Epigenome-Wide Association Study of Tic Disorders

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Tic disorders are moderately heritable common psychiatric disorders that can be highly troubling, both in childhood and in adulthood. In this study, we report results obtained in the first epigenome-wide association study (EWAS) of tic disorders. The subjects are participants in surveys at the Netherlands Twin Register (NTR) and the NTR biobank project. Tic disorders were measured with a self-report version of the Yale Global Tic Severity Scale Abbreviated version (YGTSS-ABBR), included in the 8th wave NTR data collection (2008). DNA methylation data consisted of 411,169 autosomal methylation sites assessed by the Illumina Infinium HumanMethylation450 BeadChip Kit (HM450k array). Phenotype and DNA methylation data were available in 1,678 subjects (mean age = 41.5). No probes reached genome-wide significance ($p < 1.2 \times 10^{-7}$). The strongest associated probe was cg15583738, located in an intergenic region on chromosome 8 ($p = 1.98 \times 10^{-6}$). Several of the top ranking probes ($p < 1 \times 10^{-4}$) were in or nearby genes previously associated with neurological disorders (e.g., *GABBRI, BLM*, and *ADAM10*), warranting their further investigation in relation to tic disorders. The top significantly enriched gene ontology (GO) terms among higher ranking methylation sites included anatomical structure morphogenesis (GO:0009653, $p = 4.6 \times 10^{-15}$) developmental process (GO:0032502, $p = 2.96 \times 10^{-12}$), and cellular developmental process (GO:0048869, $p = 1.96 \times 10^{-12}$). Overall, these results provide a first insight into the epigenetic mechanisms of tic disorders. This first study assesses the role of DNA methylation in tic disorders, and it lays the foundations for future work aiming to unravel the biological mechanisms underlying the architecture of this disorder.

■ **Keywords:** Tourette syndrome, tics, epigenetics, DNA methylation, EWAS

Tic disorders form a broad spectrum encompassing four different clinical entities: Tourette syndrome (TS), chronic (motor or vocal) tic disorder, transient tic disorder, and tic disorder not otherwise specified. Tics are characterized by sudden, rapid, motor movements, or vocalizations, performed in a ritualized, recurrent, and stereotypical fashion (DSM-IV-TR; American Psychiatric Association, 2000).

Multiple lines of evidence suggest that both genetic and interacting environmental factors are causes underlying the etiology of these phenotypes (Paschou, 2013). Twin and family studies have shown that the prevalence of TS or chronic tic disorders among first-degree relatives of affected individuals varies between 15% and 53% (Towbin, 2010). Furthermore, heritability estimates on tic disorders or TS range between 0.28 and 0.56 (Anckarsäter et al., 2011;

Bolton et al., 2007; de Haan et al., 2015; Lichtenstein et al., 2010; Mathews & Grados, 2011; Ooki, 2005). However, most genetic and clinical studies so far have been hampered by small sample sizes, ambiguity in phenotype definitions, and

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TABLE 1

Number of Participants Included in the Analysis

	MZ twins		DZ/DOS twins		Relatives	
	Male (case/control)	Female (case/control)	Male (case/control)	Female (case/control)	Male (case/control)	Female (case/control)
N	241 (30/211)	680 (74/606)	213 (31/182)	404 (38/366)	63 (8/55)	77 (7/70)
Age (SE)	42.48 (14.00)	40.57 (12.08)	37.61(10.11)	38.46 (11.04)	42.83 (14.10)	41.96 (12.45)

Note: MZ = monozygotic twins, DZ = dizygotic twins, DOS = dizygotic opposite-sex twins.

difficulties in disentangling genetic and familial environmental effects. Thus, molecular genetic studies have thus far not yet yielded robust findings (Pauls et al., 2014).

The study of epigenetic mechanisms on a genome-wide scale in humans represents the bridge between disease susceptibility and gene expression variation. It is known that epigenetic mechanisms regulate gene expression, and that these epigenetic mechanisms change during life, up- and down-regulating different genes in response to external environmental conditions. DNA methylation at CpG dinucleotides is the most studied epigenetic mechanism in humans. It regulates gene expression by targeting, for example, promoters and enhancers, and its pattern may change as a consequence of external and internal stimuli (Hackett et al., 2013). DNA methylation is also involved in other biological processes such as genomic imprinting, where CpG sites are methylated based on their parental origin, which are different between the paternal and maternal branch (Liu et al., 2010), and chromosome X inactivation, where one copy of the female X chromosome is inactivated (Heard et al., 1997). Genomic imprinting has also been suggested to be involved in TS in a study evaluating parental gender-influenced differences in childhood TS phenotype. Greater motor tic complexity was associated with maternal transmission, whereas higher frequency in vocal tics was associated with paternal transmission (Lichter et al., 1995).

EWAS have thus far been used to reveal altered methylation patterns in several complex disorders such as obesity, diabetes, schizophrenia, and autism (Dempster et al., 2011; Moore et al., 2014; Rakyan et al., 2011; Wockner et al., 2014). This approach is becoming increasingly accessible and it is likely that DNA methylation is also involved in other neurological disorders. Currently, no EWAS of tic disorders have been performed. These studies may clarify underlying mechanisms that differentially regulate genes in individuals manifesting tics.

