

LETTER TO THE EDITOR

Replication of association between a SLITRK1 haplotype and Tourette Syndrome in a large sample of families

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Tourette Syndrome (TS) is a neuropsychiatric disorder of complex etiological background, with multiple genes interacting with environmental factors to lead to the onset of symptoms. Although multiple genetic loci have been implicated in TS etiology, to date, no single chromosomal region has been consistently replicated as a TS susceptibility region.¹ The implication of a member of the SLIT and TRK family of proteins (SLITRK1) in TS etiology² has spurred intense debate, with the significance of the original findings being questioned, and multiple studies suggesting that, if this gene is indeed involved in TS etiology, it may only account for a very small fraction of cases. Here, studying one of the largest family samples that have been analyzed for TS to date, we further investigate the contribution of SLITRK1 in TS risk, and show significant over-transmission of alleles and haplotypes to affected individuals, by a rate of about 1.5.

SLITRK1 is a type-I transmembrane protein with an extracellular leucine-rich repeat domain homologous to SLIT and a short intracellular domain lacking the tyrosine phosphorylation motifs that are found among the other members of SLITRK family.³ It has been shown to control neurite outgrowth and it is expressed in the embryonic and postnatal brain, including the cortex, thalamus and the basal ganglia, reflecting the neuroanatomical regions most commonly implicated in TS.⁴ By studying mouse, monkey and human brains, Stillman *et al.*⁵ recently showed that SLITRK1 expression in the striatum is high during early brain development but significantly diminished later. This suggests a possible role of SLITRK1 in establishing the corticostriatal circuitry, in concordance with the neurodevelopmental hypothesis for TS etiology. Furthermore, it has been demonstrated that SLITRK1 interacts with 14-3-3 proteins, which are ubiquitously expressed phosphorylation-binding proteins that regulate several important cellular processes, including cell proliferation, neuronal migration and membrane excitability.⁶

Studying a TS patient with a *de novo* inversion of chromosome 13, Abelson *et al.*² mapped the 13q33.1 breakpoint ~350 kb from SLITRK1. By resequencing this gene in a cohort of 174 individuals with TS, two additional mutations were discovered: a single-nucleotide deletion that led to a frameshift and a prematurely truncated protein (varCDF), and a missense mutation in the 3' untranslated region (variant

321-var321) predicted as a binding site for microRNA hsa-miR-189. These mutations were absent in over 3600 control samples.² Subsequent mutation screens of SLITRK1 were unable to find these two reported variants in individuals with TS of European and Japanese origin, while three additional extremely rare exonic variants were reported in these studies.^{7–9} Direct testing for var321 in almost 2000 individuals with TS of mixed European, Ashkenazi and Costa Rican origin,^{10,11} and 322 patients with obsessive-compulsive disorder (OCD),¹² only found the variant in a total of seven patients with TS, as well as one Ashkenazi and one European control. It was reported that var321 was over-represented in the Ashkenazi population, suggesting that population stratification had led the original study to a false-positive result.¹⁰ However, additional population genetics studies of an independent set of seven TS patients carrying var321, argued against population stratification confounding the original data.¹³ Furthermore, novel mutations in the SLITRK1 gene have been found, that co-segregate with OCD spectrum disorders.¹⁴

Undertaking a different approach, a recent study tested for association of the TS phenotype with three common tagging single-nucleotide polymorphisms (SNPs; tSNPs) spanning SLITRK1.¹⁵ The study included 154 nuclear families from Canada, with one or more affected children, with a total of 208 affected children for the association analyses. Patients were ascertained using DSM-III-R criteria. None of the patients carried any of the variants reported by Abelson *et al.*² However, significant associations with SNP rs9593835 as well as two three-marker haplotypes were identified, suggesting, for the first time, that there is a common TS risk factor of low penetrance in linkage disequilibrium (LD) with the associated marker and/or haplotypes.¹⁵ The study concluded that confirmation studies were needed in order to support these preliminary findings.

Following up on the results of Miranda *et al.*,¹⁵ we studied a large sample of European trios with TS (one individual affected with the disorder and their parents) for the three tSNPs, which were selected using the HapMap CEPH European population as reference for SLITRK1 variation (rs9593835, rs9531520 and rs9546538). It should be noted that SNPs rs9593835 and rs9546538 are in very high LD ($r^2 = 0.98$, Supplementary Table 1). A total of 222 trios were studied, originating from Hungary (57 trios), Italy (43 trios), Poland (27 trios) and Germany (95 trios). Samples of Polish, Italian and Hungarian origin were collected as part of the efforts of a multinational

consortium (TSGeneSEE: the Tourette Syndrome Genetics–Southern and Eastern Europe Initiative, supported by the Tourette Syndrome Association, USA and COST Action BM0905). TS was ascertained according to DSM-IV-TR criteria for Italy and Hungary, and by DSM-IV criteria for Poland. The German families have been previously described¹⁶ and ascertained using DSM-III-R criteria. Differences between DSM-III-R, DSM-IV and DSM-IV-TR are minimal and therefore, although studied samples were collected at different times, we expect very little (if any) heterogeneity among patients; the upper age of onset is 18 in DSM-IV (and DSM-IV-TR) and 21 in DSM-III-R, and the ‘marked distress’ criterion, possibly pointing to more severe cases, only appears in DSM-IV¹⁷ (applied only for the 27 Polish families). DNA samples were genotyped using the fluorescently based KASPAR assay, as developed by KBiosciences, Hoddesdon, Herts, UK. Genotyping results for all SNPs conformed to Hardy–Weinberg equilibrium proportions and Mendelian inheritance within families.

