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HLA class II haplotype and sequence analysis support a role for *DQ* in narcolepsy

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Abstract A systematic haplotype and sequencing analysis of the *HLA*-DR and -*DQ* region in patients with narcolepsy was performed. Five new $(CA)_n$ microsatellite markers were generated and positioned on the physical map across the *HLA*-DQB1-DQA1-DRB1 interval. Haplotypes for these new markers and the three *HLA* loci were established using somatic cell hybrids generated from patients. A four-marker haplotype surrounding the *DQB1*0602* gene was found in all narcolepsy patients, and was identical to haplotypes observed on random chromosomes harboring the *DQB1*0602* allele. Eighty-six kilobases of contiguous genomic sequence across the region did not reveal new genes, and analysis of this sequence for single nucleotide polymorphisms did not reveal sequence variation among *DQB1*0602* chromosomes. These results are consistent with other studies, suggesting that the *HLA*-DQ genes themselves are among the predisposing factors in narcolepsy.

Introduction

Narcolepsy is a debilitating, lifelong sleep disorder that typically arises during early adulthood (Aldrich 1993). The abnormal patterns of REM sleep and associated clinical features observed in narcoleptics suggest that the disease affects fundamental mechanisms of sleep regulation (Aldrich 1993). The measured frequency of the disease ranges from 1 in 4000 in North American and European Caucasians to 1 in 600 in Japan (Mignot 1997). There is no cure for the disease, and current treatments with amphetamines and antidepressants are purely symptomatic and largely unsatisfactory (Nishino and Mignot 1997).

The pathophysiological mechanisms underlying narcolepsy are unknown. Neurochemical studies suggest abnormal monoaminergic and cholinergic transmission in both humans and in a dog model of the disorder (Aldrich et al. 1994; Nishino and Mignot 1997). There have been no clear demonstrations of tissue damage associated with the disease in humans (Erlich and Itabashi 1986), but recent results in the dog model have shown increased microglial major histocompatibility complex class II expression in the white matter and neuronal degeneration in the amygdala and in the basal forebrain around the time of disease onset (Siegel et al. 1995; Tafti et al. 1996).

A clue to the molecular basis of narcolepsy has come from genetic association studies. Since the early 1980s, narcolepsy has been known to be strongly associated with specific alleles of genes in the *HLA* class II region on chromsome 6p21 (Juji et al. 1984). This was first observed with protein-based marker systems (Andreas-Zietz et al. 1987; Billiard et al. 1986; Juji et al. 1984; Mueller-Eckhardt et al. 1986; Neely et al. 1986; Poirier et al. 1986), and subsequently with more specific DNA-based systems (Mignot et al.1994, 1997; Rogers et al. 1997). Recent studies have demonstrated that 85%–100% of Causasian and Japanese patients with narcolepsy/cataplexy are positive for the *HLA* class II alleles *DQB1*0602*, *DQA1*0102*, and *DRB1*1501* (Lin et al.1992; Mignot et al. 1994; Rogers et al. 1997). Similarly, American Black patients exhibit a $>90\%$ frequency of the *DQB1*0602/DQA1*0102*, but exhibit reduced association at the *DRB1* locus (Mignot et al. 1994, 1997; Rogers et al. 1997). These results suggest the presence of a narcolepsy susceptibility factor in the immediate *DQB1/DQA1* region. Sequencing of the *DQ* and

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Table 1 Primer sequences for markers developed in this study. Markers are listed by GDB-assigned D-segment numbers for microsatellite markers, and by STS name for other markers. Size ranges are given for microsatellites. Alleles are listed as size variations for microsatellites, or DGGE/RFLP polymorphisms detected in STSs 2E3, 2D7, 2C10, and 9–10 (see Materials and methods). GDB accession numbers are listed in the last column

