Int J Mol Epidemiol Genet 2012;3(1):91-95
www.ijmeg.org /ISSN:1948-1756/IJMEG1202003

Original Article

An examination of *TOR1A* variants in recurrent major depression

Heining FA, Langguth B, Eichhammer P, Domani M, Hajak G, Sand PG

Department of Psychiatry, University of Regensburg, Germany

Received February 20, 2012; accepted February 25, 2012; Epub Feb 28, 2012; Published March 15, 2012

Abstract: Background: Observations of comorbid depression in subjects with primary dystonia have suggested a dual role for the TOR1A gene in mood disorders and movement disorders. We conducted a systematic search for carriers of the ΔGAG deletion and for other variants in TOR1A exon 5 among 414 Caucasian subjects with recurrent major depression from the Upper Palatinate. Findings: Allele frequencies were determined for 27 TOR1A diallelic markers, including two novel synonymous substitutions (L262L and E310E) in the region encoding the torsinA C-terminus, plus four novel variants in the gene's 3'UTR. No carriers of the ΔGAG deletion were observed. When data were compared to previously examined control populations, no significant allelic associations were noted after corrections for multiple testing. Conclusions: The present study adds to the spectrum of *TOR1A* mutations but provides no evidence of a common genetic predisposition to DYT1 dystonia and recurrent major depression.

Keywords: DYT1, TOR1A, torsion dystonia, recurrent major depression, genetic variation

Introduction

Early-onset, primary dystonia (DYT1) is a rare condition marked by involuntary, sustained muscle contractions that typically initiate in the lower limbs, and that follow an autosomal dominant mode of inheritance. The disease has been linked to *TOR1A*, the gene encoding torsin A on chromosome 9q34, and segregates with a 3bp deletion, ΔGAG [1]. Observations on psychiatric comorbidity in dystonia patients have suggested that DYT1 and major depression may share genetic risk factors [2]. This view is in line with a mouse model of DYT1 featuring alterations in serotonergic transmission [3], a hallmark of depressive illness. Finally, hypometabolism in the subgenual cingulate cortex of ΔGAG deletion carriers would appear to match brain metabolic changes observed in depressed subjects [4].

However, several open issues remain that warrant a follow-up on the proposed dual role of the *TOR1A* gene. Firstly, depressed mood in dystonia may be a reaction to impaired motor function rather than a preexisting condition. The early age of onset in DYT1 precludes the use of temporal parameters to distinguish primary from secondary depression, and data are lacking to support dystonia severity as a surrogate marker of secondary depression [2]. Secondly, the majority of reports on comorbid affective illness in dystonia refer to patients whose mutation carrier status was not ascertained [5]. Finally, the hypothesis of a shared genetic etiology of primary dystonia and major depression implies the existence of common variants that act as risk modifiers and account for differences in heritability of the two conditions. To shed more light on the putative impact of *TOR1A* genetic variants on non-motor phenotypes, we therefore screened for the ΔGAG deletion and other known or novel gene variants in subjects with a diagnosis of recurrent major depression.

Materials and methods

Subjects

414 caucasian in- and outpatients from the Upper Palatinate region of eastern Bavaria (160 men and 254 women, age 48.2 ± 13.2yrs, mean ± SD) who presented consecutively to

our department between 1998 and 2005, provided written informed consent and were included in the study. The study protocol was approved by the local Ethics Committee at the University of Regensburg. For inclusion in the study, a clinical diagnosis of recurrent major depression by at least two experienced psychiatrists was required according to DSM IV criteria.

Mutation screening

Genomic DNA was extracted from lymphocytes using standard procedures prior to amplification of the region harboring the ΔGAG deletion in the *TOR1A* terminal exon. Briefly, a 847bp amplicon was generated using the following oligomers: 5'-TGG ATG AAC AGC ACC TTG TT-3' (forward) and 5'-GGA CCA TCC TGG GAC AGA-3' (reverse). PCR products were purified with ExoSAP-IT (GE Healthcare, Freiburg, Germany) for Sanger sequencing and for the identification of DNA variants by comparison with the human genome reference (Genome Reference Consortium Build 37, June 2011 release). Multiple sequence alignments were conducted with DNA Dynamo 1.0 (Blue Tractor Software, UK).

