S. Jenisch
E. Westphal
R.P. Nair
P. Stuart
J.J. Voorhees
E. Christophers
M. Krönke
J.T. Elder
T. Henseler

Linkage disequilibrium analysis of familial psoriasis: identification of multiple disease-associated MHC haplotypes

Key words:
psoriasis; HLA antigens; HLA haplotypes; linkage; linkage-disequilibrium

Acknowledgments:
We gratefully acknowledge Clauda Gier, Hilke Clasen and Dorit Schuster for skilled assistance with DNA-based HLA typing, Angela Schulz, Corinna Greve, Bettina Marxsen, Marion Schromm and Anke Siemsen for expert serological HLA typing, and Winfried Lenk and Jaques Horstmann for their assistance with clinical evaluation. We also thank Wolfgang Müller-Ruchholtz for advise and support. This research was supported by award DFG-WE 905/1–1 from the German Research Foundation (EW, TH, SJ) and by USPHS awards P30 HG00209–03 and R01 AR4274–01 (JTE, PS, RN).

Abstract: Although psoriasis vulgaris (PsV) is strongly associated with certain human leukocyte antigens, the pathogenetic nature of these associations remains elusive. The objectives of this study were: (i) to determine whether HLA loci directly determine susceptibility or merely serve as markers for the susceptibility allele; and (ii) to identify additional disease-associated haplotypes. By applying maximum likelihood linkage disequilibrium analysis (LDA) in cases vs. controls, we found the susceptibility gene to be more strongly associated with specific HLA haplotypes than with their component alleles. Stronger linkage disequilibrium between PsV and HLA alleles was detected at HLA-C and HLA-B than at DRB1 and DQB1. Parametric linkage analysis accounting for marker-trait disequilibrium in psoriasis vulgaris pedigrees yielded most significant results for a locus close to HLA-B and -C. Furthermore, we found that susceptibility is linked to at least three different ancestral HLA haplotypes; among them, HLA-Cw7-B8-DRB1*0301-DQB1*02 is linked to PsV for the first time. These results identify a major PsV susceptibility locus in the immediate vicinity of, but distinct from HLA-B or HLA-C, and suggest that multiple disease alleles have arisen during human evolution.

Authors' affiliations:

S. Jenisch[1],
E. Westphal[1],
R.P. Nair[2],
P. Stuart[2],
J.J. Voorhees[2],
E. Christophers[3],
M. Krönke[1],
J.T. Elder[2,4,5],
T. Henseler[3,5]

[1]Department of Immunology, University of Kiel, Kiel, Germany,

[2]Department of Dermatology, University of Michigan, Ann Arbor, Michigan, USA,

[3]Department of Dermatology, University of Kiel, Kiel, Germany,

[4]Department of Radiation Oncology (Cancer Biology), University of Michigan, Ann Arbor, Michigan, USA,

[5]Co-senior authors

Correspondence to:
Stefan Jenisch
Institute of Immunology
University of Kiel
Michaelisstrasse 5
24105 Kiel
Germany
E-mail:
jenisch@immunologie.uni-kiel.de

Received 20 July,
accepted for publication 11 November 1998

Tissue Antigens . ISSN 0001-2815

Tissue Antigens 1999: **53**: 135–146
Printed in Denmark .

Psoriasis vulgaris (PsV) is a chronic inflammatory skin disease affecting 1–2% of the Caucasian population (1). Although keratinocyte hyperproliferation is a cardinal feature of PsV, increasing evidence also indicates that immune system activation plays a central role in its pathogenesis (reviewed in (2)). Suggestive of a primary immune pathogenesis, two different animal models demonstrate that dysregulated (3) or superantigen-activated T cells (4) induce PsV-like skin syndromes in mice, and the administration of a toxin selective for activated T-lymphocytes results in clinical improvement (5). Association of PsV with HLA antigens was first described in 1972 (6, 7). Since then, numerous phenotypes at the HLA-A, -B, -C,-DRB1, DQA1 and DQB1 loci have been associated with PsV, with HLA-Cw6 demonstrating the strongest association in most investigations (reviewed in (8)). Given the immunological background of

PsV, it has been proposed that HLA molecules are directly involved in its etiology (9, 10). However, at present, the primary cause of PsV remains elusive.

Several partial or complete genome scans in PsV have appeared in recent years (11–14). Two of these scans revealed evidence for a gene in the HLA region, as expected given the strength of known HLA associations (11, 13). However, the evidence for linkage to HLA region markers in these studies was surprisingly limited. In a recent investigation, we studied the inheritance of HLA alleles themselves, rather than that of microsatellite markers mapping to the MHC (15). In that study, we demonstrated that alleles at HLA-C, -B, -DRB1 and -DQB1 belonging to the ancestral haplotype (AH) 57.1 (16) are linked to PsV in the presence of strong linkage disequilibrium (LD). That study also showed that incorporation of marker-trait LD markedly increased the ability to detect linkage in parametric linkage analysis, and served to localize the gene to the HLA-B/-C region. It did not, however, identify the susceptibility gene itself, nor did it exclude the presence of disease allele(s) on haplotypes other than AH 57.1.

The current study utilizes a German cohort consisting of 80 families with 300 affected members, assembled on the basis of juvenile onset in the proband. This cohort is nearly twice the size of the German subgroup analyzed in our recent study of PsV linkage to HLA markers in U.S. and German families (15). The objectives of the present study were: (i) to determine whether the HLA loci under study directly determine susceptibility or merely serve as markers for the susceptibility allele; and (ii) to identify additional disease-associated haplotypes. We find that susceptibility is linked to at least three different ancestral HLA haplotypes, and that LD with the trait is stronger for these haplotypes than for their component alleles. One of these haplotypes, HLA-Cw7-B8-DRB1*0301-DQB1*02, is linked to PsV for the first time. We also confirm the conclusion of our previous study (15) that the disease allele resides in the vicinity of the class I-class III junction. Taken together, these results strongly suggest that the susceptibility determinant is not encoded by any of the HLA genes tested, and that multiple disease alleles have arisen at the trait locus during the evolution of modern humans.

Material and methods

Ascertainment of probands, families and controls

Psoriasis patients with early onset (<40 years) were identified from billing records, referrals, or clinic files, and used to identify families with at least two affected members. All individuals were examined by a dermatologist and considered to be affected if they displayed two or more characteristic skin, scalp, nail or joint lesions (1). Parents and siblings of affected sib pairs were collected whenever possible. The study group obtained using these criteria consisted of a total of 646 HLA-typed individuals (300 affected) belonging to 80 German families, yielding a total of 886 evaluable meioses. Selected characteristics of the study cohort are presented in Table 1. A group of healthy, unrelated local blood donors served as the control group for estimations of LD (see below). This group consisted of 86 unrelated local blood donors fully typed for HLA-A, -B, -C, -DRB1 and -DQB1, and an additional 49 blood donors typed only for DRB1 and DQB1.

