EWAS of monozygotic twins implicate a role of mTOR pathway in pathogenesis of tic spectrum disorder

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Introduction and aim

Tic spectrum disorder (TSD) is an umbrella term which includes the childhood-onset Gilles de la Tourette syndrome (GTS) and chronic tic disorder (CTD). They are considered highly heritable, yet the genetic components remain largely unknown, and epigenetic changes brought on by environmental factors are hypothesized to contribute to the phenotype. In the present study, we aimed to investigate the epigenetic contribution to TSD pathogenesis by performing an exploratory analysis of the genome-wide DNA methylation patterns in whole blood samples of 16 monozygotic twin pairs. Eight of these were discordant and six were concordant for TSD, while two pairs were asymptomatic.

Methods

- Illumina MethylationEPIC arrays.
- Identification of differentially methylated probes (DMPs) using a linear mixed effects model with twin pair as a random effect.
- Identification of differentially methylated regions (DMRs).
- Function and pathways enrichment analysis of the identified DMPs.

Table 1: Top results (DMPs) from the site-specific analysis

Rank	CpG site	Position (GRCh37/hg19)	*p*-value	FDR	Closest genes (distance to TSS)
1	cg00425865	chr9:135820111	1,49E-06	0,48	*TSC1* (-104) / *GFI1B* (-826)
2	cg16051558	chr14:50235537	1,98E-06	0,48	*KLHDC2* (+1,212) / *NEMF* (+84,383)
3	cg15728744	chr1:5917533	2,29E-06	0,48	*NPHP4* (+134,997)
4	cg18868182	chr10:60086874	2,54E-06	0,48	*UBE2D1* (-7,860) / *CISD1* (+58,057)
5	cg00703598	chr2:88480233	4,94E-06	0,58	*THNSL2* (+9,255) / *TEX37* (-343,935)
6	cg02228383	chr20:56934684	5,33E-06	0,58	*VAPB* (-29,493) / *RAB22A* (+49,933)
7	cg06538333	chr16:23445078	5,39E-06	0,58	*COG7* (+19,422) / *SCNN1B* (+131,488)
8	cg08283932	chr20:43280707	7,36E-06	0,63	*ADA* (-325)
9	cg20974961	chr6:49500569	7,54E-06	0,63	*GLYATL3* (+32,899) / *RHAG* (+103,982)

Table 2: Top results (DMRs) from the regional analysis

Rank	Position (GRCh37/hg19)	Length (bp)	Length (sites)	SLK adj. *p*	Sidak adj. *p*	Closest genes (Distance to TSS)
1	chr1:75198768-75199178	410	8	7,60E-06	0.001	*CRYZ (+119) / TYW3 (+133)*
2	chr7:3227262-3227333	71	3	9,01E-06	0.091	*SDK1 (-113,782) / CARD11 (-143,719)*
3	chr3:138067848-138068014	166	6	3,07E-05	0.131	*MRAS (+1,392) / ESYT3 (-85,524)*
4	chr15:93353059-93353199	140	3	7,35E-05	0.328	*CHD2 (-89,929) / FAM174B (-153,941)*
5	chr19:17830341-17830453	112	3	7,61E-05	0.402	*MAP1S (+236)*
6	chr13:41635362-41635513	151	4	1,05E-04	0.409	*WBP4 (+28)*
7	chr8:1765217-1765388	171	7	1,54E-04	0.494	*ARHGEF10 (-6,839) / CLN8 (+53,375)*

Table 3: Results from the enrichment analysis

ONTOLOGY	TERM	Binom Raw P-Value	Binom FDR Q-Value	Binom Fold Enrichment	Binom Observed Region Hits
GO Cellular Component	Insulin receptor substrate binding	3.88E-4	4.33E-2	2.25	23
GO Biological Process	Positive regulation of hormone metabolic process	9.20E-6	1.12E-3	2.70	26
	Positive regulation of hormone biosynthetic process	1.56E-5	1.79E-3	3.08	20
	Regulation of protein ubiquitination involved in ubiquitin-dependent protein catabolic process	5.10E-4	2.65E-2	2.36	20
MSigDB Pathway	Phosphoinositides and their downstream targets	7.62E-4	3.14E-2	2.07	25
	PTEN is a tumor suppressor that dephosphorylates the lipid messenger phosphatidylinositol triphosphate	1.13E-3	3.73E-2	2.39	17

Results

- No DMPs with genome-wide significance.
- Several DMPs (Table 1) and DMRs (Table 2) with suggestive significance.
- Many of the identified genes have previously been associated with neuropsychiatric disorders (*TSC1, NEMF, ADA, SDK1, CHD2* and *CLN8*).
- The top genes identified (*TSC1* and *CRYZ*/*TYW3*), as well as three of the enriched cellular functions and pathways (Table 3), have previously been implicated in, or related to, the PI3K/AKT/mTOR pathway.

Conclusions

The results of our study implicate epigenetic deregulation of the PI3K/AKT/mTOR pathway as a contributing risk factor in TSD pathogenesis. Genes in this pathway have on several occasions been associated with GTS, and mTOR signalling has been implicated in a broad range of neuropsychiatric disorders including autism.

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