Statistical analysis

STATA 8.0 (Stata Corporation, College Station, TX, USA) was used for descriptive statistics, and for conducting tests of allelic association. *TOR1A* allele frequencies from reference populations were retrieved from the literature and from human variation databases for comparison with the present data using Fisher's exact test. The level of statistical significance was set at $p<0.05$ and a Bonferroni correction was applied to account for multiple comparisons. Linkage disequilibrium and conformity of genotype distributions with the Hardy-Weinberg equilibrium (HWE) was measured with HaploView 4.2.

Results

We identified two variants in the coding region and nine variants in the *TOR1A* 3'-untranslated region (3'UTR) with minor allele frequencies ranging from 0.001 to 0.229 (**Table 1**). Of these, the coding variants (L262L and E310E), and four variants in the gene's 3'UTR (G1192A, G1200A, G1411T, and T1415G) have not been previously described. E310E maps to a highly conserved region encoding the C-terminal α-helical subdomain α6 which is disrupted by the ΔGAG deletion. Genotype distributions of all eleven polymorphic variants conformed to the HWE ($p>0.48$). Out of 2048 possible haplotypes, only three common haplotypes were inferred (**Figure 1**).

Ten additional variants listed in dbSNP were monomorphic in this population: rs10988523, rs17849354, rs148036363, rs143571401, rs11546836, rs148849547, rs55800846, rs1045414, rs1045440, and rs60745320. Finally, we observed no carriers of the ΔGAG deletion or of other known mutations within the region targeted (**Table 1**).

For 17 *TOR1A* variants, reference allele frequencies in healthy Cauacasian populations were obtained from the literature and from dbSNP (**Table 1**). When these were compared to the respective frequencies in patients diagnosed with recurrent major depression, only one variant (rs1182) gave an uncorrected p value of <0.05, but this finding did not survive corrections for multiple testing. Still, a weak modulatory effect could not entirely be ruled out for rare variants. According to power simulations based on the entire sample of depressed patients and all caucasian control populations, we should require over 60,000 depressed patients and over 1,950,000 healthy controls in order to exclude a modifying role of the ΔGAG deletion on allelic risk with a statistical power of 0.8.

Discussion

The present study extends the mutation spectrum at the *TOR1A* locus but offers little support for a dual role of this gene in dystonia and recurrent major depression. Two female carriers of silent mutations, L262L and E310E aged 38 and 55 years, respectively, did not present with any clinical signs of dystonia. The negative association finding for all *TOR1A* variants investigated in recurrent major depression is in line with eight genome-wide linkage analyses none of which have produced any noteworthy signals for major depression at 9q34 (reviewed by Middeldorp et al. [6]). On the one hand, we acknowledge that minor effects of rare variants on the risk for recurrent major depression cannot be entirely dismissed and more *TOR1A* vari-

Table 1. Observed genotype and allele frequencies for the *TOR1A* sequence screened in subjects with recurrent major depression (N=414) and tests for allelic association using Caucasian control populations. Eleven confirmed variants are shaded.