Applying the transmission test for LD as implemented in Haploview,¹⁸ we found SNPs rs9593835 and rs9546538 to be nominally associated with the disorder, whereas the same three-SNP haplotype as reported by Miranda *et al.*¹⁵ (haplotype TCT) was also over-transmitted in our sample (Table 1). Analysis by individual populations is shown in Supplementary Tables 2 and 3 and does not reveal heterogeneity among populations. The rare CCT haplotype, which was also found associated with TS in the Canadian sample, was not observed in our populations, whereas the CCC haplotype showed an indication of under-transmission to TS patients. Joint analysis of our novel and the Canadian dataset (total of 376 families) greatly boosted the significance of association, with positive results for SNPs rs9593835 and rs9546538 still retaining significant *P*-values after 1000 permutation tests were performed (Table 1). Positive association results became even stronger when the haplotype test was performed, with the TCT haplotype showing strong over-transmission to TS patients and the protective signal of the Canadian population CCT haplotype also remaining statistically significant in the combined dataset (Table 1).

In an effort to further dissect the associated haplotype, two additional SNPs were genotyped in our TSGeneSEE and German samples (rs3164 and rs9546539). As the initially analyzed SNPs were selected as tSNPs by Miranda *et al.*,¹⁵ they are in high LD with these additional SNPs as well (Supplementary Table 1). Results did not offer any additional information as, despite the additional SNPs that were genotyped, the TS-associated haplotype TCT, could not be further dissected. The most common haplotype was found to still be over-transmitted to TS patients at a statistically significant level based on the analysis of the German sample alone as well as our complete TSGeneSEE sample (Supplementary Table 4). It should be noted that, as Speed *et al.*¹⁹ have recently demonstrated, variation at

Table 1 Transmission test for linkage disequilibrium for SLITRK1 variation tSNPs and haplotypes

rs#	Over-transmitted	TSGeneSEE and German (222 families)			Canadian sample (154 families)			Joint dataset (376 families)							
		T:U ^a	χ^2	P-value	Permutation P-value	T:U	χ^2	P-value	Permutation P-value	T:U	χ^2	P-value	Permutation P-value		
rs9593835	T	103:76	4.073	0.0436	0.2020	92:56	8.757	0.0031	0.0080	195:132	12.138	5.0E-4	<0.001		
rs9531520	C	71:62	0.609	0.4352		54:37	3.176	0.0747		124:99	2.803	0.0941			
rs9546538	T	109:81	4.126	0.0422	0.1950	75:54	3.419	0.0645		184:135	7.527	0.0061	0.0160		
Haplotype	Haplotype frequency	T:U	χ^2	P-value	Permutation P-value	Haplotype frequency	T:U	χ^2	P-value	Permutation P-value	Haplotype frequency	T:U	χ^2	P-value	Permutation P-value
TCT	0.70	110:81	4.403	0.0359	0.0750	0.71	97:69	9.478	0.0021	0.0060	0.70	207:3:140	13.036	3.0E-4	<0.001
CTC	0.19	66:75.5	0.632	0.4268		0.17	37:2:53.9	3.042	0.0811		0.18	103.2:129.4	2.936	0.0866	
CCC	0.12	32:51.5	4.574	0.0325	0.0590	0.08	29.5:33.1	0.211	0.646		0.10	62:84.9	3.575	0.0587	
CCT	NA					0.04	6.2:25.4	11.679	6.0E-4	0.0030	0.01	6.5:24.6	10.61	0.0011	0.0030

Abbreviations: TSGeneSEE, Tourette Syndrome Genetics–Southern and Eastern Europe Initiative; tSNPs, tagging single-nucleotide polymorphisms.

^aTransmitted:untransmitted ratio of allele or haplotype.

The TSGeneSEE sample consists of TS (Tourette Syndrome) trios of Hungarian, Polish and Italian origin. The permutation *P*-value was computed after 1000 permutations of the datasets.

SLITRK1 demonstrates an unusual pattern, with one non-ancestral haplotype being the single most common haplotype worldwide. The globally most common haplotype differs from the ancestral haplotype by two single-nucleotide polymorphisms (rs3164 and rs9546538) and is the most common haplotype in every population, ranging in frequency from 38.9 to 86%. Surprisingly, our results indicate that this haplotype also confers elevated risk for the development of TS symptoms.

On the other hand, the three-site haplotype that provided some indication for protection for TS in our sample is actually associated with allele combinations at rs3164, rs9546538 and rs9546539 that are observed most commonly in Africa (~20% in African Americans) and are, in fact, extremely rare in Europe (<5%; <http://alfred.med.yale.edu>). If this association holds in larger samples, it will have important implications for explaining possible frequency differences of the disorder around the world.

In conclusion, through analysis of a novel family sample and meta-analysis of a total of 376 nuclear families with TS, we show evidence of association of the disorder with variation across SLITRK1. Our study clearly suggests that the role of SLITRK1 in TS etiology may have been previously underappreciated. It should be noted that the SNPs that we find significantly associated with TS are also in high LD ($r^2 > 0.3$) with a cluster of SNPs in a non-genic region about 200 kb upstream of the start of SLITRK1, indicating a possible involvement of SLITRK1 regulatory variants in TS etiology and possibly warranting further genotyping and investigation of this region in association to TS (Supplementary Table 5). In fact, the original translocation breakpoint identified by Abelson *et al.*² in a TS patient, was actually located 350 kb from the SLITRK1-coding region, in concordance with a hypothesis of a positional effect, resulting in altered regulation of expression, as a contributor to risk for TS. It is possible that, so far, variation in SLITRK1 regulatory regions may have been missed resulting in underestimation of the contribution of this gene to TS risk.

Conflict of interest

The authors declare no conflict of interest.

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