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Strong Allelic Association between the Torsion Dystonia Gene (*DYT1*) and Loci on Chromosome 9q34 in Ashkenazi Jews

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Summary

The *DYT1* gene responsible for early-onset, idiopathic torsion dystonia (ITD) in the Ashkenazi Jewish population, as well as in one large non-Jewish family, has been mapped to chromosome 9q32-34. Using (GT)n and RFLP markers in this region, we have identified obligate recombination events in some of these Jewish families, which further delineate the area containing the *DYT1* gene to a 6-cM region bounded by loci *AK1* and *ASS*. In 52 unrelated, affected Ashkenazi Jewish individuals, we have found highly significant linkage disequilibrium between a particular extended haplotype at the *ABL-ASS* loci and the *DYT1* gene. The 4/A12 haplotype for *ABL-ASS* is present on 69% of the disease-bearing chromosomes among affected Jewish individuals and on only 1% of control Jewish chromosomes ($\chi^2 = 91.07$, $P << .001$). The allelic association between this extended haplotype and *DYT1* predicts that these three genes lie within 1–2 cM of each other; on the basis of obligate recombination events, the *DYT1* gene is centromeric to *ASS*. Furthermore, this allelic association supports the idea that a single mutation event is responsible for most hereditary cases of dystonia in the Jewish population. Of the 53 definitely affecteds typed, 13 appear to be sporadic, with no family history of dystonia. However, the proportion of sporadic cases which potentially carry the A12 haplotype at *ASS* (8/13 [62%]) is similar to the proportion of familial cases with A12 (28/40 [70%]). This suggests that many sporadic cases are hereditary, that the disease gene frequency is greater than 1/15,000, and that the penetrance is lower than 30%, as previously estimated in this population. Most affected individuals were heterozygous for the *ABL-ASS* haplotype, a finding supporting autosomal dominant inheritance of the *DYT1* gene. The *ABL-ASS* extended-haplotype status will provide predictive value for carrier status in Jewish individuals. This information can be used for molecular diagnosis, evaluation of subclinical expression of the disease, and elucidation of environmental factors which may modify clinical symptoms.

Introduction

Idiopathic torsion dystonia (ITD) is a neurological disease of unknown etiology and is characterized by sustained muscle contractions causing twisting and repetitive movements of the limbs, trunk, and neck, which can progress to abnormal postures (Fahn et al. 1987). The prevalence of the disease has been estimated at 1/160,000 in the general population (Zeman and Dyken 1967) and is approximately 5–10 times more common in the Ashkenazi Jewish population, where its prevalence has been estimated at 1/23,000–1/15,000 (Eldridge 1970; Zilber et al. 1984). Most forms of inherited dystonias appear to exhibit autosomal dominant transmission (for review, see Muller and Kupke

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1990), although an X-linked recessive form has been described (Lee et al. 1976; Fahn and Moskowitz 1988; Kupke et al. 1990). Early studies suggested an autosomal recessive mode of inheritance in the Jewish population (Eldridge 1970), but recent evidence has supported an autosomal dominant mode of transmission with a penetrance of 30%–40% (Zilber et al. 1984; Bressman et al. 1989; Pauls and Korczyn 1990; Risch et al. 1990).

A gene causing dystonia in two different populations—i.e., one large non-Jewish family (penetrance of 75%) and 12 Ashkenazi Jewish families (penetrance of 30%)—has been mapped to chromosome 9q32-34 by genetic linkage analysis using DNA markers (Ozelius et al. 1989; Kramer et al. 1990). In both cases the disease gene was located in an approximately 30-cM region flanked by marker loci *D9S26* and *D9S10*. Although the penetrance and frequency of the disease gene are different in these two populations, the disease manifestations are very similar, characterized by childhood onset in a limb, with progression to generalized impairment in its most severe expression. It is assumed that the same gene (*DYT1*) is responsible for dystonia in both populations and that either different mutations or modifying factors underlie these two groups' susceptibility to the disease state.

