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Genetic evidence for an association of the TOR1A locus with segmental/focal dystonia

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Abstract

Polymorphisms in the TOR1A/TOR1B region have been implicated as being associated with primary focal and segmental dystonia. In a cohort of subjects with either focal or segmental dystonia affecting the face, larynx, neck or arm we report a strong association of a single nucleotide polymorphism (SNP), the deletion allele at the Mtdel SNP (rs3842225) and protection from focal dystonia. In contrast, we did not find an association of either allele at the D216H SNP (rs1801968) with focal or segmental dystonia in the same cohort.

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Keywords

focal dystonia; segmental dystonia; torsinA; single nucleotide polymorphisms

Introduction

Adult onset focal dystonia accounts for about 90% of all cases of dystonia with a prevalence estimated at 30/100,000 in the general population [1,2]. Adult onset primary dystonia is probably more complex genetically than childhood onset forms and the role of genes in the etiology of the various adult clinical subtypes is still under study. Several large studies of focal dystonia have reported positive family histories in 2–25% of cases [3–9], suggesting that a substantial fraction of adult-onset cases may have a hereditary component. Genetic studies to determine the mode of inheritance using segregation analysis conclude that focal dystonia is inherited as an autosomal dominant trait with a reduced penetrance of about 12–15%[8, 10, 11]. Although the overall risk to first-degree relatives of affected individuals is low, several large multiplex (more penetrant) families have been described[12–14]. However, the vast majority of cases of focal primary torsion dystonia appear to be sporadic or limited to small families that by themselves are not informative for linkage studies.

Rare monogenic diseases can sometimes help elucidate genetic risk factors for common complex diseases. There are several examples of genes in which rare mutations give rise to rare diseases with Mendelian inheritance and common variants (single nucleotide polymorphisms; SNPs) in those same genes are associated with common, sporadic diseases with similar phenotypes[15]. This has been substantiated for both the alpha synuclein gene in Parkinson's disease and the amyloid precursor protein gene in Alzheimer's disease [16, 17]. Similarly, polymorphisms in the genes that underlie Mendelian forms of dystonia may contribute to the risk of developing focal dystonia. A major cause of primary childhood onset dystonia is a three base pair deletion (GAG) in the TOR1A gene[18]. Recent studies implicate polymorphisms in the TOR1A genomic region as being associated with adult onset, mainly focal dystonias. In cases of idiopathic sporadic dystonia in the Icelandic population, a significant association was observed with a haplotype spanning the TOR1A gene[19]. However, two studies from Germany failed to replicate this association[20, 21]. In contrast, a study involving Italian and North American blepharospasm cohorts revealed an association in the Italian group with the same risk allele seen in the Icelandic population but no such association was found in the American group[22]. However, when these same two cohorts were stratified based on risk of spread of dystonia, both showed a similar association with a SNP in the 3' untranslated region (UTR) of TOR1A. Finally, a group of Austrian and German patients with predominantly focal dystonia showed a strong association with two SNPs in the 3' UTR of the TOR1A gene[23]. But, rather than being a risk haplotype for the development of dystonia, as reported in the previously studied populations, these SNPs showed a strong protective effect against the development of dystonia in the Austrian and German cohort.

In addition, a TOR1A variant at nucleotide position 688 (in the cDNA, known as D216H) that changes an amino acid appears to have functional consequences[18, 24] and may influence the penetrance of DYT1 dystonia[25, 26, 27]. Assessment of this variant in association studies of focal dystonia patients has yielded conflicting results; in two studies no association was found [21, 23] whereas a recent report did find an association in focal patients having a family history of dystonia [28]. Although inconsistent, the results support a role for genetic variability in the TOR1A genomic region as a risk factor in the development of late onset, focal dystonia that may also depend on the specific dystonia subtype and whether or not there is a family history of dystonia. Thus, we genotyped a large series of North Americans with

predominantly focal laryngeal or cervical dystonia for two known TOR1A SNPs to further investigate the role of the TOR1A locus in the development of adult onset dystonia.

Methods

Patients and Controls

Subjects were recruited from dystonia clinics in New York and Boston. Diagnosis and confirmation that these subjects had focal or segmental dystonia was established according to published criteria[29] by neurologists trained in movement disorders. The presence of a family history of dystonia was determined, for first and second-degree relatives, as previously described[14]. Subjects were considered to have a positive family history of dystonia if the first or second-degree relative with dystonia either provided medical records confirming the diagnosis or was examined by one of the clinical investigators who participated in this study (NS, R S-P, MB, SB). Subjects were considered to have a negative family history of dystonia if they specifically denied such a history or were unable to present either confirmatory medical records for review or the family member for examination. The local institutional review boards approved the study and all participating individuals gave informed consent. Control samples, consisting of one hundred three European Caucasian DNA samples were obtained from the CEPH collection.

Molecular analysis

DNA was extracted from white blood cells using the Purgene procedure (Gentra Systems, Minneapolis, MN). Two SNPs in the TOR1A gene were selected for study based on previous reported associations; the D216H SNP (rs1801968) that is within exon 4[25] and the Mtdel SNP (rs3842225) that is within the 3' UTR[19]. The primers for amplification and the genotyping method used were as previously reported[25].