SNP identifier	chr9 position	major>minor alleles	predicted TOR1A protein variant	MAF in recurrent major depression	MAF in unrelated Caucasian controls (N)		$p_{association}$ ($p_{corrected}$)
rs10988523	132,576,494	C>T	F252F	0.000	0.000 (60)	[a]	n.s.
					0.000 (150)	[9]	n.s.
rs17849354	132,576,475	G>C	D259H	0.000	0.000 (150)	[9]	n.s.
C786A (novel)	132,576,464	C>A	L262L	0.001	0.000 (812)	[10]	n.s.
rs148036363	132,576,427	A>G	K275E	0.000	0.0004 (3510)	[b]	n.s.
-	132,576,387	G>A	R288Q	0.000	0.000 (150)	[9]	n.s.
rs143571401	132,576,363	A>G	I296V	0.000	0.0001 (3510)	[b]	n.s.
-	132,576,359	A>C	V297V	0.000	0.0001 (3510)	[b]	n.s.
-	132,576,344-6	GAG>del	E302del	0.000	0.000 (150)	[9]	n.s.
					0.000 (812)	[10]	n.s.
					0.00004 (12,000)	[11]	n.s.
					0.000 (521)	[12]	n.s.
G930A (novel)	132,576,320	G>A	E310E	0.001	0.000 (150)	[9]	n.s.
-	132,576,313-16	AGAG>del	E312STOP325	0.000	0.0006 (812)	[10]	n.s.
rs11546836	132,576,293	C>T	C319C	0.000	0.000 (150)	[9]	n.s.
-	132,576,266-83	G>A	F323_Y328del	0.000	0.000 (812)	[10]	n.s.
rs148849547	132,576,260	C>T	Y330Y	0.000	0.0004 (3510)	[b]	n.s.
-	132,576,244		-	0.000	0.0001 (3510)	[b]	n.s.
rs1182	132,576,060	G>T	-	0.229	0.244 (521)	[12]	n.s.
					0.177 (254)	[13]	0.025 (0.725)
					0.221 (242)	[14]	n.s.
					0.157 (99)	[15]	0.026 (0.754)
					0.210 (46)	[c]	n.s.
					0.236 (36)	[d]	n.s.
G1192A (novel)	132,576,058	G>A	-	0.004	-		-
G1200A (novel)	132,576,050	G>A	-	0.001	-		-
rs1183	132,576,037	C>G	-	0.078	0.075 (60)	[e]	n.s.
					0.062 (24)	[f]	n.s.
					0.069 (36)	[g]	n.s.
rs55800846	132,576,034	G>A	-	0.000	-		-
rs1045414	132,576,024	A>G	-	0.000	-		-
rs1045440	132,575,860	T>C	-	0.000	-		-
G1411T (novel)	132,575,839	G>T	-	0.003	-		-
rs35153737	132,575,837	G>del	-	0.227	-		-
rs60745320	132,575,835-6	TT>TCT	-	0.000	-		-
T1415G (novel)	132,575,835	T>G	-	0.035	-		-
T1422del	132,575,828	T>del	-	0.026	-		-
rs1045441	132,575,797	T>A	-	0.078	0.075 (60)	[h]	n.s.
					0.069 (36)	[i]	n.s.

all alleles refer to the transcribed strand, MAF = minor allele frequency. [a]dbSNP ss19032613; [b]reference population of European ancestry from the NHLBI GO Exome Sequencing Project. Data retrieved with the Exome Variant Server (URL: http://evs.gs.washington.edu/EVS/), accessed Feb. 2012; [c]dbSNP ss43890027; [d]dbSNP ss123594382; [e]dbSNP ss10540562; [f]dbSNP ss23585341; [g]dbSNP ss123594377; [h]dbSNP ss2160149; [i]dbSNP ss123594365.