Here we used highly informative (GT)n polymorphisms (Litt and Luty 1989; Weber and May 1989) for several loci which lie between *D9S26* and *D9S10*, including genes for gelsolin (*GSN*) (Kwiatkowski and Perman 1991), the abl oncogene (*ABL*) (Kwiatkowski 1991), and argininosuccinate synthetase (*ASS*) (Kwiatkowski et al 1991*a*), as well as RFLPs for the adenylate kinase-1 gene (*AK1;* Bech-Hansen et al. 1989; Schuback et al. 1991), to identify recombination events in the Jewish families that localize the *DYT1* gene to a less than 6-cM region flanked by the *AK1* and *ASS* loci. We have also evaluated allelic association between *DYT1* and alleles at *AK1, ABL, ASS,* and *D9S10* in the 12 original Ashkenazi families analyzed by Kramer et al. (1990) and at *ABL* and *ASS* in an additional 40 Ashkenazi affecteds and 81 control Ashkenazim. The combined data set yields significant evidence for allelic association between *DYT1* and an extended haplotype at the *ABL-ASS* loci.

Material and Methods

Neurologic Examination and Family Material

A series of 53 Ashkenazi Jewish individuals (from 52 families) affected with classic ITD were ascertained for the present study, from a computerized data-base file of patients followed either by the Movement Disorder Group at Columbia Presbyterian Medical Center (50 families) or through the Dystonia Medical Research Foundation (two families). Clinical and pedigree information on these families has been reported in detail elsewhere (Bressman et al. 1989). The criteria for the diagnosis of primary versus secondary dystonia and the method of evaluation were the same as described elsewhere (Bressman et al. 1989; Kramer et al. 1990). In brief, a neurological history and standardized examination were performed by a neurologist trained in analysis of movement disorders. In 45 of 52 families all members were examined regardless of affected status. In the remaining seven families only the probands were examined, and they were considered as sporadic cases. Video examinations were performed according to a standardized protocol and were reviewed by at least two neurologists who were blind to the status and identity of the subject. A final determination of "definite," "probable" or "possible" dystonia, or "unaffected" was made (Bressman et al. 1989); only those individuals designated as definitely affected were classified as affected in the present study. Forty of these affecteds were from pedigrees with other affected members, and the other 13 appeared to be sporadic, with no family history of dystonia. The mean age at onset of dystonia was 17 years, with a range of 6–66 years. The median age at onset was 12 years.

The criteria for designation as Ashkenazi Jewish were the same as described elsewhere (Bressman et al. 1989). In the present study, 47 of 53 individuals had 100% Ashkenazi Jewish ancestry, three individuals had 3/4 Ashkenazi Jewish grandparents, two had 2/4 Ashkenazi Jewish grandparents, and one had only 1 Ashkenazi Jewish grandparent. In these latter six individuals there was no evidence for inheritance of the dystonia gene through a non-Ashkenazi ancestor.

Unrelated, unaffected individuals with 100% Ashkenazi Jewish ancestry and no family history of dystonia were included as controls. These came from three sources: spouses married into the dystonia families and genetically unrelated to the affecteds (n = 28), staff neurologists (n = 11), and unaffected members of families in which other members were affected with familial dysautonomia (n = 42). This latter disease is a rare, autosomal recessive sensory neuropathy in which all classic cases are of Ashkenazi Jewish descent (Axelrod and Pearson 1984; Maayan et al. 1987). Controls from the dysautonomia families manifested no dystonic symptoms, as evaluated by Dr. Felicia Axelrod (NYU Medical Center). The other control

subjects were evaluated using the same examination procedures as described above for affected individuals, except for nine of the staff neurologists, who were not given neurologic exams.

DNA Methods, Probes, and Polymorphism Analysis

Blood was obtained by venipuncture from consenting family members. DNA was extracted from lymphoblast lines (Anderson and Gusella 1984) or whole blood according to a method described elsewhere (Gusella et al. 1979; Breakefield et al. 1986). Southern blot analysis was carried out using standard techniques. DNA probes were labeled with [^{32}P]dATP (3,000 Ci/mmol; Amersham) by random oligonucleotide priming (Feinberg and Vogelstein 1984) and hybridized to filters as described elsewhere (Ozelius et al. 1989). Six RFLP marker probes on 9q were used: CRI-L659 (*Taq*I) for locus *D9S26* (Donis-Keller et al. 1987), pAK1B3.25 (*Taq*I and *Ban*I) for the *AK1* locus (q32-34) (Bech-Hansen et al. 1989; Schuback et al. 1991), ASSG1 (*Hin*dIII) and ASSG3 (*Pst*I) for the *ASS* locus (q34) (Northrup et al. 1989); pMCT136 (*Pst*I) for VNTR locus *D9S10* (q34) (Lathrop et al. 1988), and EFD126.3 (*Msp*I) for VNTR locus *D9S7* (Nakamura et al. 1987).