Locus/STS name	Size (bp)	PCR primers	Alleles	GDB ID
D6S2445	162–198	AATATGATGGAAGAAGTAATCCAG GGATTACAGGTATAAGCCATTG	6	GDB:5886533
D6S2444	142–158	GAGCCAAGAACCCAGCATTC GGAAGGATTCTAAATAGGGGAG	8	GDB:5886535
D6S2443	169–203	CCATACCAAAGTAAAACCCAG GAGGATGAAGGGAAATTAGAG	9	GDB:5886534
D6S2447	178–200	TTGAGAGGTGTGCATGTTAC GCATTTCTCTTCCTTATCACTTC	9	GDB:5886537
D6S2446	168–200	GAGAATCCAGCATATTGGAG CACTATCATTAAATTTGCTTTCCAC	13	GDB:5886536
D6F374S1	~190	CGGGATTGAGAGGATTCTAAATG GTGAGAGCTTCACAGTGCAG	Many	GDB:5886538
2E3	246	GTAGGGAGTTGTAATAGGTTTG TTTGGTGGGTCATTATGTATTATC	3	GDB:5886539
2D7	437	GGAAAGGAATGTTATGCTCTG AGGAGTGGGGTGTTATTG	2	GDB:5886540
2C10	3496	GAGGTGTGTTGGAAAGAG GTAGGATGGGATTGGATTG	3	GDB:5886542
9-10	593	TGAGCACAGTGGATTGAG TGGTAGTTGTCAGGAAGG	3	GDB:5886541

DRB1 genes from narcoleptic and control individuals has revealed no polymorphisms that correlate with the disease (Lock et al. 1988; Mignot et al. 1994; Uryu et al. 1989). Since *DQB1*0602/DQA1*0102* is a very common haplotype in all populations (Fernandez-Vina et al. 1991; Yasunaga et al. 1996), the predictive value of these markers with respect to disease is low.

In the current study, we took a systematic approach to identify genetic variation across the narcolepsy-associated *HLA* class II region. By developing and mapping novel polymorphic markers in conjunction with existing *HLA* markers, we investigated whether a *DQB1*0602* haplotype of higher predictive value can be distinguished in narcoleptic versus control subjects.

Materials and methods

Human subjects and cell line subjects were 23 patients with narcolepsy-cataplexy, identified from the University of Michigan Sleep Disorders Center database. All subjects had definite cataplexy characterized by brief episodes of bilateral muscle weakness, which were triggered by emotion and were not associated with loss of consciousness. Sixteen had been tested with a Multiple Sleep Latency Test (MSLT), and the other seven subjects were all over the age of 50 with long histories of unambiguous narcolepsy/cataplexy. Fourteen of the 16 had MSLT results typical for narcolepsy (mean sleep latency of less than 5 minutes and two or more Sleep Onset REM periods). After informed consent, 10–35 ml whole blood was obtained by venipuncture. Genomic DNA from blood was prepared as previously described (Wolff et al. 1992). Immortalized cell lines were established using standard procedures and hybrid cell lines containing chromosome 6 were produced as previously described (Jackson 1994). Cell lines containing individual homologues of chromosome 6 were selected based on their containing only one allele of a genetic marker on chromosome 6 known to be heterozygous in that individual. Random human samples were from two sources. In cases where the phase of markers was desired, genomic DNA from 26 individuals of the grandparental generation of the CEPH/Utah families were used (Coriell Cell Repositories, Camden, NJ). Additional Caucasian random individuals were sampled from Mercator Genetics, Inc.

HLA typing

HLA class II typing was performed using available typing systems for *HLA*-DQB1, DQA1, and *DRB1* (Olerup and Zetterquist 1992; Olerup et al. 1993), which are based on polymerase chain reaction (PCR) assays with allele-specific oligonucleotide primers (Dynal Inc., Lake Success, NY), according to the manufacturer′s instructions. Allele frequencies were compared with published population studies Begorich et al. 1992; Fernandez-Vina et al. 1991).