Here, we report on the first EWAS of tic disorders performed in a population-based sample from the NTR.

Material and Methods

Subjects' Demographics

Subjects in this study are participants in the NTR biobank Project (Willemsen et al., 2010, 2013). Since 1991, the NTR has been collecting information on a broad range of phenotypes in twins and family members (Willemsen et al., 2013). In total, 3,264 peripheral blood samples from 3,221 NTR participants have been assessed for genome-wide methylation data. After quality control (QC) on the methylation data, the total final selection comprised 3,089 samples, for a total of 3,057 individuals (32 subjects had methylation data for two time points). For a complete description of the entire methylation dataset from the NTR, please see Van Dongen et al. (2015).

In the present study, we analyzed tic data from the 2008 wave of collection in a subset of individuals in whom genome-wide methylation data were available. A total of 1,678 individuals (twins, siblings, and parents) from 1,057 families were included in the analysis. Table 1 gives an overview of the subjects entered in the analysis and demographics. Zygosity was assessed by DNA polymorphisms as described by van Beijsterveldt et al. (2013). The study was approved by the Central Ethics Committee on Research involving human subjects of the VU University Amsterdam.

Phenotype

Tics were measured using an abbreviated 12-item self-report version of the YGTSS, the latter being a well-validated interview with a high internal consistency (Cronbach's alpha > 0.90; Leckman et al., 1989). The YGTSS-abbreviated (YGTSS-ABBR) contains the 12 most frequently occurring tics, assessing their occurrence: *never* (0), *< than 1 year ago* (1), *between 15 years ago* (2) or *as a child* (3). Three additional questions are asked on age at onset, duration, and severity, to enable establishing a probable diagnosis according to DSM-IV-TR criteria (American Psychiatric Association, 2000). Table 2 shows the YGTSS-ABBR questionnaire used for measuring tics. A diagnosis of probable chronic tic disorder was established if the person had: (1) one or more chronic motor or one or more vocal tics, that (2) occurred before age 21, and (3) had been present for >1 year. Probable TS diagnosis was established when two or more motor and one or more vocal tics were reported that occurred before age 21 and had lasted for >1 year, and probable transient tic disorder was established when motor and/or vocal tics had occurred before age 21 for less than one year. From these categories, we derived a dichotomous variable on absence or presence of a probable tic disorder diagnosis — chronic tic disorder, transient tic disorder or TS, as referenced by the Tourette Syndrome Classification Study Group (1993). An extensive genetic analysis on the heritability of tic disorders has been performed (Zilhão et al., 2015). Since smoking is known to have an effect on DNA methylation (Lee & Pausova, 2013), we controlled for smoking status in

TABLE 2

Yale Global Tic Severity Scale Abbreviated

1	Please indicate whether you have ever had any of the following involuntary sudden nervous tics (several answers possible):	Never	0–1 years ago	1–5 years ago	More than 5 years ago
a	Eye movements: for example, blinking, rolling, squinting				
b	Nose movements: for example, nose twitching, broadening nostrils				
c	Lip or mouth movements: for example, chewing, licking, pouting				
d	Head shaking				
e	Shoulder or neck movements				
f	Arm or hand movements: for example, rapid 'purposeless' bending, stretching				
g	Squeaking or whistling noises				
h	Growling, throat clearing, coughing, sniffing				
i	'Purposeless' cursing or utterance of rude or obscene language				
2	If you have suffered from any of the above tics:				
a	At what age did you first exhibit any of these phenomena?	_____years (age)			
b	Have you ever suffered from these tics for more than a year at a time?	___ No___ Yes			
c	How often did you have tics in the period that you suffered from them most?				
		___not daily			
		___ daily, but tic-free for most of the day			
		___daily, but tic-free periods of 3 hours not uncommon			
		___daily, with tic-free periods of at most half an hour			

the epigenome-wide association analysis. Smoking status was assessed at the moment of blood draw by interview.

Infinium HumanMethylation450 BeadChip Data

DNA methylation was assessed with the Infinium HumanMethylation450 BeadChipKit (Illumina, Inc.; Bibikova et al., 2011). Genomic DNA (500ng) from whole blood was bisulfite treated using the ZymoResearch EZ DNA Methylation kit (Zymo Research Corp, Irvine, CA, USA) following the standard protocol for Illumina 450K micro-arrays, by the department of Molecular Epidemiology from the Leiden University Medical Center (LUMC), The Netherlands. Subsequent steps (i.e., sample hybridization, staining, scanning) were performed by the Erasmus Medical Center micro-array facility, Rotterdam, the Netherlands. QC and processing of the blood methylation dataset has been described in detail previously (Van Dongen et al., 2015). In short, a number of sample-level and probe-level quality checks were performed. Sample-level QC was performed using MethylAid (van Iterson et al., 2014). Probes were set to missing in a sample if they had an intensity value of exactly zero, or a detection p-value > .01, or a bead count < 3. After these steps, probes that failed based on the above criteria in >5% of the samples were excluded from all samples (only probes with a success rate ≥95% were retained). Probes were also excluded from all samples if they mapped to multiple locations in the genome (Chen et al., 2013), and/or had a SNP within the CpG site (at the C or G position) irrespective of minor allele frequency in the Dutch population (Genome of the Netherlands Consortium, 2014). Only autosomal methylation sites were analyzed in the EWAS. The

methylation data were normalized with Functional Normalization (Fortin et al., 2014) and normalized intensity values were converted into beta (β)-values. The β-value represents the methylation level at a site, ranging from 0 to 1 and is calculated as

$$\beta = \frac{M}{M + U + 100},$$

where M = methylated signal, U = unmethylated signal, and 100 represents a correction term to control the β-value of probes with very low overall signal intensity. After QC, from an initial total of 485,577 methylation sites, the final total selection of methylation sites was 411,169.

