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Torsion Dystonia Genes in Two Populations Confined to a Small Region on Chromosome 9q32-34

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Summary

Idiopathic torsion dystonia (ITD) is characterized by sustained, involuntary muscle contractions, frequently causing twisting and repetitive movements or abnormal postures. Most familial forms of ITD display autosomal dominant inheritance with reduced penetrance. Linkage analysis has been previously used to localize a dystonia gene to the 9q32-34 region in a large non-Jewish family and in a group of Ashkenazi Jewish families. Utilizing GT repeat polymorphisms from this region, here we demonstrate that the gene causing dystonia in Ashkenazi Jews can be localized to the 11-cM interval between AK1 and D9S10. Linkage analysis in the non-Jewish family is also consistent with occurrence of the gene in this region, although positive lod scores extend over a >20-cM interval in that family. These results set the stage for positional cloning of the dystonia gene. Currently there are no known candidate genes in this region.

Introduction

Torsion dystonia is a syndrome characterized by sustained, involuntary muscle contractions, frequently causing twisting and repetitive movements or abnormal postures (Fahn 1988). The occurrence of dystonia in the absence of either known disease (e.g., Wilson disease) or identifiable biochemical or pathologic disorder is classified as idiopathic (primary) torsion dystonia (ITD).

The nosology of the idiopathic dystonias is complex, because of the occurrence of dystonia in several ethnically distinct populations, with clinical features varying in age at onset, tendency to diurnal fluctuation, specific manifestations, and response to pharmacologic therapies (Lee et al. 1976; Segawa et al. 1976; Bressman et al. 1988, 1989; deYebenes et al. 1988; Gimenez-Roldan et al. 1988). The disease occurs in both hereditary and sporadic forms. Inherited forms of dystonia generally display autosomal dominant transmission, although X-linked (Lee et al. 1976) and possible autosomal recessive (Eldridge 1970) forms have been described. ITD occurs at highest frequency among Ashkenazi Jews (1/15,000) (Zilber et al. 1984; Bressman et al. 1989) and is less common in non-Jewish populations (1/160,000) (Zeman and Dyken 1967). Disease expression is highly variable. Onset in childhood usually portends development of severe, disabling, generalized dystonia, whereas onset in adulthood is frequently more restricted or segmental in its expression.

Our group previously demonstrated genetic linkage between a gene for dystonia and DNA markers in the chromosome 9q32-34 region in a large non-Jewish kindred (Ozelius et al. 1989) and in a group of Ashkenazi Jewish families (Kramer et al. 1990). Both of these forms of hereditary dystonia present with early-onset disease, usually progressing to generalized dystonia. These forms differ in ethnic origin and in penetrance, which is .30 and .75 in the Jewish and non-Jewish groups, respectively (Kramer et al. 1988; Bressman et al. 1989; Ozelius et al. 1989; Risch et al. 1990). The DNA markers giving strongest linkage were *GSN* (gelsolin) and *ASS* (argininosuccinyl synthetase) in the non-Jewish family and the Jewish families, respec-

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tively. The *GSN* and *ASS* markers have been mapped to 9q32-34 by physical and genetic means (Kwiatkowski et al. 1988, 1989; Northrop et al. 1989), with recombination distance between them being 14 cM. The gene causing dystonia in the non-Jewish family has been designated DYT1. Whether dystonia in the Jewish population is caused by the same or a second locus has not been ascertained.

Here we use linkage analysis to delimit this dystonia gene(s) to the region between AK1 and D9S10 (a region which spans <11 cM) on chromosome 9q34. We also provide additional evidence that the same locus is responsible for dystonia in these two populations.

Subjects and Methods

Patients, Families, and Clinical Evaluation

Twelve Ashkenazi Jewish families and one large family of mixed non-Jewish ancestry (family R) were analyzed in this study. Clinical and pedigree information on these families has been reported in detail (Ozelius et al. 1989; Kramer et al. 1990).

The method of evaluating patients and family members for dystonia has also been described in detail (Bressman et al. 1989; Risch et al. 1990). In brief, standardized neurologic examinations were performed by neurologists experienced in evaluating dystonia. Of those individuals included in the analysis, all but two also underwent videotaping of the examination. Subsequently, videotape exams were reviewed by examiners blinded to the identity of the subjects.