HLA typing

DNA was prepared from peripheral blood lymphocytes or immortalized lymphoblast suspensions (17). HLA-A and -B were typed serologically by the lymphocytotoxicity test (LCT) (18). Allele frequencies were calculated from the observed HLA-A and -B serotypes assuming Hardy Weinberg equilibrium. HLA-C alleles were determined by polymerase chain reaction with sequence-specific primers (PCR-SSP) (19), and HLA-DRB1 alleles were assigned by group-specific PCR followed by hybridization with sequence-specific oligonucleotide probes (PCR-SSOP) according to the 11th International HLA Workshop DNA typing reference protocol (20). DQB1 specificities were determined by PCR-SSP (21) and the alleles of the DQB1*05 and *06 groups were subsequently estimated by PCR-SSOP using 11th International HLA Workshop probes (20). Ambiguous hybridization patterns were validated by sequencing-based typing (SBT), using group-specific PCR primers tailed with the -21M13 universal primer (5′-tgt aaa acg acg gcc agt-3′) to amplify the sequence in question, followed by sequencing on an ABI 373A DNA sequencer (Applied Biosystems, Foster City, CA, USA), using fluorescent-labeled M13 sequencing primers and Amplitaq FS® polymerase (Perkin-Elmer-Cetus, Norwalk, NJ, USA).

Estimation of HLA associations

HLA phenotypes of the psoriatic case group were derived from 96 unrelated, diseased founders of the families under investigation and compared to those obtained from the unrelated local controls described above. Deviations of observed HLA allele frequencies between cases and controls were determined from 2×N contingency tables, and significance was tested using a Monte Carlo approach involving 5,000 simulations (22).

General characteristics of the study cohort

A. Proportion of subjects affected

Affected/total male	136/302	45.03%
Affected/total female	164/344	47.67%
Affected/total individuals	300/646	46.44%

B. Number of affected individuals per family

Number	affected	Number of families
	2	22
	3	21
	4	19
	5	6
	6	3
	7	7
	8	1
	12	1
total:	300	80

Table 1

Linkage disequilibrium analysis (LDA)

In order to estimate the likelihood L of LD and the corresponding LD parameters under the genetic model chosen for our linkage studies (see below), two sets of calculations were performed using the case-control option of the EH program (23, 24). The first set of calculations involved individual HLA loci vs. the trait locus, and will be referred to here as two-locus LDA. The second set of calculations involved pairs of adjacent HLA loci taken together with the trait locus, and will be referred to as three-locus LDA. Significance levels for two-locus LDA were estimated from χ^2 values ($=2 \ln[L]$) and their number of degrees of freedom (df) using the CHIPROB linkage utility (23). The hypotheses defining the three possible outcomes of three-locus LDA are H0 (no association), H1 (markers associated independently of disease), and H2 (markers and disease associated). The relevant hypothesis is H2–H1, because LD between the markers is assumed (24). Therefore, the relevant distribution is χ^2 (H2-H1)=χ^2 (H2)−χ^2 (H1). Due to the extensive polymorphism of the HLA loci analyzed, the number of df exceeded by several times the number of individuals present in the case and control groups combined ($df=n_1 \cdot n_2 \ldots \cdot n_i - i + 1$; where i is number of loci investigated and n_i is the number of alleles at locus i) (23). Therefore, it was necessary to estimate three-locus LDA significance levels by Monte Carlo methods (22, 23). Repeated simulations were performed to generate random genotype tables having the same marginal totals as the observed data. Because random simulation of HLA genotypes calculated for each locus separately does not take disequilibrium between the HLA loci into account, we used the haplotype frequencies determined for the control population as described above to generate sets of random haplotypes, from which the simulated genotypes were extracted. One thousand sets of genotypes, each the size of the case group, were generated and used as input for repeated runs of the EH program. We then counted the number of times that a χ^2 value determined by the observed data was equaled or exceeded by the simulated data. The P-value corresponding to the 95% confidence interval was determined from this count using the BINOM linkage utility (23).

Linkage studies

We selected a dominant genetic model, the use of which is well-supported by prior studies of the inheritance of HLA in PsV (6, 25, 26). We assumed a disease allele frequency of 0.03, penetrances $f_{DD}=f_{Dd}=0.3=0.3$ and $f_{dd}=0.001$, a disease prevalence ϕ of 1.9%, and the proportion of cases due to nongenetic causes (R) of 5% (24). These values fit Hardy-Weinberg equilibrium and are reasonable in view of the estimated psoriasis prevalence in Germany (1) and the existence of a distinct form of adult-onset psoriasis displaying several characteristics of the spondyloarthropathies (27). Two-point

Two-locus LDA: Maximum likelihoods and significance determinations

Table 2

Locus:	HLA-A	HLA-C	HLA-B	HLA-DR	HLA-DQ
ln(L) for H1[1]	−867.23	−599.56	−1110.06	−875.15	−706.17
ln(L) for H0[2]	−881.18	−638.36	−1155.16	−891.88	−720.44
χ^2 for H1-H0[3]	27.90	77.60	90.20	33.46	28.54
df	28	32	50	24	14
P-value	n.s.	0.000012	0.00043	0.01	0.012

[1] L=likelihood of obtaining observed results given H1 (marker and trait locus associated)
[2] L=likelihood of obtaining observed results given H0 (marker and trait locus not associated)
[3] χ^2 value for H1–H0=−2 ln [L (H0)/L (H1)]=2 [ln L (H1)–ln L (H0)]
df: degrees of freedom

Two-locus LDA: Haplotype frequencies *Table 3*

HLA allele	d/HLA[1]	D/HLA[1]	Allelic association[2]	Component of
A1	0.1637	0.0075	0.044	AH 8.1, 57.1
A2	0.2323	0.0124	0.051	
A24	0.0766	0.0036	0.045	
Cw6	0.0895	0.0183	0.170	AH 57.1
Cw7	0.2785	0.0051	0.018	AH 8.1, others
B8	0.0929	0.0031	0.032	AH 8.1, others
B13	0.0275	0.0066	0.194	AH 13.1
B44	0.1159	0.0026	0.022	AH 44.1, 44.2
B57	0.0237	0.0103	0.303	AH 57.1
DRB1*0301	0.0953	0.0031	0.032	AH 8.1, others
DRB1*13	0.0916	0.0038	0.040	
DRB1*0701	0.0971	0.0145	0.130	AH 57.1
DQB1*06	0.2314	0.0046	0.019	
DQB1*02	0.1789	0.0093	0.049	AH 13.1, others
DQB1*0301	0.1412	0.0040	0.028	AH 8.1, others
DQB1*0303	0.0498	0.0097	0.163	AH 57.1

[1] Marker-trait-locus haplotype frequencies calculated under the assumption of allelic association. Only haplotypes occurring at least three times in the case group are shown. D denotes disease allele, d denotes non-disease allele(s)
[2] Defined as D/HLA−(D/HLA+d/HLA)

