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RESEARCH

Fine Localization of the Torsion Dystonia Gene (*DYT1*) on Human Chromosome 9q34: YAC Map and Linkage Disequilibrium

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The *DYT1* gene, which maps to chromosome 9q34, appears to be responsible for most cases of early-onset torsion dystonia in both Ashkenazic Jewish (AJ) and non-Jewish families. This disease is inherited in an autosomal dominant mode with reduced penetrance (30%–40%). The abnormal involuntary movements associated with this disease are believed to be caused by unbalanced neural transmission in the basal ganglia. Previous linkage disequilibrium studies in the AJ population placed the *DYT1* gene in a 2-cM region between the loci *D9S62a* and *ASS*. A YAC contig has now been created spanning 600 kb of this region including *D9S62a*. The location of the *DYT1* gene has been refined within this contig using several new polymorphic loci to expand the linkage disequilibrium analysis of the AJ founder mutation. The most likely location of the *DYT1* gene is within a 150 kb region between the loci *D9S2161* and *D9S63*.

Torsion dystonia is a movement disorder characterized by sustained muscle contractions, frequently causing twisting and repetitive movements or abnormal postures (Fahn 1988). Primary or idiopathic torsion dystonia (ITD) has unknown etiology and comprises a number of clinically and genetically distinct syndromes. At least six different autosomal genes can cause clinically distinct forms of ITD, all of which are inherited in a dominant fashion with reduced penetrance and variable expressivity (for review, see Gasser et al. 1992; Kramer et al. 1995). The gene *DYT1*, which underlies early-onset generalized dystonia, the most severe form of dystonia, maps to chromosome 9q34 (Ozelius et al. 1989; Kramer et al. 1990, 1994). The gene *GCH1*, which is responsible for dopa-responsive dystonia (including the Segawa

variant), has been mapped to chromosome 14q21–22 (Nygaard et al. 1993; Endo et al. 1995) and encodes the enzyme GTP cyclohydrolase I, which is the rate-limiting enzyme for synthesis of tetrahydrobiopterin, the cofactor for tyrosine hydroxylase that converts tyrosine to dopa (Ichinose et al. 1994). A gene for one form of paroxysmal dystonia has been mapped to chromosome 2q (Fink et al. 1996; Fouad et al. 1996), and some types of late-onset focal dystonia are caused by genes on chromosomes 18p (Leube et al. 1996) and 8 (L. Almasy, S.B. Bressman, D. deLeon, P.E. Greene, G. Heiman, B.A. Ford, D. Raymond, A.C. Jones, H. Shen, P.L. Kramer, unpubl.). Genes responsible for other hereditary types of dystonia, including rapid-onset (Dobyns et al. 1993), myoclonic (Kurlan et al. 1988; Kyllerman et al. 1990), and other late-onset focal dystonias (Forsgren et al. 1988; Bressman et al. 1994a) have not been mapped yet.

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The *DYT1* gene appears to be responsible for most cases of early-onset ITD in both Jewish and non-Jewish families (Kramer et al. 1990, 1994). The incidence of this form of dystonia has been estimated to be ~5- to 10-fold greater in the Ashkenazi Jewish (AJ), as compared to non-Jewish populations or non-Ashkenazi population (Zeman and Dyken 1967; Eldridge 1970; Korczyn et al. 1980; Bressman et al. 1989). This increased incidence results from a founder mutation that was introduced into the AJ population some 20–30 generations ago and probably originated in Lithuania or Byelorussia; this mutation can be identified by a haplotype of alleles at polymorphic loci surrounding it (Risch et al. 1995). The frequency of this founder mutation is estimated between 1/6000 and 1/2000 in individuals of AJ descent with 30% penetrance of the disease phenotype (Bressman et al. 1989; Risch et al. 1990, 1995). Initial linkage disequilibrium studies in affected AJ individuals indicated that the *DYT1* gene was located within a short distance of the argino-succinate synthetase (*ASS*) locus (Ozelius et al. 1992a). Using six polymorphic markers spanning a 3-cM region, including the *ASS* gene (centromere-*D9S62a/b*-*D9S63*-*ASS*-*ABL*-*D9S64*-telomere), the highest lod scores were obtained with *D9S62a/b*-*D9S63* and the strongest disequilibrium with *D9S63* in 54 AJ families with early-onset torsion dystonia (Risch et al. 1995), suggesting that *DYT1* is in between *D9S62* and *ASS*, in close proximity to *D9S63*.

