Am. J. Hum. Genet. 59:1097–1107, 1996

# Genetic Linkage of Familial Granulomatous Inflammatory Arthritis, Skin Rash, and Uveitis to Chromosome 16

G. Tromp,[1,*] H. Kuivaniemi,[1,*] S. Raphael,[3] L. Ala-Kokko,[1] A. Christiano,[2] E. Considine,[1] R. Dhulipala,[1] J. Hyland,[2] A. Jokinen,[1] S. Kivirikko,[2] R. Korn,[1] S. Madhatheri,[1] S. McCarron,[1] L. Pulkkinen,[2] H. Punnett,[3] K. Shimoya,[1] L. Spotila,[1] A. Tate,[1] and C. J. Williams[1]

Departments of [1]Biochemistry and Molecular Biology and [2]Dermatology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, and [3]Department of Pediatrics, St. Christopher's Hospital for Children and Temple University School of Medicine, Philadelphia

## Summary

**Blau syndrome (MIM 186580), first described in a large, three-generation kindred, is an autosomal, dominantly inherited disease characterized by multiorgan, tissue-specific inflammation. Its clinical phenotype includes granulomatous arthritis, skin rash, and uveitis and probably represents a subtype of a group of clinical entities referred to as "familial granulomatosis." It is the sole human model with recognizably Mendelian inheritance for a variety of multisystem inflammatory diseases affecting a significant percentage of the population. A genomewide search for the Blau susceptibility locus was undertaken after karyotypic analysis revealed no abnormalities. Sixty-two of the 74-member pedigree were genotyped with dinucleotide-repeat markers. Linkage analysis was performed under a dominant model of inheritance with reduced penetrance. The marker D16S298 gave a maximum LOD score of 3.75 at $\theta = .04$, with two-point analysis. LOD scores for flanking markers were consistent and placed the Blau susceptibility locus within the 16p12-q21 interval.**

## Introduction

An autosomal, dominantly inherited syndrome characterized by granulomatous skin rash, uveitis, and arthritis with camptodactyly was described by Blau in 1985. With further characterization of the disease, both in the large kindred originally described (Blau 1985; Raphael et al. 1993) and in other families with Blau syndrome (Pastores et al. 1990; de Chadarevian et al. 1993), the disease has been considered a subset of familial granulomatosis (S. Raphael, unpublished data). Although the disease has a variable age at onset, most patients present with symptoms during their first 2 decades of life, and disease onset is unlikely to occur after the 4th decade.

Blau syndrome represents a unique constellation of symptoms and pathological findings, which distinguishes it from a variety of classic, multisystem inflammatory diseases such as sarcoidosis and inflammatory arthridites (Raphael et al. 1993). With regard to sarcoidosis, no subject has yet manifested sarcoid-like lung disease. Likewise, the phenotypic expression of the disease does not appear to diminish with time as has been described for classic sarcoidosis. Finally, the fact that the disease is inherited in an autosomal dominant manner further distances it from the sarcoidosis disease complex.

Blau syndrome is also distinct from both juvenile- and adult-onset rheumatoid arthritis (RA). The pathological and histopathological findings of granulomatous inflammation are unique, and the presence of camptodactyly in association with arthritis is characteristic of the syndrome (Raphael et al. 1993). Although rash may be present in RA, the absence of elevated levels of IgM rheumatoid factor in patients with Blau syndrome is a distinguishing feature. It is also noteworthy that, although a few subjects of the kindred reported here had low antinuclear antibody titers, none had clinically significant titers (de Chadarevian et al. 1993). Last, Raphael et al. (1993, p. 847) reported that "segregation analysis revealed that the expression of the disease in the kindred did not segregate with any HLA haplotype."

Interestingly, the members of the Blau syndrome kindred presented here exhibit many of the disparate clinical findings reported in numerous, unrelated patients with multisystem inflammatory disease (North et al. 1970; Laxer et al. 1986). The availability of such a kindred, displaying an autosomal dominant inheritance pattern, provides a unique opportunity to define, at the molecular level, the etiology and pathogenesis of self-directed inflammation in humans.

Received January 18, 1996; accepted for publication August 5, 1996.

Address for correspondence and reprints: Dr. Charlene J. Williams, Division of Rheumatology, Department of Medicine, 508 BLSB, 233 South Tenth Street, Philadelphia, PA 19107.

*Present affiliation: Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit.



