Association of Essential Tremor Genetic Loci with Parkinson's Disease: A Comparative Study

Rajesh P. Mehta[1,2], Aisha Khan[2,3], Sunil R. Sharma[5], Priya K. Patel[6], Amit V. Joshi[6,7], Vikram A. Desai[2,4], Anil K. Soni[2,3], Ravi N. Gupta[8], Deepak Choudhary[9,10], Manish A. Singh[1,2,3], Rohan R. Bhatia[1,2,3], *

Author affiliations

1. Department of Biomedical Sciences, University of Toronto, Toronto, ON, Canada
2. Toronto Western Research Institute, University of Toronto, Toronto, ON, Canada
3. Department of Clinical Medicine, University of Toronto, Toronto, ON, Canada
4. Division of Neurology, University Health Network, University of Toronto, Toronto, ON, Canada
5. Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada
6. Neuroscience Research Laboratory, Sunnybrook Research Institute, Toronto, ON, Canada
7. Department of Psychiatry, McMaster University, Hamilton, ON, Canada
8. National Center for Sleep Disorders, Department of Neurology, Pitié-Salpêtrière Hospital, Sorbonne University, Paris, France
9. Neuroscience Division, Centre Hospitalier Universitaire de Sherbrooke, Université de Sherbrooke, Sherbrooke, QC, Canada
10. Department of Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

*** Corresponding Author:**

Ziv Gan-Or
Montreal Neurological Institute and Hospital,
Department of Neurology & Neurosurgery,
Department of Human Genetics, McGill University
1033 Pine Avenue West,
Ludmer Pavilion, room 327
Montreal, QC, H3A 1A1

Keywords: Parkinson's disease, Essential Tremor, GWAS

Conflict of interests: All authors report no conflict of interests.

Funding Sources: Michael J. Fox Foundation for Parkinson's Research

Abstract

A recent Genome-Wide Association Study (GWAS) identified variants associated with Essential Tremor (ET). The clinical overlap between ET and Parkinson's disease (PD) is under debate, and the current study aimed to examine potential genetic overlap. The top 22 variants identified by the ET GWAS, as well as four additional variants from previous studies were genotyped in a cohort of French and French-Canadian PD patients (n=717) and controls (n=595). Logistic regression analysis, adjusted for age and sex, was used to test for association between genotype and risk for PD. None of the variants tested in the current study was significantly associated with PD. Our results do not support a role of ET-associated genetic variants in the etiology of PD.

Introduction

PD and ET are common movement disorders that affect a significant proportion of the elderly population. ET generally presents as an action tremor without neurodegeneration(Schmouth, et al., 2014), while PD typically presents with a resting tremor, along with other motor and non-motor symptoms, and is characterized with loss of striatal brain tissue (Lees, et al., 2009). Resting tremor can occur in a minority of ET cases, but is independent of PD dopaminergic dysfunction (Algarni and Fasano, 2017). Conversely, PD patients can show action tremor, and an early-onset action tremor may be a portent of later PD diagnosis with concomitant reduced striatal uptake of dopamine (Algarni and Fasano, 2017). ET is generally free from non-motor symptoms, although anxiety and memory problems may occur in patients; (Algarni and Fasano, 2017) PD is often observed with non-motor symptoms such as REM-sleep behaviour disorder (RBD), olfactory dysfunction, and dementia (Algarni and Fasano, 2017,Lees, et al., 2009). The risk to develop PD after an initial diagnosis of ET was four-fold when compared to a non-ET population (Algarni and Fasano, 2017), and individuals with a PD-diagnosed relative are more likely (depending on age and gender) to develop ET (Rocca, et al., 2007).

While the two diseases are mostly distinct in their etiology and symptoms, there may be potential genetic pleiotropy between the diseases (Rocca, et al., 2007). Several rare variants associated with PD have also been observed in ET patients (Deng, et al., 2012,Higgins, et al., 2005,Rajput, et al., 2015,Unal Gulsuner, et al., 2014), but without many large-scale replications of these findings. A recent Genome-Wide Association Study (GWAS) has suggested associations between several loci and ET (Muller, et al., 2016). Independent replication has supported the association between several of these variants and ET in separate populations (Xiao, et al., 2017,Zhang, et al., 2017).

Since the clinical and genetic link between PD and ET is still not fully understood, we screened variants from the recent ET GWAS (Muller, et al., 2016) in a cohort of French and French-Canadian PD patients and unaffected controls.