Genomic clones

DNA substrates for the development of microsatellite markers were the yeast artificial chromosomes (YACs) A148A7 and B1D12, which were previously shown to span the class II region (Kozono et al. 1991). These YACs were sized using Bio Rad (Hercules, CA) CHEF gels (Chu et al. 1986) and found to be 360 kilobases (kb) (A148A7) and 375 kb (B1D12) in size. Additional YACs spanning the class II region (clones 410A3, 283E9, 405C3, 98E6, and 129B9) were from the CEPH map of the region (Abderrahim et al. 1994). P1 clones p312g9 and p797a11 were isolated by virtue of their containing a sequence-tagged site (STS; primers GH26 and GH27) in the *DQA1* gene (Scharf et al. 1986), and were obtained from the screening service at Genome Systems Inc. (St. Louis, MO).

Microsatellite marker development and genotyping

DNA from YACs A148A7 and B1D12 was used to construct a cosmid library in the modified sCos vector sCos-dRI (Gillett et al. 1996); a gift of J. Yu, University of Washington. *Eco* RI fragments containing polymorphic $(CA)_n$ repeats were identified using standard methods (Litt and Browne 1994; Weber 1990), and then subcloned and sequenced on both strands. PCR primers flanking the $(CA)_n$ repeat were designed using the program OSP (Hillier and Green 1991). The marker *D6S273* was from Research Genetics (Huntsville, AL), and maps to the *HLA* class III region near the complement factor *B* and *C4* genes, based on reference to the MIT/Whitehead database (Hudson et al. 1995). All markers were assayed according to standard conditions