0002-9297/96/5905-0019$02.00

## Subjects, Material, and Methods

### *Family Studies and Collection of Material*

The family that was the subject of the current study included 62 participating members, 16 of whom were affected with Blau syndrome. Fifty-seven of the participating members were genotyped for the genomewide screening; the remaining five were genotyped for chromosome 16 markers only. The family is nonconsanguineous. The diagnosis of Blau syndrome was based on any one or combination of the following (fig. 1): (1) persistent inflammation of any joint(s) or tendon(s) or both, characterized by marked edema and giant cyst formation or biopsy-proved granulomatous joint inflammation or both; (2) ophthalmologist-diagnosed anterior- or posterior-tract uveitis or both, in one or both eyes at any age, in the absence of trauma or any other identifiable cause; and (3) persistent rash characterized by biopsy-proved granulomatous inflammation (Blau 1985; Raphael et al. 1993). Most affected patients were examined personally by S.R. A 10–20-ml sample of blood was obtained from each participating member, for genomic DNA extraction or establishment of lymphocyte cultures or both. The appropriate informed consent was obtained from all subjects.

### *DNA Microsatellite Marker Analyses*

DNA was extracted from whole blood or from Epstein-Barr virus–transformed lymphoblasts on an automated DNA extractor, under conditions specified by the manufacturer (GENEPURE 341; ABI). DNA samples were stored at −20°C. Polymorphic dinucleotide-repeat markers (Weber 1990; Weber and May 1991) were analyzed by PCR (Saiki et al. 1985) amplification of 30 ng of genomic DNA in a 20-μl reaction containing 30 ng (2–5 pmol) of each primer (purchased from Isogen Bioscience or Research Genetics), 1.5 mM $MgCl_2$, 250 μM of each nucleotide, and 0.5 units of *Taq* polymerase (Perkin Elmer Cetus). One of the primers was labeled at the 5′ terminus, with $\gamma$-$^{32}$P-ATP (Maniatis et al. 1982). Thermal cycling was performed in a Perkin-Elmer model 9600 thermocycler, under annealing conditions recommended for each primer. The amplified PCR products were resolved on 6% denaturing polyacrylamide gels (Sequagel-6; National Diagnostics) and were visualized by autoradiography (Maniatis et al. 1982).

### *Linkage Analyses*

Linkage analyses and simulations were performed with the FASTLINK versions (Cottingham et al. 1993; Dwarkadas et al. 1994; Schaffer et al. 1994) of the LINKAGE and SLINK packages of programs (Lathrop and Lalouel 1984; Lathrop et al. 1984; Ott 1989; Weeks et al. 1990*b*). The order and intermarker distances used were as published elsewhere (Shen et al. 1994).

## Results

### *Clinical Characterization of Blau Syndrome*

The clinical symptoms of Blau syndrome have been described in detail elsewhere (Blau 1985; Pastores et al. 1990; de Chadarevian et al. 1993; Raphael et al. 1993). In brief, affected members may present with one or more manifestations of arthritis with camptodactyly, giant synovial cysts, uveitis, and skin rash. Figure 1*A* depicts the hand and forearm of a family member with arthritis. Joint swelling is both articular and periarticular, and giant synovial cysts are evident. Usually joint swelling is not associated with destructive changes. Synovial granulomas are noncaseating and have few distinguishing features. They consist predominantly of activated T-cells and large epithelioid mononuclear cells, with occasional multinucleated giant cells (de Chadarevian et al. 1993). They are enveloped by a thin mantle of CD8-positive lymphocytes, the majority of which are also CD4 positive (de Chadarevian et al. 1993). Many granuloma cells express HLA class II antigens and interleukin-2 receptors. There is no evidence of immunoglobulin deposition within the granulomas. Figure 1*B* illustrates the severe retinal inflammation seen in an affected family member. In early stages, uveitis is primarily in the anterior tract and, in later stages, progresses to the posterior tract. Cellular infiltrates eventually become granulomatous. Most family members have required treatment with systemic or intraocular corticosteroids or both, to control their symptoms. Retinal detachment, severe glaucoma, and subsequent blindness may occur.

Skin-rash lesions are localized to the upper and mid dermis. Rash may appear episodically on any part of the body and may vary in severity (fig. 1*C*). Granulomas in the skin contain an admixture of cells, as do the synovial granulomas. For most affected members, titers for antinuclear antibodies and levels of the IgG, IgM, and IgA antibody classes were within the normal range, as were values for rheumatoid-factor production and blood-cell counts (de Chadarevian et al. 1993). There was no evidence of hypercalcemia, and Kveim skin tests for sarcoidosis were nonreactive by visual inspection (Raphael et al. 1993).