DNA Methods, Probes, and Polymorphism Analysis

Blood was obtained, by venesection, from consenting family members. DNA was prepared from either peripheral blood leukocytes or established lymphoblastoid cell lines by standard methods (Ozelius et al. 1989). Southern blot analyses were performed according to methods described elsewhere (Ozelius et al. 1989). Two polymorphic marker probes on 9q were used: pMCT136 (PstI) for random VNTR locus *D9S10* (q34) (Lathrop et al. 1988) and pAK1B3.25 (*Taq*I and *Ban*I) for the adenylate kinase-1 locus (*AK1;* q32-34) (Bech-Hansen et al. 1989; Zuffardi et al. 1989; D. E. Schuback, L. Ozelius, and X. O. Breakefield, unpublished observations).

Analysis of allele status for the *GNS, ABL,* and *ASS* GTn repeat polymorphisms was performed using oligonucleotide primer pairs CAGCCAGCTTTGGAGACAAC and TCGCAAGCATATGACTGTAA (GSN), TTTACACCTTCACCCAGAGA and GGCTGTGTTCAGTTAAACGT (ABL), and TGGGGAGCTATAAAAATGAC and GGTTGGCCTAAGAAAACCAT (ASS) (Kwiatkowski 1991; Kwiatkowski et al. 1991; Kwiatkowski and Perman 1991) in the PCR to amplify the repeat-containing region. Reaction volumes were 10 μl and contained 0.2 mM each of dATP, dGTP, and dTTP; 2.5 μM dCTP; 4 ng each oligonucleotide; 0.08 μl ^{32}P dCTP (3,000 mCi/mm); and 0.05 μl *Taq* polymerase (Perkin Elmer–Cetus). Thermal controller settings were 94°C for 1.5 min; 25 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and 72°C for 9 min. Amplified products were analyzed by electrophoresis of a 2-μl aliquot of the preparation on 6% acrylamide, 8 M urea, sequencing-type gels. Gels were dried and exposed to Kodak XAR film, without screen, for 4–24 h for preparation of autoradiographs.

Linkage Map of 9q32-34, and Linkage Analysis

A linkage map of 9q32-34 was created using the markers GSN, AK1, ABL, ASS, and D9S10 (MCT136) and a panel of 56 Venezuelan reference pedigrees containing 631 individuals (Haines et al. 1990; Ozelius et al. in press). The map is shown in figure 1, and the marker order shown has odds >1,000:1 over the next most likely order, as determined by analysis using MAPMAKER (Lander et al. 1987), except for the placement of ABL, for which the odds were only 100:1 over placement of ABL telomeric to ASS.

Two-point linkage analysis was performed using the LIPED program, version 3 (Ott 1976; Hodge et al. 1979), which incorporates age correction. Autosomal dominant inheritance of a rare gene (frequency 0.01%) was assumed, and penetrance was estimated at .30 in the Jewish families and .75 in the non-Jewish family. Parameters for the age correction were based on empirical age-of-onset data on affected individuals in the two populations discussed here. Allele frequencies for the various markers used were estimated by counting alleles in the married-in-individuals, both in these two populations and in the Venezuelan reference pedigrees.

Multipoint analysis was done by using the LINK-

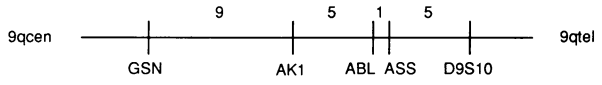

Figure 1 Map of 9q34. Distances are indicated in centi-Morgans.

MAP program from the LINKAGE computer package, version 5.03 (Lathrop and Lalouel 1988). Because of the multiple alleles found with several of these markers, and because of the extent of family material, two separate multipoint analyses were performed using subsets of the DNA markers with the dystonia trait.

Results and Discussion

Table 1 presents the results of pairwise linkage analysis between a panel of DNA markers from 9q32-34 and dystonia in the non-Jewish family R and in the Jewish families. Strong positive scores were seen for the *GSN, ABL,* and *ASS* GT repeat markers, with maximum values, in one or both populations, at 0% recombination. The highest lod scores were observed with *ABL* in the non-Jewish family and with *ASS* in the Jewish families. The non-Jewish family exhibited a broad region of positive lod scores at 0% recombination (extending from *GSN* to *ASS*), indicating that over that entire interval there were no crossover events in this particular pedigree. Analysis of the 12 Jewish families indicated that there were five, one, zero, one, and three likely crossover events between, respectively, the *GSN, AK1, ABL, ASS,* and *D9S10* markers and the dystonia trait in different family members.