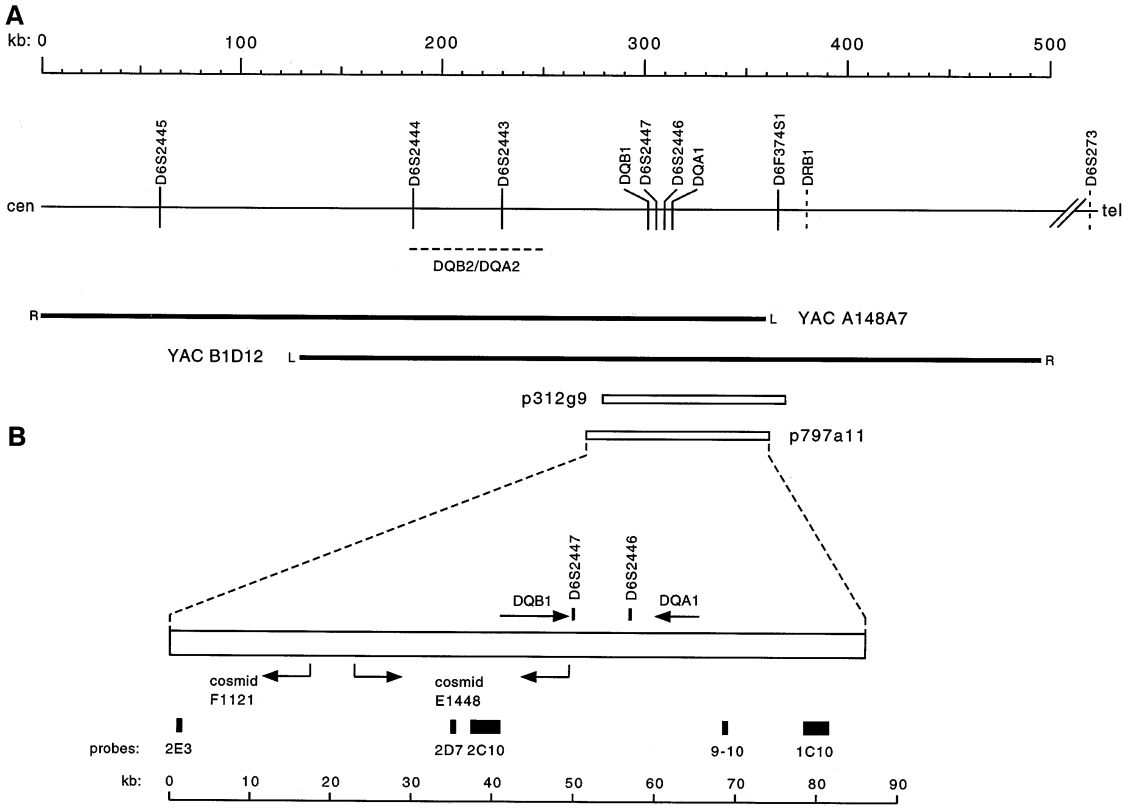

Fig. 1 A Map of the *HLA* class II region and clones used in this study. Scale bar in kb is shown at the *top* of the Figure. The second line indicates the positions of loci used as genetic markers in this study that were determined by RARE cleavage, indicated as *vertical lines*. Probes adjacent to markers *D6S2447* and *D6S2446* gave two positions when used in RARE cleavage experiments due to sequence similarity to loci in the *DQB2* region. The actual positions of these markers were confirmed through genomic sequencing. The position of *D6F374S1* as determined by RARE cleavage and analysis of its sequence (data not shown) indicate that it derives from the *DRB1/DRB6* pseudogene region. The positions of *DQB2*, *DQA2*, *DRB1*, and *D6S273* are based on references to other maps (Hudson et al. 1995; Kozono et al. 1991) and are thus approximate, and represented by *dashed lines*. The sizes and approximate endpoints of the indicated YAC (*heavy lines*) and P1 (*open bars*) clones are shown. The *left* and *right* ends of the YAC clones are indicated by "L" and "R", respectively. **B** Schematic representation of the sequence of p797a11. The 86 kb of genomic sequence is represented by the *open bar*. The positions of the *DQB1* and *DQA1* genes and the size and direction of transcription are represented *above* the bar, as are the positions of *D6S2447* and *D6S2446*. The positions at which the genomic sequence of p797a11 overlaps sequences from cosmids F1121 and E1448 (Sanger Centre, UK) are indicated. The positions of the probes 2E3, 9–10, 2D7, 2C10, and 1C10 that were used as assays for sequence polymorphism are indicated *below* the bar (see Materials and methods). With the exception of probe 1C10, which detected an *Msp* I polymorphism in the *DQB1*0602* chromosome from Black patient NY14 that was not detected among the other *DQB1*0602* chromsomes used in this study, all *DQB1*0602* chromosomes were identical, whether from narcoleptic or random subjects

(Weber and May 1989). Sequence analysis and position of *D6F374S1* indicated that it derived from the *DRB1/DRB6* pseudogene region. Data from this marker was not used for scoring, as it amplified multiply duplicated sequences from this region (data not shown). Oligonucleotide sequences for markers developed in this study are shown in Table 1. Additional information for these markers such as allele frequencies and PCR conditions have been deposited with GDB.

Marker mapping

Physical map positions of new genetic markers were established using RecA-assisted restriction endonuclease (RARE) cleavage (Ferrin and Camerini-Otero 1991). *Eco* RI subclones containing $(CA)_n$ repeats were sequenced at the insert/vector junction to identify the genomic sequence adjacent to the *Eco* RI site. Oligonucleotides from this sequence were used in RARE cleavage experiments as described (Gnirke et al. 1994; Iadonato and Gnirke 1994). *Eco* RI sites adjacent to $(CA)_n$ repeat markers were initially positioned on the YACs A148A7 and B1D12, and were confirmed on a second set of YACs that also span the class II region (410A3, 283E9, 405C3, 98E6, 129B9).

Genomic sequencing and computer analysis

The P1 clone p797a11 was used as a substrate for genomic sequencing, which was performed as described (Ruddy et al. 1997). Finished sequence was analyzed using the gene prediction program GRAIL (Uberbacher et al. 1996) and by searching the sequence against GenBank using BLAST (Altschul et al. 1990). The 86 kb sequence of p797a11 has been deposited in GenBank (accession number U92032). Based on GenBank comparisons, the *HLA* type of p797a11 is most likely *DQB1*0201*, *DQA1*0501*.