Statistical Analysis

EWAS was performed using linear regression under an additive model correcting for principal components (PCs) and covariates. PCs were calculated from the methylation data after QC and normalization. The PCA plot calculated can be seen in Supplementary Figure S2. Supplementary Figure S3 provides the correlation plot between the first 20 PCs and the covariates (monocyte count, eosinophils count, neutrophils count, array row number, smoking status, age, and sex) in our dataset. Generalized estimation equation (GEE) models were used to test whether tics were associated with DNA methylation. In the final model, DNA methylation level was used as outcome with the following predictors: tics, top five PCs, smoking status, eosinophil percentage, and monocyte percentage. Additional models were tested to evaluate the inflation factor with different covariates (Supplementary material). Sex, neutrophils count,
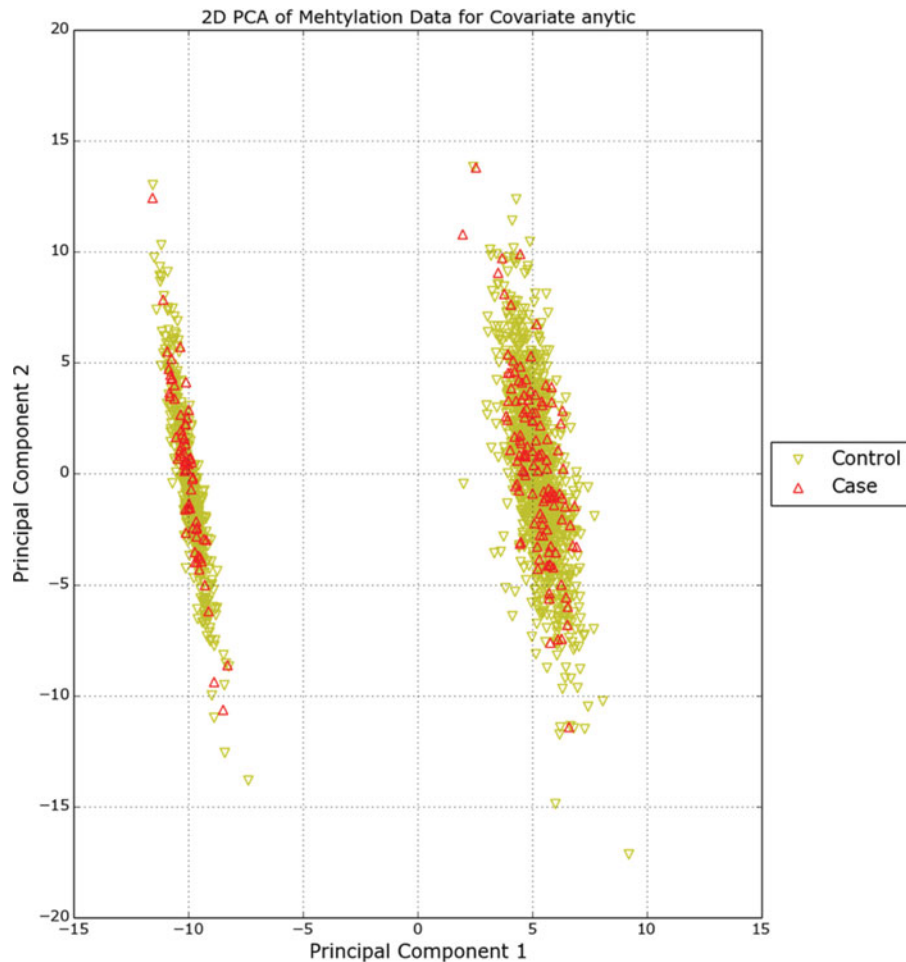
FIGURE 1

(Colour online) Two-dimensional PCA plot labeled by case-control status.

