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Susceptibility to Relapsing-Progressive Multiple Sclerosis Is Associated with Inheritance of Genes Linked to the Variable Region of the TcR β Locus: Use of Affected Family-Based Controls

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Summary

We tested the hypothesis that susceptibility to relapsing-progressive (RP) (but not to relapsing-remitting [RR]) multiple sclerosis (MS) is associated with a gene linked to the TcR β-chain variable region delimited by the Vβ8-*Bam*HI and Vβ11-*Bam*HI RFLP alleles in DRw15$^+$ MS patients, using a contingency-table test of patient data and affected family–based controls. Control alleles and haplotypes were composed of parental marker alleles and haplotypes not transmitted to the affected child, in 90 simplex and 31 multiplex families from British Columbia. A total of 6,164 alleles at 11 loci were segregated through families of probands with RP MS or RR MS. The Vβ8–Vβ11 subhaplotype frequencies in the DRw15$^+$ RP MS (but not RR MS) patients differed from control frequencies, because of an increase of the 2-1 subhaplotype (P = .02). Vβ8-*Bam*HI and Vβ11-*Bam*HI allele frequencies (P = .05 and .009, respectively) in the DRw15$^+$ RP MS (but not RR MS) patients differed from control frequencies. The Vβ1–Vβ8 subhaplotype frequencies in the DRw15$^-$ RP MS (but not RR MS) patients differed from control frequencies (P = .03), with a significantly increased frequency of the 1-1 subhaplotype (P = .01; RR = 7.1) in RP MS versus RR MS patients. Susceptibility to RP MS is associated both with a recessive inheritance of a gene linked to the 3′ (Vβ11) end of the 2-1 subhaplotype defined by the Vβ8-*Bam*HI and Vβ11-*Bam*HI alleles in DRw15$^+$ patients and with a gene, located on the 1-1 subhaplotype, defined by the Vβ1-*Taq*I and Vβ8-*Msp*I alleles of the TcR β-chain complex in DRw15$^-$ patients.

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Introduction

Liability (Falconer 1989) to develop multiple sclerosis (MS; MIM 126200 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?126200]) is partly determined by an underlying genetic susceptibility due to the inheritance of at least two unlinked gene complexes (Ebers at al. 1986, 1996; Risch 1987; Sadovnick et al. 1993; Ebers 1994; Haines et al. 1996; Sawcer et al. 1996). There may be multiplicative interactions involved between these loci (Beall et al. 1989, 1993; Kellar-Wood et al. 1995; Epplen et al. 1997). The liability is also determined by modulation of the expression of these genes through the immune system and by exposure to environmental factors. Major candidate-gene complexes include the HLA locus (specifically the DR, DQ, and DP loci; reviewed in Tiwari and Terasaki 1985; Olerup and Hillert 1991; Haegert and Marrosu 1994; Hillert 1994), which encodes self-molecules, which present processed peptides to the protein products on T cells encoded by two other candidate gene complexes, the TcR α- and β-chain loci (reviewed in Beall et al. 1989, 1993; Haegert and Marrosu 1994; Hauser 1995).

The TcR is a heterodimer comprising α and β chains, each of which is divided into variable (V) and constant (C) regions (Rowen et al. 1996). We originally showed association of the TcR β-chain genes with susceptibility to MS in chronic progressive (CP), primarily DR2$^+$ (DRw15$^+$), MS patients (Beall et al. 1987*a*, 1989). A nonparametric linkage study of 40 affected sib pairs (ASPs) showed linkage between this locus and susceptibility (Seboun et al. 1989). The two major animal models of the disease that have been studied most extensively—experimental allergic encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus (TMEV)–induced demyelinating disease—also provide strong evidence that the TcR β-chain locus is a major susceptibility gene if coinherited with another susceptibility locus, H-2 (equivalent to the HLA locus of the human) (Melvold et al. 1987; Rodriguez et al. 1992, 1994; Goverman et al. 1993).

There have been a number of investigations of the TcR β-chain locus and susceptibility to MS. Case-con-

trol studies have yielded either positive (Beall et al. 1989, 1993; Charmley et al. 1991; Martinez-Naves et al. 1993; Epplen et al. 1997) or negative (Fugger et al. 1990; Hillert et al. 1991; Vandevyver et al. 1994; Wei et al. 1995; Droogan et al. 1996) results. Linkage studies using nonparametric methods also have yielded either positive (Seboun et al. 1989; Wood et al. 1995; Haines et al. 1996) or negative (Lynch et al. 1991; Ebers et al. 1996; Sawcer et al. 1996) results.

The classic way to determine whether genes confer susceptibility to MS is to determine whether they are linked or associated with the disease (reviewed in Thomson 1991). Previous linkage studies that used parametric methods may be inappropriate in the study of diseases with multigenic inheritance (Thomson 1991). Previous association studies that used population-based controls have been plagued with possible experimental bias due to the mismatching of controls and patients with regard to ethnic diversity (Ebers and Sadovnick 1994). These problems can be lessened by the use of affected family–based controls (AFBACs) (Falk and Rubinstein 1987; Thomson 1988, 1991, 1995; Thomson et al. 1989; Beall et al. 1993, 1995, 1996; Beall and Hockertz 1993; Schaid and Sommer 1993; Spielman et al. 1993; Ebers 1994; Ebers and Sadovnick 1994; Khoury 1994; Suarez and Hampe 1994; Hockertz et al. 1996; Risch and Merikangas 1997; S. S. Beall, unpublished data).

In addition to the aforementioned methodological problem, there is also a problem introduced by the very nature of MS. Approximately 10% of cases will undergo a "benign" course—that is, either start as relapsing remitting (RR) and be virtually normal after 22 years or allow the affected individual to walk, with aids, 35 years after onset (Weinshenker et al. 1989). Approximately 15% of cases will be chronically progressive (CP); 85% of cases start as RR, and then more than half of this latter group enters a relapsing-progressive (RP) phase after 10 years of disease (Weinshenker et al. 1989). The population of MS patients may thus actually constitute a continuum that ranges from MS patients whose disease remains relatively benign (i.e., RR) as they age to MS patients whose disease progresses quickly. The particular point that each patient occupies on the continuum will be determined by the genes inherited and by the environment encountered. Therefore, the disparity of results reported in the past may also be explained by the fact that different groups of patients with different severities of the disease have been studied. We (Beall et al. 1987*a*, 1989, 1993; Charmley et al. 1991; Beall and Hockertz 1993; Hockertz et al. 1996) have been especially interested in the RP and CP patients, since they have the worst prognosis and should be given priority of understanding and treatment.

