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Analysis of D216H Polymorphism in Argentinean Patients With Primary Dystonia

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Abstract: The D216H polymorphism (rs1801968) in *TOR1A* has been suggested as a risk factor for developing primary dystonia in German subjects not carrying the deletion c.904-906delGAG (ΔGAG). However, this association could not be confirmed in other populations with different ethnic backgrounds. The purpose of this study is to evaluate the D216H polymorphism in an Argentinean cohort of 40 patients with primary dystonia and 200 unrelated control subjects. The authors could observe a significantly higher frequency of the H216 variant in dystonic patients lacking ΔGAG as compared with controls.

Keywords: Argentina, D216H, primary torsion dystonia, rs1801968

INTRODUCTION

Primary torsion dystonia (PTD) is a clinically and genetically heterogeneous disorder. Early-onset dystonia has mainly being associated with deletion c.904-906delGAG (ΔGAG) in the *TOR1A* gene, with a reduced penetrance ranging between 30% and 40%. In addition, D216H polymorphism (rs1801968) in *TOR1A* has been proposed as a potential penetrance modulator of ΔGAG (Risch et al., 2007). Besides, variant H216 was described as a risk factor for dystonia in patients not carrying ΔGAG in Germany (Bruggemann et al., 2009). However; the results obtained with Chinese and Indian patients showed that allele H216 contribution to the risk of primary dystonia was not confirmed (Naiya et al., 2006; Chen et al., 2012).

The aim of this study was to evaluate the D216H polymorphism in primary dystonia patients not carrying ΔGAG and control subjects in Argentina.

METHODS

Subjects

PTD patients were classified according to established criteria (Burke et al., 1985) at the Parkinson's Disease and Movement Disorders Program, Department of Neurology, Hospital de Clínicas José de San Martín. Institutional ethical boards approved the study and all subjects signed written informed consent statements. There were 40 index cases, 19 male and 21 female, lacking ΔGAG and displaying focal (48.4%), segmental (25.8%), hemidystonic (3.2%), or generalized (22.6%) primary dystonia. The age of onset was 27.8 ± 18.5 years. Out of these 40 patients, 19 had a positive family history: 12/19 had one affected first- or second-degree relative, 6/19 had two, and 1/19 had three. The affected status of first- or second-degree family members has been determined by direct examination. Likewise, we included 39 relatives and 200 unrelated Argentinean donors as controls. Family history was regarded as positive when at least one first- or second-degree relative had dystonia.

Genetic Analysis

Genotyping of rs1801968 and ΔGAG in DYT1 were performed by Real Time Polymerase Chain Reaction (RT-PCR) followed by High Resolution Melting (HRM) analysis (Rotor-Gene 6000; Corbett Research, Sydney, Australia). The primers were 5'AAACCCTGTCCTTACCCACTG3' (forward), 5'GCTTGATGTCTTCCCTCTGC3' (reverse) for rs1801968

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and 5'CTAAAAATGTGTATCCGAGTGGAAAT3' (forward), 5'GAAAACTCTCTCCTCTTTGG3' (reverse) for c.904-906delGAG. Both sets of primers were designed using Primer 3 program.

All reactions were performed using high-quality DNA, in quantities to attain input amounts of 10 ng/μL (Plexor; Promega, Madison, WI, USA), 200 nM dNTPs, 1.5 mM $MgCl_2$, 5 × buffer GoTaq Colorless (Promega), 0.3 pmol of the intercalary fluorescent dye Syto 9 (Life Technologies, Applied Biosystems, Foster City, CA, USA), and 10 pmol of each primer. PCR cycling conditions are as follows: 95°C for 2 min, 40 cycles at 95°C for 30 s, annealing for 20s (55°C for ΔGAG or 59°C for rs1801968), 72°C for 30 s; 72°C for 6 min. The final HRM temperature ramp was established from 76°C to 83°C at 0.2°C/s increments.

Patients were evaluated for matrilineal ancestry by means of mitochondrial DNA haplogrouping by RT-PCR-HRM (Zuccarelli et al., 2011). Statistical analysis was based on Fisher's test (two-tailed *p* value) (www.graphpad.com).

RESULTS AND DISCUSSION

Allele distribution met Hardy-Weinberg equilibrium. Table 1 summarizes allele and genotype frequencies observed for D216H.

The H216 variant frequency detected in unrelated control Argentinean subjects was 9.75%. In contrast, index cases lacking ΔGAG exhibited a significantly higher frequency (20%) as compared with controls ($P = 0.0124$). Furthermore, when we divided the index cases according to their family history, we noticed a slight increase in H216 frequency in patients with positive family history (23.7%) ($P = 0.025$) but not for idiopathic cases (16.7%) as compared with controls.

Regarding ethnicity, the mitochondrial lineage was used to define the ethnic distribution of index cases: 45% of patients belonged to Native American ethnic group and the remaining 55% exhibited mostly European ancestry. This figure is concordant with published data for central Argentina (Corach et al., 2010). The H216 variant frequency was similar between the two cohorts with different ethnic ancestries.

Table 1. Allele and genotype frequencies of locus H216.

Subjects	Total no. of cases	H/D	D/D	H216 allelic frequency
Index cases	40	16	24	0.20[a]
Positive family history patients	19	9	10	0.237[b]
Idiopathic patients	21	7	14	0.167
Relatives	39	10	29	0.147
Symptomatic relatives	15	5	10	0.167
Nonsymptomatic relatives	24	5	19	0.104
Controls	200	39	161	0.0975

Compared with controls: [a]$P = 0.0124$; [b]$P = 0.025$.

In addition to index cases, we also assessed a cohort of 39 relatives, none of them carrying ΔGAG. No statistically significant increase of H216 variant frequency (16.7%) was observed in clinically affected family members as compared with nonsymptomatic relatives (10.4%) and control subjects (9.75%).

Our results demonstrate a significantly higher frequency of H216 variant in PTD patients not carrying the ΔGAG as compared with control subjects in Argentina. Likewise, this difference is only statistically significant for patients with a positive family history. These results are in agreement with those previously reported for familial cases in Germany (Bruggemann et al., 2009), in which variant H216 was identified as a risk factor of primary dystonia. However, they are different from those reported in Chinese and Indian populations (Naiya et al., 2006; Chen et al., 2012). This could be accounted for by different ethnic backgrounds or a relationship between positive family history and idiopathic cases: in this study, we have 47.5% index cases with family history in Argentina as compared with 8.1% in China or 3.4% in India.

To our knowledge, this is the first genetic study of D216H polymorphism in DYT1 on PTD in Argentineans. Since the study was performed in a small cohort of patients, further epidemiological studies are necessary to confirm the effect of H216 allele as a risk factor for dystonia.

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