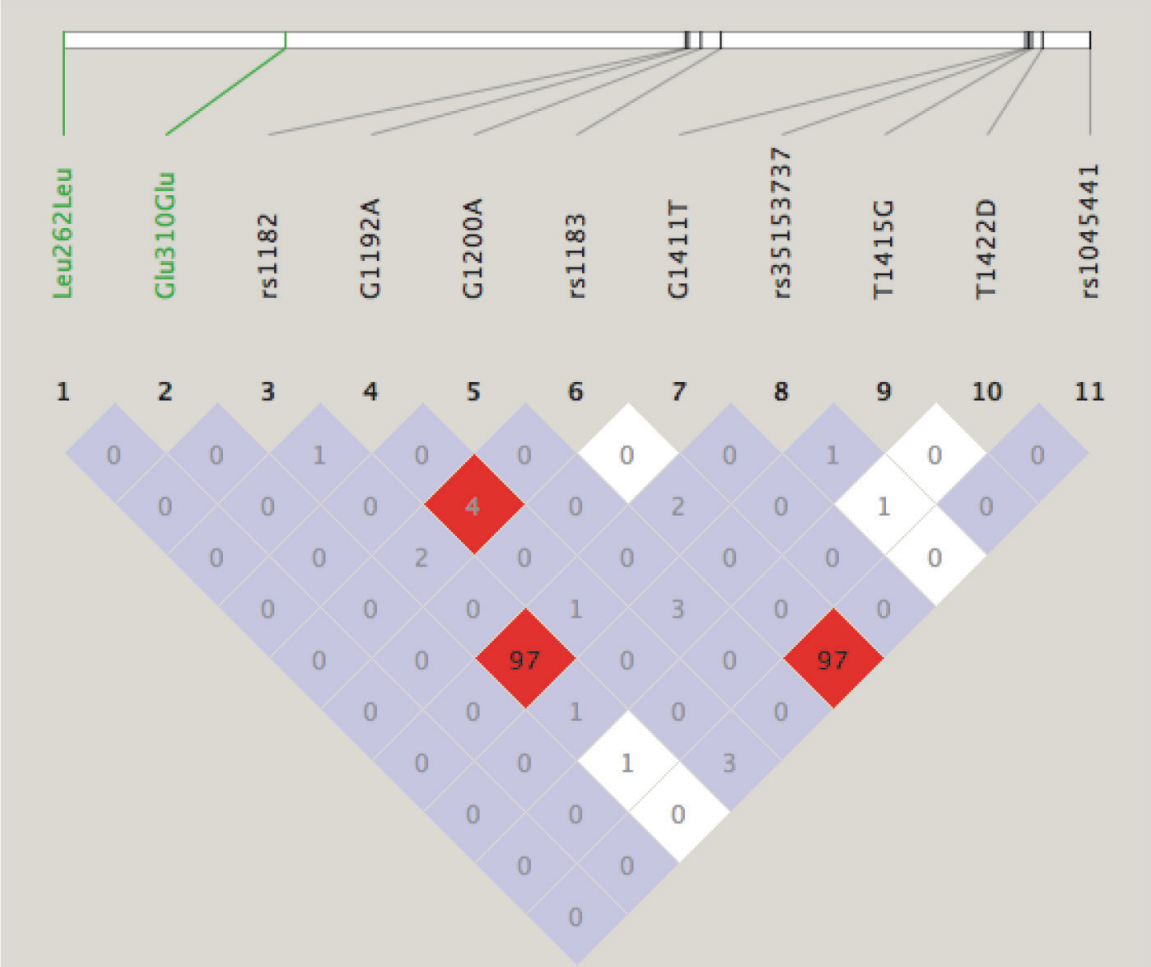

Figure 1. (**A**) LD plot and R^2 x100 values for the eleven *TOR1A* variants identified

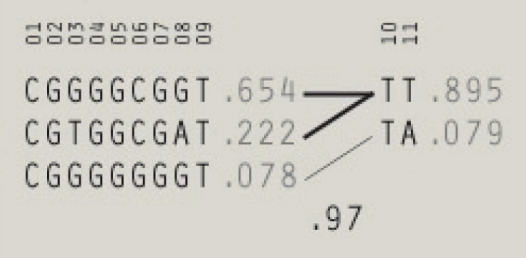

Figure 1. (**B**) common haplotypes inferred from these variants in the population under study.

ants exist than were genotyped. On the other hand, the previously claimed association may stem from overestimating the prevalence of recurrent major depression in ΔGAG deletion carriers [2], owing to the relatedness of subjects, a disproportionately small number of non-carriers, the criteria applied to distinguish non-manifesting from manifesting subjects and, finally, to investigator blinding issues. Severe dystonia pervades many aspects of life that are routinely addressed in screenings for depression, e.g. the level of functioning, family and social relationships. Therefore, despite the use of telephone interviews, such assessments are liable to disclose the handicap.

We note that under the hypothesis of a shared etiology in dystonia and major depression, one should expect some crossmodal activity of antidepressants in dystonia. However, trials of antidepressants and structurally related compounds in dystonia have disappointed [7]. Instead, dystonia appears to be precipitated or worsened by many antidepressants [8]. Non-pharmacological antidepressant treatment has

also failed to produce lasting effects on the motor phenotype [5].

Taken together, the existing data call for a cautious stand on the alleged contribution of *TOR1A* to the etiology of recurrent major depression, in addition to its established impact on DYT1.

Authors' contributions

PGS designed the study and wrote the protocol. FAH conducted the experiments and assisted PGS with statistical analysis. PE supervised the recruitment of subjects who took part in the study. BL, MD and GH managed the literature searches, analyzed the data and helped to draft the manuscript. All authors contributed to and have approved the final manuscript.

Address correspondence to: Dr. Philipp G Sand,Department of Psychiatry, University of Regensburg, Universitaetsstrasse 84, 93053 Regensburg, Germany Tel: +49 - 941 - 944 8955; Fax: + 49 - 941 - 944 8956; E-mail: philipp.sand@klinik.uni-regensburg.de

References

[1] Ozelius LJ, Hewett JW, Page CE, Bressman SB, Kramer PL, Shalish C, de Leon D, Brin MF, Raymond D, Corey DP, Fahn S, Risch NJ, Buckler AJ, Gusella JF, Breakefield XO. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. Nat Genet 1997; 17: 40-48.