In this study we have assembled a YAC contig (~600 kb), including a number of cosmids, which spans the *DYT1* region from *D9S62a* to a new marker, *D9S2163*, which is telomeric to *D9S63*. We have defined and mapped seven polymorphic marker loci in this region and used a set of 11 marker loci between and including loci *D9S62a* and *ASS* to define the most likely location of the *DYT1* gene by linkage disequilibrium studies in AJ families. This high-resolution genetic and physical map of the chromosomal region bear-

ing the *DYT1* gene paves the way for identification of this and other genes in the region.

RESULTS

YAC Map

A yeast artificial chromosome (YAC) contig was constructed for the region implicated by initial linkage disequilibrium studies spanning loci *D9S62a* and *D9S63* (Fig. 1). Initial screening of YAC libraries with these genetic markers and *D9S62b* resulted in two YAC contigs around loci *D9S62a/b* and *D9S63*. Alignment of the clones and ordering of markers was based on the presence or absence of each marker in the clones as assessed by PCR or hybridization. To extend these contigs, *Alu*-PCR products and end clones were generated from these YACs and used to identify overlapping YACs, including 8H12 and 21B5. To link the two contigs, end clones were isolated from YACs 5H7 and 8H12. These clones did not identify any new YACs but did hybridize to the

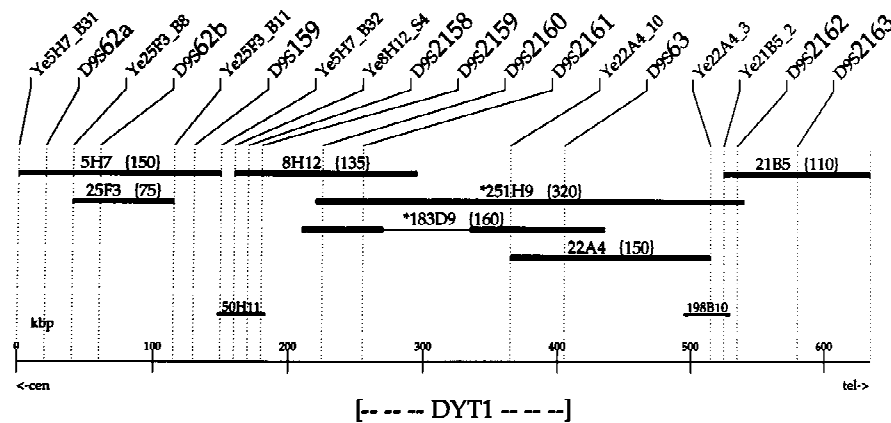


Figure 1 Physical map from markers *D9S62a* to *D9S2163* containing the *DYT1* gene. Markers (SSR and end clones) used to screen YACs and cosmids are represented by a label at the *top* of the map and a vertical line. YAC and cosmid clones are drawn to scale under the loci that they contain. YACs are indicated by bold horizontal lines with a corresponding name and size. YAC names preceded by asterisks are CEPH coordinates, and the remaining YACs are from the flow-sorted chromosome 9 library. The thin line in YAC 183D9 indicates a region of deletion. YAC clone lengths were estimated by pulsed-field gel electrophoresis and are in brackets next to the YAC names. Cosmids are indicated by smaller horizontal lines. The cosmids were obtained from a chromosome 9-specific cosmid library generated at Lawrence Livermore National Laboratory and should be preceded by the designation LL09NC01. Cosmids were given the average size of 40 kb. Distances between markers are estimated based on their presence or absence in the clones and the clone lengths. A kilobase scale is shown at the *bottom*. The minimal region containing the *DYT1* gene, as defined by haplotype analysis in the AJ population, is indicated by the bracketed region at the *bottom*.

same cosmid, LL09NC0150H11, allowing joining of the contigs around *D9S62a/b* and *D9S63*. To get a more accurate estimate of the size of the contig, because of the discrepancy in the size of YACs 251H9 and 183D9 covering the same region, the end clones from YACs 22A4 and 21B5 were isolated and hybridized to both the YAC and cosmid libraries. Again, no new YACs were identified, but cosmid LL09NC01198B10 was found to link YACs 22A4 and 21B5 and overlap 251H9. The resultant YAC physical map has one gap that is covered by a cosmid (LL09NC0150H11), contains 10 simple sequence repeat (SSR) markers, and spans a physical distance of ~600 kb on chromosome 9q34 based on YAC sizes estimated by pulsed-field gel electrophoresis, and assuming an average size of cosmids (40 kb).