Analysis of (GT)n polymorphisms for the *GSN, ABL,* and *ASS* loci was carried out on genomic DNA by using oligonucleotide primer pairs described elsewhere (Kwiatkowski 1991; Kwiatkowski et al. 1991*a;* Kwiatkowski and Perman 1991) and PCR to amplify the repeat-containing region. Reaction volumes were 10 μl and contained 0.2 mM each of dATP, dCTP, and dTTP; 2.5 μM dGTP; 4 ng each oligonucleotide; 0.08 μl ^{32}P dGTP (3,000 mCi/mM); 0.05 μl *Taq* polymerase; and 1 × reaction buffer (Perkin Elmer Cetus). Thermal controller settings were 94°C for 1.5 min; 25 cycles, each of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and 72°C for 10 min. Amplified products were analyzed according to a method described by Kwiatkowski et al. (1991*a*).

Statistical Methods

A common measure of the strength of an association between a disease and a particular marker allele is the odds ratio. An odds ratio significantly greater than 1.0 indicates that the particular allele under consideration is more frequent in patients compared with controls, whereas a ratio less than 1.0 indicates a decreased frequency among patients. The significance of an odds ratio is measured by a χ^2 test. We examined association between the *DYT1* gene and alleles at closely linked loci according to the methods that Fleiss (1981) and Thomson (1981) described for calculating odds ratios. We considered one system at each of four marker loci: the (GT)n polymorphisms at *ASS* and *ABL* and the RFLPs at *AK1* and *D9S10.* Our initial sample comprised 13 affected individuals and 16 unrelated, normal Ashkenazi individuals from the original families on which our linkage studies were based (Kramer et al. 1990). This odds-ratio analysis does not require knowledge of linkage phase for affected individuals and is thus a preliminary analysis of allelic association, because normal chromosomes are combined with disease allele–bearing chromosomes among the affected group. Sample composition for the four markers is given in table 1.

More rigorous allelic-association analysis according to methods described by Chakravarti et al. (1984) was done with those markers for which the odds ratio suggested evidence of an association with *DYT1.* We considered the (GT)n polymorphism at *ABL* and a three-system haplotype at *ASS;* specifically, eight alleles are defined by the (GT)n, and two alleles are defined by each of the two RFLPs, for a total of 32 possible haplotypes at *ASS.* Including both the 13 affected and the 16 normal cases from above, our sample contained a total of 53 affected individuals and 81 controls. For this analysis, alleles (or haplotypes, as for *ASS*) at linked markers on disease-bearing chromosomes from affected individuals were compared with those on nondisease chromosomes from controls. Among the 53 affecteds, we were able to unambiguously deduce disease-bearing chromosome in 26 individuals. Among controls, we could haplotype 73 individuals. Thus, the analysis for *ASS* is based on a sample of 26 disease-bearing chromosomes and 146 control chromosomes. Similar samples were analyzed for *ABL* and for the extended *ABL-ASS* haplotype (table 2).

Table 1

Sample Composition for Preliminary Association Analysis between *DYT1* and Closely Linked Marker Loci

	Marker			
	ABL	*ASS*	*AK1*	*D9S10*
No. of affected individuals	13	13	13	13
No. of control individuals	16	16	12	11

NOTE.—The number of chromosomes on which the analysis is based is twice the number of individuals.

Table 2

Allelic Association Summary

Locus	Associated Allele	% of Affected Chromosome[a]	% of Control Chromosome[a]	χ^2	Relative LD (D')
ASS	A12	69% (26)	1.4% (146)	92.53 ($P < .001$)	.688
ABL	4	92% (26)	63% (162)	7.44 ($P < .01$)	.792
ASS/ABL ..	4A12	69% (26)	.75% (134)	91.07 ($P < .001$)	.686

[a] Numbers in parentheses are number of chromosomes analyzed.

