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The Ataxia-Telangiectasia Gene (ATA) on Chromosome 11 Is Distinct from the ETS-1 Gene

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Summary

We have studied the segregation of an RFLP detected with a human ETS-1 genomic probe in 25 families containing members affected with ataxia-telangiectasia (AT) and in 27 families from the Centre d'Etude du Polymorphisme Humain (CEPH) panel. We have recently mapped a gene for AT to 11q22-23 by linkage to the markers THY1 and D11S144. Multipoint linkage analysis of the CEPH families indicated that ETS-1 is located on chromosome 11q approximately 19.2 centimorgans telomeric to THY1. Analysis of the segregation of ETS-1 alleles in AT families yields strongly negative LOD scores, excluding an AT gene from a region extending 15 cM to either side of ETS-1. Multipoint mapping of ETS-1, D11S144, THY1, and AT also excludes the possibility that an AT gene is telomeric to ETS-1.

Introduction

Ataxia-telangiectasia (AT) is a rare human autosomal recessive disease characterized by cerebellar ataxia, hypersensitivity to ionizing radiation, chromosomal instability, immunodeficiency, and a greatly elevated incidence of cancer, particularly of the lymphoid system, in affected individuals (Boder 1985). Heterozygotes also have an increased cancer incidence (Swift et al. 1987; Pippard et al. 1988), and their cultured fibroblasts display a sensitivity to radiation that is intermediate between that of normal and affected individuals (Paterson 1985). Heterodikaryons formed through the fusion of fibroblasts from unrelated affected individuals sometimes show normal radiation sensitivity. This observation permits the subdivision of AT patients into four complementation groups: A (55% of cases), C (28% of cases), D (14% of cases), and E (3% of cases) (Jaspers et al. 1989). Some variant groups (V1 and V2) share only subsets of the clinical features of AT (Curry et al. 1989).

We have recently mapped an AT gene, corresponding to at least complementation group A (ATA), to the chromosome region 11q22-23 (Gatti et al. 1988). Significant LOD scores were observed between AT and two markers in this region, THY1 and D11S144 (pYNB3.12), in a set of 31 AT families with one or more affected members. THY1 and D11S144 map approximately 10 centimorgans (cM) apart on chromosome 11, with D11S144 centromeric (Charmley et al., in press). Three-point mapping of the ATA gene relative to these two markers indicates that ATA is unlikely to lie between them (odds of 600:1 against that location) (Gatti et al. 1988). However, the data are insufficient to determine whether ATA is telomeric to THY1 or centromeric to D11S144. Since these markers lie a substantial distance apart on the chromosome 11 map, it is important to resolve this ambiguity.

By in situ hybridization and analysis of somatic cell hybrids containing recombinant 11 chromosomes, the human ETS-1 gene, a marker that could potentially help settle this issue, has been localized to the chromosome region 11q23-24 (de Taisne et al. 1984; Diaz et al. 1986; Griffin et al. 1986; Sacchi et al. 1986*b*; Morris et al. 1988; Budarf et al. 1989; Kennedy et al. 1989; Yunis

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et al. 1989). The human ETS-1 gene was originally identified because of its homology to the 5′ end of the *v-ets*-transforming sequence present in the avian erythroblastosis virus E26 (Watson et al. 1985). The human ETS-1 gene encodes a protein of 51 kD which is expressed at high levels in B and T lymphocytes but not in cells of the myeloid or erythroid lineages (Chen 1985; Fujiwara et al. 1988). Given the susceptibility to lymphoid cancers in AT patients, ETS-1, a human homologue of the v-ets oncogene located in the 11q23 region and known to be expressed in T and B cells, is a viable candidate gene for AT as well as a potentially useful marker for further fine mapping of AT.

We have determined the segregation of an ETS-1 RFLP in families from the Centre d'Etude du Polymorphisme Humain (CEPH) collection of pedigrees (Dausset 1986). This has allowed us to map ETS-1 relative to other markers on the primary linkage map of 11q22-23 that we have previously linked to AT (Charmley et al., in press). We have further analyzed the segregation of this ETS-1 RFLP in a set of 34 AT families. These studies exclude an AT gene from a region of 30 cM surrounding the ETS-1 locus and strongly suggest that ATA is centromeric to ETS-1.

Material and Methods

DNA Markers

The probe pHE 5.4, obtained from the American Type Culture Collection, contains a 5.4-kb genomic *Eco*RI fragment derived from the ETS-1 gene. This insert was isolated by *Eco*RI digestion and gel purification.