and age were not included due to their correlation with the top five PCs (Supplementary Figure S3). Basophil percentage was not included because it showed little variation between subjects, with a large number of subjects having 0% of basophils. Smoking status was coded as '0 = non-smoker', '1 = former-smoker', '2 = current-smoker'. GEE uses cluster robust standard errors with family ID as cluster variable, and thus standard errors are robust for the presence of related individuals in the sample. A permuted p-value was calculated by random sampling of the phenotype ($n = 10,000$), defining the proportion of permutations meeting or exceeding our p-value estimate based on the actual data. CpGs with p-values $< 1.2 \times 10^{-7}$ (Bonferroni correction of 0.05/411169 — autosomal sites) were considered statistically significant.
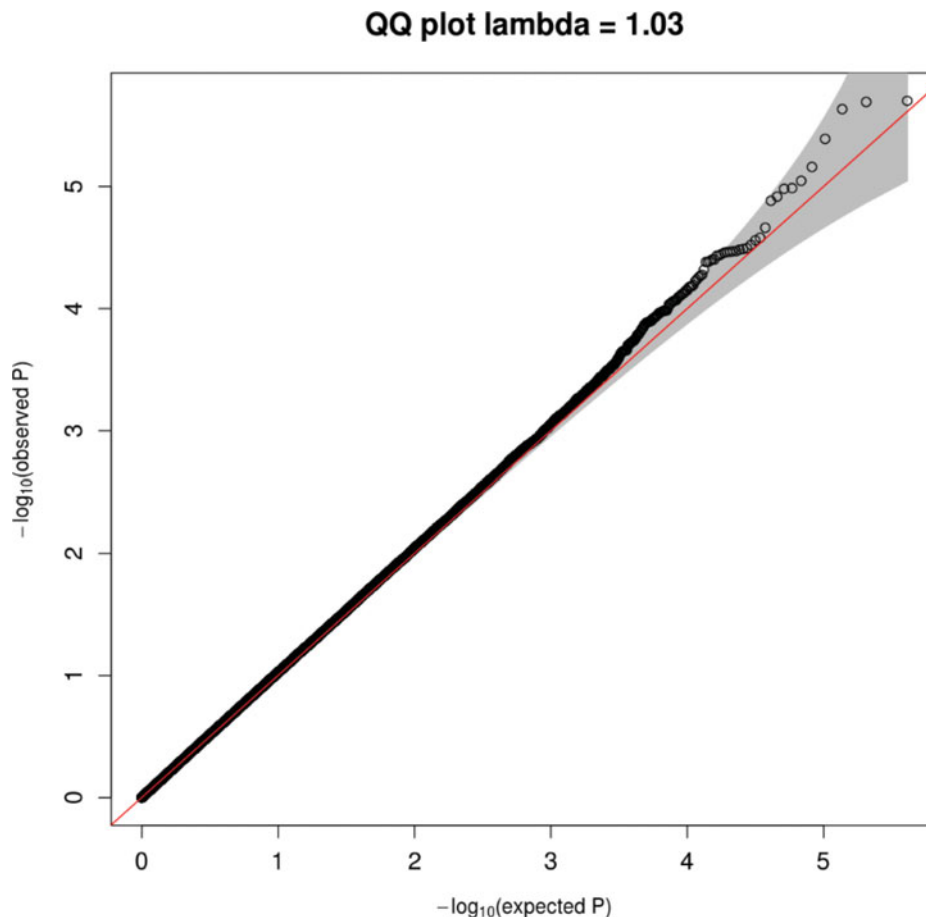
Enrichment of Gene Ontology Terms

Enrichment of GO terms among methylation sites having a stronger association with tics was done by ranking all methylation sites based on the EWAS p-value and the resulting ranked gene list was supplied to the online software tool GOrilla (Eden et al., 2009). The GO tool GOrilla takes into account the rank of the gene whereby p-value cut-off is not required in creating the gene list. A false discovery rate (FDR) q-value <0.05 was considered for a GO term to be statistically significant.

Results

After QC, the final dataset consisted of 411,169 autosomal CpG sites, for 1,678 individuals (188 cases and 1,490 controls). The variation in the data captured by the first two PCs is shown in Figure 1. It can be seen that the main contributors to variation in DNA methylation are not associated with the phenotype. The first component represented sex and the second component correlated strongly with neutrophil count. Supplementary Figure S1 shows the

QQ plot lambda = 1.03

FIGURE 2

(Colour online) QQ plot of *p*-values from GEE model with top five PCs and covariates tics, smoking status, eosinophils, monocytes, and array row.

distribution of genome-wide methylation level for all individuals included in the analysis.

The quantile-quantile (QQ) plot based on the EWAS model is shown in Figure 2. The inflation factor (λ) is 1.03, indicating that the results are not stratified.

None of the CpG sites passed the Bonferroni *p*-value threshold ($p = 1.2 \times 10^{-7}$) for the association with Tics. Table 3 shows all CpG sites with a *p*-value $< 1 \times 10^{-4}$ ($N = 57$). Permutation tests for all of these 57 CpG sites resulted in a permutation *p*-value $< .05$, indicating that these CpG sites did not occur by chance. As an example, the distribution of permutation *p*-values for the top nine CpGs is shown in Supplementary Figure S7. The Manhattan plot can be seen in Figure 3 with the top 57 CpG sites highlighted in green. Figure 4 shows the methylation level in cases and controls for our top 15 CpGs.

Gene Ontology Enrichment Analysis

GO enrichment analysis identified a large number of GO terms that were significantly enriched (Supplementary