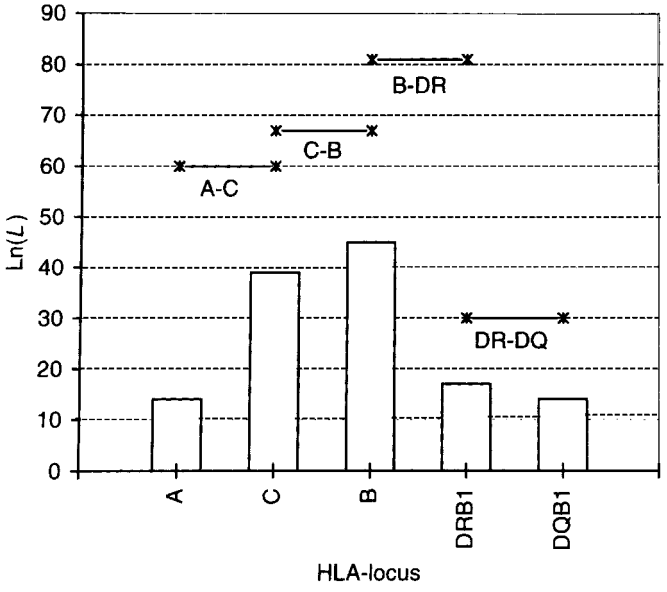

Fig. 1. **Likelihoods (*L*) of LD between the trait locus and individual HLA loci vs. two-locus HLA haplotypes.** Bars represent the Ln(*L*) for disequilibrium between the trait and individual HLA loci, and horizontal lines represent the Ln(*L*) for disequilibrium between the trait locus and the indicated two-locus HLA haplotypes. For two-locus LDA, the hypothesis of allelic association was conditioned against the hypothesis of no association. For three-locus LDA, the hypothesis of both markers and trait locus associated was conditioned against the hypothesis of markers associated with each other independent of disease.

parametric linkage calculations were performed using MLINK, ILINK (28) and HOMOG (23). Pairwise log likelihood odds ratios (lod scores) were calculated for five HLA loci (HLA-A, HLA-C, HLA-B, HLA- DRB1 and HLA-DQB1). LD information was incorporated into parametric linkage analysis by substituting marker-trait locus haplotype frequencies under the "define haplotype option" in MLINK AND ILINK, as described (15, 24). The haplotype frequencies used were the maximum likelihood estimates determined by two-locus LDA. For some very rare HLA alleles not found in the control populations but present in the study group, two-locus LDA yielded a haplotype frequency of zero. In such cases, the trait-marker locus haplotype frequencies were set to the lowest possible non-zero value (1×10^{-7}).

Results

HLA allele frequency distributions differed significantly between affected founders and the healthy control population for all HLA loci investigated (HLA-A: $\chi^2=35.25$, $P<0.001$; HLA-B: $\chi^2=88.15$, $P<0.001$; HLA-C: $\chi^2=80.09$, $P<0.001$; HLA-DRB1: $\chi^2=34.42$, $P<0.001$; HLA-DQB1: $\chi^2=23.31$, $P<0.005$). Unaffected founders differed significantly from healthy controls only for HLA-C ($\chi^2=23.33$, $P<0.05$).

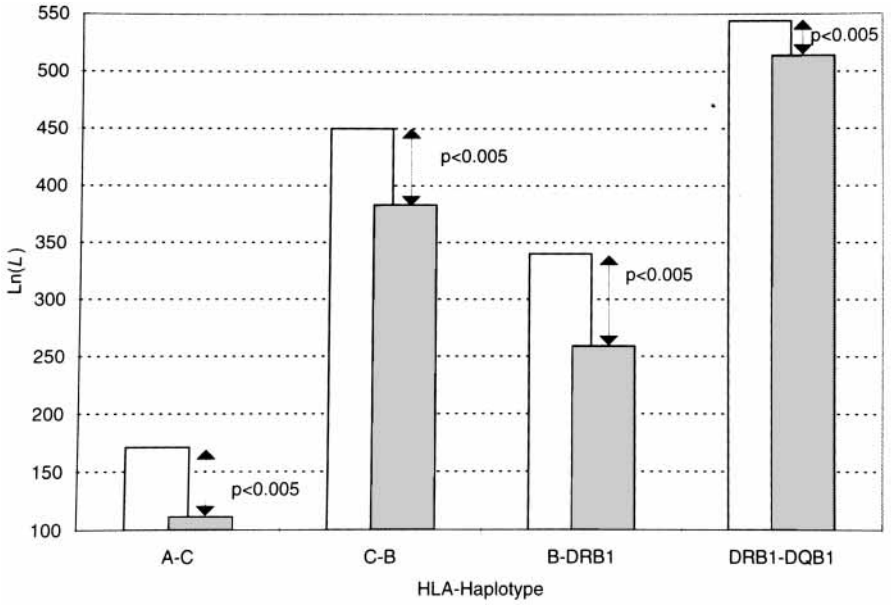

***Fig. 2.* Likelihoods (*L*) determined by three-locus LDA.** Bars indicate the ln(*L*) of allelic association of the indicated HLA haplotypes dependent upon (open bars) or independent of (hatched bars) association with the trait locus. Significance levels are determined by Monte Carlo simulation. All likelihood values shown are conditioned against the hypothesis of no association between the marker loci.

The likelihood and strength of LD between individual HLA loci and the trait locus was estimated by two-locus LDA. The maximum likelihoods and significance values are given in Table 2, and the corresponding marker-trait haplotype frequencies are given in Table 3. Disequilibrium with the trait locus was most significant for HLA-B and HLA-C, less significant for DRB1 and DQB1, and not significant for HLA-A. The Class I alleles A2, A1, A24, Cw6, Cw7, B8, B13, and B57 displayed the greatest haplotype frequencies with the disease allele, as did the Class II alleles DRB1*0701, DRB1*0301, DRB1*1301/02, DQB1*0303, DQB1*0201/02, and DQB1*06 (Table 3). The allelic association (defined here as the percentage of a given haplotype positive for the disease allele) was highest for B57, followed by B13, Cw6, and DQB1*0303. Although the disease allele was frequently found on haplotypes positive for Cw7, B8, DQB1*02 and DRB1*0301, the allelic association of these markers was low, due to the high frequency of the respective alleles in the control population. Except for A2, B44 and DRB1*1301/2, all alleles found at high frequency on chromosomes carrying the disease allele at the trait locus could be assigned to established ancestral HLA haplotypes. These included AH 57.1 (Cw6-B57-DRB1*0701-DQB1*0303), AH 13.1 (Cw6-B13-DRB1*0701-DQB1*02), and AH 8.1 (Cw7-B8-DRB1*0301-DQB1*02).