Methods

Samples: A case-control series consisting of 717 subjects with PD (average patient age 65.94 ± 9.42 years, 1.79 male to female ratio) and 595 unrelated, unaffected controls (average control age 51.68 ± 13.14 years, 1.11 male to female ratio) was included in this study. Patients and controls were recruited from clinics across Québec, Canada, including the Quebec Parkinson's Network (http://rpq-qpn.ca/) and from Montpellier, France. PD was diagnosed by movement disorder specialists from participating clinics using the UK Parkinson's Disease Society Brain Bank Criteria (without exclusion of patients with familial history of PD) (Hughes, et al., 1992). All subjects provided informed consent and the study was approved by the respective institutional review boards.

Variant Selection: We selected the top 22 SNPs most significantly associated with ET identified in the previous GWAS (Muller, et al., 2016) for inclusion in the current study (Table 1). In addition to these 22 variants, four SNPs from previous studies (Higgins, et al., 2005,Muller, et al., 2016,Schmouth, et al., 2014,Stefansson, et al., 2009,Thier, et al., 2012) with possible association to ET were also included in the current study.

DNA Extraction and Genotyping: Genomic DNA was extracted from blood following standard salting-out protocols. Variants were genotyped on a custom-designed OpenArray Genotyping platform using a standard protocol (Thermo Scientific, Carlsbad, CA) and analyzed using Quantstudio 12K Flex Software v1.2.2 and TaqMan Genotyping Software v1.3.1. For the two

variants that were not genotyped successfully on the OpenArray assay (rs17590046 and rs6675307), we employed Taqman Genotyping (Thermo Scientific, Carlsbad, CA) following standard protocols.

Statistical Analysis: Quality control and statistical analysis was performed using PLINK 1.9 (Chang, et al., 2015). Samples were removed from analysis if per-sample call rate was lower than 0.90. Hardy Weinberg equilibrium (HWE) was assessed, with a threshold of $p < 1.0 \times 10^{-4}$ for deviation from HWE. Binary logistic regression was used to test for association of the selected SNPs with PD, with age and sex included as covariates. Association was considered significant below a Bonferroni multiple testing threshold of $p < 0.05/26$ (0.0019). Power analysis was performed, and this cohort had 80% power to detect risk variants with odds ratios (ORs) of 1.2158 – 1.42 and protective variants with ORs of 0.7365 – 0.8225 (calculated with minor allele frequencies of 0.10 – 0.50 in patients).

Results

Of the 1312 samples assayed, 1113 had no missed genotyping calls. A total of 56 samples (0.036) were excluded from subsequent analysis as they had genotyping call rates < 0.90. Hence, a total of 679 PD patients and 577 controls were included in the regression model, and the final genotyping call rate across all samples was 0.995. No variants significantly deviated from HWE (all had $p > 0.01$). Table 1 details the association of the ET SNPs with PD in our cohort. Following multiple testing correction, none of the variants were found to be significantly associated with PD (Table 1). Further supporting lack of association, all the tested SNPs had corrected $p>0.05$ in PDgene (www.pdgene.org).

Discussion

Our results do not support pleiotropy between ET and PD, based on the top 22 SNPs identified in the ET GWAS (Muller, et al., 2016), and four other SNPs from previous studies (Higgins, et al., 2005,Schmouth, et al., 2014,Stefansson, et al., 2009,Thier, et al., 2012). As the recent GWAS identified loci associated with ET, if a significant overlap between ET and PD exists, there should be some variants that are common to both diseases. As patients who where initially diagnosed as ET have a risk to be later diagnosed as having PD (Lees, et al., 2009), variants observed in both diseases may be predictive for this conversion. However, we did not find evidence for this possibility. Furthermore, when examining the PDgene database (www.pdgene.org), which summarizes data from large PD GWAS including 13,708 PD patients and 95,282 controls, all the tested SNPs had corrected *p* values of >0.05, further demonstrating lack of pleiotropy between ET and PD.

Two *LINGO1* variants, rs9652490 and rs11856808, were included in the current study. Each of these SNPs were previously suggested to be involved in ET and PD risk (Deng, et al., 2012,Vilarino-Guell, et al., 2010), but were not significantly associated with PD in the current study. Further supporting the lack of association of these two SNPs with PD; both SNPs have $p>0.05$ in the PDgene database. Interestingly, one of the top ET-associated SNPs is located near the *RAB29* gene within the *PARK16* locus, which is strongly associated with PD. However, rs823141, possibly associated with ET (OR 1.17, 95%CI 1.10-1.25, $p=1.54\text{x}10^{-6}$ in the ET study), is not associated with PD in our cohort, and only has nominal association ($p<0.05$) in the PDgene database. Of note, that the direction of effect is opposite within this locus, as in ET the minor allele is associated with increased risk, and in PD it is associated with decreased risk. It is therefore

possible that different alleles or genetic risk factors are associated with ET and PD within this locus, and further genetic analysis is needed.

