of Mendelian inheritance (50). A functional study of *lnc-NR2F1* identified its role in neuronal maturation in vitro through expression regulation of a network of genes that have been

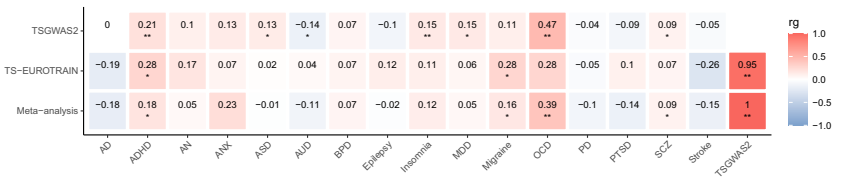

Figure 2. Genetic correlations with TS. The genetic correlations were estimated with bivariate linkage disequilibrium score regression (23). We showcase the correlations between 3 TS studies (TS-EUROTRAIN, TS-EUROTRAIN/TSGWAS2 meta-analysis, and TSGWAS2) and 16 psychiatric and neurological traits (see Table S2 for full list of studies and abbreviations). The number in each square denotes the correlation *rg*. *Shows the correlations with nominal $p < .05$. **Denotes the correlations that were identified as statistically significant after Benjamini-Hochberg false discovery rate correction ($\alpha = 0.05$). AD, Alzheimer's disease; ADHD, attention-deficit/hyperactivity disorder; AN, anorexia nervosa; ANX, anxiety disorders; ASD, autism spectrum disorder; AUD, alcohol use disorder; BPD, bipolar disorder; GWAS, genome-wide association study; MDD, major depressive disorder; OCD, obsessive-compulsive disorder; PD, Parkinson's disease; PTSD, posttraumatic stress disorder; SCZ, schizophrenia; TS, Tourette syndrome.

linked to autism (50). Functional studies of the *NR2F1* gene have also indicated its critical role in neurodevelopment through investigations into human and mouse haploinsufficiency (51), insertion of point mutations in mouse models that lead to excitatory/inhibitory neuronal imbalance (52) and the study of knock-out mouse models (53). Notably, in the absence of *NR2F1*, an imbalance between oligodendrocytes and astrocytes develops, leading to postnatal hypomyelination and astrogliosis (51).

NR2F1 is a highly conserved orphan nuclear receptor that is a regulator of transcription. It belongs to the steroid/thyroid hormone nuclear receptor superfamily, which is involved in a wide range of roles, including cell differentiation, cancer progression, and central and peripheral neurogenesis (54). A multitude of pathogenic variants have been identified in *NR2F1*, leading to Bosch-Boonstra-Schaaf optic atrophy syndrome and autosomal dominant neurodevelopmental disorder (55). *NR2F1* is also known by its historical name, *COUP-TF1*; it is a target of the androgen receptor, along with *SOX9* and *OCT4* (56). *NR2F1* interacts with *SOX9* (56,57) and represses a host of targets in multiple tissues, including *CYP17A1*, oxytocin gene *OXT*, and *OCT4* (58). Especially in the case of *CYP17A1*, encoding for a key enzyme of steroid biosynthesis, *NR2F1* and *SF-1* exert opposing effects as repressor and activator, respectively (59).

A limitation of our study is that we did not replicate our findings in independent samples due to lack of data availability. Future studies will help evaluate the magnitude of the contribution from the locus implicated by our study. Nevertheless, we sought to validate our results through means of heritability correlation patterns and polygenic risk scoring. Heritability analysis in the TS-EUROTRAIN dataset indicated that a large proportion of TS h^2_{SNP} can be attributed to common variants ($h^2_{SNP} = 0.4385$), in concordance with the estimate ranges found in previous investigations (4,7). We suspect that the lower SNP heritability ($h^2_{SNP} = 0.3504$) that we observed in meta-analysis could be due to the heterogeneity associated with the additional samples and different populations that were included in the meta-analysis. Polygenic prediction in the TS-EUROTRAIN cohort using the TSGWAS2 results as discovery achieved significant predictive levels on par with the intercohort predictive Nagelkerke's R^2 levels in the previous TS GWAS (7) and substantially increased by more than an order of magnitude compared with tic prediction in a general population cohort (12).