Tables T1–T3). Top enriched GO Processes include developmental process (GO:0032502, $p = 3.98e^{-16}$, FDR *q*-value $= 2.69e^{-12}$), cellular developmental process (GO:0048869, $p = 4.35e^{-16}$, FDR *q*-value $= 1.96e^{-12}$), single-organism developmental process (GO:0044767, $p = 6.57e^{-23}$, FDR *q*-value $= 2.22e^{-12}$), and other GO terms related to development. Brain processes, including regulation of neuron projection guidance (GO:0097485, $p = 8.33e^{-11}$, FDR *q*-value $= 5.11e^{-08}$), axon guidance (GO:0007411, $p = 8.33e^{-11}$, FDR *q*-value $= 4.89e^{-08}$), and neuron differentiation (GO:0030182, $p = 1.15e^{-10}$, FDR *q*-value $= 6.24e^{-08}$) were also significantly enriched. The top most enriched GO component was cell junction (GO:0030054, $p = 6.73e^{-09}$, FDR *q*-value $= 1.09e^{-05}$), followed by the neuron-specific GO components neuron part (GO:0097458, $p = 4.39 \times 10^{-07}$, FDR *q*-value $= 1.78e^{-04}$), synapse part (GO:0044456, $p = 5.13 \times 10^{-06}$, FDR *q*-value $= 1.39e^{-03}$), postsynaptic density (GO:0014069, $p = 1.67e^{-05}$, FDR *q*-value $= 3.87e^{-03}$) and dendrite (GO:0030425, $p = 1.04e^{-04}$, FDR *q*-value $= 1.05e^{-02}$). GO components related to histone modification, including *MOZ/MORF* histone
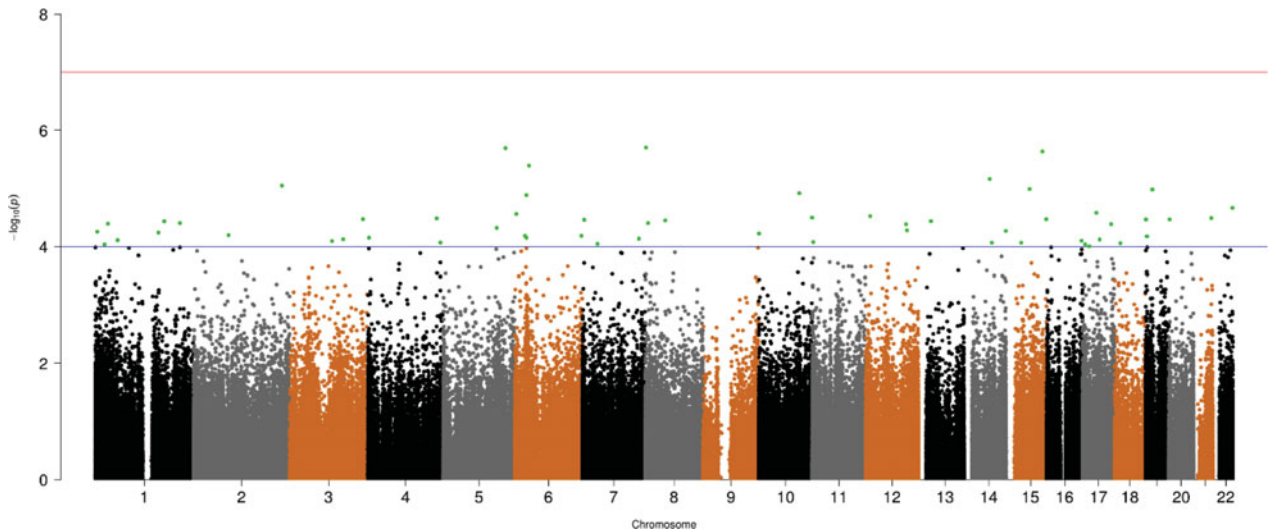
FIGURE 3

Manhattan plot showing the *p*-values of genome-wide CpG sites. The red line is the genome-wide threshold and the blue line is the suggestive threshold ($p < 1 \times 10^{-4}$). Top CpG sites are highlighted in green.
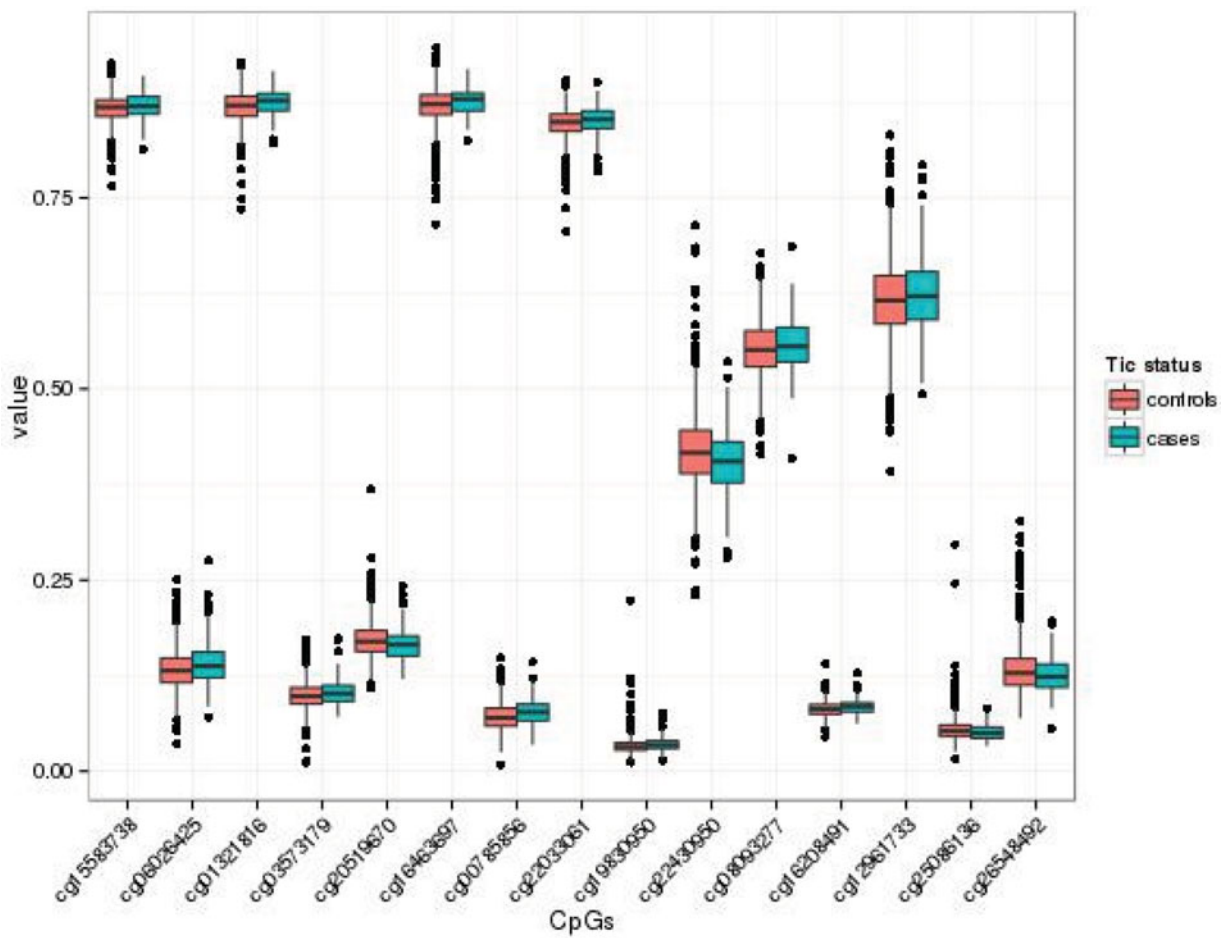