The current study was not designed to test rare variation as a common cause of PD and ET. Genes such as *DNAJC13* (Rajput, et al., 2015) and *HTRA2* (Unal Gulsuner, et al., 2014) may have a role in the pathology of both diseases, but these genes were not identified by the large-scale ET (Muller, et al., 2016) or PD (Nalls, et al., 2014) GWASs and their role in familial PD still requires further replication. While the current study does not support a common role for genetic variants in PD and ET, further study is required to understand the potential link between PD and ET.

Acknowledgements

We thank the patients and controls for their participation in the study. This work was financially supported by the Michael J. Fox Foundation. GAR holds a Canada Research Chair in Genetics of the Nervous System and the Wilder Penfield Chair in Neurosciences. We thank Daniel Rochefort, Pascale Hince, Helene Catoire, Cynthia Bourassa, Pierre Provencher, Cathy Mirarchi and Vessela Zaharieva for their assistance. We thank the Quebec Parkinson's Network and its members (http://rpq-qpn.ca/) for their collaboration.

References

Algarni, M., Fasano, A. 2017. The overlap between Essential tremor and Parkinson disease. Parkinsonism Relat Disord. doi:10.1016/j.parkreldis.2017.07.006.

Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., Lee, J.J. 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 4, 7. doi:10.1186/s13742-015-0047-8.

Deng, H., Gu, S., Jankovic, J. 2012. LINGO1 variants in essential tremor and Parkinson's disease. Acta Neurol Scand 125(1), 1-7. doi:10.1111/j.1600-0404.2011.01516.x.

Higgins, J.J., Lombardi, R.Q., Pucilowska, J., Jankovic, J., Tan, E.K., Rooney, J.P. 2005. A variant in the HS1-BP3 gene is associated with familial essential tremor. Neurology 64(3), 417-21. doi:10.1212/01.WNL.0000153481.30222.38.

Hughes, A.J., Daniel, S.E., Kilford, L., Lees, A.J. 1992. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55(3), 181-4.

Lees, A.J., Hardy, J., Revesz, T. 2009. Parkinson's disease. Lancet 373(9680), 2055-66. doi:10.1016/S0140-6736(09)60492-X.

Muller, S.H., Girard, S.L., Hopfner, F., Merner, N.D., Bourassa, C.V., Lorenz, D., Clark, L.N., Tittmann, L., Soto-Ortolaza, A.I., Klebe, S., Hallett, M., Schneider, S.A., Hodgkinson, C.A., Lieb, W., Wszolek, Z.K., Pendziwiat, M., Lorenzo-Betancor, O., Poewe, W., Ortega-Cubero, S., Seppi, K., Rajput, A., Hussl, A., Rajput, A.H., Berg, D., Dion, P.A., Wurster, I., Shulman, J.M., Srulijes, K., Haubenberger, D., Pastor, P., Vilarino-Guell, C., Postuma, R.B., Bernard, G., Ladwig, K.H., Dupre, N., Jankovic, J., Strauch, K., Panisset, M., Winkelmann, J., Testa, C.M., Reischl, E., Zeuner, K.E., Ross, O.A., Arzberger, T., Chouinard, S., Deuschl, G., Louis, E.D., Kuhlenbaumer, G., Rouleau, G.A. 2016. Genome-wide association study in essential tremor identifies three new loci. Brain 139(Pt 12), 3163-9. doi:10.1093/brain/aww242.