Marker-allele frequencies were estimated by simple gene counting in the control sample. The presence of significant allelic association was tested using a χ^2 test of homogeneity (described in Chakravarti et al. 1984 and applied in Kerem et al. 1989). The degree of association was measured by relative linkage disequilibrium, or D', described by Thomson (1981) and Chakravarti et al. (1984).

A panel of 62 Venezuelan reference pedigrees (Haines et al. 1990) containing over 700 individuals was used to construct a linkage map of the critical area on chromosome 9q32-34 containing the markers *D9S26* (L659), *GSN, AK1, ABL, ASS, D9S10* (MCT136), and *D9S7* (EFD126.3) (Ozelius et al. 1991). All data were entered into a computer file by using the LIPIN data-management program (Troffater et al. 1986). Analysis was performed using the MAPMAKER program (version 1.0) (Lander et al. 1987) by employing the strategy described by Haines et al. (1990).

Results

Crossover Events

The order of markers in the q32-q34 region of chromosome 9 is shown in figure 1. The chromosomal order as well as the genetic distance between markers was determined using 62 Venezuelan reference pedigrees. Loci were placed at the given positions with odds of at least 1,000:1 over any other position in the map (Ozelius et al., in press). The order for *ABL* and *ASS* could not be determined because no recombinations were observed between these markers in this data set. (GT)n polymorphisms at the *GSN, ABL,* and *ASS* loci, as well as RFLPs at *AK1* and at *D9S10,* were used to score obligate recombination events between these loci and the *DYT1* gene in affected individuals in Ashkenazi Jewish families. In the 12 families described elsewhere (Kramer et al. 1990), we observed five, one, zero, and two obligate crossovers with *GSN, AK1, ABL,* and *D9S10,* respectively, as well as a probable crossover at *ASS.* Two-point lod scores and multipoint analysis presented by Kwiatkowski et al. (1991*b*) support these results, showing that the most likely position for the *DYT1* gene is midway between *AK1* and *ASS,* with a lod score of 4.43 at a recombination fraction of 0.

The top panel of figure 2 shows a Jewish pedigree in which several recombination events have occurred. The most relevant event for determining the position of the disease gene is in individual II-3. This cross occurs between *AK1* and *ASS* on the disease chromosome (the *ABL* polymorphism is uninformative), and it places the disease gene distal to *AK1.* The bottom panel of figure 2 shows a "probable" crossover event which places the disease gene proximal to *ASS.* In this case, affected members III-2 and III-4 do not share a common haplotype at the *ASS* locus (again the *ABL* polymorphism is uninformative). The cross itself appears to have occurred in an individual two or three generations preceding the affected individuals. It cannot be ruled out that two different disease genes, associated with different *ASS* haplotypes (A12 and C10), have entered from two different branches of the family and that no crossover occurred in the family. However, this seems unlikely, both because there is no fam-

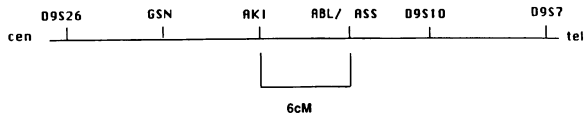

Figure 1 Genetic map of chromosome 9q in region surrounding *DYT1* gene. The order and distance of marker loci were determined using 62 Venezuelan reference pedigrees. The map was generated using the computer program MAPMAKER (version 1.0) with each marker placed on the map with odds of at least 1,000:1 over any other position on the map (Ozelius et al. 1991).