In the past we have also focused on DRw15$^+$ patients, since (1) these patients constitute the majority, and (2) it was in these patients that we reported the initial association between susceptibility to the progressive form of MS and the TcR β-chain locus, a hypothesis that has received recent support from a nonparametric linkage study (Kellar-Wood et al. 1995; Wood et al. 1995) and a very large case-control study (Epplen et al. 1997). It is not unexpected therefore that previous studies that did not stratify patients as to HLA class II type would show results different from our own previously published results (Beall et al. 1987*a*, 1989, 1993; Charmley et al. 1991).

The results of our previous studies (Beall et al. 1987*a*, 1989, 1993) support the following hypothesis. In the TcR β-chain V region, a gene(s) may exist that predisposes to progression of disease if this gene is coinherited with a DRB1*1501 (DR2 or DRw15)–DQB1*0602 (DQ1 or DQw6) HLA class II haplotype. This gene will be at the 3′ (Vβ11) end of a subhaplotype delimited by the Vβ8 and Vβ11 DNA polymorphisms. To avoid previous biases (see above), the hypothesis should be tested by use of AFBACs. The present study was designed to test this hypothesis.

Subjects and Methods

Subjects

As of 1995, 3,683 patients had been registered in the MS clinic in Vancouver, which is believed to be the largest MS clinic in the world (Redekop 1995). It is also unique in that (1) clinical data for all probands are stored in a computerized database (COSTAR [computer-stored ambulatory record) (Paty et al. 1994), (2) the diagnosis of MS is made by neurologists specialized in the diagnosis of MS, (3) clinical data are updated at each patient visit and are entered into COSTAR, and (4) pedigrees are updated annually by a clinical geneticist (Sadovnick 1994). Because this is a population-based clinic, it is free from ascertainment bias that may occur in clinics that are part of tertiary-care referral centers. After the proposed study was approved by the institution's ethics committee, blood was obtained, in coded fashion, from simplex families (those in which one nuclear-family member has MS) and multiplex families (those in which more than one nuclear-family member is affected with MS).

Blood was collected from 90 simplex families (which can be utilized for calculation of risk and control allele, genotype, and haplotype frequencies) and from 31 multiplex families comprising ASP, parent-child, and parent–child–sib-pair families (which also can be used as a source of control alleles, genotypes, and haplotypes). Of the simplex families, 80 contained both parents, and 10 had one parent and an unaffected sib available for study. Alleles were typed in the families in a blinded fashion

and, after alleles and haplotypes were determined, the code was broken as to simplex or multiplex status. In addition, at that time, COSTAR (Paty et al. 1994) was accessed to see which of the children was the affected proband and which type of MS (i.e., RR or RP) the proband had. Because this is a family-based association test, the control genotype was defined as the two alleles not passed to the proband (Lander and Schork 1994). In this study, 68% of the probands of the simplex families were RR MS, and 32% of the probands were RP MS. All probands fulfilled criteria for definite diagnosis of MS (Poser et al. 1983).

DNA Preparation and Cataloguing

Genomic DNA was prepared from peripheral blood lymphocytes from venous blood from the proband and first-degree relatives, as described elsewhere (Beall et al. 1987*b*). Each subject was assigned a unique eight-digit ID number, which, together with experimental data, was entered into a computer heuristic, CHIAARRA (computerized heuristic of Internet accessible alleles for relational retrieval and analysis), that we have designed (Beall et al. 1996; S. S. Beall, M.K. Hockertz, D. Studney, and D.W. Paty, unpublished data). This heuristic permits automatic calculation of experimental variables for molecular genotyping and semiautomated entry and analysis of segregated allele data. The heuristic also effectively links the phenotypic (clinical) data, genetic (clinical) data, and genotypic (molecular genotype) data within a system of distributed relational databases and database-analysis programs, which can be viewed universally by way of a PC with access to the Internet (Beall et al. 1996; S. S. Beall, M.K. Hockertz, D. Studney, and D.W. Paty, unpublished data).

Probes

Plasmids containing cDNA inserts for DR β and DQ β HLA class II exons and for DQ α class II exons have been described elsewhere (Beall et al. 1995). In addition, we have previously described plasmids containing cDNA inserts for TcR Vβ7, TcR Vβ1, TcR Vβ8, and TcR Vβ11 exons (Beall et al. 1989, 1993). cDNA isolation from the plasmids was as described elsewhere (Beall et al. 1987*b*), and radioisotope labeling from gel-purified inserts of these cDNAs was also as described elsewhere (Beall et al. 1989, 1993).

Southern Blot Analysis

DNA samples from patients and from their first-degree relatives were digested with the restriction enzymes *Bam*HI, *Taq*I, and *Msp*I. Southern blots were prepared as described elsewhere (Beall et al. 1989). Hybridization in a rotisserie oven (Hybaid) was performed under conditions described elsewhere (Beall et al. 1993). After a washing, blots were stripped and hybridized sequentially with the HLA DR β, TcR Vβ7, TcR Vβ1, TcR Vβ8, and TcR Vβ11 probes. To detect the HLA DR and TcR polymorphisms, filters were washed and autoradiography was performed as described elsewhere (Cox et al. 1988; Beall et al. 1989, 1993, 1995).