Markers

Eleven SSR polymorphisms within the *DYT1* region were used to align YAC contigs and to carry out linkage disequilibrium studies in affected AJ individuals. Four of these SSRs have been described and placed on a genetic map previously; in order from centromere to telomere they are: *D9S62a*, *D9S62b*, *D9S63*, and *ASS* (Kwiatkowski et al. 1992; Ozelius et al. 1992b). *D9S62a* and *D9S62b* lie within the same 40-kb cosmid (Henske et al. 1993). No recombinations have been observed between *D9S62a/b* and *D9S63*, in >800 meiotic events evaluated in the large Venezuelan reference pedigrees (VRPs) and our AJ dystonia families (Ozelius et al. 1992b; Risch et al. 1995). Results of marker-to-marker linkage analysis gives a maximum likelihood estimate for the recombination fraction between the *D9S62a/b*-*D9S63* cluster and *ASS* of ~1.8 cM (Risch et al. 1995).

Six new polymorphic markers were generated from YACs and cosmids in the *DYT1* region (Table 1). The other marker, *D9S159*, was described previously by Weissenbach et al. (1992) but repositioned compared to previous genetic maps as part of this study. These markers comprise four (GT)_n repeats (*D9S2158*, *D9S2159*, *D9S2163*, and *D9S159*); two tetranucleotide repeats [*D9S2160* (ATAG)_n and *D9S2161* (GATA)_n]; and a trinucleotide repeat [*D9S2162* (AAT)_n]. The (GT)_n polymorphism *D9S2158* was isolated from a cosmid overlapping the end of YAC 183D9; another, *D9S2159*, from a cosmid that spans YACs 8H12 and 5H7 (Fig. 1); and the third, *D9S2163*, from a cosmid that hybridized to *Alu*-PCR products from YAC 21B5. *D9S2160* and *D9S2162* were isolated from YACs 183D9 and 21B5, respectively, by sequencing cloned *Alu*-PCR products from them. *D9S2161* was identified in a cosmid

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that hybridized to an *Alu*-PCR product from YAC 8H12. Allele frequencies for these seven new markers were determined in the AJ population (Table 1). Allele frequencies for the four previous markers, *D9S62a*, *D9S62b*, *D9S63*, and *ASS*, in this population are provided in Risch et al. (1995).

Linkage Disequilibrium

Based on haplotype analysis in the immediate chromosomal region surrounding *DYT1*, it is clear that most cases of early-onset dystonia in the AJ population result from a founder mutation (Ozelius et al. 1992a; Bressman et al. 1994b; Risch et al. 1995). Here we have determined the haplotype associated with the founder mutation for 11 polymorphic markers, including eight (GT)_n repeats (*D9S62a/b*, *D9S63*, *ASS*, *D9S2159*, *D9S159*, *D9S2158*, and *D9S2163*), two tetranucleotide repeats (*D9S2160* and *D9S2161*), and a trinucleotide repeat (*D9S2162*). The allele associated with the *DYT1* founder mutation at each of these markers, its percentage in affected and control AJ chromosomes, and the linkage disequilibrium parameter δ are given in Table 2 for all markers except *D9S2161* and *D9S2163*. These markers were identified later and used to confirm recombination events. The 11 markers from *D9S62a* to *ASS* define a haplotype, portions of which were observed in 94% (64/68) of unrelated AJ individuals with early-onset dystonia (Table 3 illustrates these 64 chromosomes with all or part of the founder haplotype). In families with more than one affected individual, a definite disease-bearing chromosome could be identified (38/64); however, when there was only one affected member in a family, only a probable disease-bearing chromosome could be assigned (26/64; Table 3). The full haplotype (all 11 markers) was not seen in any of 280 phased chromosomes from AJ controls (Table 4), although two of the control chromosomes do carry a portion of the founder haplotype from *D9S62a* to *D9S63* (7 markers). When these two chromosomes were typed for the two new loci (*D9S2161* and *D9S2163*) neither carried the associated allele at these loci. Some marker combinations in controls appeared at an increased frequency, suggesting that there may be linkage disequilibrium between them, particularly for the 4-4-4-16 haplotype at markers *D9S2158*, *D9S2159*, *D9S2160*, and *D9S63*, which lie in the 150-kb region believed to contain the *DYT1* gene (see below; total of 19 occurrences vs. 11.9 expected).