Polymorphism analysis

STSs designed from the p797a11 sequence were amplified from genomic samples using either standard PCR (probes 2E3, 2D7, 9–10) or long-range PCR (probe 2C10), using the TaKaRa LA PCR kit (PanVera Corp., Madison, WI) according to the manufacturer's instructions. Sequences of primers for these STSs are shown in Table 1. Standard size STSs were analyzed for sequence polymorphism using denaturing gradient gel electrophoresis (DGGE), using gel systems and temperature control tanks from the C.B.S. Scientific Company, Inc.

Table 2 Genotyping data from 23 narcolepsy patients. Genotyping information is listed for the 23 narcolepsy patients used in this study. Numbers denote allele sizes in bp, except for *DQB1*, *DQA1*, and *DRB1*, where alleles are denoted according to the standard nomenclature for each respective typing system. Patients for which somatic cell hybrid lines were established for each homologue of chromosome 6 are indicated with a (*), and the haplotype from the individual homologue is listed. Markers which define the four-marker haplotype in common among all patients are boxed

Patient	Race	D6S2445	D6S2444	D6S443	DQB1	D6S2447	D6S2446	DQA1	DRB1	D6S273
NY1	C	180/164	148/144	199/169	0501/0602	182/182	186/184	0101/0102	01/1501	136/134
NY2	C	164/164	152/144	197/169	03/0602	198/182	184/172	04/0102	11/1501	136/134
NY3	C	164/164	152/144	197/169	0402/0602	190/182	184/182	04/0102	08/1501	136/132
NY4	C	164/164	152/148	201/197	03/0602	190/182	184/178	03/0102	04/1501	136/132
NY5	C	164/162	144/144	179/169	03/0602	196/182	184/170	04/0102	12/1501	136/134
*NY6	B	164	142	179	03	196	170	04	11	136
		164	144	169	0602	182	184	0102	11	132
*NY7	C	164	152	195	0602	182	184	0102	1501	136
		162	152	171	02	178	188	04	03	140
*NY8	C	162	144	171	03	196	178	0302	04	134
		162	152	197	0602	182	184	0102	1501	132
NY9	C	164/162	152/144	197/169	03/0602	198/182	184/172	04/0102	11/1501	136/134
*NY10	C	162	144	169	0602	182	184	0102	1501	136
		162	144	169	03	198	202	0201	07	136
NY11	C	164/162	152/152	197/171	02/0602	182/178	188/184	0501/0102	0301/1501	140/136
*NY12	C	164	152	199	0602	182	184	0102	1501	136
		162	152	195	0602	182	184	0102	1501	138
*NY13	C	164	158	179	02	192	204	0201	07	130
		164	152	197	0602	182	184	0102	1501	136
*NY14	B	194	144	179	0602	182	184	0102	11	136
		164	144	169	0501	182	182	0101	1001	132
*NY15	C	164	152	197	0602	182	184	0102	1501	136
		164	152	195	0602	182	184	0102	1501	140
*NY16	C	164	152	197	0602	182	182	0102	1501	136
		162	144	169	0602	182	184	0102	1501	136
*NY17	C	164	152	197	0602	182	184	0102	1501	136
		164	142	169	0501	182	186	0101	01	134
NY18	C	164/164	152/152	197/197	0602/0602	182/182	184/184	0104/0102	1501/1501	136/136
*NY19	C	162	144	169	0602	182	184	0102	1501	136
		164	144	169	0602	182	184	0102	1501	136
NY20	C	164/162	152/144	197/169	03/0602	198/182	184/172	0501/0102	11/1501	136/134
NY21	C	162/162	152/144	171/169	02/0602	182/178	188/184	0501/0102	0301/1501	140/136
NY22	C	180/162	148/144	201/169	03/0602	190/182	184/178	0301/0102	04/1501	136/134
*NY23	B	190	144	179	03	196	170	0501	11	134
		188	148	199	0602	182	184	0102	1503	138