For the Jewish and non-Jewish families, two sets of multipoint analyses were performed: (1) one with the dystonia locus, GSN, and AK1 and (2) a second with dystonia, AK1, ASS, and D9S10. Results are presented graphically in figure 2. With respect to the Jewish families, the DYT1 gene is clearly excluded from the region spanning GSN to AK1 (fig. 2*a*). The most likely position of the DYT1 gene is midway between AK1 and ASS (lod score 4.43; fig. 2*b*), very close to ABL (which was not included in the analysis because it was relatively uninformative for linkage). This is entirely consistent with previous results for these Ashkenazi families (Kramer et al. 1990); furthermore the 1-lod-unit confidence interval has been reduced from 18 cM to 6 cM.

Analyses in the non-Jewish family clearly reflect the fact that no recombinations were observed between the dystonia locus and GSN, AK1, or ASS. Although the highest lod score (4.87) occurs at ASS, GSN and AK1 fall within the 1-lod-unit confidence interval.

These data provide strong evidence that, in the Jewish families studied here, the dystonia gene is located within the 11-cM interval flanked by AK1 and D9S10,

Table 1

Pairwise Linkage Analyses of Dystonia with Markers on Chromosome 9q

	Lod Score at θ =					
Gene Symbol Marker	.00	.05	.10	.20	.30	.40
GSN (GT repeat):						
Family R	3.99	3.73	3.45	2.76	1.92	.91
Jewish family	−8.77	.68	1.31	1.37	.97	.47
Total	−4.78	4.41	4.76	4.13	2.89	1.38
AK1:						
Family R	−.12	.00	.04	.04	.03	.01
Jewish family	−6.48	−.72	−.02	.28	.18	.04
Total	−6.60	−.72	.02	.32	.21	.05
ABL (GT repeat):						
Family R	5.11	5.06	4.78	3.91	2.72	1.27
Jewish family	−1.33	1.19	1.06	.80	.55	.29
Total	−6.44	6.25	5.84	4.71	3.27	1.56
ASS (GT repeat):						
Family R	4.65	4.58	4.36	3.59	2.50	1.15
Jewish family	3.60	4.72	4.24	3.03	1.80	.76
Total	8.25	9.30	8.60	6.62	4.30	1.91
D9S10 (MCT136):						
Family R	−4.37	1.15	1.44	1.37	.96	.38
Jewish family	−3.26	−1.04	−.47	−.07	.01	.00
Total	−7.63	.11	.97	1.30	.97	.38

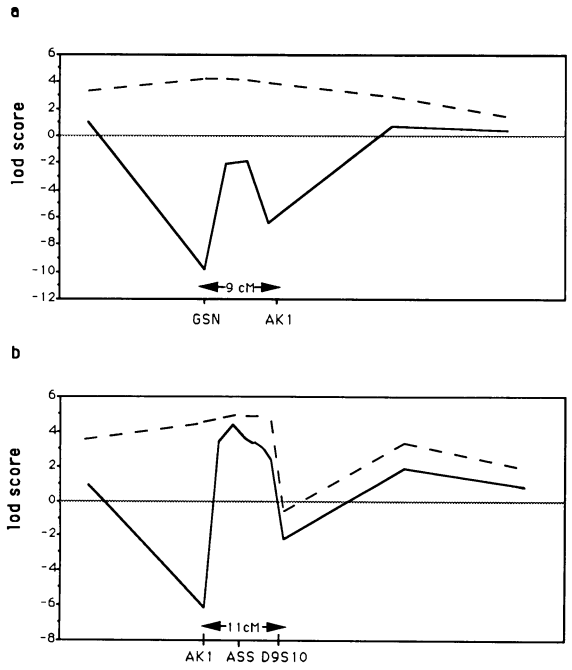

Figure 2 Multipoint analysis of dystonia and 9q markers in 12 Ashkenazi Jewish families (——) and in one large non-Jewish family (---). The location map summarizes lod scores for dystonia at various map positions in a fixed marker map of GSN and AK1 (*a*) and of AK1, ASS, and D9S10 (*b*). Genetic distances are given in centiMorgans (cM) and are calculated on the basis of sex-average recombination estimates.

with odds of 21,878:1 that the gene actually lies near the center of the 6-cM interval between AK1 and ASS. Although the ethnic backgrounds of the two populations studied are distinct, and although the penetrance of the condition also differs (being .75 in the non-Jewish family and .30 in the Jewish families), our results are consistent with the same gene, or two related genes linked to each other, causing the disease in these two groups. The difference in penetrance may be explained by the occurrence of distinct mutations in the same gene in the two populations.