The pedigree for the Blau syndrome family that was the subject of the present study is shown in figure 2. Details concerning the variability of phenotype and age-dependent onset of symptoms are discussed below.

### *Linkage Analysis*

Initial analyses were performed with a simple affected/unaffected dominant model with a slightly reduced penetrance. A large number of unaffected individuals who were <30 years of age were classified as unknown, and therefore the model forfeited considerable power. Subsequently, a more complex model was developed that took

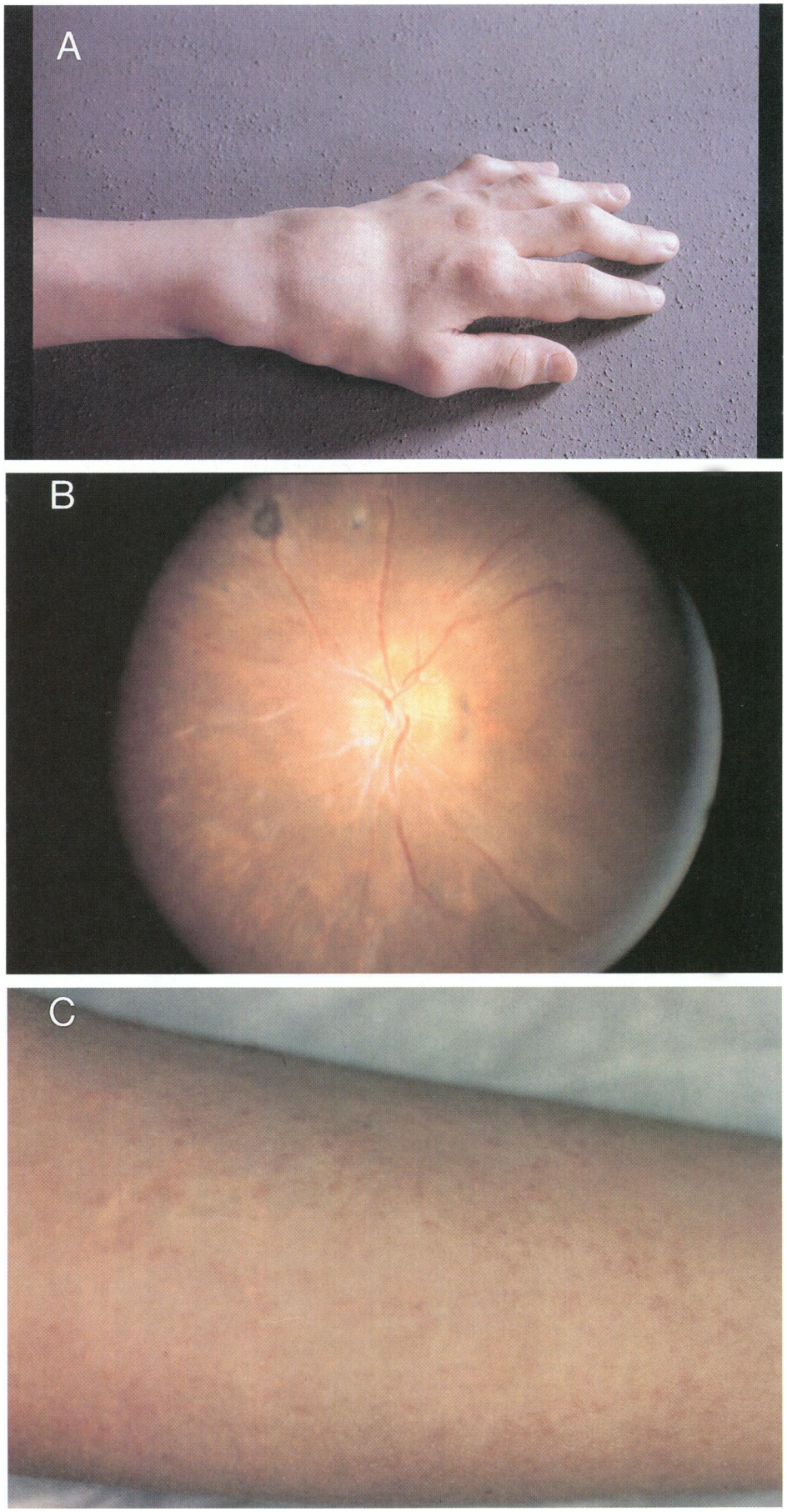


**Figure 1** Manifestations of Blau syndrome in the family. *A*, Camptodactyly and large synovial cysts in the wrist of a family member with granulomatous arthritis. *B*, Retina of the left eye of a family member with uveitis. Multifocal chorioretinal lesions, several pigmented scars, and marked perivascular sheathing are visible. *C*, Forearm of a family member with skin rash.