(Del Mar, CA), and techniques as described (Myers et al. 1988). *Dde* I and *Alu* I polymorphisms were detected in the 2C10 STS on standard agarose or polyacrylamide gels. The 3 kb clone 1C10 from p797a11 was used as a probe on Southern blots of human genomic DNA to detect *Msp* I polymorphisms according to standard techniques.

Results

Five microsatellite markers developed and mapped in the HLA class II region

Our objective in the current study was to identify genetic variation across the entire *HLA* class II region and to measure allelic associations with narcolepsy. As a first step, we set out to develop polymorphic markers across the region using two well-characterized YACs (clones A148A7 and B1D12) that span the region, as shown in Fig. 1 (Kozono et al. 1991). We chose to develop simple tandem repeat polymorphisms (STRPs) of the $(CA)_n$ dinucleotide class, as they are among the most common form of polymorphic repeat in the human genome (Beckmann and Weber 1992). Using YACs A148A7 and B1D12 as starting material, six independent $(CA)_n$ markers were isolated and characterized (see Materials and methods). The physical positions of these markers were determined using Rec-A-assisted restriction endonuclease cleavage, or RARE cleavage, (Ferrin and Camerini-Otero 1991), a technique for mapping individual *Eco* RI sites relative to the end of a YAC (Gnirke et al. 1994; Iadonato and Gnirke 1994). The six $(CA)_n$ markers were positioned over a region extending approximately 300 kb centromeric from the *DRB1* region (Fig. 1). All distances and locations were confirmed by performing RARE cleavage on a second set of YACs from the class II region isolated from the CEPH library, an independent source of YACs (Abderrahim et al. 1994).

These markers were tested on 23 narcolepsy/cataplexy patients and 26 random individuals of Caucasian descent in order to determine allele frequencies. Genotyping data from *D6F374S1* could not be scored, due to apparent amplification of multiply duplicated sequences and was not used for further analysis. In total, scorable $(CA)_n$ markers were developed at five loci through the class II region (Fig. 1). All five markers displayed multiple alleles (Table 1), and were highly polymorphic (heterozygosity $>65\%$). Notably, all 23 narcolepsy patients carried allele 182 of marker

Table 3 Haplotypes of *DQB1*0602* chromosomes from narcoleptic and random individuals. Alleles are listed as in Table 2. Haplotypes were established from narcolepsy patient hybrid cell lines or from phased CEPH individuals. The four-marker haplotype associated with *DQB1*0602* is underlined. Haplotypes at flanking markers were not conserved. One homologue from patient NY16 in which allele 182 of marker *D6S2446* presumably arose by mutation is indicated by (*)

	Number	*D6S2444*	*D6S2443*	*DQB1*	*D6S2447*	*D6S2446*	*DQA1*	*DRB1*	*D6S273*
Patient	15	Variable[a]	Variable[a]	0602	182	184	0102	Variable[a]	Variable[a]
	1	152	197	0602	182	182*	0102	1501	136
Random	3	152	197	0602	182	184	0102	ND	ND
	2	144	169	0602	182	184	0102	ND	ND

[a] See Table 2
ND: not determined

D6S2447 and allele 184 of marker *D6S2446* (see below). During subsequent analysis, we discovered that markers *D6S2447* and *D6S2446* are identical to previously characterized *DQCAR*, and *DQCARII* markers, respectively (Macaubas et al. 1995; Mignot et al. 1995a, 1997). The *D6S2447* PCR product is 79 base pairs (bp) longer than reported *DQCAR* alleles, whereas *D6S2446* alleles are 16 bp shorter than *DQCARII* alleles. The pattern of allelic association of *D6S2447* and *D6S2446* with *HLA* DQ and *DR* observed in this study is identical to that reported with *DQCAR* and *DQCARII* (data not shown).