PCR-Based RFLP Analysis

The TcR Vβ6.1, TcR Vβ6.3, TcR Vβ6.7, and TcR Vβ6.10 dinucleotide repeat polymorphisms were determined as described elsewhere (Charmley and Concannon 1993). To detect the Vβ8-*Bam*HI polymorphism (Concannon et al. 1987), PCR primers AP6′ (5′-CCT TGC CAC ATA ATT TAA CAC-3′) and 8154 (5′-TGC CAA CAA AAG AGA AGG GC-3′) were developed (GenBank accession numbers L36092 and L36190 [http://www.ncbi.nlm.gov/]), which allowed detection of this polymorphism by use of PCR-based methods. To detect the Cβ-*Bgl*II polymorphism (Robinson and Kindt 1985), PCR primers SB20 (5′-GGA ATG GAT AAG ATG ACT TC-3′) and SB21 (5′-AAT CCT CTT TGA TGG TTC TAC C-3′) were developed, which allowed detection of this polymorphism. To detect the Cβ *Kpn*I polymorphism (Perl et al. 1989), PCR primers SB22 (5′-GGC TCA ATG TCC TTA CAA AGC-3′) and SB23 (5′-ATG TTC CAT CCC CTG CCC CCA TCA TC-3′) were developed. Genomic DNA was then amplified, with the AP6′ and 8154 primers, for 35 cycles of 1 min at 92°C, 1 min at 55°C, and 1½ min at 72°C. To detect the Cβ-*Bgl*II and *Kpn*I polymorphisms, genomic DNA was amplified with the specified primers for 35 cycles of 1 min at 92°C, 1 min at 60°C, and 1 min at 72°C, in a total volume of 20 μl. All reactions were performed in a Perkin Elmer Cetus model 480 DNA thermocycler by standard procedures. The "hot start" technique was used for the SB22 and SB23 primers, to avoid primer-dimer formation. All PCRs are now performed using of Ultra Therm *Taq* polymerase (0.05 units/reaction). After genomic DNA amplification, 2–4 units of requisite restriction enzyme was added to 5 μl of each PCR, and the product was electrophoresed through a 2% agarose gel.

Segregation of Alleles and Analysis of Data

Autoradiographs, sequencing gels, and agarose-gel patterns were analyzed, and alleles were segregated through the simplex and multiplex pedigrees. All alleles followed Mendelian segregation. For determination of risk genotypes and alleles, data from simplex families were utilized. Control genotypes were constructed from alleles not passed either to the proband (Lander and Schork 1994), in the case of simplex families, or to affected sibs from unaffected parents, in the case of multiplex families. For families with only one living parent, the alleles of the deceased parent could be reconstructed

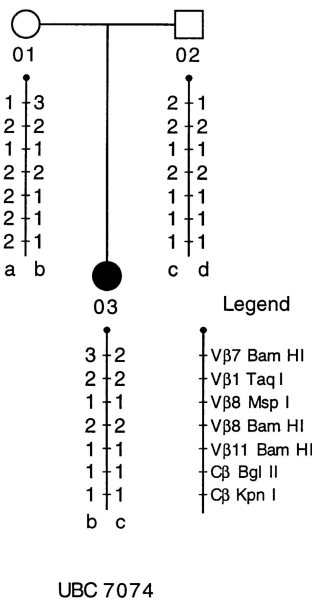

Figure 1 Assignment of at-risk and control alleles and subhaplotypes, by use of AFBACs. Dinucleotide repeat polymorphisms were used to help establish phase but were not utilized in the genotype or haplotype analysis. In this illustrative example of one of the MS simplex families from the University of British Columbia, phase has been established by use of seven markers (see legend beside the pedigree and correlate with the previously published map of the TcR β-chain locus [Beall et al. 1993]). Proband 7074-03 inherits the maternal haplotype 3-2-1-2-1-1-1 (b) and the paternal haplotype 2-2-1-2-1-1-1 (c). Risk alleles for Vβ11 are 1,1, and control alleles are those carried on the a and d control haplotypes (chromosomes not passed to the proband), which are equivalent to 2,1. The proband inherits the disease subhaplotypes 2-1 and 2-1 delimited by the Vβ8-*Bam*HI and Vβ11-*Bam*HI markers (further described by Beall et al. [1987*a*, 1989, 1993]). In this manner, 6,164 alleles were scored through the families, to obtain the data in tables 1–3.

on the basis of the unaffected sib. In the rare instances when this was not possible, the genotype of the unaffected sib was used as the control genotype (Field et al. 1991). After data were tabulated, genotype and allele frequencies, as well as Hardy-Weinberg equilibrium (HWE) calculations, were determined by use of a customized analysis program built within CHIAARRA (Beall et al. 1996; S. S. Beall, M.K. Hockertz, D. Studney, and D.W. Paty, unpublished data).

Statistical Analysis

The χ^2 contingency-table test for heterogeneity was used for comparisons of allele, genotype, and haplotype frequencies in patients versus controls, by use of standard methods (Crow 1986). The smallest classes were combined, so that expected numbers were not less than five in the χ^2 test, although this may be overly conservative (Cochran 1954). We used one-tailed tests and did not apply Bonferroni corrections (Ingelfinger et al. 1983) to obtain probability tests of previous hypotheses; we used two-tailed tests and applied Bonferroni corrections to obtain probability tests of new hypotheses.

Results

Determination of Genotype, Allele, and Subhaplotype Frequencies in the HLA Class II and TcR β-Chain Loci

As an initial step, the 90 simplex families were typed at the HLA class II locus, as described elsewhere (Bidwell et al. 1987; Cox et al. 1988; Horne and Keown 1993; Beall et al. 1995), and will form part of the data set of a separate report. A total of 52% of the probands of the simplex families in this study were DRw15$^+$.

Genotypes were determined at nine TcR V-region loci and at two C-region loci, in 90 simplex families and 31 multiplex families (for alleles and subhaplotypes used in the analysis, see fig. 1). A total of 6,164 alleles were manually segregated through the families in a coded fashion (HLA status, individuals affected with MS, and RR/RP status were unknown). After segregation of alleles, they were entered into CHIAARRA (Beall et al. 1996; S. S. Beall, M.K. Hockertz, D. Studney, and D.W. Paty, unpublished data). COSTAR (Paty et al. 1994) was then accessed, and the code was broken as to affected status. Genotype frequencies and allele frequencies were then determined (tables 1 and 2). All genotypes were in HWE (table 1). Subhaplotypes (Beall et al. 1993) were determined at each portion of the TcR β-chain locus (fig. 1). Subhaplotype frequencies were calculated in a way analogous to that described above for genotype and allele frequencies and are presented in table 3.