Statistical analysis

Data file preparation and statistical analyses were performed using R version 2.6.0 (The R Foundation for Statistical Computing), PLINK version 0.99[30] and our own perl scripts. SNP genotype distributions were tested for deviation from Hardy-Weinberg expectations in the control group using Pearson's χ^2 tests. Observed genotypes counts were not significantly different from expected counts at either SNP ($p > 0.05$). Allele frequency differences between case and control groups were evaluated using 2-by-2 contingency tables; p-values were generated using two-tailed χ^2 tests. Comparisons were made between the control group and 1) all cases; 2) cases of focal or segmental dystonia involving the neck; 3) cases of focal or segmental dystonia involving the larynx excluding the neck; 4) all cases stratified by confirmed family history of dystonia.

Results

We studied DNA samples from a total of 263 unrelated patients of mixed European descent with primary focal or segmental dystonia involving the face, jaw, larynx, arm and/or neck (Table 1). This cohort contained 183 females and 80 males. Age of onset of dystonia was 21 years or older for all subjects (Average age of onset = 45 years, range of onset: 21– 72 years) with an average disease duration of 10.4 years and a range of disease duration from 1 to 47 years. Two hundred sixteen subjects had focal dystonia (97 laryngeal, 86 cervical, 25 cranial and 8 brachial) with an average disease duration of 9.9 years and a range of disease duration from 1 to 42 years. The remaining 47 subjects had segmental dystonia involving 2 or more body regions. Those with dystonia of the trunk or legs were excluded from this study. Sixty-seven of the subjects had a confirmed family history of dystonia, which represents 25.5% of the total cohort. Of the 67 subjects with a positive family history of

dystonia, 54 (80.6%) had a first degree relative with dystonia. The TOR1A GAG deletion was excluded in all dystonia patients. The CEPH control cohort contained 50 males and 53 females. Information regarding age at the time of DNA collection was available for 40 of the control subjects, who had an average age at collection of 45.5 years with a range in age from 30 to 63 years.

We examined two SNPs, rs3842225 (also known as MtDel or gdel) and rs1801968 (also known as D216H) in the TOR1A locus in the 263 patients and 103 CEPH control subjects (summarized in Table 2). We identified a significant association between the presence of the deletion allele at the Mtdel SNP and protection from developing focal and segmental dystonia (n = 263, p = 0.007, OR = 0.59). No significant association was found between the D216H allele and the risk of developing dystonia in this cohort nor could we confirm the previously reported association with D216H even when we stratified our cohort based on a positive family history of dystonia (n=67, p=0.99, OR=0.997) however, the sample size is small. As we found no evidence for an association with the D216H SNP and development of focal/segmental dystonia, we focused on the Mtdel SNP only in further analyses, described below.

To determine if the potential protective role of the Mtdel SNP differed among dystonia subtypes, we analyzed the frequency of this SNP in those with focal or segmental dystonia involving the neck. We found a significant association between the presence of the deletion allele at the Mtdel SNP and protection from developing dystonia involving the neck (n = 116, p = 0.002, OR = 0.48). When we analyzed the frequency of this SNP in a different sub-group of patients, those with focal or segmental dystonia of the larynx excluding any involvement of the neck, we found a trend toward a significant association between the presence of the deletion allele at the Mtdel SNP and protection from developing dystonia (n = 109, p = 0.05, OR = 0.63). In the remaining sub-groups, those with dystonia of the facial muscles and/or arm without neck or laryngeal involvement (n = 26) and those with dystonia of the arm alone (n = 10), there were too few samples to test.

To determine if the presence of a confirmed family history of dystonia affects the potential protective role of the deletion allele at the Mtdel SNP, we stratified based on family history and found a statistically significant association between patients with no family history of dystonia and the presence of the deletion allele at the Mtdel SNP again, showing protection from developing focal and segmental dystonia in this population (n = 196,p = 0.001, OR = 0.50).

Discussion

In this study we test one of the largest cohorts of focal and segmental dystonia cases reported on to date for association with SNPs in the TOR1A locus. Our cohort also includes one of the largest numbers of laryngeal dystonia cases that has been reported to date. We demonstrate a strong association between the Mtdel SNP (rs3842225) located in the 3’UTR of the TOR1A gene and focal/segmental dystonia in a North American population of mixed European descent. This association remains when we divide the sample by site of dystonia, as the presence of the del allele in the Mtdel SNP confers protection against the risk of developing focal/segmental dystonia involving the neck and there is a trend toward a significant association in cases involving the larynx but excluding the neck. In addition, when we stratify based on family history, the presence of the del allele in the Mtdel SNP also confers protection against the risk of developing focal/segmental dystonia in those cases with a negative family history of dystonia. In contrast, no association between the D216H SNP and focal/segmental dystonia was found in our cohort.