*Narcoleptics share a common haplotype around the DQB1*0602 gene that is indistinguishable from control haplotypes*

To determine the genetic variation present at the class II loci, the 23 narcoleptic patients were typed at the three *HLA* genes *DQB1*, *DQA1*, and *DRB1* using available PCR-SSP systems. Results are shown in Table 2. Consistent with previously reported studies, all Caucasian patients carried *DQB1*0602* and *DQA1*0102* in association with *DRB1*1501*. All Black patients were also *DQB1*0602* and *DQA1*0102* but carried more variable *DRB1* types (i.e., *DRB1*11* and *DRB1*1503*).

To establish the phase for all the markers used in this study, somatic cell hybrids carrying single homologues of chromosome 6 were established for 12 of the narcolepsy patients used in this study, which allows the unambiguous assignment of alleles to a specific haplotype (Table 2). This analysis revealed that allele 182 of marker *D6S2447* and the 184 allele of marker *D6S2446* were in nearly complete linkage disequilibrium with the *DQB1*0602/DQA1*0102* narcolepsy susceptibility haplotype. Overall, the 0602/182/184/0102 haplotype was observed on 15 separated chromosomes derived from 12 patients, and inferred in the remaining 11 patients (Table 2). One chromosome (from patient NY16) carried *D6S2446* allele 182 on an otherwise identical *DQB1*0602* chromosome (Table 3), presumably a rare allele that arose through mutation at the $(CA)_n$ locus. At markers flanking the immediate *DQ* region (*D6S2445*, *D6S2444*, and *D6S2443* centromeric, and *DRB1* and *D6S273* telomeric), haplotypes were not conserved (Table 2).

To compare the haplotypes observed in narcolepsy patients with those from random individuals, we identified individuals with a *DQB1*0602* allele from the CEPH/Utah families. Because these individuals are in families, one can follow the inheritance pattern of the individual chromsomes to establish phase for markers. The results of this analysis are shown in Table 3. The *HLA*/microsatellite haplotypes observed in these individuals do not differ from those observed in narcolepsy patients. These results suggest that the pattern of linkage disequilibrium is the same in narcoleptics and controls with *DQB1*0602*, and thus at this level of resolution there does not appear to be a narcolepsy-specific "subhaplotype" among *DQB1*0602* chromosomes.

Sequencing and polymorphism studies did not reveal narcolepsy-specific sequence variation or other genes in the DQ region

To approach the identification of additional polymorphism that is not reflected in the *HLA*/microsatellite haplotype, we determined the complete sequence of a P1 clone that spans the *DQ* region and used this as a tool to develop assays for sequence polymorphism (Fig. 1). This P1 clone extends from ~40 kb centromeric of *DQB1* to ~40 kb telomeric of *DQA1*. Analysis of the 86 kb of complete genomic sequence by BLAST searches and the gene identification program GRAIL identified the genes for *DQB1* and *DQA1*, but gave no evidence for other genes in the region (Fig. 1). Sequence variation was assayed either by testing STSs from multiple individuals on gel based systems to detect polymorphism (see Materials and methods), or by detection of restriction fragment-length polymorphisms (RFLP) on Southern blots of genomic DNA from multiple individuals. In total, these techniques were used to assay 7.8 kb of genomic sequence. Polymorphisms were detected in all five probes among various haplotypes (Table 1), and four of the five showed no polymorphism among *DQB1*0602* chromosomes (Fig. 1). Probe 1C10, which detected an *Msp* I RFLP between *DQB1*0602* chromosomes from Caucasians (all *DQB1*0602/DRB1*1501*-positive) and Black patient NY14 (*DQB1*0602/DR11*-positive), may define a telomeric boundary of the region of identity. Although this analysis confirms the existence of a high degree of polymorphism among various *HLA* haplotypes, it does not provide evidence for sequence variation among *DQB1*0602* chromosomes in the immediate *DQ* region.

