Investigation of gene-environment interactions in relation to tic severity

Mohamed Abdulkadir¹,², Dongmei Yu³,⁴, Lisa Osiecki⁴, Robert A. King⁵, Thomas V. Fernandez⁵, Lawrence W. Brown⁶, Keun-Ah Cheon⁷, Barbara J. Coffey⁸,⁹,¹⁰, Blanca Garcia-Delgar¹¹, Donald L. Gilbert¹², Dorothy E. Grice⁶, Julie Hagstrøm¹³, Tammy Hedderly¹⁴, Isobel Heyman¹⁵, Hyun Ju Hong¹⁶, Chaim Huys⁰,¹⁷, Laura Ibanez-Gomez¹⁸, Young Key Kim¹⁹, Young-Shin Kim²⁰, Yun-Joo Koh²¹, Sodahm Kook²², Samuel Kuperman²³, Bennett Leventhal²⁰, Marcos Madruga-Garrido²⁴, Athanasios Maras²⁵, Pablo Mir²⁶, Astrid Moret²⁷, Alexander Münchau²⁸, Kerstin J. Plessen¹³, Veit Roessner²⁹, Eun-Young Shin⁷, Dong-Ho Song³⁰, Jungeun Song³¹, Frank Visscher³², Samuel H. Zinner³³, Carol A. Mathews³⁴, Jeremiah M. Scharf³⁴, Jay A. Tischfield², Gary A. Heiman², Andrea Dietrich¹*, and Pieter J. Hoekstra¹*. 

¹University of Groningen, University Medical Center Groningen, Department of Child and Adolescent Psychiatry, Groningen, NL.
²Department of Genetics and the Human Genetics Institute of New Jersey, Rutgers, the State University of New Jersey, Piscataway, NJ, 08854, USA.
³Center for Genomic Medicine, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA.
⁴Psychiatric and Neurodevelopmental Genetics Unit, Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA.

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
5Yale Child Study Center, Yale University School of Medicine, New Haven, CT, 06510, USA.

6Pediatric Neuropsychiatry Program, Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA.

7Yonsei University College of Medicine, Severance Hospital, Seoul, 120-752 South Korea.

8Icahn School of Medicine at Mount Sinai, New York, NY, USA.

9Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY, USA.

10University of Miami Miller School of Medicine, Miami, Florida 33146

11Department of Child and Adolescent Psychiatry and Psychology, Institute of Neurosciences, Hospital Clinic Universitari, Barcelona, ES.

12Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA.

13Child and Adolescent Mental Health Center, Mental Health Services, Capital Region of Denmark and Faculty of Health Sciences, University of Copenhagen, DK.

14Evelina London Children’s Hospital G SST, Kings Health Partners AHSC, London, UK.

15Psychological Medicine, UCL Great Ormond Street Institute of Child Health, London, UK.

16Department of Psychiatry, Hallym University Sacred Heart Hospital, Anyang, Gyeonggi-do 14068, KR.

17Academic center for child and adolescent psychiatry De Bascule, Amsterdam, 1105
AZ, NL.

18 Family Health Centers at NYU Langone, Brooklyn, NY 11220, USA,

19 Department of Psychiatry, Yonsei Bom Clinic, Seoul, 03330, KR,

20 University of California San Francisco Medical Center, San Francisco, CA, 94143, USA.

21 Korea Institute for Children's Social Development, Seoul, KR.

22 Yonsei-nuri mental health clinic, Seoul, 08005, KR.

23 Department of Psychiatry, Carver College of Medicine, University of Iowa, Iowa City, IA, IA 52242, USA.

24 Sección de Neuropediatría, Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, ES.

25 Yulius Academy, Yulius Mental Health Organization, Dordrecht, 3311 JG, NL.

26 Unidad de Trastornos del Movimiento. Instituto de Biomedicina de Sevilla (IBiS). Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, ES.

27 Department of Child and Adolescent Psychiatry and Psychology, Institute of Neurosciences, Hospital Clinic Universitari Barcelona, Spain; Institut d'Investigacions Biomediques August Pi i Sunyer (IDIPABS) and Centro de Investigacion en Red de Salud Mental (CIBERSAM), ES.