Analysis of the distribution of marker alleles in affected AJ individuals who bear only a portion of

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Marker	PCR primers ^a	Allele	Allele frequency	Size range ^c	Heterozygosity
D9S159 ^{b,d} (GT) _n	AGCTGGAATGAGTGCTGGGC GAAGGGCTGGTGTTCGCCTA	0	0.01	116-134	0.81
		2	0.01		
		4	0.12		
		6	0.01		
		8	0.06		
		10	0.03		
		12	0.21		
		14	0.22		
		16	0.27		
		18	0.05		
		20	0.02		
D9S2158 (GT) _n	GACTCTTGAGCAACCAACTG TGGTCCAAACCCAGCTTCAG	0	0.02	116-132	0.78
		2	0.16		
		4	0.35		
		6	0.24		
		8	0.09		
		10	0.05		
		12	0.04		
		14	0.04		
16	0.01				
D9S2159 (GT) _n	GGTAGTCCCTGTAAACGTC GCCCAGCTGGCAGACACTTC	-6	0.01	144-160	0.67
		-4	0.03		
		-2	0.04		
		0	0.08		
		2	0.04		
		4	0.53		
		6	0.07		
		8	0.17		
10	0.03				
D9S2160 (ATAG) _n	ACCCTGTGTCAAGGAAATGC CATGAGCCAATTATATGGCA	-4	0.01	84-124	0.75
		0	0.01		
		1	0.01		
		2	0.08		
		3	0.17		
		4	0.23		
		5	0.38		
		6	0.11		
D9S2161 ^e (GATA) _n	CAATGTCGTGCTAAACTACAG CATAGATAGAGATAGGCAG	1	0.10	152-180	0.79
		2	0.28		
		3	0.13		
		4	0.18		
		5	0.28		
		6	0.02		
		7	0.01		
D9S2162 (AAT) _n	CAAGAGCAAGACCCTGTCTCG GATCAGTCTACTACTCAGTGC	-1	0.01	91-112	0.76
		0	0.03		
		1	0.13		
		2	0.32		
		3	0.06		
		4	0.32		
		5	0.13		
6	0.01				
D9S2163 ^e (GT) _n	CCACCGAGAGCCTCTGAGTC CAAGAACACTCTGCAGTAC	-14	0.18	157-183	0.81
		-12	0.02		
		-10	0.02		
		0	0.17		
		2	0.11		
		4	0.28		
		6	0.18		
		8	0.02		
		10	0.01		
		12	0.01		

^aOligonucleotide primer sequences are listed 5' → 3'.
^bFrequencies based on 280 AJ control chromosomes.
^cRange of allele sizes given in base pairs.
^dThis marker was generated by Weissenbach et al. (1992).
^eFrequencies based on 120 AJ control chromosomes.

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Table 2. Linkage Disequilibrium Between 9q34 Markers and the *DYT1* Gene in the AJ Population

Locus	Associated allele	Percent affected chromosomes ^a	Percent control chromosomes ^b	δ
<i>D9S62a</i>	2	90 (36/40)	5 (14/280)	0.894
<i>D9S62b</i>	8	90 (36/40)	11 (32/280)	0.887
<i>D9S159</i>	12	95 (38/40)	21 (60/280)	0.937
<i>D9S2158</i>	4	90 (36/40)	35 (98/280)	0.846
<i>D9S2159</i>	4	92.5 (37/40)	54 (150/280)	0.837
<i>D9S2160</i>	4	95 (38/40)	23 (65/280)	0.935
<i>D9S63</i>	16	92.5 (37/40)	11 (32/280)	0.916
<i>D9S2162</i>	4	92.5 (37/40)	31 (87/280)	0.891
<i>ASS</i>	12	82.5 (33/40)	10 (29/280)	0.806

^aDisease-bearing chromosomes from unrelated AJ affecteds (in parentheses).
^bPhased, unrelated chromosomes from AJ controls (in parentheses).