Nalls, M.A., Pankratz, N., Lill, C.M., Do, C.B., Hernandez, D.G., Saad, M., DeStefano, A.L., Kara, E., Bras, J., Sharma, M., Schulte, C., Keller, M.F., Arepalli, S., Letson, C., Edsall, C., Stefansson, H., Liu, X., Pliner, H., Lee, J.H., Cheng, R., International Parkinson's Disease Genomics, C., Parkinson's Study Group Parkinson's Research: The Organized, G.I., andMe, GenePd, NeuroGenetics Research, C., Hussman Institute of Human, G., Ashkenazi Jewish Dataset, I., Cohorts for, H., Aging Research in Genetic, E., North American Brain Expression, C., United Kingdom Brain Expression, C., Greek Parkinson's Disease, C., Alzheimer Genetic Analysis, G., Ikram, M.A., Ioannidis, J.P., Hadjigeorgiou, G.M., Bis, J.C., Martinez, M., Perlmutter, J.S., Goate, A., Marder, K., Fiske, B., Sutherland, M., Xiromerisiou, G., Myers, R.H., Clark, L.N., Stefansson, K., Hardy, J.A., Heutink, P., Chen, H., Wood, N.W., Houlden, H., Payami, H., Brice, A., Scott, W.K., Gasser, T., Bertram, L., Eriksson, N., Foroud, T., Singleton, A.B. 2014. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat Genet 46(9), 989-93. doi:10.1038/ng.3043.

Rajput, A., Ross, J.P., Bernales, C.Q., Rayaprolu, S., Soto-Ortolaza, A.I., Ross, O.A., van Gerpen, J., Uitti, R.J., Wszolek, Z.K., Rajput, A.H., Vilarino-Guell, C. 2015. VPS35 and DNAJC13 disease-causing variants in essential tremor. Eur J Hum Genet 23(6), 887-8. doi:10.1038/ejhg.2014.164.

Rocca, W.A., Bower, J.H., Ahlskog, J.E., Elbaz, A., Grossardt, B.R., McDonnell, S.K., Schaid, D.J., Maraganore, D.M. 2007. Increased risk of essential tremor in first-degree relatives of patients with Parkinson's disease. Mov Disord 22(11), 1607-14. doi:10.1002/mds.21584.

Schmouth, J.F., Dion, P.A., Rouleau, G.A. 2014. Genetics of essential tremor: from phenotype to genes, insights from both human and mouse studies. Prog Neurobiol 119-120, 1-19. doi:10.1016/j.pneurobio.2014.05.001.

Stefansson, H., Steinberg, S., Petursson, H., Gustafsson, O., Gudjonsdottir, I.H., Jonsdottir, G.A., Palsson, S.T., Jonsson, T., Saemundsdottir, J., Bjornsdottir, G., Bottcher, Y., Thorlacius, T., Haubenberger, D., Zimprich, A., Auff, E., Hotzy, C., Testa, C.M., Miyatake, L.A., Rosen, A.R., Kristleifsson, K., Rye, D., Asmus, F., Schols, L., Dichgans, M., Jakobsson,

F., Benedikz, J., Thorsteinsdottir, U., Gulcher, J., Kong, A., Stefansson, K. 2009. Variant in the sequence of the LINGO1 gene confers risk of essential tremor. Nat Genet 41(3), 277-9. doi:10.1038/ng.299.

Thier, S., Lorenz, D., Nothnagel, M., Poremba, C., Papengut, F., Appenzeller, S., Paschen, S., Hofschulte, F., Hussl, A.C., Hering, S., Poewe, W., Asmus, F., Gasser, T., Schols, L., Christensen, K., Nebel, A., Schreiber, S., Klebe, S., Deuschl, G., Kuhlenbaumer, G. 2012. Polymorphisms in the glial glutamate transporter SLC1A2 are associated with essential tremor. Neurology 79(3), 243-8. doi:10.1212/WNL.0b013e31825fdeed.

Unal Gulsuner, H., Gulsuner, S., Mercan, F.N., Onat, O.E., Walsh, T., Shahin, H., Lee, M.K., Dogu, O., Kansu, T., Topaloglu, H., Elibol, B., Akbostanci, C., King, M.C., Ozcelik, T., Tekinay, A.B. 2014. Mitochondrial serine protease HTRA2 p.G399S in a kindred with essential tremor and Parkinson disease. Proc Natl Acad Sci U S A 111(51), 18285-90. doi:10.1073/pnas.1419581111.

Vilarino-Guell, C., Wider, C., Ross, O.A., Jasinska-Myga, B., Kachergus, J., Cobb, S.A., Soto-Ortolaza, A.I., Behrouz, B., Heckman, M.G., Diehl, N.N., Testa, C.M., Wszolek, Z.K., Uitti, R.J., Jankovic, J., Louis, E.D., Clark, L.N., Rajput, A., Farrer, M.J. 2010. LINGO1 and LINGO2 variants are associated with essential tremor and Parkinson disease. Neurogenetics 11(4), 401-8. doi:10.1007/s10048-010-0241-x.