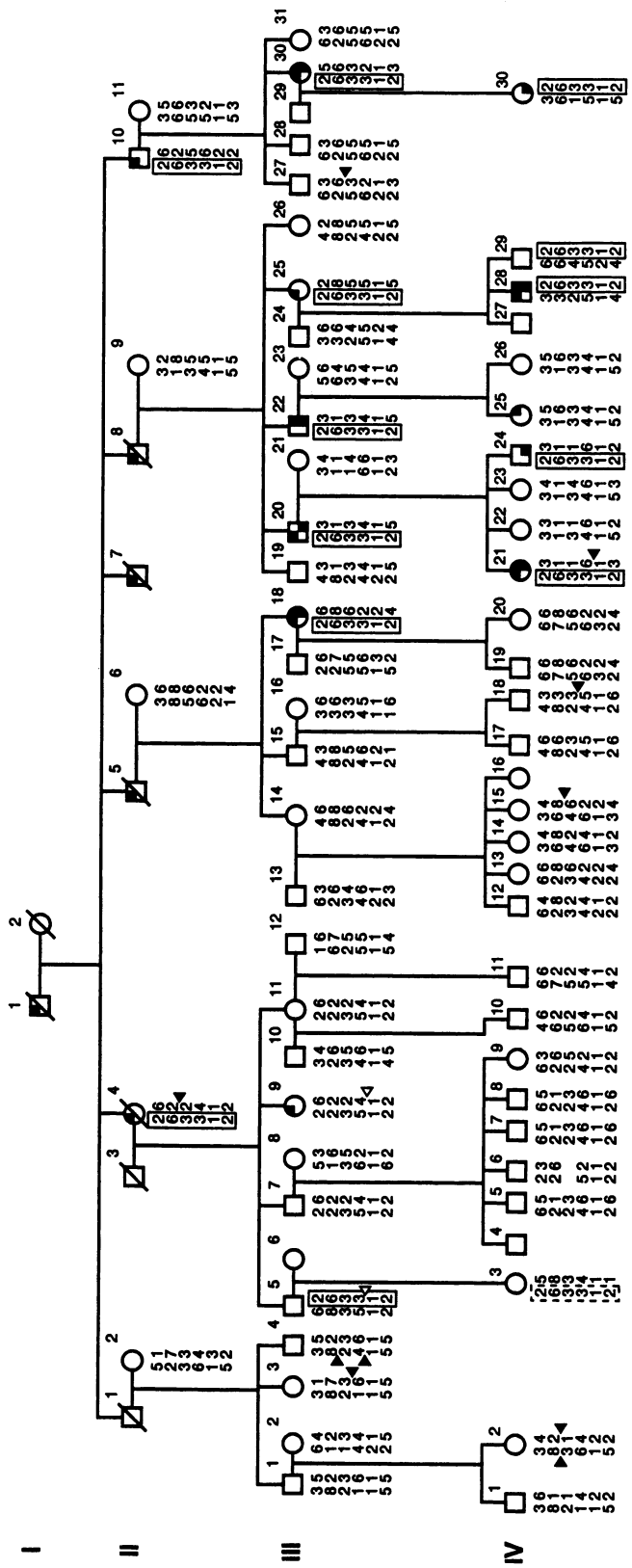


**Figure 2** Blau syndrome pedigree. Unblackened symbols are unaffected members. Partially blackened symbols denote symptoms as follows: blackened upper-left quadrant, arthritis and camptodactyly; blackened upper-right quadrant, uveitis; and blackened lower-right quadrant, skin rash. The genotypes for markers in the pericentromeric region of chromosome 16 are indicated beneath each genotyped family member. The markers are (top to bottom) D16S298, SPN, D16S411, D16S308, D16S416, and D16S419. The disease haplotype is boxed. A blackened arrowhead indicates recombination of parental alleles; and the unblackened arrowhead indicates possible recombination of parental alleles. In the nuclear family of parents II-3 and II-4, member III-5 has apparently inherited the disease haplotype and yet is unaffected in his 4th decade. Also, member III-9 is affected but has not inherited the disease haplotype. One or both may have an unobservable recombination in the region of D16S416 or D16S419. In the absence of recombination, III-5 would represent nonexpression of the phenotype, and III-9 would represent a phenocopy. A second member diagnosed as affected who did not inherit the disease haplotype is IV-25, who probably is a phenocopy. Member IV-29, who was scored as unaffected during the study, inherited the disease haplotype and possibly represented a double recombinant or incomplete penetrance. It is noteworthy that he developed granulomatous skin rash subsequent to this study. The status of member IV-30, which changed during the study, after she developed skin rash, is correctly reflected in the pedigree and in the analysis.

**Table 1**

**Age-at-Onset Classes in Pedigree**

| Age (years) | No. | Cumulative Total | Individual Designation[a] |
|---|---|---|---|
| 0–10 | 6 | 6 | III-18, III-30, IV-21, IV-24, IV-26, IV-28, IV-29[b], IV-30[c] |
| 11–15 | 5 | 11 | II-4, II-5, II-10, III-20, III-25 |
| 16–20 | 4 | 15 | I-1, II-7, II-8, III-9 |
| 21–30 | 1 | 16 | III-22 |

[a] From figure 2.

[b] Developed symptoms after the study; consequently, data on this individual were not included in those used to generate the analysis model.

[c] Developed symptoms during the study; consequently, data on this individual were not included in those used to generate the analysis model.

into account the age at onset as well as other factors, such as phenocopies and diagnostic uncertainty (see Ott 1991; Terwilliger and Ott 1994). All data presented are those obtained with the more complex model. The following facts were considered: (1) the age at onset varied considerably (table 1); (2) Blau syndrome comprises symptoms in three distinct tissue/organ systems, and some affected individuals had