the founder haplotype allows finer assessment of the location of the *DYT1* gene (Table 3). Although variations at any one allele could result from de novo mutations ("slippage" events of unknown mechanism) of the repeat element at that site, variations in a linear set of adjacent alleles indicate a recombination event, which could have occurred in any of the meiotic events from the time of the founder mutation to the generation of the affected individual under study. On the telomeric side, there are nine chromosomes in affected individuals that showed recombinations between *D9S63* and *ASS* (chromosome categories 2–10; Table 3), four are directly observed recombination events that occurred in the generation under study (Table 3, footnote c), whereas the other five represent historic recombinations that occurred in a preceding generation. One of these historic recombinations was also seen with *D9S2162* (Table 3, category 10), but five others (three historic, and two observed) were homozygous 4 at this locus. The new marker *D9S2163* was used to type all of the telomeric recombinant chromosomes. Three of the chromosomes that were homozygous 4 at the *D9S2162* locus had the associated allele at *D9S2163*, suggesting that the recombination occurred between *D9S2163* and *ASS*. However, the other two crosses had different alleles at marker *D9S2163*, placing the *DYT1* gene proximal to this locus. The chromosome that recombined at the *D9S2162* locus also crossed with the *D9S2163* marker, consistent with placement of the disease gene proximal to *D9S2162*. One chromosome (Table 3, category 11) that displayed a historic cross between *D9S2160* and *D9S63* was homozygous 4 at

D9S2162, suggesting the possibility of a slippage event at *D9S63*. This chromosome also recombined at *D9S2163*, however, thus confirming that a recombination event most likely occurred proximal to locus *D9S2163* and probably proximal to *D9S63* as well. The closest distal marker to *DYT1* thus appears to be *D9S63*. On the centromeric side of *DYT1* there are six putative historic recombination events between *D9S62b* and *D9S63* (Table 3, chromosome categories 12–17). One of these chromosomes (Table 3, category 12) recombines with all six markers, thus placing the disease gene distal to the *D9S2160* locus. Another chromosome (Table 3, category 13) has a historic recombination with five of the six markers, placing the gene distal to *D9S2159*, but *D9S2160* is homozygous 4. When these two chromosomes were typed for *D9S2161*, neither had the associated allele, supporting *D9S2161* as the proximal border. Three other chromosomes (categories 14–16) reveal historic recombinations with more proximal markers and retained the associated allele at *D9S2161*. These events are consistent with positioning the *DYT1* gene distal to the *D9S2161* locus. However, another chromosome (Table 3, category 17) had the associated alleles 12-4-4-4 between *D9S159* and *D9S2160* but did not have the associated allele at *D9S2161*. This would place the *DYT1* gene proximal to *D9S2161*, a position that would appear to be in conflict with the other two crosses. However, this 12-4-4-4 haplotype is seen on 2% of control AJ chromosomes, whereas the associated haplotype 16-4-12 between markers *D9S63* and *ASS*, seen in chromosomes 12 and 13 (Table 3), was never seen on any control AJ chromosomes (Table

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Table 3. Haplotype Analysis on AJ Disease Chromosomes

Chromo- some category	Locus														ASS	DBC ^a	PDBC ^b	Total
	← proximal							distal →										
	<40 kb	70 kb	100 kb	<40 kb	150 kb	130 kb	<40 kb	D9S2162	D9S2163	D9S2163	D9S2163	D9S2163	D9S2163	D9S2163				
1	2	8	12	4	4	4	4	5	16	16	4	4	8	12	32	17	49	
2	2	8	12	4	4	4	4	5	16	16	4*	4*	8	2	1	0	1	
3	2	8	12	4	4	4	4	5	16	16	4*	4*	8	2	1	0	1	
4	2	8	12	4	4	4	4	5	16	16	4	4	8	8	0	1	1 ^c	
5	4	8	14	6	8	4	4	3	16	16	4*	4*	8	10	1	0	1 ^{c,d}	
6	2	8	12	4	4	4	4	5	16	16	4	4	8	2	0	1	1	
7	2	8	12	4	4	4	4	5	16	16	4	4	0	10	1	0	1 ^c	
8	2	8	12	4	4	4	4	5	16	16	4*	4*	4	2	0	1	1	
9	2	8	12	4	4	4	4	5	16	16	4*	4*	2	2	0	1	1 ^c	
10	2	8	12	4	4	4	4	5	16	16	1	4	4	2	1	0	1	
11	2	8	12	4	4	4	4	5	12	12	4*	6	6	2	0	1	1	
12	4	12	10	6	6	6	7	1	16	16	4	4	8	12	0	1	1	
13	4	8	14	6	8	4*	3	16	16	16	4	4	8	12	1	0	1 ^d	
14	4	8	16	6	4	4*	5	16	16	16	4	4	8	12	0	1	1	
15	4	8	8	4	4	4	4	5	16	16	4	4	8	2	0	1	1	
16	4	12	12	4	4	4	4	5	16	16	4	4	8	12	0	1	1	
17	4	10	12	4	4	4	4	3	2	2	2	2	6	8	1	0	1	

(*) Homozygous.
^a(DBC) Definite disease-bearing chromosome.
^b(PDBC) Probable disease-bearing chromosome of phased haplotype carriers.
^cThese chromosomes contain defined recombination events between D9S2163 and ASS.
^dTwo chromosomes from the same family.