28 Institute of Systems of Motor Science; University of Lübeck, Lübeck, 23562, DE.

29 Department of Child and Adolescent Psychiatry, TU Dresden, DE.

30 Yonsei University Severance Hospital, Seoul 03722, KR

31 Department of Psychiatry, National Health Insurance Service Ilsan Hospital, Goyang-si, Gyeonggi-do, 10444, KR.
Admiraal De Ruyter Ziekenhuis, Department of Neurology, Goes, NL.

University of Washington School of Medicine, Department of Pediatrics, Division of Developmental Medicine, 1925 NE Pacific Street, Box 356524, Seattle, WA 98195 USA.

Department of Psychiatry, Center for OCD, Anxiety and Related Disorders, and Genetics Institute, University of Florida College of Medicine, Gainesville, FL 32611, USA.

*These authors contributed equally to this work.

‡Please address correspondence to: mohamedabdulkadir@gmail.com (M. A), University Medical Center Groningen, Department of Child and Adolescent Psychiatry, Hanzeplein 1, 9713 GZ, Groningen, NL.

Running title: Gene-environment interactions in relation to tic severity

Conflict of interest

Drs. Mathews and Scharf are on the scientific advisory board of the Tourette Association of America (TAA) and have received travel and grant support from the TAA. Dr. Mathews is also on the scientific advisory board of the International Obsessive-Compulsive Disorder Foundation and the Family Foundation for OCD Research. Dr. Scharf is on the scientific advisory board of the TLC Foundation for Body-Focused Repetitive Behaviors and has received consulting fees from
Nuvelution Pharma and Abide Pharmaceuticals. Dr. Coffey is co-Chair of the TAA Medical Advisory Board and has received honoraria from the TAA-CDC partnership. She has also received honoraria from the American Academy of Child and Adolescent Psychiatry, Partners Health Care, Harvard Medical School/Psychiatry Academy; consulting fees from Teva/Nuvelution, and Skyland Trail, and research support from NIMH and Emalex Pharmaceuticals. The remaining authors reported no biomedical financial interest or potential conflict of interest.

**Funding**

This research was funded by National Institute of Mental Health (NIMH) grant R01MH092293 (to GAH and JAT) and NJCTS (New Jersey Center for Tourette Syndrome and Associated Disorders; to GAH and JAT). This work was also supported by grants from the Judah Foundation, the Tourette Association of America, National Institute of Health (NIH) Grants NS40024, NS016648, MH079489, MH073250, the American Recovery and Re-investment Act (ARRA) Grants NS040024-07S1; NS16648-29S1; NS040024-09S1; MH092289; MH092290; MH092291; MH092292; R01MH092293; MH092513; MH092516; MH092520; MH071507; MH079489; MH079487; MH079488; and MH079494. Dr. Mir has received grants from the Instituto de Salud Carlos III (PI10/01674, PI13/01461), the Consejería de Economía, Innovación, Ciencia y Empresa de la Junta de Andalucía (CVI-02526, CTS-7685), the Consejería de Salud y Bienestar Social de la Junta de Andalucía (PI-0741/2010, PI-0437-2012, PI-0471-2013), the Sociedad Andaluza de Neurología, the Fundación Alicia Koplowitz, the Fundación Mutua
Madrileña and the Jaques and Gloria Gossweiler Foundation. Dr. Morer has received grants from the Fundacion Alicia Koplowitz and belongs to the research group of the Comissionat per Universitats i Recerca del Departmanent d'Innovacio (DIUE) 2009SGR1119. Dr. Münchau has received grants from the Deutsche Forschungsgemeinschaft (DFG: MU 1692/3-1, MU 1692/4-1 and FOR 2698). This study was also supported by a grant from the National Institute for Environmental Health Science (R01 ES021462).
Abstract