In order to determine whether or not any of the tested HLA loci might be the direct determinant of susceptibility, we also performed three-locus LDA. For all HLA intervals investigated, the log likelihoods for LD between the trait locus and pairs of adjacent HLA marker loci determined by three-locus LDA were much greater than those determined by two-locus LDA (Fig. 1). Moreover, the maximum likelihood values for LD between each of the four pairs of contiguous HLA loci were much greater when estimated under the assumption of LD with the trait locus (Fig. 2). To evaluate the significance of the results shown in Fig. 2, we performed three-locus LDA on 1,000 sets of simulated control genotypes and compared the resulting χ^2 values to those obtained using the observed data.

Three-locus LDA: Monte Carlo significance tests[1]

	A–C			C–B			B–DRB1			DRB1–DQB1		
	S_{min}	S_{max}	obs.	S_{min}	S_{max}	obs.	S_{min}	S_{max}	obs.	S_{min}	S_{max}	obs.
H2	278	406	342	662	829	899	561	969	681	1197	1367	1089
H1	251	363	222	637	804	765	506	640	519	1266	1353	1029
H2-H1	22	52	120	13	38	134	40	76	162	5	24	60

[a] Simulated χ^2 values for H2, H1 and H2–H1 after 1,000 Monte-Carlo simulations, vs. observed (obs.) values for H2, H1; and H2–H1. H1: markers associated; H2: markers and disease associated. S_{min} and S_{max} denote minimum and maximum simulated values obtained under each hypothesis

Table 4

Three-locus LDA: Haplotype frequencies[1]

Table 5

Allele at locus 1	Allele at locus 2	d/HLA	D/HLA	Allelic association[2]	Component of
A2	Cw6	0.032	0.009	0.220	
A1	Cw6	0.019	0.004	0.174	AH 57.1
A1	Cw7	0.093	0.002	0.021	AH 8.1, others
Cw6	B57	0.008	0.011	0.579	AH 57.1
Cw6	B13	0.031	0.006	0.162	AH 13.1
Cw7	B8	0.088	0.002	0.022	AH 8.1
B57	DRB1*0701	0.012	0.009	0.429	AH 57.1
B13	DRB1*0701	0.015	0.005	0.250	AH 13.1
DRB1 *0701	DQB1*0303	0.023	0.009	0.281	AH 57.1
DRB1 *0701	DQB1*02	0.075	0.005	0.063	AH 13.1
DRB1 *13	DQB1*06	0.083	0.004	0.046	
DRB1 *0301	DQB1*02	0.098	0.003	0.030	AH 8.1, others

[1] Marker-trait-locus haplotype frequencies, calculated under the hypothesis H2–H1 in Table 4 (i.e., allelic association between the marker loci is given). Haplotypes occurring at least three times in the case group are shown. D denotes disease allele, d denotes non-disease allele(s)

[2] defined as D/HLA÷(D/HLA+d/HLA)

For each pair of contiguous HLA loci analyzed, none of the 1,000 simulations reached the χ^2 values achieved by the actual data (Table 4). Since the sample-based 95% confidence interval of P for zero observations after 1,000 runs extends from zero to 0.003, this analysis yields significance at the $P \leq 0.005$ level for all intervals investigated.

The most common three-locus haplotypes determined to contain the disease allele at the trait locus are given in Table 5. The strongest allelic associations between two-locus HLA haplotypes and the trait locus were observed for Cw6-B57, B57-DRB1*0701, and DRB1*0701-DQB1*0303, all of which are components of the ancestral haplotype AH 57.1. Cw6-B13, B13-DRB1*0701, and DRB1*0701-DQB1*02, all of which are components of AH 13.1, yielded consistently lower allelic associations. The third ancestral haplotype to be represented was AH 8.1, which encompasses three HLA haplotypes identified by three-locus LDA: A1-Cw7, Cw7-B8, and DRB*0301-

Lod scores calculated by two-point linkage analysis, with and without incorporation of marker-trait haplotype frequencies

		Z (θ)						
	Locus	0.0	0.1	0.2	0.3	0.4	Z_{max}	θ_{max}
	HLA-A	−8.79	−1.11	0.66	0.83	0.44	0.87	0.265
without	HLA-C	−4.48	1.36	2.24	1.75	0.83	2.24	0.198
haplotype	HLA-B	−13.61	−1.70	1.18	1.53	0.88	1.58	0.271
frequencies	HLA-DRB1	−11.21	−1.13	1.03	1.20	0.66	1.26	0.263
	HLA-DQB1	−11.97	−2.20	0.09	0.53	0.31	0.53	0.298
	HLA-A[1]	−6.69	3.50	4.86	4.07	2.29	5.13	0.195
with	HLA-C	−0.39	11.52	11.48	8.83	4.79	12.23	0.144
haplotype	HLA-B	−8.02	9.41	11.38	9.51	5.49	11.39	0.189
frequencies	HLA-DRB1	−7.95	4.48	6.16	5.27	3.03	6.16	0.203
	HLA-DQB1	−11.13	1.08	3.41	3.34	2.03	3.58	0.243

[1] HLA-A did not yield significant LD with the trait locus. Haplotype-based lod-scores for this locus are shown only for completeness

Table 6

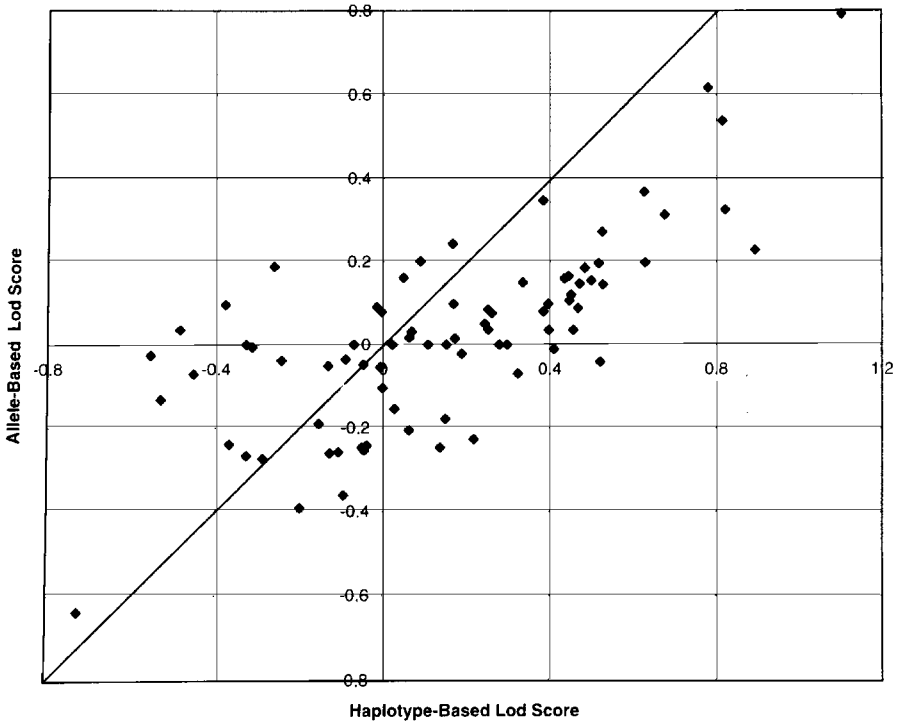

Fig. 3. **Scatterplot of allele-based vs. haplotype-based parametric two-point linkage lod scores for individual families, HLA-C, dominant model, θ=0.144.** Points below the diagonal line represent families for whom the lod score increased after incorporating marker-trait disequilibrium data.