FIGURE 4

Average methylation levels of the top CpGs in cases versus controls.

TABLE 3

Top CpG Sites in the EWAS With *p*-value <0.0001

CpG site	CHR	CpG Location (bp)	*p*-value	Δβ	[Nearest] gene	Gene location
cg15583738	8	2176944	$1.98e^{-06}$	0.00408	—	—
cg06026425	5	157284650	$2.03e^{-06}$	0.008678	CLINT1	5q33.3
cg01321816	15	91358514	$2.32e^{-06}$	0.006536	BLM	15q26.1
cg03573179	6	36165382	$4.07e^{-06}$	0.003791	BRPF3	6p21.31
cg20519670	14	65172006	$6.91e^{-06}$	-0.00561	PLEKHG3	14q23.3
cg16463697	2	223886480	$8.97e^{-06}$	0.006082	KCNE4	2q36.1
cg00785856	15	59041883	$1.03e^{-05}$	0.005749	ADAM10	15q21.3
cg22033061	19	17531746	$1.04e^{-05}$	0.003424	FAM125A/MVB12A	19p13.11
cg19830950	10	102729375	$1.21e^{-05}$	0.002377	SEMA4G	10q24.31
cg08093277	6	29595299	$1.31e^{-05}$	0.006787	GABBR1	6p22.1
cg12961733	22	50165244	$2.17e^{-05}$	0.009437	BRD1	22q13.33
cg22430950	17	35166190	$2.62e^{-05}$	-0.0146	—	—
cg16208491	6	4021748	$2.76e^{-05}$	0.003249	PRPF4B	6p25.2
cg14830166	12	11821908	$3.01e^{-05}$	-0.01192	ETV6	12p13.2
cg23261919	10	135072960	$3.20e^{-05}$	0.002744	ADAM8	10q26.3
cg08451325	21	44720984	$3.25e^{-05}$	0.003124	SNF1LK/SIK1	21q22.3
cg15234400	4	174453166	$3.29e^{-05}$	-0.01027	NBLA00301/HAND2-AS1	4q34.1
cg21923992	3	185825749	$3.38e^{-05}$	0.007345	ETV5	3q27.2
cg07394446	15	100881458	$3.39e^{-05}$	-0.00446	ADAMTS17	15q26.3
cg19391247	20	2360385	$3.42e^{-05}$	0.00292	TGM6	20p13
cg07975472	19	1503610	$3.44e^{-05}$	0.003127	ADAMTSL5	19p13.3
cg23572228	7	4923575	$3.48e^{-05}$	0.006102	RADIL	7p22.1
cg25086136	8	50823124	$3.56e^{-05}$	-0.00347	SNTG1	8q11.21
cg06872019	13	31588778	$3.66e^{-05}$	-0.012	C13orf26/TEX26	13q12.3
cg20240091	1	175044916	$3.69e^{-05}$	0.00516	TNN	1q25.1
cg17750334	1	214776613	$3.96e^{-05}$	-0.00386	CENPF	1q41
cg14752139	8	7328654	$3.96e^{-05}$	0.003239	DEFB104B	8p23.1
cg16650530	1	32538413	$4.06e^{-05}$	0.002929	TMEM39B	1p35.2
cg23221732	17	72383708	$4.14e^{-05}$	-0.00432	GPR142	17q25.1
cg03704355	12	102270112	$4.15e^{-05}$	0.002634	DRAM/DRAM1	12q23.2
cg26548492	5	135170171	$4.79e^{-05}$	-0.00723	LOC153328/ SLC25A48	
cg21651356	12	104685539	$5.27e^{-05}$	-0.01119	TXNRD1	12q23.3
cg11597832	14	105993747	$5.39e^{-05}$	0.003214	TMEM121	14q32.33
cg19403269	1	5569798	$5.57e^{-05}$	-0.01835	—	—
cg24688563	1	160388964	$5.77e^{-05}$	0.006028	VANGL2	1q23.2
cg18556420	10	864596	$5.99e^{-05}$	-0.01245	LARP5/LARP4B	10p15.3
cg26116669	2	88654640	$6.39e^{-05}$	0.007397	—	—
cg01927730	6	168955764	$6.56e^{-05}$	-0.00385	SMOC2	6q27
cg23066982	6	26204463	$6.58e^{-05}$	0.002922	HIST1H4E	6p22.2
cg01490283	19	4066033	$6.70e^{-05}$	0.003977	ZBTB7A	19p13.3
cg21879791	6	29594830	$7.07e^{-05}$	0.003829	GABBR1	6p22.1
cg15347627	4	2941570	$7.08e^{-05}$	0.004751	NOL14/NOP14	4p16.3
cg01703966	7	143207845	$7.36e^{-05}$	-0.00416	LOC285965	
cg19511664	3	135685098	$7.53e^{-05}$	0.002758	PPP2R3A	3q22.2
cg11155621	17	43238118	$7.60e^{-05}$	0.003843	HEXIM2	17q21.31
cg07477602	1	56961319	$7.82e^{-05}$	0.002334	PPAP2B	1p32.2
cg00473985	16	87670568	$7.94e^{-05}$	0.001967	JPH3	16q24.2
cg04747445	3	107241417	$8.09e^{-05}$	0.004325	BBX	3q13.12
cg27135510	11	2423571	$8.43e^{-05}$	0.001627	TSSC4	11p15.5
cg02525719	4	183728549	$8.59e^{-05}$	0.01153	ODZ3/TENM3	4q34.3
cg19497750	14	70588881	$8.63e^{-05}$	-0.00479	SLC8A3	14q24.2
cg22112443	15	37393989	$8.66e^{-05}$	0.002057	MEIS2	15q14
cg21281009	18	14748298	$8.79e^{-05}$	-0.01545	ANKRD30B	18p11.21
cg01119319	7	38356808	$9.04e^{-05}$	0.014511	TARP	7p15-p14
cg24419101	17	6484720	$9.17e^{-05}$	0.002819	KIAA0753/TXNDC17	17p13.1
cg25203007	1	24126017	$9.22e^{-05}$	0.00304	GALE	1p36.11
cg08490349	17	17086207	$9.92e^{-05}$	0.002258	M-RIP/MPRIP	17p11.2