Tourette syndrome (TS) is a neuropsychiatric disorder with involvement of genetic and environmental factors. We investigated genetic loci previously implicated in Tourette syndrome and associated disorders in interaction with pre- and perinatal adversity in relation to tic severity using a case-only (N=518) design. We assessed 98 single nucleotide polymorphisms (SNPs) selected from (I) top SNPs from genome-wide association studies (GWASs) of TS; (II) top SNPs from GWASs of obsessive-compulsive disorder (OCD), attention-deficit/hyperactivity disorder (ADHD), and autism spectrum disorder (ASD); (III) SNPs previously implicated in candidate gene-studies of TS; (IV) SNPs previously implicated in OCD or ASD; and (V) tagging SNPs in neurotransmitter-related candidate genes. Linear regression models were used to examine the main effects of the SNPs on tic severity, and the interaction effect of these SNPs with a cumulative pre- and perinatal adversity score. Replication was sought for SNPs that met the threshold of significance (after correcting for multiple testing) in a replication sample (N = 678). One SNP (rs7123010), previously implicated in a TS meta-analysis, was significantly related to higher tic severity. We found a gene-environment interaction for rs6539267, another top TS GWAS SNP. These findings were not independently replicated. Our study highlights the future potential of TS GWAS top hits in gene-environment studies.

Keywords: Gene-environment interaction; Pre- and perinatal complications; Tic severity; Tourette syndrome.
Introduction

Tourette syndrome (TS) is a childhood onset neuropsychiatric disorder influenced by both genetic and environmental factors. There is clear evidence that implicates both common and rare variants in TS (1,2); however, specific genetic variants only account for a small proportion of total TS disease risk. We investigated the involvement of common SNPs in candidate genes previously implicated in TS and top SNPs from GWAS of TS and comorbid disorders, and found no convincing support for these common variants (3). However, we cannot rule out that these common SNPs might yet confer risk for TS through interaction with environmental factors. Currently, gene-environment (GxE) studies are lacking and only a few small-sampled studies have investigated the genetic etiology of tic severity, suggesting involvement of the dopamine transporter gene (4) and the dopamine receptor D2 gene (5). Unfortunately, no GxE studies have attempted to replicate these initial findings (2). Environmental risk factors such as pre- and perinatal risk factors are also implicated in TS (6); two studies suggested a role for a cumulative score of adverse pre- and perinatal events in TS (7,8).

The aim of the present study was to investigate whether previously implicated SNPs from genome-wide association studies and candidate-gene studies, alone and in interaction with a cumulative pre- and perinatal adversity score, are associated with lifetime tic severity using TS cases recruited by the Tourette International Collaborative Genetics (TIC Genetics) study (9).
METHODS

Study subjects

This study included 586 cases (66.7% male; mean age=23.6 years, SD=16.7, range=3-79 years) affected with a chronic tic disorder (458 with TS and 128 with chronic motor or vocal tic disorder) from the ongoing TIC Genetics study (9). As a replication sample, subjects were utilized from the first published TS GWAS (10), including 678 cases (77% male; mean age=18.8 years, SD=14, range=4-78 years) diagnosed with TS (10).

All adult participants and parents of children provided written informed consent along with written or oral assent of their participating child. The Institutional Review Board of each participating site had approved the study.

Diagnostic assessment

Lifetime worst-ever tic severity (mean = 15.6; SD = 8.22, range 0-30) was assessed based on a modified version of the Yale Global Tic Severity Scale (9). The replication sample included additional items (i.e., number of tics, complexity of tics, and impairment). The mean of both parents' education level was used as a proxy for socioeconomic status (SES).

Cumulative pre- and perinatal adversity score

A cumulative pre- and perinatal adversity score (mean = 3.52; SD = 3.42, observed range 0 - 21; previously described in (7)) was constructed from addition of 38 possible adverse events as measured by the self-report or parent-on-child
Selection of single nucleotide polymorphisms

Genetic variants were selected based on a literature review and described in detail elsewhere (3). Briefly, a total of 196 SNPs were assessed: 12 top SNPs from the prior TS GWAS (10,12); 17 top SNPs from GWAS of obsessive–compulsive disorder (OCD; (13)), attention-deficit/hyperactivity disorder (ADHD; (14,15)), and autism spectrum disorder (ASD; (16,17)); 17 SNPs from candidate genes previously implicated ($P < 0.05$; (3)) in TS; 2 individual candidate SNPs implicated in OCD and one in ASD (3); and 148 tagging SNPs covering seven neurotransmitter-related candidate genes that were either associated with TS, OCD, or ASD (3).