Allelic heterogeneity with this 9q locus is recognized, on the basis of linkage analysis, for three other forms of dystonia with distinct clinical features. These forms are dystonia with responsiveness to l-dopa and with Parkinsonian features (Kwiatkowski et al. 1991), dystonia responsive to alcohol and with myoclonic features (J. Wahlstrom, L. Ozelius, P. Kramer, G. Holmgren, M. Kyllerman, U. Drugge, and X. O. Breakefield, unpublished observations), and X-linked dystonia occurring in the Philippines (Kupke et al. 1990). However, it is possible that this same gene(s) from 9q34 is responsible for most other cases of "classic" dystonia, what has been called "childhood-onset without marked diurnal variation" (Fahn 1989; Muller and Kupke 1990).

Our analysis illustrates the utility of GTn repeat polymorphisms in linkage analysis studies. These polymorphisms can be highly informative (Weber 1990) and are widely distributed over the human genome, occurring, on average, every 50–100 kb (Litt and Luty 1989; Weber and May 1989). Indeed, the polymorphisms used in the present study were specifically sought within the *GSN, ABL,* and *ASS* loci to improve the quality of the linkage-data relative to these loci in the dystonia family material available. All three markers consist of at least eight alleles with heterozygosity .67–.83 (Kwiatkowski 1991; Kwiatkowski et al. 1991; Kwiatkowski and Perman 1991). Such highly informative markers are particularly useful in linkage analysis of relatively isolated populations such as Ashkenazi Jews, in which conventional two-allele markers often have limited utility. In this particular case, the ABL GT polymorphism was also not very informative in the Jewish population (table 1).

This narrow localization of the disease gene by identification of flanking markers (*AK1* and *D9S10*) sets the stage for identification of the disease gene by positional cloning techniques. Previously, we have considered gelsolin and dopamine β-hydroxylase as potential candidates for the dystonia gene (Ozelius et al. 1989). Gelsolin is excluded by the current results, at least in the Jewish population. Dopamine β-hydroxylase, which maps telomeric to *D9S10* (Ozelius et al., in press), has been excluded, in these and two other forms of dystonia, by other studies (Schuback et al., in press). The only remaining known genes in this region are the ABL oncogene and the argininosuccinate synthetase (ASS) gene, neither of which appears to be a likely candidate gene for a neurologic disorder whose pathophysiology is presumably localized to the basal ganglia.

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References

Bech-Hansen NT, Marshall KJ, Krause SL (1989) TaqI RFLP in human adenylate kinase-1 gene region on chromosome 9. Nucleic Acid Res 17:4004

Bressman SB, deLeon D, Brin MF, Risch N, Burke RE, Greene PE (1989) Idiopathic dystonia among Ashkenazi Jews: evidence for autosomal dominant inheritance. Ann Neurol 26:612–620

Bressman SB, deLeon D, Brin MF, Risch N, Shale H, Burke RE, Greene PE, et al. (1988) Inheritance of idiopathic torsion dystonia among Ashkenazi Jews. Adv Neurol 50: 45–56

deYebenes JG, Moskowitz CB, Fahn S, Saint-Hilaire MH (1988) Long-term treatment with levodopa in a family with autosomal dominant torsion dystonia. Adv Neurol 50:101–111

Eldridge R (1970) The torsion dystonias: literature review: genetic and clinical studies. Neurology 20:1–78

Fahn S (1988) Concepts and classification of dystonia. Adv Neurol 50:1–8

——— (1989) Clinical variants of idiopathic torsion dystonia. J Neurol Neurosurg Psychiatry 52 [Suppl]: 96–100

Gimenez-Roldan S, Delgado G, Marin M, Villanueva JA, Mateo D (1988) Hereditary torsion dystonia in gypsies. Adv Neurol 50:73–81

Haines J, Ozelius L, McFarlane H, Menon A, Tzall S, Martiniuk F, Hirschhorn R, et al (1990) A genetic linkage map of chromosome 17. Genomics 8:1–6

Hodge SE, Morton LA, Tideman S, Kidd KK, Spence MA (1979) Age-of-onset correction available for linkage analysis (LIPED). Am J Hum Genet 31:761–762

Kramer PL, deLeon D, Ozelius L, Risch N, Bressman SB, Brin MF, Schuback DE, et al (1990) Dystonia gene in Ashkenazi Jewish population located on chromosome 9q32-34. Ann Neurol 27:114–120

Kramer P, Ozelius L, Brin M, Fahn S, Kidd KK, Gusella J (1988) The molecular genetics of an autosomal dominant form of torsion dystonia. Adv Neurol 50:57–66