Previous genotype data for the markers STMY (stromelysin), CD3, D11S132, D11S144 (MCT128.1 or pYNB3.12), D11S29, D11S133, D11S138, THY1, and D11S83 are described by Charmley et al. (in press). In these data, genotypes for RFLPs detected with the CD3 epsilon, gamma, and delta genes were combined into single haplotypes. Similarly, genotypes for D11S133 and D11S138 were combined, and the new hybrid locus is referred to as S133/S138 hereafter. Note also that the probe pYNB3.12 (D11S148), originally reported as linked to ATA (Gatti et al. 1988), is identical to the probe MCT128.1 (D11S144) and is referred to as D11S144 throughout the present report.

Southern Blots

Five-microgram aliquots of genomic DNA were digested with either a 3–5-fold excess of *Sac*I or a 10–12-fold excess of *Xba*I in buffer and temperature conditions specified by the manufacturers, separated on 1% agarose gels, and transferred to either Zeta-probe (Bio-Rad) or Biotrace (Gelman Scientific) nylon membranes by vacuum blotting in 0.4 N NaOH. DNA probes were labeled with ^{32}P-dNTPs by the random priming method (Feinberg and Vogelstein 1984). Hybridizations were carried out in plastic boxes on a rocker platform in a 42°C incubator in the hybridization solution described by Gatti et al. (1984). Final stringency washes were in 0.1 × SSC/0.1% SDS at 65°C for 30 min.

Families

The majority of AT families studied here have been described elsewhere (Gatti et al. 1988). They include nine American families of varied ethnic origin designated AT#, two Italian families designated IAT#, and 14 Turkish families designated TAT#. All of the Turkish families, as well as the American Amish family AT012, have inbred affected members. Families AT006, AT010, AT021, TAT05, and TAT13-18 have only one affected member. All other families have multiple affected siblings. In most cases, clinical diagnoses of AT were confirmed by demonstration of radiation-resistant DNA synthesis (Young and Painter 1989). High-molecular-weight DNA derived from the members of 40 well-characterized normal pedigrees were provided by CEPH (Dausset 1986).

Linkage Analysis

We assessed the extent of linkage disequilibrium between *Sac*I and *Xba*I alleles at the ETS-1 locus by a χ^2 comparison of observed haplotype frequencies with expected frequencies under linkage equilibrium.

The ordering of marker loci other than ETS-1 in the 11q22-23 region is described by Charmley et al. (in press). The linkage calculations carried out there and in the present report have all relied on the computer program MENDEL (Lange et al. 1988). MENDEL is fully able to account for the high degree of inbreeding occurring in the majority of the AT families studied. However, to increase computational efficiency, only two- and three-point linkage calculations were undertaken in the AT families. In the CEPH families it was possible to perform multipoint mapping with many more markers.

Results

We screened six unrelated individuals with the ETS-1 probe pHE 5.4. Their genomic DNA was cut with 12 different restriction enzymes—*Ava*I, *Bgl*I, *Bgl*II,

*Bst*EII, *Eco*RV, *Hin*dIII, *Kpn*I, *Msp*I, *Rsa*I, *Sac*I, *Taq*I, and *Xba*I—to test for the presence of RFLP. We observed a previously reported *Xba*I RFLP (Savage et al. 1987) and an RFLP with *Sac*I (an isoschizomer of *Sst*I). The restriction-fragment lengths for *Sac*I did not correspond to those for either of two *Sst*I RFLPs reported elsewhere (Sacchi et al. 1986*a*; Kerckaert et al. 1987). Examples of the segregation of the *Xba*I and *Sac*I alleles are shown in figure 1.

Table 1 lists the allelic frequencies and observed heterozygosity for the *Sac*I and *Xba*I RFLPs detected with pHE 5.4. These were established by probing blots containing DNAs from the unrelated parents of the CEPH families. We inferred haplotypes for these parents by inspecting the genotypes of the children and grandparents. The χ^2 statistic for independence of the two different RFLPs provided no evidence for significant linkage disequilibrium between alleles at the two loci (χ^2 = 1.04 with 1 df).

Since the *Sac*I RFLP was substantially more informative than the *Xba*I RFLP, it was further typed in all informative CEPH pedigrees (27 of 40 families). *Sac*I genotypes collected from this analysis were used in multipoint mapping to determine the most likely location of ETS-1 relative to other markers (particularly THY1 and D11S144) on a provisional linkage map of the 11q22-23 region (Charmley et al., in press). Figure 2 shows the most likely location of ETS-1 relative to the markers D11S144, THY1, S133/S138, and D11S83, with the odds against alternative locations noted. In this most likely order, ETS-1 is approximately 19 cM telomeric to THY1.