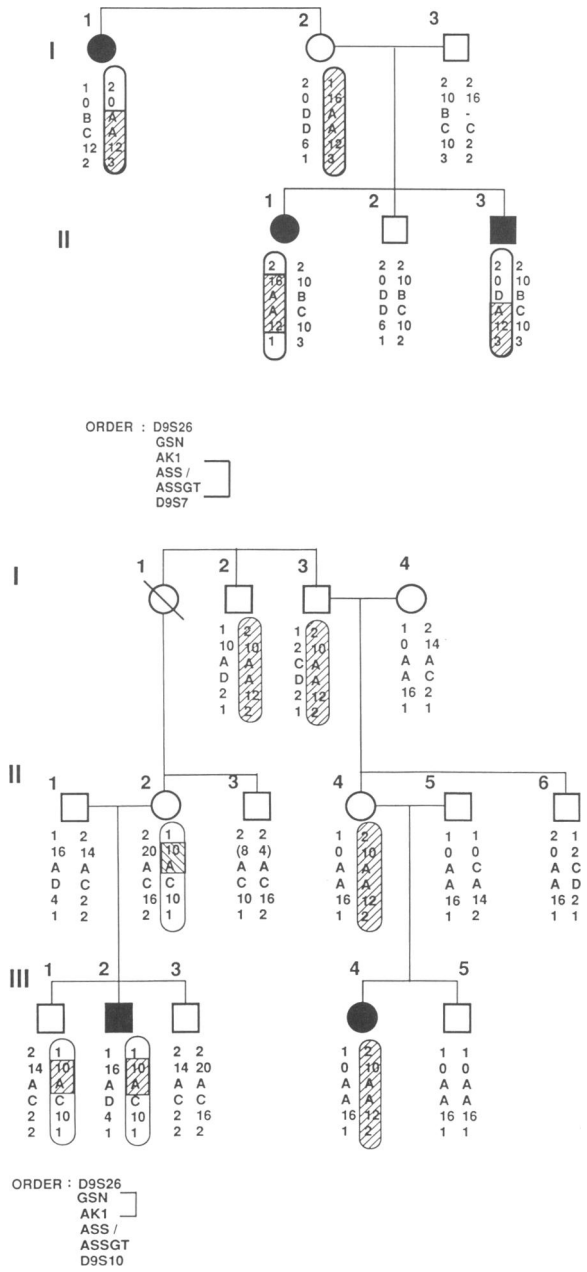

Figure 2 Segregation of alleles in 9q34 region in affected families. The order of marker loci is given in the lower left of each panel, from centromeric side to telomeric side (top to bottom). Alleles for these loci were phased by following their inheritance in these families. A dash or parentheses indicates that the allele status was not clear or unphasable, respectively. The disease-bearing chromosomes are circled and indicated by diagonal stripes. The top panel shows that the only allele shared by all four obligate gene carriers in this family is the "A12" haplotype of *ASS*. The bottom panel shows that only two alleles—the "10" allele of *GSN* and the "A" allele of *AK1*—are shared among five obligate gene carriers in this family.

ily history of dystonia in any of the married-in individuals in this family and because dystonia is a rare disease. In the Venezuelan pedigrees, the distance between *AK1* and *ASS* was calculated to be 6 cM (fig. 1); the *DYT1* gene thus lies within this interval.

Allelic Association

We calculated odds ratios as preliminary measures of association between DYT1 and four closely linked markers: *ABL, ASS, AK1,* and *D9S10.* As described in the Statistical Methods section above, for the initial sample we selected one affected individual (generally the proband) from each of the 12 original Jewish families on which our linkage results were based (Kramer et al. 1990). However, in the family which represents a probable crossover event between *ASS* and *DYT1* (fig. 2, *bottom*) we included both affected members (III-2 and III-4). This resulted in a sample of 13 affected individuals. The control sample consisted of 16 Ashkenazim from the original families, who were genetically unrelated either to any affected members or to each other (table 1). Of the 26 *ASS* (GT)n alleles represented among the affected individuals, 12 were allele 12 (106 bp using primers described in Kwiatkowski et al. 1991*a*), whereas in controls only 2 of the 32 *ASS* alleles were 12. The odds ratio is 12.86 (χ^2 = 10.39, $P < .008$). Of the 26 *ABL* alleles among affected individuals, 22 were allele 4 (141 bp using primers in Kwiatkowski 1991), compared with 19 of the 32 control alleles. The corresponding odds ratio is 3.76 (χ^2 = 3.28, $.10 < P < .05$). There was no evidence for an association between *DYT1* and any of the alleles at *AK1* or *D9S10;* the highest χ^2 values obtained were .73 and 1.0, respectively.

The nonrandom association between *DYT1* and alleles at *ASS* and *ABL* warranted collection of additional families. In all, 53 affected individuals (from 52 unrelated families) and 81 controls were typed both for the three polymorphisms at *ASS* and for the (GT)n polymorphism at *ABL.* Of the 53 affected individuals, 38 could be haplotyped at *ASS;* of these, the disease-bearing chromosome could be identified in 26. Of the 81 control individuals, 73 could be haplotyped at *ASS.* Thus, for this analysis the sample consisted of 26 disease-bearing chromosomes and 146 control chromosomes.