Xiao, B., Deng, X., Ng, E.Y., Tio, M., Prakash, K.M., Au, W.L., Tan, L., Zhao, Y., Tan, E.K. 2017. GWAS-linked PPARGC1A variant in Asian patients with essential tremor. Brain 140(4), e24. doi:10.1093/brain/awx027.

Zhang, Y., Zhao, Y., Zhou, X., Li, K., Yi, M., Guo, J., Yan, X., Tang, B., Sun, Q. 2017. Assessment of Three New Loci from Genome-wide Association Study in Essential Tremor in Chinese population. Sci Rep 7(1), 7981. doi:10.1038/s41598-017-08863-5.

Table 1. ET-associated variants in 679 Parkinson's disease patients and 577 Controls.

CHR:POS	dbSNP	NT	GENE	MAF (A)	MAF (U)	OR (95% CI)	*p*-value
1:205741426	rs823141	T/C	RAB29	0.480	0.455	1.089 (0.8956-1.325)	0.3919
1:90646872	rs6675307	G/A	intergenic	0.240	0.221	1.039 (0.8226-1.312)	0.7484
2:12186335	rs893787	T/C	LOC100506457	0.509	0.482	1.093 (0.8981-1.331)	0.3744
2:19901053	rs34533275	T/C	intergenic	0.319	0.299	1.01 (0.8184-1.246)	0.9264
2:203750049	rs10189499	T/C	WDR12	0.360	0.332	1.078 (0.8793-1.322)	0.4691
2:220050707	rs11680709	A/G	HS1-BP3	0.425	0.406	1.085 (0.8903-1.323)	0.4180
2:235807629	rs6431308	A/C	intergenic	0.241	0.232	1.132 (0.8951-1.432)	0.3007
3:113890815	rs6280	T/C	DRD3	0.312	0.296	1.052 (0.8504-1.302)	0.6390
3:151563759	rs10935878	T/A	AADACL2-AS1	0.290	0.263	1.203 (0.9646-1.5)	0.1011
4:177242959	rs4690686	C/T	SPCS3	0.430	0.382	1.125 (0.9227-1.372)	0.2440
4:24362541	rs17590046	T/C	PPARGC1A	0.200	0.215	0.9571 (0.7489-1.223)	0.7258
4:5128159	rs10937625	T/C	STK32B	0.247	0.271	0.8843 (0.7071-1.106)	0.2813
4:79421963	rs1496588	T/C	FRAS1	0.483	0.486	0.9839 (0.8115-1.193)	0.8691
6:33778964	rs9394169	G/A	intergenic	0.474	0.462	1.097 (0.901-1.335)	0.3573
7:115554668	rs2402000	C/T	intergenic	0.247	0.234	1.091 (0.8651-1.375)	0.4627
7:75348306	rs11770686	T/A	HIP1	0.474	0.497	1.027 (0.8477-1.244)	0.7862
8:18308810	rs10109552	G/T	intergenic	0.276	0.258	1.03 (0.8285-1.28)	0.7916
10:66483216	rs1915613	C/T	intergenic	0.244	0.240	0.9068 (0.7207-1.141)	0.4042
10:68845715	rs12764057	T/G	CTNNA3	0.419	0.418	0.9336 (0.7649-1.14)	0.4996
10:68850419	rs10822974	A/G	CTNNA3	0.496	0.497	0.9439 (0.7716-1.155)	0.5748
10:68917164	rs7903491	G/A	CTNNA3	0.414	0.400	1.112 (0.9092-1.359)	0.3019
11:35329615	rs3794087	G/T	SLC1A2	0.240	0.254	0.9561 (0.7662-1.193)	0.6911
12:28974648	rs10843247	T/C	intergenic	0.286	0.259	1.094 (0.8785-1.361)	0.4234
15:77963887	rs9652490	A/G	LINGO1	0.200	0.195	0.982 (0.771-1.251)	0.8832
15:77972770	rs11856808	T/C	LINGO1	0.340	0.304	1.139 (0.924-1.404)	0.2225
18:59274791	rs11152303	G/A	intergenic	0.255	0.241	1.087 (0.8681-1.361)	0.4671

Key: CHR, Chromosome; POS, position (hg19); dbSNP, identifier in dbSNP; NT, nucleotide change; MAF, Minor allele frequency; A, affected; U, unaffected; OR, odds ratio; CI, confidence interval.