Note: CHR = chromosome; Δβ, Difference in mean methylation β-values between cases and controls; bp, base pairs.

acetyltransferase complex (GO:0070776, $p = 4.21e^{-05}$, FDR *q*-value $= 6.20e^{-03}$) and *H3* histone acetyltransferase complex (GO:0070775, $p = 4.21e^{-05}$, FDR *q*-value $= 5.68e^{-03}$), were significantly enriched. The top enriched GO function was protein binding (GO:0005515, $p = 1.10e^{-17}$, FDR *q*-value $= 4.55e^{-14}$) followed by many functions involving DNA binding.

Discussion

In this work, we present the first genome-wide epigenetic analysis on tics/tic disorders. Although no methylation site achieved significance at the genome-wide threshold, gene-ontology analysis of the top hits revealed enrichment in brain-specific and developmental processes. Thus, our findings provide interesting targets for further analysis.

Two of our top CpGs (cg08093277 and cg21879791, ranked 11 and 41) are located near the *GABBR1* gene (39670 bp and 39201 bp, at 5′, respectively), which represents a very interesting target for further analysis. *GABBR1* encodes a subunit of the GABA receptor, the major inhibitory neurotransmitter in the central nervous system (CNS). GABA acts at brain inhibitory synapses where it binds transmembrane receptors of both presynaptic and postsynaptic neurons. Notably, *GABBR1* has been previously associated with autism, schizophrenia, tremor, and obsessive-compulsive disorder (Fatemi et al., 2009; Hegyi, 2013; Luo et al., 2012; Richter et al., 2012).

The involvement of GABA in TS is well documented in the extant neurobiological literature. Tics have been associated with reduced basal ganglia volume and reduced cortical thickness in motor and sensorimotor areas controlling the facial, orolingual, and laryngeal muscles (Sowell et al., 2008). Studies of brain activity using PET or fMRI in TS subjects compared to controls have implicated dysfunctional striatum and thalamus, as well as cortical regions. The medium-sized spiny neurons in the basal ganglia have GABAergic inhibitory projections to the substantia nigra and Globus Pallidus (Ribak et al., 1979). It is hypothesized that the tonic activity of the striatum acts to inhibit unwanted motor patterns (Albin & Mink, 2006). Treatment strategies have aimed at increasing GABA levels (Awaad, 1999; Mink, 2001) to counteract decreased inhibitory output resulting in excessive activity in frontal cortical areas. Although not all studies showed significant results, one randomized double-blind study administering a GABA β-receptor agonist in TS resulted in reduced tic severity (Singer et al., 2001).