The distribution of *ASS* haplotypes in control and affected chromosomes is illustrated in figure 3. Of the 32 possible *ASS* haplotypes, 20 occurred in normal and/or affected individuals. Twenty separate tests

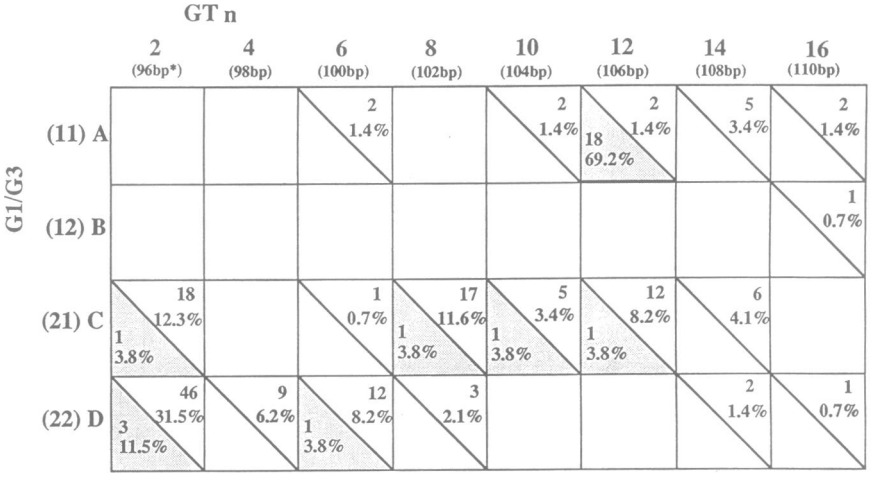

Figure 3 Frequency of *ASS* alleles in control and disease-bearing chromosomes. Haplotype status for *ASS* was determined using both the (GT)n alleles (2–16, horizontal dimension) and two RFLPs (alleles A–D, vertical dimension; length of the product is as amplified by ASS-A and ASS-C primers from Kwiatkowski et al. 1991*a*). A total of 146 control chromosomes (2 from each of 73 individuals) and 26 disease-bearing chromosomes (1 from each of 26 individuals) were evaluated. The number of chromosomes bearing a particular allele is indicated in the boxes, with the % of that group (controls are in the upper right half of each box; and disease-bearing individuals are in the shaded, lower left half of each box).

were carried out to compare the number of individuals having haplotype X with the number of individuals having haplotype not-X, in both control and affected groups. Each *P* value was multiplied by 20 to account for all the tests. The comparison which contributed most significantly to the χ^2 value was that for haplotype A12. Among the affecteds, 69% (18 of 26 chromosomes) bore the A12 haplotype, whereas only 1.4% of control chromosomes had this haplotype; this is remarkably significant, as indicated by $\chi^2 = 92.53$, uncorrected $P < 10^{-21}$, and corrected $P < 10^{-19}$ (table 2). The relative linkage disequilibrium, or D', is equal to .688. Figure 3 clearly indicates that the excess of the A12 haplotype in affecteds corresponds most notably to a relative decrease in the number of D2 haplotypes compared with that in controls.

For *ABL* we could identify the disease-bearing chromosome in 26 of the original 53 affected individuals. The control group consisted of 81 individuals (162 chromosomes). Six alleles occurred in the control and affected individuals. As for the *ASS* analysis described above, six separate tests were performed for allele X versus allele not-X, in affecteds and controls, and *P* values were multiplied by 6 to account for all these tests. The major contribution to this χ^2 analysis was made by allele 4. Of the 26 affected chromosomes, 24 (92%) carried the 4 allele; of the 162 control chromosomes, 102 (63%) carried the 4 allele; the χ^2 value for the difference between affected and controls is 7.44, uncorrected $P = .003$, corrected $P = .01$, and $D' = .792$ (table 2). This is not as significant as the results for *ASS;* however, this is to be expected, since the 12 allele at *ASS* is very rare, whereas the 4 allele at *ABL* is very common in the Ashkenazi population. This could also indicate either that *ABL* is farther from *DYT1* than is *ASS* or that a recombination event took place shortly after the mutation occurred.