DQB1*02. For AH 57.1, the strongest allelic associations were observed over the HLA-B/C-interval, whereas the HLA-B-DRB1-interval yielded the strongest association for AH 13.1. In the case of AH 8.1, allelic associations were much lower and no particular interval was predominant.

To extend the results of the foregoing case-control tests to our pedigrees, we applied the HLA marker-trait locus haplotype frequencies defined by two-locus LDA to parametric linkage analysis. The lod scores obtained for all individuals are summarized in Table 6. In the absence of marker-trait disequilibrium, the largest maximum lod score observed using ILINK was $Z_{max}=2.24$ for HLA-C at a recombination fraction theta (θ)=0.198. However, the replacement of allele frequencies by marker-trait-locus haplotype frequencies resulted in highly significant lod scores for HLA-B and HLA-C, the highest value observed being $Z_{max}=12.23$ for HLA-C at $\theta=0.144$. The lod scores for HLA-DRB1 and -DQB1 also increased, but did not reach the levels observed for HLA-B and -C. For all analyzed markers and haplotypes, admixture tests for genetic heterogeneity were carried out (23). No statistically significant evidence for heterogeneity was found (data not shown).

Parametric two-point lod scores for HLA-C at $\theta=0.144$ (the θ value yielding Z_{max}) are presented on a family-by-family basis in Fig. 3. As illustrated by the preponderance of points under the diagonal line, most lod scores increased after application of LD information (increased in 58, decreased in 20, and unchanged in 2 out of 80 pedigrees). In most of the pedigrees in which lod scores increased, phase-unknown matings could be found. In several others, we found affected sibs that were HLA-different, and in at least two families affected grandchildren shared no HLA haplotypes with their diseased grandparents. Representative examples of such pedigrees are shown in Fig. 4.

Discussion

Linkage studies seek to identify disease genes by virtue of cosegregation of the trait and marker genotypes within families. Such analyses can normally detect genes of large effect with reasonable power over large distances (up to 20 cM). However, due to the compact nature of the MHC, which spans only about 4 cM and yet contains at least 200 genes (29), linkage methods that count recombinations within families have proven to be of limited use for disease gene mapping within the MHC. In contrast, studies looking for associations between particular marker alleles and the disease phenotype in a set of unrelated individuals can be more powerful, when the marker is less than 1 cM apart from the disease locus (30). For PsV, numerous such association studies have been performed, typically using HLA phenotypes or genotypes as markers, but non-HLA-genes like TAP or TNF have also been investigated (31, 32).

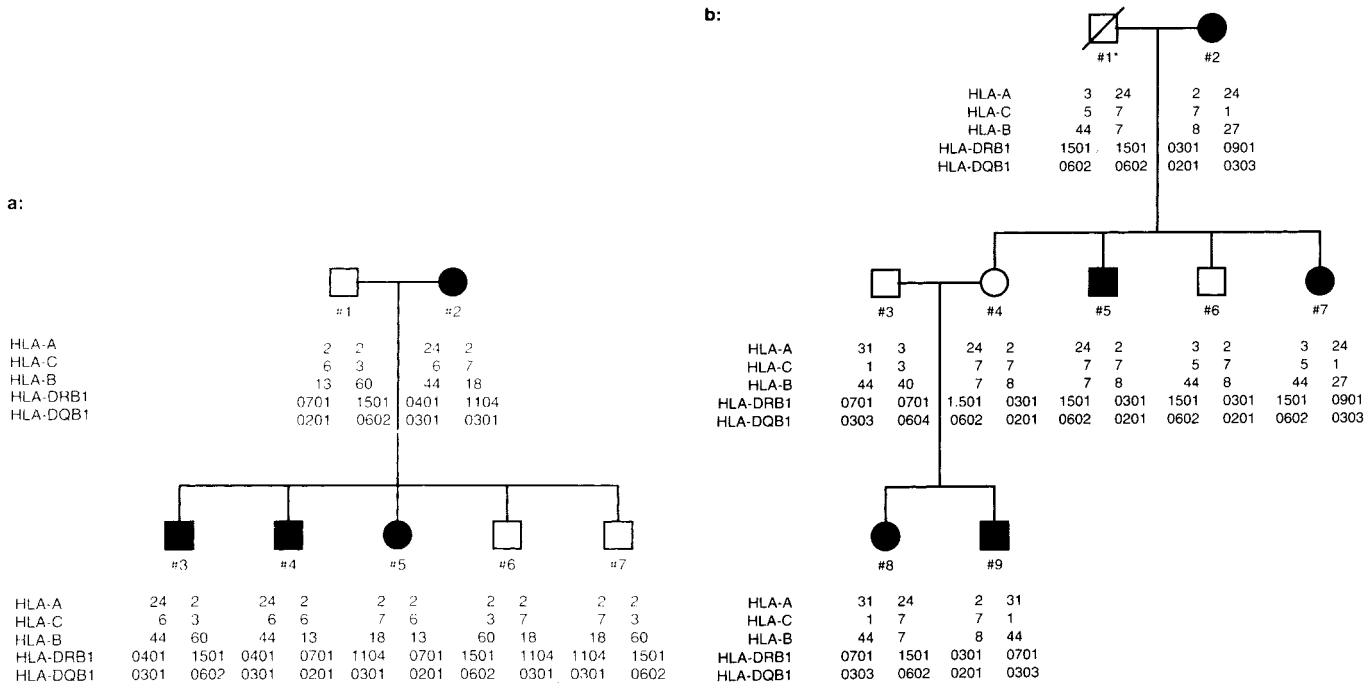

***Fig. 4.* Examples of pedigrees in which LOD score values increased after considering marker-trait linkage disequilibrium (LD).** (A). In this pedigree, linkage is unlikely without consideration of LD, since the affected offspring (#3, #4, #5) do not share at least one common HLA-C allele (or HLA haplotype) identical by descent from their affected mother (#2). Under the assumption of LD, linkage is much more likely, since there is now a higher probability that the disease allele was transmitted to individual #5 from the healthy father (#1), on the chromosomal segment containing the paternal HLA-Cw6 allele. In this pedigree, the lod score for HLA-C increased from −0.25 to 0.13 upon consideration of LD. (B). In this pedigree, linkage is unlikely without consideration of LD because one of the affected grandchildren (#8) does not share an HLA haplotype with the affected grandmother (#2), and because affected children are HLA-different (#5 and #7). In addition, this pedigree is uninformative for HLA-C because #4 is homozygous for HLA-Cw7, and therefore the phase of inheritance for the HLA-C alleles of individuals #8 and #9 is unknown. When marker-trait disqequilibrium information is used, HLA-C and HLA-DQB1 yield moderately positive results, because we have found the Cw7 and Cw1 alleles to be in weak LD, and the DQB1*0303 allele to be in moderate LD with the trait. Note that these alleles are transmitted on different haplotypes (founders #2 and #3). In this pedigree, the lod score for HLA-C increased from 0 (uninformative) to 0.03 upon consideration of disequilibrium; for HLA-DQB1 from −0.05 to 0.3. For the other loci, lod scores are negative, even when disequilibrium information is considered. (*): Not HLA-typed; the haplotypes are estimated from the HLA data of the children and the mate.