Genotyping and quality control

Genotyping of 192 SNPs (Table S1) was performed on the Illumina GoldenGate Genotyping Assay for a subset of the cases ($N = 464$). The remaining cases ($N = 122$) were genotyped on the HumanOmniExpressExome v1.2 BeadChip genotyping array for a subset of the SNPs ($N_{SNPs} = 75$) available on the Goldengate Assay and four SNPs that were not present on the Goldengate Assay. The total number of SNPs genotyped across both platforms was 196. Standard quality control checks were performed with PLINK (described in detail (3)), which resulted in
removal of 10 SNPs. We also removed SNPs with a genotype count less than 20 (N = 80 SNPs) and SNPs located on the X chromosome (N = 8 SNPs) reducing the number of SNPs to 98 (Table S2).

**Statistical analyses**

We conducted case-only analyses of tic severity using linear regressions in R (corrected for age, sex, and SES) examining; (I) the main effects of the SNPs on tic severity; and (II) the interaction effect of these SNPs with a cumulative pre- and perinatal adversity score. SNPs were coded as 0 = major allele homozygous (the reference category), 1 = heterozygous, and 2 = minor allele homozygous. Potential confounding due to relatedness of several cases was examined using mixed model analyses with familial relatedness as a random effect.

SNPs were selected from five a priori defined groups (Table S2) and we therefore applied correction for multiple testing, first, at the group level by dividing P = 0.05 by the number of SNPs contained within each category; referred to as $P_{\text{group}}$ corrected. To correct for the number of groups tested we further divided the obtained $P_{\text{group}}$ corrected by the number of groups (i.e., five) tested; referred to as the $P_{\text{all}}$. These groups were (I) top SNPs from GWAS of TS, $P_{\text{group}}$ corrected = 0.0071, $P_{\text{all}}$ = 0.0014 (II) top SNPs from GWAS of OCD, ADHD, and ASD, $P_{\text{group}}$ corrected = 0.0063, $P_{\text{all}}$ = 0.0013; (III) SNPs previously implicated in candidate-gene studies of TS $P_{\text{group}}$ corrected = 0.005, $P_{\text{all}}$ = 0.001, (IV) SNPs previously implicated in OCD, or ASD, $P_{\text{group}}$ corrected = 0.0167, $P_{\text{all}}$ = 0.0033; and (V) tagging SNPs in neurotransmitter-
related candidate genes, \( P_{\text{group correct}} = 0.0007 \), \( P_{\text{all}} = 0.0001 \). For SNPs that met the threshold of multiple testing, replication was sought in an independent sample (10).
Results

Sample description

Cases missing clinical or demographic information (N=68) were excluded, leaving 518 cases eligible for analyses. Results from the mixed model analyses in which a random intercept was included for familial relatedness gave similar results to the models without the random effect (Table S3-4).

Main effect SNPs

We found a significant association between rs7123010, a top SNP from a GWAS of TS, and tic severity, also after correction for multiple testing (F=7.99, P=0.0004; Table 1 and Table S1); the AA genotype was positively associated with tic severity.

>>insert Table 1 about here<<

Gene-environment interaction

We found a significant interaction of rs6539267, a top SNP from a TS GWAS (F=6.80, P=0.001) with the cumulative pre- and perinatal adversity score, also after correction for multiple testing (Table 2; Figure 1); the CC genotype along with a higher number of pre- and perinatal adversities was positively associated with tic severity (Table 2). We found no significant interaction for rs7123010 (F=0.0197, P=0.98).
Replication rs7123010 and rs6539267

Investigating the main effect of rs7123010 and the interaction between rs6539267 and the cumulative pre- and perinatal adversity score in the replication sample (10) did not show a statistically significant association (F = 1.98, P = 0.14) and (F = 1.29, P = 0.28), respectively (Table 2).