We then constructed haplotypes with both *ABL* and *ASS* markers and examined allelic association between *DYT1* and the 4/A12 haplotype. Of those chromosomes for which linkage phase could be determined, 69% (18 of 26) of affecteds carried the 4/A12 haplotype; only 0.75% (1 of 134) of controls carried the 4/A12. These gave values of $\chi^2 = 91.07$, uncorrected $P < 10^{-21}$, corrected $P < 10^{-19}$, and $D' = 0.686$ (corrected for multiple tests) (table 2).

Sporadic ITD

Of the 53 affecteds who were typed, 13 were classified as sporadic cases with no family history of dystonia. These were collected as singleton cases, and therefore phase could not be determined. In the sporadic cases we were also unable to determine the disease-bearing chromosome; however, we were able to tabulate the number of cases who potentially had an A12 haplotype at the *ASS* locus on one of their chromo-

somes, versus a non-A12 haplotype on either chromosome, and to compare this number with that in the definitely familial group. Sixty-two percent (8 of 13) of the sporadics could carry an A12 allele on one chromosome, while 38% (5 of 13) definitely did not carry an A12 on either chromosome (i.e., they were non-A12) (Table 3). This compares with 70% (28 of 40) A12 and 30% (12 of 40) non-A12 in the familial group. The numbers are not significantly different, suggesting that most sporadic cases are probably hereditary.

Discussion

We have identified recombination events in Ashkenazi Jewish families which confirm and extend the linkage data reported elsewhere (Ozelius et al. 1989; Kramer et al. 1990). These crossover events place the *DYT1* gene within a 6-cM region flanked by *AK1* and *ASS*. We also report strong linkage disequilibrium with a particular extended haplotype based on the *ABL* and *ASS* markers ($\chi^2 = 91.07$). These results suggest that the *DYT1* gene is closer to the *ASS* end of this 6-cM interval (i.e., is more telomeric). In light of linkage-disequilibrium results in cystic fibrosis (Kerem et al. 1989) and Friedreich ataxia (Fujita et al. 1990), this would suggest that the *DYT1* gene is within 500 kb–1 Mb of the *ABL* and *ASS* loci. However, the distance between the disease gene and these marker loci may be physically larger, because the *DYT1* mutation may not be as old as the most common mutations for cystic fibrosis or Friedreich ataxia. The very likely recombination (Fig. 2, *bottom*) between *ASS* and the disease gene would support this idea, as would the fact that no linkage disequilibrium is seen between the *ABL* and *ASS* polymorphisms on control Jewish chromosomes. In order to be associated with a particular haplotype (4/A12), the most common *DYT1* mutation in this population must have occurred more recently than the polymorphisms at the *ABL* and *ASS* loci, indicating that its high degree of association with the marker loci may be due in part to the relatively recent occurrence or introduction of the mutation in the Ashkenazi Jewish population. Together, these data suggest that the distance between the *ABL-ASS* loci and the *DYT1* gene may be more than 1 Mb. Several non-Jewish populations appear to have a dystonia gene in this same region of chromosome 9q34 (Ozelius et al. 1989; L. J. Ozelius, unpublished data) but do not share this haplotype (4/A12) on their disease-bearing chromosomes. This supports the idea that different mutations at the same gene are responsible for *DYT1* in the Jewish and non-Jewish populations.

We eliminated several individuals from the linkage-disequilibrium analysis because either the phase at their *ASS* haplotype (16 of 53 affecteds and 8 of 81 controls) or the disease-bearing chromosome (11 of 53 affecteds) could not be determined. The ambiguity in phasing derived from those individuals who were heterozygous for both the *Hin*dIII and *Pst*I RFLPs at *ASS;* that is, it was not clear whether these individuals were in coupling or repulsion phase. Some concern may arise from the potential bias in our allelic-association results that is due to eliminating these individuals. In fact, only one of the eight "unphaseable" controls could have been A12, i.e., was heterozygous at both RFLPs and carried at least one 12 allele at the (GT)n polymorphism. On the other hand, 10 of the 16 "unphaseable" affecteds could have been A12. Of the 11 for which the disease-bearing chromosome could not be determined, six definitely had the A12 haplotype on one of their chromosomes. Even if the one control is actually A12, and even if none of the affecteds are A12 on their disease-bearing chromosome, the allelic association between *DYT1* and the A12 haplotype would still be highly significant ($\chi^2 = 39.59$, $P < .008$).