Susceptibility to RP MS (but Not to RR MS), in DRw15$^+$ Patients

As the initial step in this study, we tested the hypothesis that susceptibility to DRw15$^+$ RP MS is associated with a gene linked to the region delimited by the Vβ8-*Bam*HI and Vβ11-*Bam*HI loci (Beall et al. 1987*a*, 1989, 1993). Frequencies of the subhalotypes delimited by the Vβ8-*Bam*HI and Vβ11-*Bam*HI loci (but not in the regions flanking this region) were significantly different from control frequencies, in the DRw15$^+$ RP MS portion of the data set ($\chi^2_2 = 6.19$; $P = .02$) (table 4). The major contributors to this difference were the increased representation, in the DRw15$^+$ RP MS patients, of the 2-1 subhaplotypes (67% vs. 42%) ($\chi^2_1 = 6.17$; $P = .006$; corrected $P = .018$; RR = 2.74), as well as a decreased representation of the 1-2 subhaplotype (30% vs. 51%) ($\chi^2_1 = 4.65$; $P = .02$; corrected $P = .06$; RR = .41). This effect was not seen in DRw15$^+$ RR MS patients ($\chi^2_2 = .40$; $P = .82$). Subhaplotype frequencies in DRw15$^+$ RP

Table 1

Genotype Frequencies Determined on the Basis of Vβ and Cβ Alleles in Caucasian MS Simplex and Multiplex Families from British Columbia

TcR β Genotype[a]	No. (Frequency) of with Genotype							
	DRw15+ and DRw15−	At Risk						
		DRw15+			DRw15−			
		All	RR MS	RP MS	All	RR MS	RP MS	Control
Vβ7-*Bam*HI:								
1,1	26 (.29)	14 (.30)	12 (.39)	2 (.13)	12 (.28)	6 (.20)	6 (.46)	21 (.23)
1,2	36 (.40)	17 (.36)	12 (.39)	5 (.31)	19 (.44)	15 (.50)	4 (.31)	37 (.41)
1,3	12 (.13)	7 (.15)	3 (.10)	4 (.25)	5 (.12)	4 (.13)	1 (.08)	17 (.19)
2,2	8 (.09)	4 (.09)	2 (.06)	2 (.13)	4 (.09)	3 (.10)	1 (.08)	7 (.08)
2,3	8 (.09)	5 (.11)	2 (.06)	3 (.19)	3 (.07)	2 (.07)	1 (.08)	8 (.09)
3,3	0 (.00)	0 (.00)	0 (.00)	0 (.00)	0 (.00)	0 (.00)	0 (.00)	0 (.00)
Total	90	47	31	16	43	30	13	90
HWE[b]	$\chi^2_3 = 2.18$	$\chi^2_3 = 1.29$	$\chi^2_3 = .61$	$\chi^2_3 = 1.48$	$\chi^2_3 = 1.19$	$\chi^2_3 = 2.37$	$\chi^2_3 = .66$	$\chi^2_3 = 5.29$
Vβ1-*Taq*I:								
1,1	3 (.03)	2 (.04)	1 (.03)	1 (.06)	1 (.02)	0 (.00)	1 (.08)	4 (.04)
1,2	18 (.20)	10 (.21)	3 (.10)	7 (.44)	8 (.19)	4 (.13)	4 (.31)	24 (.27)
2,2	69 (.77)	35 (.75)	27 (.87)	8 (.50)	34 (.79)	26 (.87)	8 (.62)	62 (.69)
Total	90	47	31	16	43	30	13	90
HWE[b]	$\chi^2_1 = 1.63$	$\chi^2_1 = 1.21$	$\chi^2_1 = 3.74$	$\chi^2_1 = .11$	$\chi^2_1 = .39$	$\chi^2_1 = .15$	$\chi^2_1 = .23$	$\chi^2_1 = .70$
Vβ8-*Msp*I:								
1,1	42 (.47)	23 (.49)	13 (.42)	10 (.63)	19 (.45)	16 (.55)	3 (.23)	38 (.44)
1,2	37 (.42)	21 (.45)	16 (.52)	5 (.31)	16 (.38)	8 (.28)	8 (.62)	40 (.46)
2,2	10 (.11)	3 (.06)	2 (.06)	1 (.06)	7 (.17)	5 (.17)	2 (.15)	9 (.10)
Total	89	47	31	16	42	29	13	87
HWE[b]	$\chi^2_1 = .18$	$\chi^2_1 = .39$	$\chi^2_1 = 2.64$	$\chi^2_1 = .12$	$\chi^2_1 = 1.22$	$\chi^2_1 = 3.67$	$\chi^2_1 = .74$	$\chi^2_1 = .10$
Vβ8-*Bam*HI:								
1,1	23 (.26)	12 (.26)	10 (.32)	2 (.13)	11 (.26)	7 (.23)	4 (.31)	23 (.26)
1,2	40 (.40)	18 (.38)	11 (.35)	7 (.44)	22 (.51)	15 (.50)	7 (.54)	42 (.47)
2,2	27 (.30)	17 (.36)	10 (.32)	7 (.44)	10 (.23)	8 (.27)	2 (.15)	25 (.28)
Total	90	47	31	16	43	30	13	90
HWE[b]	$\chi^2_1 = 1.08$	$\chi^2_1 = 2.39$	$\chi^2_1 = 2.61$	$\chi^2_1 = .15$	$\chi^2_1 = .02$	$\chi^2_1 = .00$	$\chi^2_1 = .14$	$\chi^2_1 = .40$
Vβ11-*Bam*HI:								
1,1	25 (.28)	16 (.34)	8 (.26)	8 (.50)	9 (.21)	7 (.23)	2 (.15)	21 (.24)
1,2	41 (.46)	19 (.40)	13 (.42)	6 (.38)	22 (.51)	17 (.57)	5 (.38)	42 (.47)
2,2	24 (.27)	12 (.26)	10 (.32)	2 (.12)	12 (.28)	6 (.20)	6 (.46)	26 (.29)
Total	90	47	31	16	43	30	13	89
HWE[b]	$\chi^2_1 = .71$	$\chi^2_1 = 1.62$	$\chi^2_1 = .77$	$\chi^2_1 = .26$	$\chi^2_1 = .03$	$\chi^2_1 = .54$	$\chi^2_1 = .29$	$\chi^2_1 = .25$
Cβ *Bgl*II:								
1,1	22 (.24)	11 (.23)	6 (.19)	5 (.31)	11 (.26)	9 (.30)	2 (.15)	30 (.34)
1,2	44 (.49)	22 (.47)	16 (.52)	6 (.38)	22 (.51)	15 (.50)	7 (.54)	38 (.43)
2,2	24 (.27)	14 (.30)	9 (.29)	5 (.31)	10 (.23)	6 (.20)	4 (.31)	20 (.23)
Total	90	47	31	16	43	30	13	88
HWE[b]	$\chi^2_1 = .04$	$\chi^2_1 = .17$	$\chi^2_1 = .06$	$\chi^2_1 = 1.00$	$\chi^2_1 = .02$	$\chi^2_1 = .003$	$\chi^2_1 = .14$	$\chi^2_1 = 1.38$
Cβ *Kpn*I:								
1,1	10 (.16)	4 (.13)	3 (.17)	1 (.08)	6 (.20)	3 (.17)	3 (.25)	10 (.17)
1,2	23 (.38)	14 (.45)	7 (.39)	7 (.54)	9 (.30)	5 (.28)	4 (.33)	31 (.53)
2,2	28 (.46)	13 (.42)	8 (.44)	5 (.39)	15 (.50)	10 (.56)	5 (.42)	18 (.30)
Total	61	31	18	13	30	18	12	59
HWE[b]	$\chi^2_1 = 1.86$	$\chi^2_1 = .01$	$\chi^2_1 = .45$	$\chi^2_1 = .46$	$\chi^2_1 = 3.48$	$\chi^2_1 = 2.12$	$\chi^2_1 = 1.18$	$\chi^2_1 = .30$