The association we identified in our population is consistent with that found in a cohort of German and Austrian subjects[23], which also showed a protective effect of the del allele in the Mtdel SNP. However, our results conflict with data from an Icelandic population and a sample of Italian blepharospasm subjects[19, 22].. Interestingly, a recent study of the D216H SNP in a German cohort of focal/segmental dystonia indicates that the H216 variant may increase the risk of developing focal dystonia in those who have a positive family history but do not carry the DYT1 mutation[28]. However, we could not confirm this in our population though only 25.5% of our sample had a positive family history of dystonia. Thus, studies in disparate populations (both ethnically and clinically) have provided contradictory data regarding the association of the Mtdel SNP in the TOR1A locus and the development of focal dystonia.

This contradictory data could be explained by ethnic genetic and/or clinical differences in the populations that have been studied. Both the Icelandic cohort and the Italian blepharospasm cohort are relatively ethnically homogeneous, and were compared to control populations taken from the same region. Thus, the finding that certain SNPs in the torsinA gene increase the risk of developing dystonia may only become clinically significant when those SNPs are expressed in a relatively homogeneous genetic background.

Additional confounding factors in the different published studies include that populations with different subtypes of dystonia or a varied distribution of dystonia were analyzed. In the Icelandic population, the majority of subjects had focal dystonia, but details regarding the clinical features of those who participated in the genetic study were not reported.[19] Similarly, both the German study that found an association between TOR1A SNPs[23, 28] and dystonia and the studies that did not replicate this finding[20, 21] analyzed distinct populations that contained subjects with a variety of different focal and segmental dystonias. A recent analysis of the rs1182 SNP in both an Italian and North American blepharospasm cohort highlights that in addition to focusing on a particular dystonia subtype, other clinical characteristics might also be important in order to elucidate associations. When originally studied, an association was only found in the Italian blepharospasm cohort. However, when the samples was further analyzed with regard to spread of the disease, the presence of the T allele (G/T or T/T) was associated with increased risk that dystonia would spread in both cohorts[31]. Thus, the relationship between a specific SNP in the torsinA gene and its contribution to the development and/or spread of dystonia may be important only in select ethnic groups and/or in the expression of a specific dystonia phenotype (i.e., cervical or blepharospasm) or features (i.e. risk of spread).

In this study, the subjects were clinically heterogeneous, including those with various body regions involved as well as patients with or without family history. We identified an association in the group overall. We also saw an effect when we stratified the group based on site of dystonia with focal/segmental cervical dystonia showing a statistically significant association and laryngeal dystonia showing a trend toward a significant association. This suggests that different genetic factors may influence the risk of developing the various dystonia subtypes. In cases with a family history of dystonia, there may be an as yet undefined gene that plays a major role in the development of dystonia, with the TOR1A gene playing a minor role in these families. Alternatively, the role of a specific TOR1A SNP may play a greater role in affecting penetrance in familial dystonia, where the genetic background is highly homogeneous. A larger sample of focal and segmental dystonia cases with a positive family history of dystonia, must be studied to gain a better understanding of the role of TOR1A SNPs in the pathogenesis of familial dystonia.

It is also possible that, because none of the SNPs associated with an increased or decreased risk of developing dystonia that have been identified to date appear to have functional

relevance, the contradictory results indicate that the tested SNPs are not the functional variants per se, but serve as genetic markers that are in strong linkage disequilibrium (LD) with the actual causal variant(s). However, because the region containing these variants is part of a large LD block spanning both the TOR1A and TOR1B genes, it will be difficult to identify these causal variants. Regardless, our results combined with results from previous studies strongly support a role for genetic variability in the TOR1A genomic region as a contributing factor in the risk of developing focal/segmental dystonia. Further experiments examining a comprehensive battery of SNPs that account for the majority of variation in the region in a much larger series of focal/segmental dystonia patients should clarify the associations. However, the identification of functional variants related to the regulation of torsinA and B expression will ultimately be needed to determine how these genes contribute to the molecular etiology of the various forms of focal dystonia.

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Table 1

Clinical characteristics of the patients whose DNA was analyzed

Gender	#	Distribution	#	Family History	#
Female	183	Focal	216	Positive	67
Male	80	Segmental	47	Negative	196

Table 2

Minor allele frequencies of SNPs in the TOR1A locus in focal and segmental dystonia cases and controls.

Marker	Alelles: Major/Minor		Minor Allele Frequency	Sample Size	P value	Odds Ratio
rs3842225 (Mtdel)	C/del	All Dystonia Cases	0.169	263		
		Controls	0.257	103	0.007	0.59
rs3842225 (Mtdel)	C/del	Neck Dystonia Cases	0.142	116		
		Controls	0.257	103	0.002	0.48
rs3842225 (Mtdel)	C/del	Larynx Dystonia Cases	0.179	109		
		Controls	0.257	103	0.05	0.63
rs3842225 (Mtdel)	C/del	Dystonia Cases w/o FH	0.148	196		
		Controls	0.257	103	0.001	0.50
rs1801968 (D216H)	G/C	All Dystonia Cases	0.108	263		
		Controls	0.142	103	0.22	0.73
rs1801968 (D216H)	G/C	Dystonia Cases w/ FH	0.1418	67		
		Controls	0.142	103	0.99	0.997

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