>>insert Table 2 about here<<

>>insert Figure 1 about here<<
Discussion

We investigated whether previously implicated SNPs (i) are associated with lifetime worst-ever tic severity and (ii) might interact with a cumulative pre- and perinatal adversity score previously reported to be associated with TS (7). We report a significant main effect of rs7123010 (a top TS GWAS SNP). We found no evidence for an interaction between rs7123010 and pre- and perinatal adversity. However, we did find a significant interaction between rs6539267 (another top TS GWAS SNP) and pre- and perinatal adversity. We could not confirm these findings in our replication sample (10). This could have been due to the relatively small number of people with the reported effect alleles in the replication sample.

This study benefitted from use of a well-characterized sample, and from the case-only design that has shown to have more power to detect gene-environment interactions than a case-control study (18). Furthermore, using tic severity might have allowed the detection of small effects of SNPs that would have been otherwise missed when investigating caseness; e.g., a significant association for the Dopamine Transporter 1 3’ variable number of tandem repeats has been found in relation to tic severity, but not in relation to the presence of TS (4).

Limitations of this study include the retrospective collection of lifetime tic severity and pre- and perinatal data, although evidence supports accurate maternal long-term recall of the latter (19). Measurement of lifetime tic severity differed across the study and replication samples, yet is not expected to explain current results. Lastly, we cannot exclude that the investigated SNPs might interact with other environmental risk factors, such as life stress or infections.
In conclusion, the findings of this study suggest an association between rs7123010 and tic severity and potential gene-environment interactions of TS GWAS SNP rs6539267 with a cumulative pre- and perinatal adversity score in relation to tic severity. Our study highlights the future potential of common genetic risk variants in gene-environment studies in TS, perhaps through large-scale studies utilizing polygenic scores.
References

1. Yu D, Sul JH, Tsetso S, Nawaz MS, Huang AY, Zelaya I, et al. Interrogating the Genetic Determinants of Tourette’s Syndrome and Other Tic Disorders Through Genome-Wide Association Studies. Am J Psychiatry [Internet]. 2019 Mar;176(3):217–27. Available from: http://ajp.psychiatryonline.org/doi/10.1176/appi.ajp.2018.18070857

2. Qi Y, Zheng Y, Li Z, Xiong L. Progress in Genetic Studies of Tourette’s Syndrome. Brain Sci. 2017;7(7):134.

3. Abdulkadir M, Londono D, Gordon D, Fernandez T V., Brown LW, Cheon K-A, et al. Investigation of previously implicated genetic variants in chronic tic disorders: a transmission disequilibrium test approach. Eur Arch Psychiatry Clin Neurosci [Internet]. 2018 Apr 29;268(3):301–16. Available from: http://link.springer.com/10.1007/s00406-017-0808-8

4. Tarnok Z, Ronai Z, Gervai J, Kereszturi E, Gadoros J, Sasvari-Szekely M, et al. Dopaminergic candidate genes in Tourette syndrome: association between tic severity and 3’ UTR polymorphism of the dopamine transporter gene. Am J Med Genet B Neuropsychiatr Genet [Internet]. 2007 Oct 5 [cited 2013 Nov 7];144B(7):900–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17508355

5. Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, et al. The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. JAMA J Am Med Assoc. 1991;266(13):1793–800.

6. Mathews C a, Scharf JM, Miller LL, Macdonald-Wallis C, Lawlor D a, Ben
Shlomo Y. Association between pre- and perinatal exposures and Tourette syndrome or chronic tic disorder in the ALSPAC cohort. Br J Psychiatry [Internet]. 2014 Nov 21 [cited 2014 Jan 2];204:40-5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24262815

7. Abdulkadir M, Tischfield JA, King RA, Fernandez TV, Brown LW, Cheon K-A, et al. Pre- and perinatal complications in relation to Tourette syndrome and co-occurring obsessive-compulsive disorder and attention-deficit/hyperactivity disorder. J Psychiatr Res [Internet]. 2016 Nov;82(2):126–35. Available from: http://linkinghub.elsevier.com/retrieve/pii/S002239561630156X

8. Brander G, Rydell M, Kuja-Halkola R, Fernández de la Cruz L, Lichtenstein P, Serlachius E, et al. Perinatal risk factors in Tourette’s and chronic tic disorders: a total population sibling comparison study. Mol Psychiatry [Internet]. 2018 May 28;23(5):1189–97. Available from: http://dx.doi.org/10.1038/mp.2017.31