[a] Allele designations are as in figure 1.
[b] χ^2Test statistic for HWE.

Table 2

Allele Frequencies Determined on the Basis of Vβ and Cβ Alleles in Caucasian MS Simplex and Multiplex Families from British Columbia

TcR β Allele[a]	No. (Frequency) of Alleles							
		At Risk						
	DRw15+ and DRw15−	DRw15+			DRw15−			
		All	RR MS	RP MS	All	RR MS	RP MS	Control
Vβ7-*Bam*HI:								
1	100 (.56)	52 (.55)	39 (.63)	13 (.41)	48 (.56)	31 (.52)	17 (.65)	104 (.53)
2	60 (.33)	30 (.32)	18 (.29)	12 (.38)	30 (.35)	23 (.38)	7 (.27)	62 (.32)
3	20 (.11)	12 (.13)	5 (.08)	7 (.22)	8 (.09)	6 (.10)	2 (.08)	29 (.15)
Total	180	94	62	32	86	60	26	195
Vβ1-*Taq*I:								
1	24 (.13)	14 (.15)	5 (.08)	9 (.28)	10 (.12)	4 (.07)	6 (.23)	33 (.17)
2	156 (.87)	80 (.85)	57 (.92)	23 (.72)	76 (.88)	56 (.93)	20 (.77)	160 (.83)
Total	180	94	62	32	86	60	26	193
Vβ8-*Msp*I:								
1	121 (.68)	67 (.71)	42 (.68)	25 (.78)	54 (.64)	40 (.69)	14 (.54)	123 (.66)
2	57 (.32)	27 (.29)	20 (.32)	7 (.22)	30 (.36)	18 (.31)	12 (.46)	63 (.34)
Total	178	94	62	32	84	58	26	186
Vβ8-*Bam*HI:								
1	86 (.48)	42 (.45)	31 (.50)	11 (.34)	44 (.51)	29 (.48)	15 (.58)	96 (.50)
2	94 (.52)	52 (.55)	31 (.50)	21 (.66)	42 (.49)	31 (.52)	11 (.42)	96 (.50)
Total	180	94	62	32	86	60	26	192
Vβ11-*Bam*HI:								
1	91 (.51)	51 (.54)	29 (.47)	22 (.69)	40 (.47)	31 (.52)	9 (.35)	87 (.46)
2	89 (.49)	43 (.46)	33 (.53)	10 (.31)	46 (.53)	29 (.48)	17 (.65)	102 (.54)
Total	180	94	62	32	86	60	26	189
Cβ-*Bgl*II:								
1	88 (.49)	44 (.47)	28 (.45)	16 (.50)	44 (.51)	33 (.55)	11 (.42)	105 (.55)
2	92 (.51)	50 (.53)	34 (.55)	16 (.50)	42 (.49)	27 (.45)	15 (.58)	87 (.45)
Total	180	94	62	32	86	60	26	192
Cβ-*Kpn*I:								
1	43 (.35)	22 (.35)	13 (.36)	9 (.35)	21 (.35)	11 (.31)	10 (.42)	55 (.43)
2	79 (.65)	40 (.65)	23 (.64)	17 (.65)	39 (.65)	25 (.69)	14 (.58)	72 (.57)
Total	122	62	36	26	60	36	24	127

[a] Allele designations are as in figure 1.

MS patients were significantly different from those in DRw15− RP MS patients ($\chi^2_2 = 5.75$; $P = .03$), with a highly significant overrepresentation of the 2-1 subhaplotype (67% vs. 35%) ($\chi^2_1 = 5.73$; $P = .008$; RR = 3.8) (table 3).

We next tested the hypothesis that the gene associated with progression of MS in DRw15+ MS patients maps nearer to the Vβ11-*Bam*HI locus than to the Vβ8-*Bam*HI locus (Beall et al. 1993). Only at the Vβ8-*Bam*HI and Vβ11-*Bam*HI loci in the DRw15+ RP MS portion of the data set were the risk-allele frequencies significantly different from control-allele frequencies, with greater differences being seen at the 3′ (Vβ11-*Bam*HI) end (table 5). Only at the Vβ11-*Bam*HI locus in the DRw15+ RP MS portion of the data set were disease-genotype frequencies significantly different from control-genotype frequencies ($\chi^2_2 = 5.12$; $P = .04$), confirming our previous observations (Beall et al. 1993), because of a significantly increased representation (50% vs. 24%) of the 1,1 genotype ($\chi^2_1 = 4.73$; $P = .02$; RR = 3.24). These findings were not seen in the DRw15− MS group (table 1). Only at the Vβ11-*Bam*HI locus were disease-genotype frequencies in DRw15+ RP MS patients significantly different from those in DRw15+ RR MS patients ($\chi^2_2 = 3.06$; $P = .04$), primarily because of an increased representation (50% vs. 26%) of the 1,1 genotype in DRw15+ RP MS patients ($\chi^2_1 = 2.75$; $P = .048$; RR = 2.88). We conclude from these results that recessive inheritance of a gene linked to the Vβ11-*Bam*HI 1 allele confers an approximately three-times-increased risk for progression from RR MS to RP MS if the patient also coinherits the DRw15+ allele.