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Table 4. Frequency of Associated Haplotype in Control AJ Chromosomes

D9S62a	Locus										Observed no.	Frequency	
	D9S62b	D9S159	D9S2158	D9S2159	D9S2160	D9S63	D9S2162	ASS	control ^a	expected ^b			
2	8	12	4	4	4	16	4	12	4	4	0	0.0	0.0
2	8	12	4	4	4	16	4		4	4	0	0.0	0.0
2	8	12	4	4	4	16	4		4	4	2 ^c	.007	0.0 ^d
	8	12	4	4	4	16	4		4	4	2 ^c	.007	0.0 ^d
		12	4	4	4	16	4		4	4	2 ^c	.007	.001 ^e
		12	4	4	4		4		4	4	6 ^c	.021	.009 ^f
	8	12	4	4	4		4		4	4	3 ^c	.011	.001 ^d
			4	4	4		4		4	4	19 ^{c,g}	.068	.04 ^f
			4	4	4	16	4		4	4	7 ^{c,g}	.025	.005 ^d
			4	4	4	16	4		4	4	2 ^g	.007	.002 ^f
						16	4	12	4	4	0	0.0	.003
						16	4	12	4	4	0	0.0	0.0

^aExpected frequencies calculated by multiplying individual allele frequencies from Table 1 and Risch et al. (1995).

^bBase on 280 phased control chromosomes.

^cThese include the same two control chromosomes.

^d $p < 0.001$.

^e $p = 0.001$.

^f $p < 0.05$.

^gThis increase in observed number suggests some linkage disequilibrium between these markers.

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4). Therefore, it seems more likely that the gene is distal to *D9S2161*. The affected individual carrying only the 12-4-4-4 portion of the founder haplotype may do so by chance and instead have a different mutation at the *DYT1* gene or at another locus. In summary, the haplotype analysis defines the most likely location of the *DYT1* gene to be proximal to *D9S63* and distal to the *D9S2161*, a distance of ~150 kb as estimated from the YAC contig.

DISCUSSION

Employing both YACs and cosmids, we have generated a genomic contig spanning a 600-kb region of chromosome 9q34, including the *DYT1* gene. Furthermore, we have extended our original linkage disequilibrium studies in the AJ population using 10 SSR loci within this contig (Ozelius et al. 1992a; Risch et al. 1995). The present study confirms that most cases of early-onset ITD in the AJ population result from a single founder mutation and reduces the genomic region bearing this gene to an area compatible with positional cloning. This detailed haplotype information will also serve to increase the accuracy of molecular diagnosis in the AJ population.

Haplotype analysis has been instrumental in pinpointing the genomic location of several disease genes, including those for Huntington's disease (MacDonald et al. 1992) and diastrophic dysplasia (Hastbacka et al. 1994). Using this approach, we have identified several apparent historic recombination events that place the *DYT1* gene most likely between the loci *D9S2161* and *D9S63* in a region of ~150 kb. This localization should be regarded with some caution, however, as changes at the edges of the haplotype could also result from mutations (slippage) at highly polymorphic repeat markers. If the mutation rate of a marker exceeds its recombination rate with the disease-causing mutation, then the majority of apparent historical crossovers involving this marker will actually be the result of de novo mutations. Slippage events should be evident among densely spaced markers because there will usually be no change of associated haplotype alleles at flanking loci. On the proximal side, there are two AJ disease-bearing chromosomes that place the disease gene distal to the *D9S2161* locus and one other that places the gene distal to *D9S2158*.