Kupke KG, Lee LV, Viterbo GH, Arancillo J, Donlon T, Muller U (1990) X-linked recessive torsion dystonia in the Philippines. Am J Med Genet 36:237–242

Kwiatkowski D (1991) A GTn dinucleotide repeat polymorphism within the ABL locus (9q34). Nucleic Acids Res 19:967

Kwiatkowski DJ, Nygaard TG, Schuback DE, Perman S, Trugman JM, Bressman SB, Burke RE, et al (1991) Identification of a highly polymorphic microsatellite VNTR within the argininosuccinate synthetase locus: exclusion of the dystonia gene on 9q32-34 as the cause of dopa-responsive dystonia in a large kindred. Am J Hum Genet 48:121–128

Kwiatkowski DJ, Ozelius L, Schuback D, Gusella J, Breakefield XO (1989) The gelsolin (GSN) cDNA clone, from 9q32-34, identifies BclI and StuI RFLPs. Nucleic Acids Res 17:4425

Kwiatkowski D, Perman S (1991) A GTn dinucleotide repeat polymorphism within the GSN locus (9q32-q34). Nucleic Acids Res 19:967

Kwiatkowski DJ, Westbrook CA, Bruns GAP, Morton CC (1988) Localization of gelsolin proximal to ABL on chromosome 9. Am J Hum Genet 42:565–572

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln FE, Newberg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage map of experimental and natural populations. Genomics 1:174–181

Lathrop GM, Lalouel J-M (1988) Efficient computations in multilocus linkage analysis. Am J Hum Genet 42:498–505

Lathrop M, Nakamura Y, O'Connell P, Leppert M, Woodward S, Lalouel J-M, White R (1988) A mapped set of genetic markers for human chromosome 9. Genomics 3: 361–366

Lee LV, Pascasio F, Fuentes F, Viterbo GH (1976) Torsion dystonia in Panay, Philippines. Adv Neurol 14:137–151

Litt M, Luty JA (1989) A hypervariable microstellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

Muller U, Kupke K (1990) The genetics of primary torsion dystonia. Hum Genet 84:107–114

Northrop H, Lathrop M, Lu S-Y, Daiger SP, Beaudet AL, O'Brien WE (1989) Multilocus linkage analysis with the human argininosuccinate synthetase gene. Genomics 5: 442–444

Ott J (1976) A computer program for linkage analysis of general human pedigrees. Am J Hum Genet 28:528–529

Ozelius L, Kramer FL, Moskowitz CB, Kwiatkowski DJ, Brin MF, Bressman SB, Schuback DE, et al (1989) Human gene for torsion dystonia located on chromosome 9q32-q34. Neuron 2:1427–1434

Ozelius L, Schuback D, Kwiatkowski DJ, Stefansson K, Breakefield XO, Gusella JF, Haines JL. Genetic linkage map of chromosome 9q32-34. Cell Genet Cytogenet (in press)

Risch NJ, Bressman SB, deLeon D, Brin MF, Burke RE, Greene PE, Shale H, et al (1990) Segregation analysis of idiopathic torsion dystonia in Ashkenazi Jews suggests autosomal dominant inheritance. Am J Hum Genet 46: 533–538

Schuback D, Kramer P, Ozelius L, Holmgren G, Forsgren L, Kyllerman M, Wahlstrom J, et al. Dopamine beta-hydroxylase gene excluded in four subtypes of hereditary dystonia. Hum Genet (in press)

Segawa M, Hosaka A, Miyagawa F, Nomura Y, Imai H (1976) Hereditary progressive dystonia with marked diurnal fluctuation. Adv Neurol 14:215–233

Weber JL (1990) Informativeness of human (dC-dA)n.(dG-dT)n polymorphisms. Genomics 7:524–530

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Zeman W, Dyken P (1967) Dystonia musculorum deformans: clinical, genetic, and pathoanatomical studies. Psychiatr Neurol Neurochir 70:77–121

Zilber N, Korczyn AD, Kahana E (1984) Inheritance of idiopathic torsion dystonia among Jews. J Med Genet 218:13–20

Zuffardi O, Caiulo A, Maraschio F, Tupler R, Bianchi E, Arrisano P, Beluffi G, et al (1989) Regional assignment of the loci for adenylate kinase to 9q32 and for a1-acid glycoprotein to 9q31-q32: a locus for Goltz syndrome in region 9q32-qter? Hum Genet 82:17–19