Haplotypes at *ABL-ASS* could be determined for 26 disease-bearing chromosomes (fig. 3); of these, 18 were 4/A12, while the other eight had various allele combinations. With the exception of the haplotypes 6/C8 and 12/D2, all the non-4/A12 haplotypes can be derived from single crossover events between *ABL* and relatively common *ASS* haplotypes in the control population. However, because the numbers are so small, and because the *ABL* 4 allele is so common in the population, it is difficult to know whether these non-4/A12 haplotypes represent different mutations or crossover events. The possibility that some of the

Table 3

Associated ASS Haplotypes in Sporadic and Familial Cases

	Sporadic	Familial
Potential A12	62% (13)	70% (40)
Non-A12	38% (13)	30% (40)

NOTE.—Numbers in parentheses are number of affecteds in each group.

non-4/A12 haplotypes are different mutations will be investigated by looking at various phenotypic and demographic differences between the 4/A12 individuals and the non-4/A12 cases. Site of onset, physical distribution of symptoms (i.e., focal, segmental, and generalized), geographic origin, and age at onset will be used to assess potential genetic heterogeneity. If, in fact, the non-4/A12 haplotypes represent crossover events, then examination of these chromosomes by additional markers in the region should further pinpoint the disease gene within this region.

The percentage of sporadic cases which appear to have an A12 haplotype is not significantly different from the percentage of familial cases with this haplotype. This suggests that most sporadic cases actually have heritable dystonia with a lack of family history, because of low penetrance (probably less than 30%) in this population and variable expressivity, whereby mild signs may go undiagnosed. This finding lends support to the idea that most cases of dystonia in the Ashkenazi Jews have arisen from a single mutation, as was predicted by a segregation-analysis study done on this population (Risch et al. 1990). It also suggests that in this population the gene frequency is at least twofold higher than previously thought and that the penetrance is lower. The haplotype data at *ASS* also confirm the conclusion made from segregation analyses—i.e., that ITD is inherited as an autosomal dominant trait in the Ashkenazi Jewish population (Zilber et al. 1984; Bressman et al. 1989; Pauls and Korczyn 1990; Risch et al. 1990). Of the 53 affected individuals typed at *ASS*, 48 (90.6%) were heterozygous for this haplotype, while five (9.4%) were homozygous. This result is consistent with dominant inheritance of the disease gene and strongly rejects the possibility of recessive inheritance, which would have yielded far more homozygotes.

The predictive value that the *ABL-ASS* haplotype has for the disease state will not only be helpful for molecular diagnosis and genetic counseling but also will allow a better clinical definition of the ITD phenotype. By consensus, overt twisting movements or postures are accepted as part of the dystonia spectrum (Fahn et al. 1987). But clinical observations in "obligate carriers" have led to the consideration of certain common nondystonic features—such as tremor (Zilber et al. 1984; Fletcher et al. 1990) and stuttering (Zilber et al. 1984)—as *formes frustes* or mild expression of the dystonia gene. We can now identify carriers of the *DYT1* gene in some Jewish families and can evaluate the incidence of mild neurologic symptoms in them. This analysis can be extended to examine environmental factors which may modify clinical symptoms.

This finding of linkage disequilibrium between the *DYT1* gene and the *ABL-ASS* extended haplotype has allowed us to narrow the region containing the disease gene, from 11 cM (Kwiatkowski et al. 1991*b*) to 1–2 cM. We are generating more markers in this region by walking from the ends of the *ABL* and *ASS* loci. We plan to assess linkage disequilibrium between these markers and the disease gene by finding (GT)n polymorphisms for each new marker. The information presented above is consistent with a single mutation causing most cases of dystonia in the Ashkenazi Jewish population. The non-A12 haplotypes seen in some affected may either represent evolutionary recombinations between the disease gene and the background haplotype (4/A12) on which the original mutation occurred or indicate other mutations. An extended haplotype encompassing additional markers in this region will enable us to locate historical recombination events within this region and thus will allow more precise localization of the *DYT1* gene, as well as some idea of when the mutation occurred in history.

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