Importantly, 8 of the top 57 associated CpG sites ($p < 1 \times 10^{-4}$) mapped to genes that have been previously associated with psychiatric or neurological disorders, some of them sharing neurodevelopmental aspects with tics (OCD, autism, bipolar disorder, schizophrenia, some forms of mental retardation), some disorders involving corticostriatal pathways similar to tics (as in the case of Parkinson's disease), and some in which the link with tics seems less clear (Alzheimer's disease) (Supplementary Table T4 summarizes the genes from our top list that have been previously associated with neurological disorders). CpG site cg06026425, located near *CLINT1,* has been linked to schizophrenia (Leon et al., 2011; Wang et al., 2010). Moreover, cg01321816, located near *BLM,* and cg00785856, located near *ADAM10*, involve genes that have been associated with Alzheimer's disease (Schrötter et al., 2013; Vassar, 2013). Another example is cg26548492, which is located near *LOC153328/SLC25A48*, a gene that has been proposed as candidate for Parkinson's disease (Liu et al., 2011). Lastly, cg2051967, which is located near *PLEKHG3,* involves a gene that has been linked to mental retardation (Lehalle et al., 2014; Lybaek et al., 2008). Furthermore, some of the top hits lead to genes with a brain-specific function. This is the case

for cg25086136, located near *SNTG1*, which is specifically expressed in the CNS (Hafner et al., 2010), and cg19830950, located near *SEMA4G*, which might play a role in cerebellar development, the cerebellum being a core structure involved in the precision of motor control (Maier et al., 2011).

Histone modification might also play a role in the tic phenotype through the *MOZ/MORF* complex histone acetyltransferase complex (Klein et al., 2014). Genes involved in histone modifications were significantly enriched in the GO analysis. Acetylation represents one of the most frequent post-translational modifications (PTM) and it is catalyzed by lysine acetyl-transferase enzymes (KAT). It neutralizes the positive charge present on the amino group of histone tails, allowing the switch from a condensed structure to a more relaxed one, which results in an enhanced level of transcription. Two of the top CpGs (cg03573179 in the *BRPF3* gene and cg12961733 in the *BRD1* gene) are pointing to this *MOZ/MORF* complex, which is formed by bromodomain PHD finger protein (*BRPF1/2/3*), inhibitor of growth 5 (*ING5*), and homolog of Esa1-associated factor 6 (*hEAE6*) (Sapountzi & Cote, 2011). *BRD1* is associated with schizophrenia and bipolar disorder (Christensen et al., 2012) and the *MOZ/MORF* complex plays a role in the regulation of the dentate gyrus, which is a brain structure extremely important for learning and memory (You et al., 2015). *BRD1*, which is a component of the histone H3 acetyltransferase activity within the *MOZ/MORF* complex, might have a role in gene expression through acetylation of histone H3 and H4 (Doyon et al., 2006). *BRD1* has also been associated with schizophrenia and bipolar disorder (Christensen et al., 2012; Severinsen et al., 2006). *BRPF3* is another component of the histone H3 acetyltransferase activity within the *MOZ/MORF* complex (Ullah et al., 2008). CpG site cg23066982 is located near the *HIST1H4E* gene, which is a member of the histone 4 family and is a part of the nucleosome core. It is known that nucleosomes pack DNA into chromatin to regulate several processes such as transcription, DNA replication, and chromosome stability.

These findings support a role for aberrant DNA methylation levels in tic disorders as part of a broader neurodevelopmental dysregulation. It is important to note that our study examined DNA methylation in blood rather than in the CNS. The relationship with DNA methylation in CNS tissue remains unclear. However, it has been suggested that inter-individual variation in DNA methylation is correlated to some extent across blood and brain tissues (Davies et al., 2012). Also, it was observed that exposure to different forms of early life traumas led to similar methylation changes in blood and brain cells (Klengel et al., 2013). It has been proposed that epigenetic changes induced early in development in particular may be present across many different tissues, because they are propagated through cell division (Feinberg & Irizarry, 2010; Jeffries et al., 2012; Mill & Heijmans, 2013).

Future studies might consider taking a trans-diagnostic neurodevelopmental approach, combining tic disorders and other neurodevelopmental disorders (including OCD, autism, ADHD, and childhood movement disorders), to disentangle common versus disorder-specific underlying methylation patterns. Finally, environmental factors assessed in a longitudinal study design should be incorporated in epigenetic studies, to investigate which environmental stressors/protectors at what age/stage of development influence DNA methylation.

This study provides a first step to unravel the role of epigenetic mechanisms in tic disorders. It should be noted that we analyzed an 'inclusive' tic phenotype definition that may obscure different underlying etiologies. Future studies of larger size or including clinical samples at the more extreme end of the tic phenotypic spectrum are required to improve statistical power. Future studies should also aim to examine different phenotypic tic dimensions (de Haan et al., 2015). Such studies hold the promise to shed light on the complex interaction between environmental and genetic factors leading to development and persistence of neuropsychiatric disorders.

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Supplementary Material

To view supplementary material for this article, please visit http://dx.doi.org/ 10.1017/thg.2015.72

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