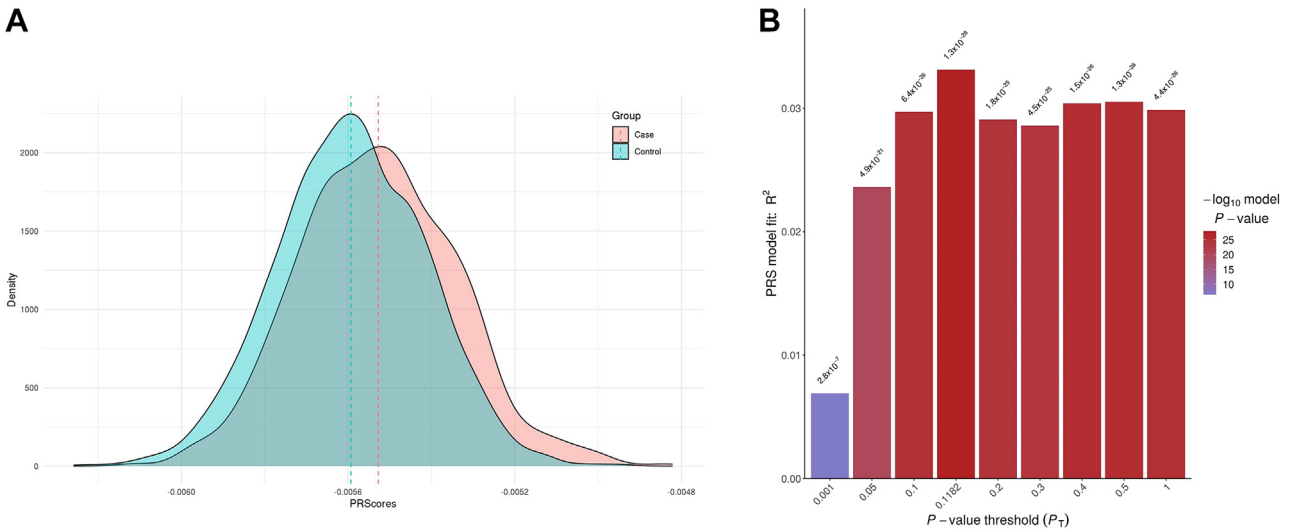

Figure 3. PRS analysis using the TSGWAS2 dataset (7) as discovery and the TS-EUROTRAIN dataset as target. The best fit *p*-value threshold was determined at $p = .1182$ (model fit $p = 1.26 \times 10^{-28}$). The maximum variance explained by the best fit model was estimated by Nagelkerke's R^2 at 3.3%. **(A)** PRS distribution comparison between cases and controls for the best fit model. **(B)** PRS histogram for each *p* value bin, including the best fit bin. PRS, polygenic risk score.

Table 3. Significant SNP-Gene Pairings Identified Through GTEx eQTL and sQTL Data

Gencode ID	Gene Symbol	SNP ID	Intron ID	*p* Value	NES	Tissue
GTEx eQTL Associations for the Top Variant in *NR2F1*						
ENSG00000175745.11	*NR2F1*	rs2453763	–	5.7×10^{-10}	0.25	Esophagus—muscularis
ENSG00000237187.8	*NR2F1-AS1*	rs2453763	–	1.9×10^{-7}	0.22	Esophagus—muscularis
ENSG00000251361.1	*CTD-2091N23.1*	rs2453763	–	2.3×10^{-4}	−0.19	Cells—cultured fibroblasts
GTEx sQTL Associations for the Top Variant in *NR2F1*						
ENSG00000251361.1	*CTD-2091N23.1*	rs2453763	93051776:93075658:clu_40848	4.6×10^{-9}	0.39	Nerve—tibial

Data from (32,33). Four significant associations were identified for SNP rs2453763, while no significant associations were identified for rs10209244. rs2453763 is an eQTL for 3 genes on 2 tissues and a sQTL for 1 gene on 1 tissue. Reported are the symbol of the associated gene, the respective associated tissue, and the normalized effect size.

eQTL, expression quantitative trait locus; GTEx, Genotype-Tissue Expression; NES, normalized effect size; SNP, single nucleotide polymorphism; sQTL, splicing quantitative trait locus.

Like most GWASs, this study does not yet have direct clinical implications. However, this work can motivate future studies that may have significant clinical impact, for example patient stratification according to genetic risk to inform clinical practice or drug discovery based on genetic loci that are identified through genetic association. Our recently described phenome-wide association analysis of TS genetic risk and a large number of clinical traits available in the UKBB represent one further step toward clinically related discoveries (60). Our study is an important stepping stone toward understanding the genetic background of TS and confirms the value of collaborative efforts toward expanding sample size and datasets available for analysis (such as the TS-EUROTRAIN, EMTICS, TSGeneSEE, and PGC initiatives). However, we still only capture a small fraction of the risk for TS attributable to common variants. Larger studies that bring together even larger datasets are necessary and warranted and are in fact currently underway, with participation from authors of this work. Increased statistical power will further enable the identification of more leads toward the elucidation of the underlying biology of TS and potential future interventions.

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