Since this is a family-based association test (Thomson 1995), the results support the hypothesis that suscepti-

bility to RP MS in DRw15$^+$ patients is associated with recessive inheritance of a gene linked to (or located on) the 3′ end of the 2-1 subhaplotype delimited by the Vβ8-*Bam*HI and Vβ11-*Bam*HI loci. This family-based association test confirms our previous hypothesis (Beall et al. 1989, 1993). The hypothesis is further strengthened by the lack of differences at other loci 5′ and 3′ to these test loci.

Susceptibility to RP MS (but Not to RR MS), in DRw15$^-$ Patients

Examination of the subhaplotype frequencies in the DRw15$^-$ portion of the data set showed that differences were found only in the region delimited by the Vβ1-*Taq*I and Vβ8-*Msp*I loci in RP MS (but not in RR MS) patients (table 4). The major contributor to the difference was a highly significant increased representation of the 1-1 and 2-2 subhaplotypes delimited by these two loci ($\chi^2_1 = 8.8$; $P = .003$; RR = 3.6), in RP MS patients compared with control subhaplotypes (table 3). We conclude from these results that coinheritance of a DRw15$^-$ major-histocompatibility-complex (MHC) class II allele with inheritance of the 1-1 and 2-2 subhaplotypes delimited by these two loci confers a 3.6-times-increased risk of progression.

For further exploration of these findings, the subhaplotype frequencies were compared between the RR MS and RP MS patients who were DRw15$^-$ (table 3). The comparison showed highly significant differences ($\chi^2_2 = 9.85$; $P = .007$). These differences were primarily due to a highly significant increase of the 1-1 subhaplotype in the MS patients who were in the progressive form of their disease, compared with the RR MS patients ($\chi^2_1 = 6.27$; $P = .01$; RR = 7.1). Comparison of allele frequencies between RR MS and RP MS patients was performed at each of the loci (Vβ1-*Taq*I and Vβ8-*Msp*I), to determine where on the 1-1 subhaplotype the gene for progression was located (table 2). Differences were greatest at the Vβ1-*Taq*I ($\chi^2_1 = 4.75$; $P = .03$; RR = 4.2) end of the subhaplotype, compared with the Vβ8-*Msp*I ($\chi^2_1 = 1.79$; $P = .18$) end of the subhaplotype. We conclude from these results that inheritance of a gene located on the 5′ end of the 1-1 subhaplotype delimited by these two loci confers a 7.1-times-increased risk for progression after an RR MS course, if there is also coinheritance of MHC class II alleles that are not DRw15.

DRw15 Allele as Not Associated with a Gene Linked to the Progression of MS

It is possible that progression of disease is associated with a gene linked to DRw15 alone. This hypothesis was tested by use of the simplex-family data set. The DRw15 allele was highly significantly associated with a gene linked with susceptibility to both RR MS ($\chi^2_1 = 18.81$; $P = .000014$) and RP MS ($\chi^2_1 = 15.02$; $P = .0001$). We conclude from these data that susceptibility to both RR MS and RP MS is associated with a gene linked to the DRw15 allele, thus supporting our earlier data from AFBACs (Beall et al. 1995). The DRw15 allele is *not,* however, involved in the progression of MS (in fact, the aforementioned data support the hypothesis that it is negatively associated with progression).

Discussion

The results described in this paper confirm and extend our original findings (Beall et al. 1987*a,* 1989, 1993; Charmley et al. 1991) by showing that epistasis between the HLA class II DRw15 allele and recessive inheritance of the upper allele (the 1 allele) of the Vβ11-*Bam*HI DNA polymorphism predisposes to RP MS (but not to RR MS). The results also support the hypothesis that epistasis between an HLA class II allele that is *not* DRw15 and inheritance of a gene located on the 5′ (centromeric) end of the 1-1 subhaplotype defined by the Vβ1-*Taq*I and Vβ8-*Msp*I DNA polymorphisms also predisposes to RP MS. Before we discuss the implications of these results, it is important to explore the controversy that has erupted around this locus and to review reasons why our previous studies, as well as linkage studies, have not been entirely reproducible.

Those TcR β-chain association studies that rely on a case-control experimental paradigm are subject to experimental bias with regard to ethnic diversity and population stratification, leading to false associations (Hodge 1993; Lander and Schork 1994; Thomson 1995). These ambiguities are lessened by the use of AFBACs, as we have done in the present study. There are, however, even more-subtle forms of bias. First, RFLPs discovered in the TcR Vβ region statistically will probably be located in either noncoding regions or introns and thus will have no basis for selection. Only if they are in linkage disequilibrium with an important coding segment will they be positively selected and thus be associated with disease. Haplotype analysis (which presumably "surrounds" at-risk genes by looking at the region between two loci) may detect regions containing susceptibility genes that would be missed by the use of noncoding region–allele analysis. Second, a case-control study, a priori, is biased in the assignment of haplotypes, because coupling phase cannot be established; only individuals who are doubly or singly homozygous at two adjacent polymorphisms will contribute haplotypes to the analysis (i.e., double heterozygotes cannot be scored). Thus, in theory, only one-half of all haplotypes can be scored. In addition, previous studies that relied on these criteria that we have proposed for assignment

Table 3

Subhaplotype Frequencies Determined on the Basis of Vβ and Cβ Alleles in Caucasian MS Simplex and Multiplex Families from British Columbia