We have seen no evidence for slippage at the *D9S2158* locus in our Jewish and non-Jewish families, but we have observed one slippage event (>400 meioses) at the *D9S2161* locus. The chances that all three of these disease-bearing chromosomes repre-

sent mutational events is highly unlikely, as they all have nonassociated alleles at the proximal markers. On the distal side there is one historic recombination event defining *D9S63* as the flanking marker. We have observed a slippage event at *D9S63* between generations in one family, and one new mutation at this locus was reported previously in the VRP (>800 total meioses) (Kwiatkowski et al. 1992), but again, the flanking markers distal to *D9S63* also show loss of associated haplotype alleles in this apparently recombinant chromosome. Thus, all of the apparent changes observed are consistent with recombination rather than slippage events.

To confirm the placement of loci and historical recombinations as well as to facilitate the positional cloning of the *DYT1* gene, a YAC and cosmid contig were constructed across a 600-kb interval containing this disease gene. The genetic distance between markers *D9S62a* and *D9S63* is small, as no recombinations have been observed between these loci in the VRP (Ozelius et al. 1992b) or in any of our dystonia families (>800 meiotic events in total). The physical map for this region, *D9S62a* to *D9S63*, spans ~300 kb. The genetic distance between *D9S62a/D9S63* and the *ASS* locus is estimated to be 1.8 cM (Risch et al. 1995). Walking from the end of the 600-kb contig toward *ASS*, we were unable to find a YAC that was also positive for YACs known to contain the *ASS* marker, suggesting that the minimal physical distance between these loci is likely to be \geq 900 kb. The minimal region most likely to bear the *DYT1* gene, as determined by linkage disequilibrium analysis in the AJ population, is ~150 kb. Assuming 100,000 genes in the haploid genome, this region would contain on average 6–7 genes.

These studies highlight the value of linkage disequilibrium studies to direct a gene search. Using this technique, we were able to reduce the interval containing the *DYT1* gene from 1.8 cM (~1.8 Mb) to ~150 kb, thus setting the stage for the eventual isolation of the disease gene.

Identification of the *DYT1* gene should provide insights into the development and the temporal and spatial organization of the basal ganglia where the defect is believed to originate (Marsden et al. 1985). Several interesting features of this disease bear on developmental plasticity of movement control in the central nervous system, including the tendency for the disorder to progress from lower to upper body regions and the positive correlation between younger age at onset and greater severity of disease (Bressman et al. 1994b). The low penetrance in this autosomal dominant syndrome suggests that other genetic and/or environmental factors can

modulate the outcome of mutations at the *DYT1* locus. Because there is no apparent neuronal degeneration associated with early-onset dystonia, eventual identification of the disease gene may lead to rational therapy.

METHODS

Examination and Family Material

Subjects for this study were ascertained from several sources, including a computerized database of patients seen by the Movement Disorder Group at Columbia University (New York, NY), patients seen at the Neurologic Institute in Moscow, contacts through the Dystonia Medical Research Foundation, and referrals from other neurologists. The criteria for diagnosis of ITD and the methods of evaluation were the same as described elsewhere (Kramer et al. 1990). To reduce heterogeneity, only affected individuals who met criteria for the early-onset form of ITD were included (Bressman et al. 1994b). These criteria are onset of symptoms prior to age 28 involving a body part at or below the level of the neck in the affected proband or in relatives of the affected proband. All patients were of AJ ancestry, as defined previously (Bressman et al. 1989). A total of 80 families was ascertained. Among these, 40 were singleton affecteds, whereas the other 40 had two or more affected relatives. Clinical and pedigree information for these families has been reported in detail elsewhere (Bressman et al. 1989, 1994b; Kramer et al. 1990; M.F. Brin, S.A. Limborska, E.D. Markova, I.A. Ivanova-Smolenskaya, L.J. Ozelius, D. deLeon, and X.O. Breakefield, in prep.). Control chromosomes were obtained from two sources; the normal (nondisease bearing) chromosome from affecteds, in cases where chromosomes could be phased, and chromosomes from unaffected individuals marrying into the families. A total of 280 control chromosomes were constructed and phased, all of which were of 100% AJ origin.

DNA Methods and Polymorphic Analysis

Venous blood samples were obtained from all participating family members after obtaining informed consent. DNA was extracted from whole blood (Gusella et al. 1979) or lymphoblast lines (Anderson and Gusella 1984). PCR analysis of SSR polymorphisms was carried out on genomic DNA using oligonucleotide primer pairs reported previously (Kwiatkowski et al. 1992; Weissenbach et al. 1992; Henske et al. 1993) or provided in Table 1. PCR conditions for amplification of SSR polymorphisms and analysis of the amplified products are described in Ozelius et al. (1992a).