Discussion

In this study, we identified a four-marker haplotype in the *DQ* region, which is strongly associated with narcolepsy, and confirmed that the disease susceptibility region does not extend to *DRB1* (Mignot et al. 1994, 1997; Neely et al. 1987; Rogers et al. 1997), or *to DQA2/DQB2* (Honda and Matsuki 1990; Mignot et al. 1997). This *DQB1*0602*-associated haplotype is indistinguishable from the *DQB1*0602* haplotypes observed on random control chromosomes, thus suggesting that a narcolepsy-specific subhaplotype does not exist among *DQB1*0602* chromosomes. It remains formally possible that thus far undetected polymorphism among *DQB1*0602* chromosomes is involved in narcolepsy, but the bulk of the genetic evidence accumulated to date argues against this possibility. In family studies, *DQB1*0602*-positive narcoleptic patients can inherit the *DQB1*0602* chromosome from either an affected or unaffected parent, suggesting that the genetic origin of the *DQB1*0602* chromosome is independent of affected status (Mignot 1997). This, together with the finding that rare non-*DR15* narcoleptic patients often carry complex recombinant haplotypes sharing only *DQA1*0102* and *DQB1*0602* (Mignot et al. 1997), rather suggests a primary role for *HLA*-DQ in disease susceptibility. As *DQB1*0602* is found in 20%–25% of the Caucasian North American population (Begouich et al. 1992; Fernandez-Vina et al. 1991), other factors must also be involved.

The fact that genomic sequencing in the *DQ* region did not reveal evidence for other candidate genes also argues in favor of a primary role for *HLA*-DQ. There has been much speculation about a possible role for the *HLA* class II system in the etiology of the disease (Carlander et al. 1993; Mignot et al. 1995b; Parkes et al. 1986), but positive evidence in support of an immune involvement remains elusive at this time. One possibility is that these particular *HLA* alleles are involved in positive selection of a subset of T cells in response to a particular environmental stimulus which then leads to tissue destruction and subsequent pathology leading to disease. Even if most studies to date have found no evidence for an autoimmune process in narcolepsy (Carlander et al. 1993; Fredrikson et al. 1990; Mignot et al. 1995b; Rubin et al. 1988), recent results have shown increased microglial *HLA* class II expression in the white matter and neuronal degeneration in the amygdala and the basal forebrain of narcoleptic canines (Siegel et al. 1995; Tafti et al. 1996). The population genetic characteristics of narcolepsy are similar to several diseases of known autoimmune etiology such as insulin-dependent diabetes mellitus and multiple sclerosis, and include low relative risk values for *HLA* susceptibility alleles (Billiard et al. 1994; Guilleminault et al. 1989; Honda and Matsuki 1990; Kales et al. 1982; Mignot 1997), and a low rate of concordance in twins (Mignot 1997; Partinen et al. 1994).

Our results also suggest that the sequence variation associated with *DQB1*0602* and narcolepsy is phylogenetically ancient. The four-marker *DQB1*0602* haplotype was observed in both Caucasians and a small number of Blacks. Although $(CA)_n$ repeats are thought to mutate at a faster rate than gene sequences, the microsatellite alleles associated with the *DQB1*0602* haplotype have been preserved in both populations, and thus variation at this level appears to predate the divergence of the races. Although the mechanisms that work to preserve this sequence variation are unclear, recent work suggests that microsatellites containing smaller numbers of $(CA)_n$ repeats are less susceptible to change than are longer repeats (Jin et al. 1996). The microsatellite alleles observed on the *DQB1*0602* haplotype are among the shorter alleles for those particular markers, and thus may be more likely to be preserved over long periods of time. It would be of interest to determine the arrangement and allelic associations of these markers in other ethnic groups as well as other primate species.

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