However, the identification of the genetic cause of these associations can cause extreme difficulties (33–35). Above and beyond the strong LD which exists within the MHC, proper interpretation of association studies depends strongly upon knowledge of population structure, including the possibility that the disease allele may have arisen independently on multiple occasions (36). If this were the case for PsV, each disease allele-bearing chromosome would be "marked" by its own characteristic HLA haplotype. However, there would be no guarantee that all chromosomes marked by that haplotype must carry the disease allele, unless the disease locus proves to be one of the HLA loci under study. Even if this were the case, the associated HLA allele might not be necessary or sufficient for development of disease (37). For instance, different alleles might interact with different antigens to provoke disease, multiple alleles might possess the ability to present a given antigen, or multiple alleles might be processed to yield the same antigenic peptide. Any of these examples would provide a plausible mechanism for invoking the involvement of multiple alleles at the disease locus (33, 38, 39).

In an effort to better understand the genetic cause of HLA associations in PsV, this study had two objectives. The first was to determine whether psoriasis is more tightly associated with particular HLA haplotypes than with their component alleles, because a primary association with one or more haplotypes would not consistent with the marker allele and the disease allele being identical. This is the first study to directly compare the strength of allele-specific vs. haplotype-specific associations in familial psoriasis. The second objective was to use the identities of the disease-associated haplotypes to strengthen our earlier conclusions about the location of the susceptibility locus within HLA (15). In pursuit of both objectives, we used a maximum-likelihood case-control approach to esti-

mate the likelihoods of LD between the PsV trait locus and individual HLA loci (two-locus LDA), and between the trait locus and each of four contiguous two-locus marker haplotypes (three-locus LDA). Finally, to make better use of the information content of our clinical material (40), the marker-trait haplotype frequencies derived from two-locus LDA were applied to parametric linkage analysis.

A critical element of all these analyses was the genetic model. Therefore, we first performed a case-control association analysis in order to guide us in the selection of suitable parameters for this model. In agreement with the published association data (reviewed in (8)), we found that affected founders differed significantly from local controls in the distribution of alleles at all HLA loci, most prominently for HLA-B and HLA-C. Interestingly, unaffected founders also yielded a significant association with the HLA-C locus. This observation could reflect either a recessive mode of inheritance, or a dominant model with high disease allele frequency and low penetrance. Previous family studies of HLA markers in PsV are most consistent with a dominant mode of inheritance (6, 25, 26). Therefore, we specified a dominant model with high disease allele frequency (3%) and low penetrance (30%). Under Hardy-Weinberg equilibrium, these parameters yield a disease prevalence of 1.9%, which is in good agreement with epidemiologic estimates of the prevalence of PsV in Germany (1).

Having selected the genetic model, we utilized several methods to determine whether the trait locus was more strongly associated with particular haplotypes ("haplotype-specific"), or with their component marker alleles ("allele-specific"). First, we compared the maximum likelihood estimates determined under two-locus vs. three-locus LDA. These comparisons revealed that the maximum likelihood estimates for LD between the trait locus and two-locus HLA haplotypes were much higher than the corresponding likelihoods for individual HLA loci (Fig. 1). The differences in likelihoods shown in Fig. 1 are striking, even though the three-locus LDA results were conditioned against the more restrictive null hypothesis of marker-marker association (see Fig. 1 legend). Second, we compared the likelihoods of LD between adjacent HLA loci independent and dependent of disease. The results demonstrated that LD between adjacent HLA loci was stronger in the presence of PsV (Fig. 2). This difference was highly significant (Table 4). Thus, both results strongly favored haplotype specificity.

Further support for haplotype-specificity was provided by the identities of the most strongly disease-associated alleles and haplotypes. The strongest disequilibria detected by two-locus LDA involved HLA-B and HLA-C. However, HLA-B yielded four associated alleles, whereas the only strongly-associated HLA-C allele was Cw6. Two of four HLA-B alleles, B57 and B13, were significantly associated with both HLA-Cw6 and the disease allele (Table 5); the two remaining HLA-B alleles, B8 and B44, were much less strongly associated. HLA-B57 (a component of AH 57.1), was much more prominent than B13 (a component of AH 13.1) in terms of allelic association. Consistent with these observations, the DQB1*0303 allele, which is also a component of AH 57.1, demonstrated much stronger LD than did DQB1*02, which is a component of AH 13.1. HLA-B37, which is also forms a haplotype with Cw6 (41), was not significantly associated with psoriasis in this study. However, B37 has been shown to be associated with psoriasis in Japan and in several other European studies (see references in (8)). Our own recent study of U.S. psoriatics yielded relative risks of 3.8 and 2.6 for B37 and B13 association, respectively, although neither result was statistically significant (15). Thus, it seems reasonable to assume that the Cw6-B13 and Cw6-B37 haplotypes also carry the risk allele in Caucasian populations, albeit at lower frequencies than does Cw6-B57. This situation appears to be reversed in the Japanese population, where Cw6-B37 carries the highest risk and is also linked to a different class II haplotype (i.e., DRB1*1001-DQB1*0501 in Japanese vs. DRB1*0701-DQB1*02 in Caucasians) (41). If Cw6 itself was a disease allele, one would expect all three of these haplotypes to be equally strongly associated with disease, irrespective of nationality. Clearly, this is not the case.

Our second objective was to better map the trait locus using disequilibrium techniques. All these studies favored localization to the centromeric class I region. Thus, the HLA-B and -C loci yielded much higher maximum likelihood estimates and marker-disease haplotype frequencies than did HLA-DRB1 and -DQB1 in two-locus LDA (Fig. 1), in three-locus LDA (Figs. 1 and 2), and in parametric linkage analysis (Table 6). Very similar results emerged from our earlier study of U.S. and German psoriatics (15), and in studies of recombinant ancestral haplotypes performed by others (42). For the two most common disease-associated ancestral haplotypes, AH 57.1 and AH 13.1, the disease-marker haplotype frequencies maximized in the vicinity of HLA-B and -C (Table 5). Given that the HLA-class III regions of AH 57.1 and AH 13.1 are clearly different (16), it is unlikely that a mutation would randomly occur on two haplotypes, both marked by Cw6 and DRB1*0701. It is therefore attractive to hypothesize that the segment containing the disease allele is common to both ancestral haplotypes. All our data point to the shared segment being closer to HLA-C than to HLA-DR. Furthermore, the existence of at least three HLA- B alleles in LD with the trait and with HLA-Cw6 strongly suggests that the disease allele resides telomeric to HLA-B.