Libraries

YACs were identified from a total human genomic YAC library constructed by the Centre d'Etudes du Polymorphisme Humain (CEPH) (Albertsen et al. 1990) and from a chromosome 9-specific YAC library constructed from flow-sorted chromosomal DNA (McCormick et al. 1993). Cosmid clones were identified from a chromosome 9-specific library constructed at the Lawrence Livermore National Laboratory (Van

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Dilla and Deaven 1990). YAC and cosmid colony filters were stamped and prepared for hybridization as described (McCormick et al. 1993; Murrell et al. 1995). Library pools for PCR screening were generated from both YAC libraries and used to amplify *Alu*-PCR products that were spotted on filters for hybridization screening with *Alu*-PCR products from individual clones.

Alu-PCR

Yeast genomic DNA containing YACs, in pools or individual clones, was amplified with *Alu* primers S (5'-GAGGTTGCAG-TGAGCCGAGAT-3') and J (5'-GAGGCTGCAGTGAGCCGTG-AT-3'), followed by purification (Murrell et al. 1995).

YAC End Isolation

YAC ends were isolated from CEPH clones by the insertion of rescue plasmids into the YAC vector (pYAC4) by homologous recombination followed by restriction enzyme digestion, circularization, and transfection into *Escherichia coli* by electroporation (Hermanson et al. 1991). End rescue from the chromosome 9-specific YAC clones involved restriction enzyme digestion, ligation, and transformation (McCormick et al. 1990; Shero et al. 1991). YAC end clones were purified on low melt agarose gels and used as hybridization probes.

Hybridization

Gel-purified YAC DNA, *Alu*-PCR products, and YAC end clones, as well as Cot1 DNA (GIBCO/BRL), and poly (dGT) (Pharmacia), were labeled by random priming (Feinberg and Vogelstein 1984). Where necessary, competition was used to saturate repeat sequences by mixing probes with 5 µg of Cot1 DNA, 2 mg of human placental DNA and 2 µg of vector DNA (pYAC4, pJS97, pJS98 or sCos) and boiling for 10–15 min followed by incubation at 65°C for 3–5 hr before addition to the hybridization buffer. Tri- and tetranucleotide 24-mers were synthesized (GIBCO/BRL) for the most common repeats and then mixed together into a cocktail. Oligonucleotides were end-labeled with T₄ polynucleotide kinase (NEB). All hybridizations were performed in Church and Gilbert buffer (0.1 mM EDTA at pH 8.0, 0.5 M sodium phosphate at pH 7.2, 7% SDS, 1% BSA) at 55°C overnight. Filters were washed and exposed to autoradiographic film, as described previously (Breakefield et al. 1986), except in the case of the end-labeled primers. These filters were washed in 1 × SSC for 15 min at 55°C, repeating as necessary.

Physical Mapping Strategy and Generation of SSR Polymorphisms

Primers corresponding to marker loci *D9S62a*, *D9S62b*, *D9S63*, and *ASS* were used to screen the YAC libraries by PCR. The YACs containing these loci were used to identify overlapping clones by hybridization of *Alu*-PCR products from the individual YAC clones to *Alu*-PCR products from pools of YAC clones representing the libraries. When overlapping clones were not identified, end clones were rescued from the YACs, hybridized back to the existing contig to distinguish the internal and extending YAC ends, and then used to screen

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the cosmid libraries. Positive cosmids were arranged into 96-well microtiter dishes, and colony filters were stamped and prepared as described (Murrell et al. 1995). The cosmid filters were probed with poly(dGT) (Pharmacia) or the tri/tetraoligonucleotide cocktail (GIBCO/BRL) to identify SSR-containing clones. These clones were digested with *Sau3a* (NEB), subcloned into Bluescript (Stratagene), and reprobed with the SSR primers. Positive clones were sequenced using Sequenase (U.S. Biochemical), and oligonucleotide primers were selected that flanked the repeat sequence (Table 1). These SSR polymorphisms were then used to identify new overlapping YAC or cosmid clones by PCR. This combination of procedures was repeated until a complete overlapping clone map was obtained covering the region bearing the *DYT1* gene, as identified by haplotype analysis.

Statistical Analysis

Marker allele frequencies were estimated by simple gene counting in the control sample. The degree of linkage disequilibrium was assessed as described in Risch et al. (1995) employing the parameter δ (Bengtsson and Thomson 1981).

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