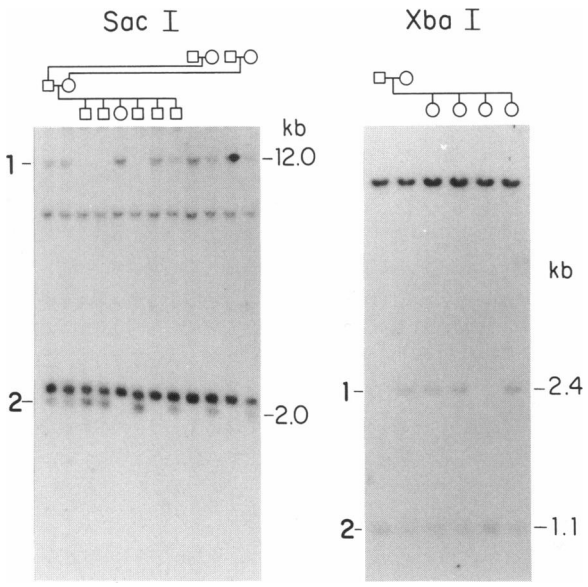

Figure 1 Hybridization patterns of ETS-1 probe pHE5.4 in *Sac*I- and *Xba*I-cut DNA. High-molecular-weight DNA from two different families was digested with *Sac*I and with *Xba*I, separated by gel electrophoresis, transferred to nylon membranes, and probed with pHE5.4 as described in Material and Methods. Family relationships are indicated at the tops of the gels. Alleles are indicated by numbers on the left, and their respective sizes (in kilobases) are indicated on the right.

Table 1

Allelic Frequencies and Observed Heterozygosity for *ETS-1* RFLPS

Enzyme	Allele (kb)	Frequency	Heterozygosity
*Xba*I.....	2.4	.096	.17
	1.1	.904	
*Sac*I	12.0	.66	.42
	2.0	.34	

The parents of affected children in 34 previously studied AT families were screened for the presence of the ETS-1/*Sac*I polymorphism. Twenty-five of these families were informative, and genotype information was collected on all available members. Table 2 indicates the LOD scores, by family, for AT and ETS-1/*Sac*I. Significantly negative LOD scores were observed in group A families alone, excluding close linkage between ATA and ETS-1. LOD scores for AT and ETS-1 in group C families alone were not of sufficient significance to draw any conclusions regarding the relationship of ATC and ETS-1. When data from all families was pooled without regard to complementation group, a LOD score of −2 excluded AT from a region of approximately 15 cM on either side of the ETS-1 locus.

The negative LOD scores for linkage between AT and ETS-1 observed in our set of AT families strongly suggested that AT is centromeric to ETS-1. To test this hypothesis, we carried out multipoint mapping of AT, ETS-1, and the previously linked markers D11S144 and THY1. Unfortunately, the absence of key family founders and the high degree of consanguinity in the Turkish AT families (families designated TAT in table 2) precluded multipoint mapping with more than three loci. Therefore, our multipoint calculations were carried out with AT, ETS-1, and either D11S144 or THY1 (but not both). The ETS-1-informative CEPH families were included in this analysis, with all individuals designated as normal homozygotes at the AT loci. These additional families provided more certainty about the

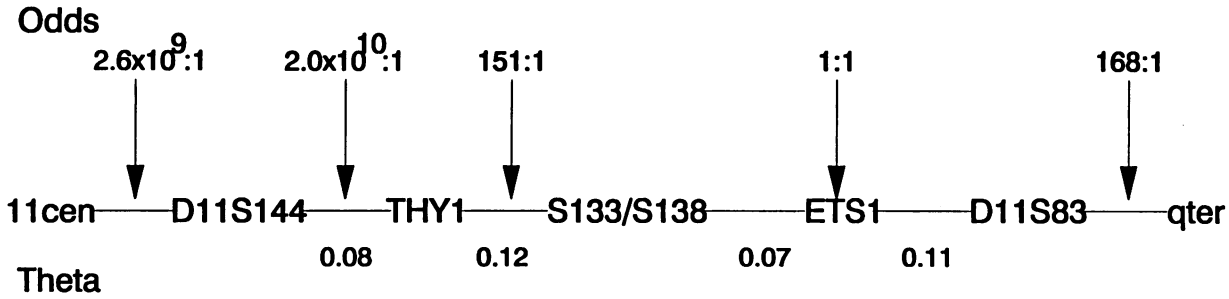

Figure 2 Maximum-likelihood map of ETS-1 vs. markers on 11q23, with odds against alternative orders. Five-point linkage maps were calculated with the order of the markers D11S144, THY1, S133/S138, and D11S83 as indicated and with the five alternative locations for ETS-1 (indicated by arrows). The most favored order is shown. Numbers in the gaps below the markers indicate maximum-likelihood estimates of the recombination fractions between adjacent markers. Values above individual arrows indicate the odds against ETS-1 being located in the associated gaps.