Two-locus and three-locus LDA identified additional alleles at various loci characterized by weak allelic associations with the trait locus, relative to AH 57.1 and AH 13.1. These alleles could not be assigned to previously described PsV-associated haplotypes. How-

ever, many of these alleles proved to be components of the ancestral haplotype AH 8.1 (A1-B8-Cw7-DRB1*0301-DQB1*02), the most frequent ancestral haplotype in the Caucasian population (41). As can be inferred from the allelic associations in Table 5, only 2–3% of AH 8.1 haplotypes were positive for the disease allele. However, due to its high frequency, AH 8.1 is found in about one-fourth of our PsV cases, including about half of the Cw6-negative cases. This finding provides additional evidence that HLA-C is not itself the disease locus, as Cw7 is also found on several other common haplotypes not in detectable LD with the trait locus. The remaining alleles (B44, DRB1*1301/2, and DQB1 *06) detected by two-locus LDA are common in Caucasians (41) but could not be assigned to a specific ancestral haplotype. Similarly, the HLA-A/-C and HLA-B/-DRB1 haplotypes found to be in *cis* to the disease allele by three-locus LDA are found on a variety of haplotypes that are common in the healthy Caucasian population (41).

Pedigrees are recognized to provide two sources of information content about the disease allele: its pattern of transmission as well as its identity (40). To utilize the disease allele transmission information available in our pedigree data, we applied the results of two-locus LDA to parametric linkage analysis, as recently described (15). Under the usual assumption of linkage equilibrium between the marker and the trait, maximum lod scores did not exceed 2.24 ($P<0.001$). Lods of this magnitude have been interpreted as only suggestive of linkage (43), and stand in obvious contrast to the LDA results, which yielded strong evidence for a susceptibility locus despite utilizing only a portion of the available information. After defining marker-trait haplotype frequencies we observed much higher lod scores, presumably because the inclusion of LD information increases the weighting given to the likelihood obtained when the disease allele is placed in *cis* to the associated marker allele (44). In agreement with our LDA results, the largest increases in lod scores were observed for HLA-B and -C (Table 6).

Our results indicate the existence of a PsV susceptibility locus in close physical proximity to HLA-B and -C. The disease allele(s) appear to be located on different haplotypes, with very different strengths of allelic association. One hypothesis suggested by these data is that disease alleles have arisen on multiple occasions throughout evolution. Under this "multiple-origins" hypothesis, LD between disease- and HLA alleles would be a function of the age of the mutation and the recombination fraction between the trait- and HLA-loci. In the case of very old disease-predisposing mutations, the gradual accumulation of recombinants results in the fragmentation of the extended ancestral haplotype(s) upon which disease allele(s) arose, while the disease-associated haplotypes would be preserved in the vicinity of the disease locus. Mutations of intermediate age may not be old enough to have accumulated a meaningful number of recombinants, resulting in similar haplotype frequencies across the entire HLA region. Such mutations would also be predicted to display lower allelic associations, since a smaller fraction of the chromosomes carrying that ancestral haplotype carries the disease allele. The most recent mutations may have arisen independently on a large number of different chromosomes, which would tend to carry the most common haplotypes by chance alone. According to this hypothesis, the oldest PsV disease allele would have arisen on AH 13.1, and then would have been propagated to AH 57.1 when this ancestral haplotype was generated by recombination. A somewhat later mutation would have occurred on AH8.1, and multiple other relatively recent mutations would have arisen independently on a large number of different haplotypes.

While the multiple-origins hypothesis is parsimonious and therefore attractive, we cannot at present exclude other explanations. For example, disease-related peptides derived from Cw6 itself could be presented to CD4+ T cells by the DQ9 heterodimer (encoded by the AH 57.1 alleles DQB1*0303 and DQA1*0201) (45). However, this would require that Cw6 peptides be presented by DQ2 in the case of AH 13.1. Also, if this were the case, more than 50% of PsV cases would have to be explained by other class I-class II interactions. Another possible explanation is the "shared epitope" hypothesis (46, 47), in which different alleles are presumed to encode the same disease-predisposing structural determinant. Some investigators have suggested that the presence of alanine at position 73 of the in the alpha 1 helix of the HLA-C molecule might be such a determinant in PsV (48). However, we found only very weak and insignificant associations between alanine-73 and psoriasis in our previous study (15), and this hypothesis cannot readily explain the haplotype dependence of the alanine-73-positive Cw6 and Cw7 associations presented in this report. In the future, more detailed linkage disequilibrium analysis of microsatellite and/or single nucleotide polymorphisms across HLA should allow more precise testing of the multiple-origins hypothesis developed in this report.