TcR β Subhaplotype[a]	No. (Frequency) of Subhaplotypes							
		At Risk						
	DRw15+ and DRw15−	DRw15+			DRw15−			
		All	RR MS	RP MS	All	RR MS	RP MS	Control
Vβ7- *Bam*HI/Vβ1-*Taq*I:								
1-1	3 (.02)	2 (.02)	0 (.00)	2 (.07)	1 (.01)	0 (.00)	1 (.04)	1 (.01)
1-2	87 (.55)	46 (.53)	36 (.64)	10 (.33)	41 (.57)	26 (.54)	15 (.63)	89 (.54)
2-1	9 (.06)	5 (.06)	2 (.04)	3 (.10)	4 (.06)	2 (.04)	2 (.08)	18 (.11)
2-2	42 (.27)	21 (.24)	13 (.23)	8 (.27)	21 (.29)	17 (.35)	4 (.17)	32 (.20)
3-1	9 (.06)	6 (.07)	3 (.05)	3 (.10)	3 (.04)	1 (.02)	2 (.08)	9 (.05)
3-2	8 (.05)	6 (.07)	2 (.04)	4 (.13)	2 (.03)	2 (.04)	0 (.00)	15 (.09)
Total	158	86	56	30	72	48	24	164
Vβ1-*Taq*I/Vβ8-*Msp*I:								
1-1	17 (.10)	10 (.11)	4 (.07)	6 (.20)	7 (.09)	2 (.04)	5 (.21)	14 (.08)
1-2	5 (.03)	3 (.03)	1 (.02)	2 (.07)	2 (.03)	1 (.02)	1 (.04)	14 (.08)
2-1	99 (.59)	54 (.61)	36 (.62)	18 (.60)	45 (.56)	37 (.66)	8 (.33)	103 (.60)
2-2	47 (.28)	21 (.24)	17 (.29)	4 (.13)	26 (.33)	16 (.29)	10 (.42)	40 (.23)
Total	168	88	58	30	80	56	24	171
Vβ8-*Msp*I/Vβ8-*Bam*HI:								
1-1	26 (.16)	14 (.16)	10 (.18)	4 (.13)	12 (.15)	9 (.17)	3 (.13)	33 (.20)
1-2	86 (.52)	48 (.56)	28 (.50)	20 (.67)	38 (.49)	28 (.52)	10 (.42)	79 (.48)
2-1	50 (.30)	23 (.27)	17 (.30)	6 (.20)	27 (.35)	17 (.31)	10 (.42)	53 (.32)
2-2	2 (.01)	1 (.01)	1 (.02)	0 (.00)	1 (.01)	0 (.00)	1 (.04)	1 (.01)
Total	164	86	56	30	78	54	24	166
Vβ8-*Bam*HI/Vβ11-*Bam*HI:								
1-1	4 (.02)	3 (.05)	2 (.03)	1 (.03)	1 (.01)	1 (.02)	0 (.00)	4 (.02)
1-2	76 (.45)	36 (.41)	27 (.47)	9 (.30)	40 (.50)	25 (.46)	15 (.58)	88 (.51)
2-1	82 (.49)	46 (.52)	26 (.45)	20 (.67)	36 (.45)	27 (.50)	9 (.35)	73 (.42)
2-2	6 (.04)	3 (.03)	3 (.05)	0 (.00)	3 (.04)	1 (.02)	2 (.08)	8 (.05)
Total	168	88	58	30	80	54	26	173
Vβ11-*Bam*HI/Cβ-*Bgl*II:								
1-1	49 (.30)	30 (.33)	18 (.30)	12 (.40)	19 (.25)	15 (.30)	4 (.15)	48 (.28)
1-2	34 (.20)	19 (.21)	10 (.17)	9 (.30)	15 (.20)	10 (.20)	5 (.19)	29 (.17)
2-1	31 (.19)	12 (.13)	9 (.15)	3 (.10)	22 (.29)	12 (.24)	7 (.27)	47 (.27)
2-2	52 (.31)	29 (.32)	23 (.38)	6 (.20)	20 (.26)	13 (.26)	10 (.38)	49 (.28)
Total	166	90	60	30	76	50	26	173
Cβ-*Bgl*II/Cβ-*Kpn*I:								
1-1	27 (.25)	15 (.27)	10 (.29)	5 (.23)	12 (.23)	6 (.19)	6 (.30)	38 (.35)
1-2	25 (.25)	12 (.21)	5 (.15)	7 (.32)	13 (.25)	11 (.34)	2 (.10)	20 (.18)
2-1	8 (.07)	4 (.07)	2 (.06)	2 (.09)	4 (.08)	2 (.06)	2 (.10)	11 (.10)
2-2	48 (.44)	25 (.45)	17 (.50)	8 (.36)	23 (.44)	13 (.41)	10 (.50)	40 (.37)
Total	108	56	34	22	52	32	20	109

[a] Allele designations are as in figure 1.

of haplotypes (Beall et al. 1987*a*, 1989, 1993) could have missed instances of recombination between adjacent loci. In this study, we in fact found instances of single and double recombination flanking the at-risk regions (data not shown). Recombination between adjacent loci (which will not be detected in case-control studies) invalidates the assignment of subhaplotypes by means of our previous (Beall et al. 1987*a*, 1989, 1993) experimental design. This problem will be most pronounced in adjacent alleles that are in the greatest amount of linkage equilibrium. Third, studies that have used "control" populations containing high allele frequencies of

Table 4

Comparison of Subhaplotype Frequencies Determined on the Basis of Vβ and Cβ Alleles in Caucasian MS Simplex and Multiplex Families from British Columbia

	DRw15+			DRw15−			DRw15+ AND DRw15−:
TcR β HAPLOTYPE	All	RP MS	RR MS	All	RP MS	RR MS	All
Vβ8-*Bam*HI/Vβ11-*Bam*HI:							
χ^2_2	2.52	6.19	.40	.43	.54	1.44	1.51
P	.14	.02	.82	.81	.76	.49	.47
Vβ11-*Bam*HI/Cβ-*Bgl*II:							
χ^2_3	6.59	7.41	4.27	.55	2.19	.53	3.64
P	.09	.06	.23	.91	.41	.91	.30
Vβ8-*Msp*I/Vβ8-*Bam*HI:							
χ^2_2	1.53	3.70	.10	.53	.91	.46	.95
P	.47	.16	.95	.77	.63	.80	.62
Cβ-*Bgl*II/Cβ-*Kpn*I:							
χ^2_3	1.88	2.45	2.08	3.06	1.56	5.45	3.61
P	.60	.48	.56	.38	.67	.14	.31
Vβ1-*Taq*I/Vβ8-*Msp*I:							
χ^2_3	2.67	4.83	3.49	4.65	9.31	4.58	5.17
P	.45	.18	.32	.20	.03	.21	.16
Vβ7-*Bam*HI/Vβ1-*Taq*I:							
χ^2_4	3.40	6.95	5.25	6.56	3.92	8.19	6.58
P	.25	.14	.26	.16	.42	.08	.16

NOTE.—Significant *P* values are underlined.