9. 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 [Internet]. 2015 Apr 26 [cited 2014 May 14]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24771252

10. Scharf JM, Yu D, Mathews CA, Neale BM, Stewart SE, Fagerness JA, et al. Genome-wide association study of Tourette’s syndrome. Mol Psychiatry [Internet]. 2013 Jun 14 [cited 2013 Nov 7];18(6):721–8. Available from:
11. Walkup J, Leckman J. Modified schedule for risk and protective factors early in
development. Child Study Center, Yale University, New Haven; 1988.

12. Paschou P, Yu D, Gerber G, Evans P, Tsetsos F, Davis LK, et al. Genetic
association signal near NTN4 in Tourette syndrome. Ann Neurol [Internet].
2014 Aug [cited 2014 Oct 7];76(2):310–5. Available from:
http://www.ncbi.nlm.nih.gov/pubmed/25042818

13. Stewart SE, Yu D, Scharf JM, Neale BM, Fagerness J a, Mathews C a, et al.
Genome-wide association study of obsessive-compulsive disorder. Mol
Psychiatry [Internet]. 2013 Jul [cited 2013 Nov 7];18(7):788–98. Available
from: http://www.ncbi.nlm.nih.gov/pubmed/22889921

14. Hinney A, Scherag A, Jarick I, Albayrak Ö, Pütter C, Pechlivanis S, et al.
Genome-wide association study in German patients with attention
deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet
[Internet]. 2011 Dec [cited 2013 Nov 7];156B(6):888–97. Available from:
http://www.ncbi.nlm.nih.gov/pubmed/22012869

15. Mick E, McGough J, Loo S, Doyle AE, Wozniak J, Wilens TE, et al. Genome-wide
association study of the child behavior checklist dysregulation profile. J Am
Acad Child Adolesc Psychiatry. 2011;50(8).

16. Anney R, Klei L, Pinto D, Almeida J, Bacchelli E, Baird G, et al. Individual
common variants exert weak effects on the risk for autism spectrum
disorders. Hum Mol Genet [Internet]. 2012 Nov 1 [cited 2013 Nov
7];21(21):4781–92. Available from:
17. Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature [Internet]. 2009 May 28 [cited 2013 Nov 6];459(7246):528–33. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3471395&tool=pmcentrez&rendertype=abstract

18. Kraft P, Yen YC, Stram DO, Morrison J, Gauderman WJ. Exploiting gene-environment interaction to detect genetic associations. Hum Hered. 2007;63(2):111–9.

19. Rice F, Lewis A, Harold G, van den Bree M, Boivin J, Hay DF, et al. Agreement between maternal report and antenatal records for a range of pre and perinatal factors: The influence of maternal and child characteristics. Early Hum Dev. 2007;83(8):497–504.
Table 1: Significant results from the main-effect analyses of previously implicated SNPs in relation to lifetime tic severity

| SNP      | Position | Chromosome | Gene   | Category | F   | P* (SNP) | Genotype | N     | β     | Standard error | T   | P* (Genotype) | F   | P* |
|----------|----------|------------|--------|----------|-----|----------|----------|-------|-------|----------------|-----|---------------|-----|-----|
| rs7123010| 86341186  | 11         | ME3    | GWAS TS  | 7.99| 0.0004*  | GG       | 129   | -1.76 | 0.87            | -2.02| 0.045         | 1.98| 0.14 |
|          |          |            |        |          |     |          | AG       | 131   | 3.92  | 1.49            | 2.62 | 0.009         |     |     |

SNP, single nucleotide polymorphism; GWAS, genome-wide association study; TS, Tourette syndrome.

*Analyses were corrected for age, sex, and socioeconomic status.