Table 2

Lod Scores for AT versus ETS-1

	Recombination Fraction					
Family	.00001	.05	.10	.20	.30	.40
Group A:						
AT010	.125	.104	.084	.049	.023	.006
AT012	.408	.452	.421	.284	.126	.010
TAT03	−4.508	−.809	−.513	−.232	−.093	−.022
Subtotal	−3.975	−.253	−.008	.101	.056	−.006
Non-group A:						
AT001[C]	−.176	−.107	−.064	−.019	−.004	−.000
AT007[C]	.125	.104	.084	.049	.023	.006
ATV20[V1]	−.176	−.137	−.140	−.056	−.024	−.006
Subtotal	−.227	−.104	−.084	−.026	−.005	.000
Unassigned:						
AT006	−.176	−.137	−.104	−.056	−.024	−.006
AT009	−4.398	−.721	−.444	−.194	−.076	−.018
AT017	−3.796	−.665	−.374	−.114	−.005	.026
AT021	−.102	−.082	−.064	−.035	−.016	−.004
IAT01	−.090	−.070	−.053	−.028	−.012	−.003
IAT04	−.051	−.041	−.032	−.018	−.008	−.002
TAT02	−1.569	−.535	−.287	−.079	−.004	.013
TAT04	−.173	−.126	−.091	−.043	−.016	−.003
TAT05	−.064	−.047	−.034	−.017	−.007	−.002
TAT08	−4.350	−.706	−.452	−.225	−.106	−.035
TAT10	−4.449	−.762	−.476	−.212	−.084	−.020
TAT13	−4.144	−.537	−.317	−.146	−.064	−.017
TAT14	−1.833	−.574	−.337	−.142	−.058	−.017
TAT15	.094	.074	.056	.029	.012	.002
TAT16	−1.110	−.505	−.303	−.126	−.051	−.016
TAT17	−1.344	−.488	−.276	−.11	−.049	−.022
TAT18	.427	.319	.224	.089	.024	.003
TAT19	−.917	−.352	−.185	−.063	−.022	−.005
TAT20	.613	.529	.447	.293	.162	.064
Subtotal	−27.432	−5.426	−3.102	−1.197	−.404	−.062
Total	−31.634	−5.819	−3.194	−1.122	−.353	−.068

distance separating the two nondisease markers. Maps generated with THY1 or with D11S144 (data not shown) were not markedly different in terms of either marker order or relative odds. Table 3 shows the most likely order of AT, THY1, and ETS-1, with maximum-likelihood ratios for alternative orders. These three-point maps exclude AT from the region telomeric to ETS-1 by odds of greater than 4,000:1.

Discussion

The long arm of human chromosome 11 is rich in potential candidates for the gene responsible for AT of complementation group A (Gatti et al. 1988; Peterson and Funkhouser 1989). In the present study, we have tested for linkage between one such gene—the human ETS-1 gene—and AT by following the segregation of an *Sac*I RFLP identified by the genomic ETS-1 probe pHE5.4 in a set of 34 affected pedigrees. Strongly negative LOD scores were observed with the ETS-1 probe whether analyzed in group A families alone (an exclusion interval of 6 cM) or in families pooled over all complementation groups (an exclusion interval of 30 cM).

While the size of this latter exclusion interval could, in part, reflect genetic heterogeneity within the population of unassigned families, it is worth noting that consistently positive LOD scores occur for the same set of families and the markers THY1 and D11S144 (Gatti et al. 1988). Furthermore, recent linkage studies utilizing D11S144 in a large group C AT pedigree suggest that the gene for AT of complementation group C (ATC) may also map to 11q22-23 (Shiloh et al. 1989). If such clustering of the ATA and ATC genes is borne out by additional marker studies, then more than 80% of all AT families could be expected to show linkage to markers in the 11q22-23 region. Our limited sample of known group C families was insufficient to draw any firm conclusions regarding the relationship of ATC and ETS-1, although the strongly negative LOD scores seen with ETS-1 in the unassigned families provided no evidence for linkage between ATC and ETS-1.

One important unresolved issue in the localization of the ATA gene is the question of whether it is located centromeric to D11S144 or telomeric to THY1. We attempted to resolve this issue by multipoint mapping of AT relative to D11S144, THY1, and ETS-1. Unfortunately, our data were insufficient to determine convincingly whether ATA is between D11S144 and THY1 or centromeric to D11S144. We did, however, find compelling evidence that ATA is centromeric to ETS-1. Future studies of AT families with other markers in the 11q22-23 region should allow us to develop a finer localization of the ATA gene.

Table 3

Maximum-Likelihood Odds Ratios for Various Gene Orders, as Determined by Multipoint Analysis

Order	Odds Ratio
11CEN–AT–THY1–ETS-1	1:1
11CEN–THY1–AT–ETS-1	5.3:1
11CEN–THY1–ETS-1–AT	4,300:1

NOTE.—Odds ratios are expressed as the odds against a given order, relative to the most preferred order.

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