References

1. Christophers E, Sterry W. Psoriasis. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. *Dermatology in General Medicine*. New York: McGraw-Hill, 1993: 489–514.
2. Christophers E. The immunopathology of psoriasis. *Int Arch Allergy Immunol* 1996: **110**: 199–206.
3. Schon MP, Detmar M, Parker CM. Murine psoriasis-like disorder induced by naive CD4+ T cells. *Nat Med* 1997: **3**: 183–8.
4. Boehncke WH, Dressel D, Zollner TM, Kaufmann R. Pulling the trigger on psoriasis [letter]. *Nature* 1996: **379**: 777.
5. Gottlieb SL, Gilleaudeau P, Johnson R et al. Response of psoriasis to a lymphocyte-selected toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nat Med* 1995: **1**: 442–7.
6. Russell TJ, Schultes LM, Kuban DJ. Histocompatibility (HL-A)-antigens associated with psoriasis. *N Engl J Med* 1972: **287**: 738–43.
7. White SH, Newcomer VD, Mickey MR, Terasaki PI. Disturbance of HL-A antigen frequeny in psoriasis. *N Engl J Med* 1972: **287**: 740.
8. Elder JT, Nair RP, Guo SW, Henseler T, Christophers E, Voorhees JJ. The genetics of psoriasis. *Arch Dermatol* 1994: **130**: 216–24.
9. Menssen A, Trommler P, Vollmer S et al. Evidence for an antigen-specific cellular immune response in skin lesions of patients with psoriasis vulgaris. *J Immunol* 1995: **155**: 4078–83.
10. Valdimarsson H, Baker BS, Jonsdottir I, Powles A, Fry L. Psoriasis: A T-cell-mediated autoimmune disease induced by streptococcal superantigens? *Immunol Today* 1995: **16**: 145–9.
11. Nair RP, Henseler T, Jenisch S et al. Evidence for two psoriasis susceptibility loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan. *Hum Mol Genet* 1997: **6**: 1349–56.
12. Tomfohrde J, Silverman A, Barnes R et al. Gene for familial psoriasis susceptibility mapped to the distal end of human chromosome 17q. *Science* 1994: **264**: 1141–5.
13. Trembath RC, Clough RL, Rosbotham JL et al. Identification of a major susceptibility locus on chromosome 6p and evidence for further disease loci revealed by a two stage genome-wide search in psoriasis. *Hum Mol Genet* 1997: **6**: 813–20.
14. Matthews D, Fry L, Powles A, Weissenbach J, Williamson R. Confirmation of genetic heterogeneity in familial psoriasis. *J Med Genet* 1995: **32**: 546–8.
15. Jenisch S, Henseler T, Nair RP et al. Linkage analysis of human leukocyte antigen (HLA) markers in familial psoriasis: strong disequilibrium effects provide evidence for a major determinant in the HLA-B/-C region. *Am J Hum Genet* 1998: **63**: 191–8.
16. Degli-Esposti MA, Leaver AL, Christiansen FT, Witt CS, Abraham LJ, Dawkins RL. Ancestral Haplotypes: Conserved Population MHC Haplotypes. *Hum Immunol* 1992: **34**: 242–52.
17. Nair RP, Guo SW, Jenisch S et al. Scanning chromosome 17 for psoriasis susceptibility: lack of evidence for a distal 17q locus. *Hum Hered* 1995: **45**: 219–30.
18. Darke C, Dyer P. Clinical HLA typing by cytotoxicity. In: Dyer P, Middleton D, eds. *Histocompatibility testing: A Practical Approach*. Oxford: Oxford University Press, 1993: 51–80.
19. Bunce M, Barnardo MCMN, Welsh KI. Improvements in HLA-C typing using sequence-specific primers (PCR-SSP) including definition of HLA-Cw9 and Cw10 and a new allele HLA-"Cw7/8v". *Tissue Antigens* 1994: **44**: 200–3.
20. Kimura A, Sasazuki T. Eleventh International Histocompatibility Workshop reference protocol for the HLA DNA-typing technique. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference.* Vol 1. Oxford: Oxford Science Publications, 1992: 397–419.
21. Bein G, Haase D, Schult J, Eiermann TH, Kirchner H. Semiautomated HLA-DQB1 typing by fluorescent dye photometry of amplified DNA on microtiter plates. *Hum Immunol* 1994: **39**: 1–8.
22. Sham PC, Curtis D. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum Genet* 1995: **59**: 97–105.
23. Ott J. *Analysis of Human Genetic Linkage*. 2nd edn. Baltimore: The Johns Hopkins University Press, 1991: 188–93.
24. Terwilliger JD, Ott J. *Handbook of human genetic linkage*. Baltimore: The Johns Hopkins University Press, 1994: 240–6.
25. Karvonen J, Tiilikainen A, Lassus A. HLA antigens in psoriasis: a family study. *Annals Clin Res* 1976: **8**: 298–304.
26. Civatte J, Lazarovici C, Ganas P et al. [HLA-A system in psoriasis: study of 31 families (author's transl.)]. *Ann Dermatol Venereol* 1977: **104**: 525–32.
27. Henseler T, Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. *J Am Acad Dermatol* 1985: **13**: 450–6.
28. Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci U S A* 1984: **81**: 3443–6.
29. Campbell D, Trowsdale J. A map of the human major histocompatibility complex. *Immunol Today* 1997: **18**: 43.
30. Risch N, Merikangas K. The future of genetic studies of complex human diseases [see comments]. *Science* 1996: **273**: 1516–17.
31. Fakler JW, Schmitt Egenolf M, Vejbaesya S, Boehncke WH, Sterry W, Eiermann TH. Analysis of TAP2 and HLA-DP gene polymorphism in psoriasis. *Hum Immunol* 1994: **40**: 299–302.
32. Hohler T, Kruger A, Schneider PM et al. A TNF-alpha promoter polymorphism is associated with juvenile onset psoriasis and psoriatic arthritis. *J Invest Dermatol* 1997: **109**: 562–5.
33. Thorsby E. HLA-associated disease susceptibility-which genes are primarily involved? *The Immunologist* 1995: **3/2**: 51–8.
34. Svejgaard A, Ryder LP. HLA and disease associations: Detecting the strongest association. *Tissue Antigens* 1994: **43**: 18–27.
35. Thomson G. HLA disease associations: models for the study of complex human genetic disorders. *Crit Rev Clin Lab Sci* 1995: **32**: 183–219.

36. Xiong M, Guo SW. Fine-scale genetic mapping based on linkage disequilibrium: theory and applications. *Am J Hum Genet* 1997: **60**: 1513–31.
37. Greenberg DA. Linkage analysis of "necessary" disease loci versus "susceptibility" loci. *Am J Hum Genet* 1993: **52**: 135–43.
38. She JX. Susceptibility to type I diabetes: HLA-DQ and DR revisited. *Immunol Today* 1996: **17**: 323–9.
39. Theofilopoulos AN. The basis of autoimmunity: Part I. Mechanisms of aberrant self-recognition. *Immunol Today* 1995: **16**: 90–8.
40. Ott J. Statistical properties of the haplotype relative risk. *Genet Epidemiol* 1989: **6**: 127–30.
41. Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T. Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference.* Vol 1. Oxford: Oxford Science Publications, 1992: 1065–220.
42. Schmitt-Egenolf M, Eiermann TH, Boehncke WH, Ständer M, Sterry W. Familial juvenile onset psoriasis is associated with the human leukocyte antigen (HLA) class I side of the extended haplotype Cw6-B57-DRB1*0701-DQA1*0201-DQB1*0303: A Population-And Family-Based Study. *J Invest Dermatol* 1996: **106**: 711–4.
43. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995: **11**: 241–7.
44. Clerget-Darpoux F. Bias of the estimated recombination fraction and lod score due to an association between a disease gene and a marker gene. *Ann Hum Genet* 1982: **46**: 363–72.
45. Parham P. Presentation of HLA class I-derived peptides: potential involvement in allorecognition and HLA-B27-associated arthritis. *Immunol Rev* 1996: **154**: 137–54.
46. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understand the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 1987: **30**: 1205–13.
47. Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M. Aspartic acid at position 57 of the HLA-DQ beta chain protects against type I diabetes: a family study [published erratum appears in *Proc Natl Acad Sci U S A* 1989: **86**: 1317]. *Proc Natl Acad Sci U S A* 1988: **85**: 8111–5.
48. Asahina A, Akazaki S, Nakagawa H et al. Specific nucleotide sequence of HLA-C is strongly associated with psoriasis vulgaris. *J Invest Dermatol* 1991: **97**: 254–8.