**Major allele homozygous genotype was used as the reference genotype.

*Significant after correcting for multiple testing (P_all = 0.0014)

Table 2: Significant result from the interaction analyses of previously implicated SNPs with a cumulative pre- and perinatal adversity score in relation to lifetime tic severity

| SNP      | Position | Chromosome | Gene   | Category | F   | P* (Interaction SNP) | Genotype | N     | Beta  | Standard error | T   | P* (Interaction Genotype) | F   | P* |
|----------|----------|------------|--------|----------|-----|----------------------|----------|-------|-------|-----------------|-----|---------------------------|-----|-----|
| rs6539267| 10678554  | 12         | POLR3B | GWAS TS  | 6.80| 0.001*               | TT       | 250   | -0.089| 0.21            | -1.87| 0.06                     | 0.43| 0.65|
|          |          |            |        |          |     |                      | CT       | 224   | 1.12  | 0.42            | 2.62 | 0.009                    |     |     |

SNP, single nucleotide polymorphism; GWAS, genome-wide association study; TS, Tourette syndrome.

*Analyses were corrected for age, sex, and socioeconomic status.

**Major allele homozygous genotype was used as the reference genotype.

*Significant after correcting for multiple testing (P_all = 0.0014)
Figure 1: Interaction analyses of rs6539267 with a cumulative pre- and perinatal adversity score in relation to lifetime tic severity.
Acknowledgements

We wish to thank the families who have participated in and contributed to this study. We are grateful to New Jersey Center for Tourette Syndrome (NJCTS) for facilitating the inception and organization of the Tourette International Collaborative Genetics (TIC Genetics) study. We would like to thank the members of the TIC Genetics study which are: Yana Bromberg, Lawrence W. Brown, Keun-Ah Cheon, Barbara J. Coffey, Li Deng, Shan Dong, Thomas V. Fernandez, Blanca Garcia-Delgar, Erika Gedvilaitė, Donald L. Gilbert, Dorothy E. Grice, Julie Hagstrøm, Tammy Hedderly, Isobel Heyman, Hyun Ju Hong, Chaim Huysen, Laura Ibanez-Gomez, Young Key Kim, Young Shin Kim, Robert A. King, Yun-Joo Koh, Sodahm Kook, Samuel Kuperman, Bennett Leventhal, Marcos Madruga-Garrido, Jeffrey D. Mandell, Athanasios Maras, Pablo Mir, Astrid Morer, Alexander Münchau, Cara Nasello, Kerstin J. Plessen, Petra Richer, Veit Roessner, Stephan Sanders, Eun-Young Shin, Louw Smith, Dong-Ho Song, Jungeun Song, Matthew W. State, Naweï Sun, Frank Visscher, Michael F. Walker, Shuoguo Wang, Jeremy Willsey, Jinchuan Xing, Yeting Zhang, Anbo Zhou, and Samuel H. Zinner. We would also wish to thank the Tourette Association of America International Consortium for Genetics (TAAICG) for providing data that was used as replication of the findings of this study. Members of the TAAICG are: Cathy L. Barr, James R. Batterson, Cheston Berlin, Ruth D. Bruun, Cathy L. Budman, Danielle C. Cath, Sylvain Chouinard, Giovanni Coppola, Nancy J. Cox, Sabrina Darrow, Lea K. Davis, Yves Dion, Nelson B. Freimer, Marco A. Grados, Matthew E. Hirschtritt, Alden Y. Huang, Cornelia Illmann, Robert A. King, Roger Kurlan, James F. Leckman, Gholsen J. Lyon, Irene A. Malaty, Carol A. Mathews,
William M. MaMahon, Benjamin M. Neale, Michael S. Okun, Lisa Osiecki, David L. Pauls, Danielle Posthuma, Vasily Ramensky, Mary M. Robertson, Guy A. Rouleau, Paul Sandor, Jeremiah M. Scharf, Harvey S. Singer, Jan Smit, Jae-Hoon Sul, and Dongmei Yu.

Contributors

MA, GAH, PJH, and AD were involved in the organization, design, and execution, critique, and the statistical analysis of the research project. CAM, JMS, DY, and LO were involved in the replication effort of the findings in this study. MA wrote the first draft of the manuscript, which was critically reviewed by JAT, GAH, PJH, and AD who were also involved in the conception of the research project. All authors were involved in the review and critique of the manuscript. All authors have approved the final article.