[2] Heiman GA, Ottman R, Saunders-Pullman RJ, Ozelius LJ, Risch NJ, Bressman SB. Increased risk for recurrent major depression in DYT1 dystonia mutation carriers. Neurology 2004; 63: 631-637.

[3] Grundmann K, Reischmann B, Vanhoutte G, Hübener J, Teismann P, Hauser TK, Bonin M, Wilbertz J, Horn S, Nguyen HP, Kuhn M, Chanarat S, Wolburg H, Van der Linden A, Riess O. Overexpression of human wildtype torsinA and human DeltaGAG torsinA in a transgenic mouse model causes phenotypic abnormalities. Neurobiol Dis 2007; 27: 190-206.

[4] Carbon M, Su S, Dhawan V, Raymond D, Bressman S, Eidelberg D. Regional metabolism in primary torsion dystonia: effects of penetrance and genotype. Neurology 2004; 62: 1384-1390.

[5] Garcia RF, Dias AG, de Freitas AR, Fontenelle LF. Short-lived response of cervical dystonia to electroconvulsive therapy. J ECT 2009; 25: 135-136.

[6] Middeldorp CM, Sullivan PF, Wray NR, Hottenga JJ, de Geus EJ, van den Berg M, Montgomery GW, Coventry WL, Statham DJ, Andrews G, Slagboom PE, Boomsma DI, Martin NG. Suggestive linkage on chromosome 2, 8, and 17 for lifetime major depression. Am J Med Genet B Neuropsychiatr Genet 2009; 150B: 352-358.

[7] Reuss R, Reuter I, Jauss M, Fischer F, Muller SC, Stolz E. Torticollis under cyclobenzaprine. Pharmacology 2009; 84: 91-92.

[8] Chong Y, Harris R, Kim WJ. Dystonia as a side effect of nonneuroleptics. J Am Acad Child Adolesc Psychiatry 1999; 38: 793-795.

[9] Xiao J, Bastian RW, Perlmutter JS, Racette BA, Tabbal SD, Karimi M, Paniello RC, Blitzer A, Batish SD, Wszolek ZK, Uitti RJ, Hedera P, Simon DK, Tarsy D, Truong DD, Frei KP, Pfeiffer RF, Gong S, Zhao Y, LeDoux MS. High-throughput mutational analysis of TOR1A in primary dystonia. BMC Med Genet 2009; 10: 24.

[10] Kabakci K, Hedrich K, Leung JC, Mitterer M, Vieregge P, Lencer R, Hagenah J, Garrels J, Witt K, Klostermann F, Svetel M, Friedman J, Kostic V, Bressman SB, Breakefield XO, Ozelius LJ, Pramstaller PP, Klein C. Mutations in DYT1: extension of the phenotypic and mutational spectrum. Neurology 2004; 62: 395-400.

[11] Frédéric M, Lucarz E, Monino C, Saquet C, Thorel D, Claustres M, Tuffery-Giraud S, Collod-Béroud G. First determination of the incidence of the unique TOR1A gene mutation, c.907delGAG, in a Mediterranean population. Mov Disord 2007; 22: 884-888.

[12] Kamm C, Asmus F, Mueller J, Mayer P, Sharma M, Muller UJ, Beckert S, Ehling R, Illig T, Wichmann HE, Poewe W, Mueller JC, Gasser T. Strong genetic evidence for association of TOR1A/TOR1B with idiopathic dystonia. Neurology 2006; 67: 1857-1859.

[13] Hague S, Klaffke S, Clarimon J, Hemmer B, Singleton A, Kupsch A, Bandmann O. Lack of association with TorsinA haplotype in German patients with sporadic dystonia. Neurology 2006; 66: 951-952.

[14] Clarimon J, Brancati F, Peckham E, Valente EM, Dallapiccola B, Abruzzese G, Girlanda P, Defazio G, Berardelli A, Hallett M, Singleton AB. Assessing the role of DRD5 and DYT1 in two different case-control series with primary blepharospasm. Mov Disord 2007; 22: 162-166.

[15] Clarimon J, Asgeirsson H, Singleton A, Jakobsson F, Hjaltason H, Hardy J, Sveinbjornsdottir S. Torsin A haplotype predisposes to idiopathic dystonia. Ann Neurol 2005; 57: 765-767.