Table 5

Comparison of Allele Frequencies Determined on the Basis of Vβ and Cβ Alleles in Caucasian MS Simplex and Multiplex Families from British Columbia

	DRw15+			DRw15−			
	All	RP MS	RR MS	All	RP MS	RR MS	DRw15+ AND DRw15−: All
Vβ11:							
χ^2_1	1.70	5.65	.01	.005	1.20	.58	.76
P	.10	.009[a]	.92	.94	.27	.45	.19
Vβ8-*Bam*HI:							
χ^2_1	.72	2.68	.00	.03	.54	.05	.18
P	.20	.05[b]	1.00	.86	.46	.82	.67
Vβ8-*Msp*I:							
χ^2_1	.76	1.80	.05	.09	1.50	.16	.14
P	.38	.18	.82	.76	.22	.69	.71
Vβ1-*Taq*I:							
χ^2_1	.22	2.20	3.02	1.37	1.37	3.99	1.02
P	.64	.14	.08	.24	.45	.05[c]	.31
Vβ7-*Bam*HI:							
χ^2_2	.67	1.04	1.11	.98	2.49	.80	.95
P	.72	.60	.57	.61	.14	.67	.62
Cβ-*Bgl*II:							
χ^2_1	1.57	.24	1.71	.30	1.41	.002	1.25
P	.21	.62	.19	.58	.24	.96	.26
Cβ-*Kpn*I:							
χ^2_1	1.06	.67	.60	1.17	.02	1.89	1.69
P	.30	.41	.44	.28	.89	.17	.19

NOTE.—Significant *P* and RR MS values are underlined.

[a] Relative Risk (RR) = 2.58.

[b] RR = 1.91.

[c] RR = 2.89.

the disease-susceptibility genes tested for may not detect a positive association (Kellar-Wood et al. 1995). Last, case-control methods that have used Southern analysis to study these questions may have been biased because of migration artifacts or partial digestions, and studies that have used PCR-based methods may have been biased because of genotyping ambiguity due to the infidelity of *Taq* polymerase. The use of family-based data (wherein segregation of alleles in Mendelian fashion must occur), as has been utilized in the present study, lessens this last experimental bias and more effectively circumvents all of the aforementioned problems of experimental design.

An important finding of this study is that, although there was a nonsignificant trend of susceptibility to MS being associated with a gene linked to the Vβ11-*Bam*HI DNA polymorphism ($\chi^2_1 = .76$; $P = .19$; table 5), the effect was more marked ($\chi^2_1 = 1.70$; $P = .10$; table 5) in the HLA class II DRw15$^+$ MS patients, supporting our original conclusions (Beall et al. 1987*a,* 1989, 1993) and others (Kellar-Wood et al. 1995; Wood et al. 1995; Epplen et al. 1997), again demonstrating epistasis between the HLA class II region and the TcR β-chain locus, in conferring susceptibility to MS. There was virtually no effect of this region in HLA class II DRw15$^-$ MS patients (table 5). It is important to note that, with regard to the Vβ11-*Bam*HI DNA polymorphism, there was a highly significant effect in the RP MS subset ($\chi^2_1 = 5.65$; $P = .009$; RR = 2.58), whereas there was no effect in the RR MS subset ($\chi^2_1 = .01$; $P = .92$) (table 5). Our findings support recent findings by Wei et al. (1995), who, in their population of primarily RR MS patients, have also failed to see in this region an effect. This gene is located at the 3′ end of the 2-1 subhaplotype defined by the Vβ8-*Bam*HI and Vβ11-*Bam*HI alleles and is recessively inherited.

We have also shown that, in HLA class II DRw15$^-$ MS patients, susceptibility to the progressive form of the disease is associated with a gene linked to the Vβ1-*Taq*I DNA polymorphism, which is located on the 1-1 subhaplotype defined by the Vβ1-*Taq*I and Vβ8-*Msp*I alleles. These findings are similar to those recently reported by Epplen et al. (1997). It will be important to define first of all which allele (other than DRB1*1501) is a secondary risk allele, by use of the method of relative predispositional effects (Payami et al. 1989). Next, it will be of interest to look at these initial results in an expanded data set selected for that secondary risk allele.

This study also supports recent findings with the two most completely studied animal models of MS, EAE- and TMEV-induced demyelination (Dal Canto et al. 1995). Goverman et al. (1993) constructed transgenic mice that expressed genes encoding a rearranged T cell receptor specific for myelin basic protein and that developed EAE spontaneously in a nonsterile environment (but not in a sterile environment). Similarly, Rodriguez et al. (1992, 1994) showed that strains of mice with massive deletions of the TcR β-chain complex develop severe demyelination if they also inherit the appropriate H-2 complex.

The results of the present study may explain the disparate results that were found in the recent worldwide genome search for susceptibility genes in MS (Ebers et al. 1996; Haines et al. 1996; Sawcer et al. 1996). Our original experimental design (Beall et al. 1987*a,* 1989) studied severely affected (CP and RP) MS patients, which may have expedited the discovery of genes that are involved in progression (Beall et al. 1993; present study) but that may not have been found had the whole population of MS patients been analyzed. Recent work supports this hypothesis. Mahtani et al. (1996) have recently completed, in Finnish families, a genome scan for susceptibility to type 2 diabetes. By selecting patients who were phenotypically more severely affected, they uncovered genes originally missed in the population as a whole. The disparate results reported recently by three groups (reviewed in Bell and Lathrop 1996) regarding genetic susceptibility to MS may be due to phenotypic differences. Tertiary-care referral centers utilized by the U.S. medical-care system may follow patients with more-severe forms of MS and thus may uncover different genes, compared with centers that follow patients with a wide spectrum of disease. Supporting this hypothesis is the fact that the U.S. group (Haines et al. 1996) targeted the TcR β-chain complex (or a gene closely linked to it) as a possible candidate gene other than HLA.

It is understood that a certain percentage of RR MS patients will enter a progressive phase after an unknown period. Only by periodically updating our data set will we be able to detect differences in genotype-phenotype correlation. In this population, which is free from ascertainment bias, we estimate that in 10% of our patients the disease will be benign (Weinshenker et al. 1989). Conversely, certain of our RR MS patients will undergo a severe secondary progressive course. It will be extremely important to define these populations more precisely at the genetic level, to look at how genetic susceptibility influences the course of disease over time. Our system of the semirelational database COSTAR and the heuristic CHIAARRA (Beall et al. 1996; S. S. Beall, M.K. Hockertz, D. Studney, and D.W. Paty, unpublished data)—a system that, by use of input phenotypic parameters from COSTAR, automatically updates experimental analysis of genetic data—will facilitate this process.

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