only one of the symptoms (fig. 2); and (3) despite strict diagnostic criteria, phenocopies exist for all the symptoms. When the study was initiated, there were 16 affected individuals; a small infant (IV-30 in fig. 2) developed symptoms during the study. The affected individuals could be grouped into five categories: those with either arthritis with camptodactyly, uveitis, or skin rash alone; those with uveitis and skin rash; and those with all three symptoms. Age-dependent penetrance was incorporated into the model by estimating the penetrance from the pedigree data. With only 16 affected individuals in the pedigree and five different categories of combinations of symptoms, the age-dependent penetrance could be estimated only on an affected/unaffected basis. The cumulative relative frequency ($F(x)$) of the four age classes—0–10, 11–15, 16–20, and 21–30 years of age (table 1)—was plotted against age, and the midpoint value of $F(x)$ for a particular class was used to compute the penetrances (penetrance = $1 - F(x)$). The estimated penetrance for the class of 21–30 years of age was adjusted up from .03 to .07, and the penetrance of the unaffecteds $\geq$31 years of age was set to .05, to allow for the possibility of a carrier showing no sign of the disease even at advanced age (table 1). In-laws were considered definitely unaffected with no probability of carrying the disease allele, on the basis of phenotype (table 1). After individual IV-30 was diagnosed as affected, her phenotypic status was changed from unaffected to affected; however, no changes were made to the model, in terms of age-dependent penetrances.

To account for phenocopies and diagnostic uncertainty, a liability class was assigned to each category of affected individual (table 2). Those with all three symptoms were considered definitely affected, with no probability of being affected without having the disease allele. The arthritis in this pedigree produced large synovial cysts and camptodactyly. RA constitutes the majority of inflammatory arthridites and, in terms of the arthritis alone, is an alternative diagnosis (Lawrence et al. 1989; Felson 1993). RA is, however, a common disorder, and few members in the pedigree were subjected to synovial biopsy to verify the granulomatous nature of the arthritis. Incidence estimates for RA vary considerably by population (O'Sullivan and Catchcart 1972; Linos et al. 1980; Aho et al. 1989; Chan et al. 1993; Felson 1993). Estimates for the outbred American population $\geq$18 years of age are as high as 60/100,000/year for females and 22/100,000/year for males (Chan et al. 1993). On the other hand, the highest prevalence of RA, ~2%, has been recorded in Finland (Aho et al. 1989; Felson 1993). For lack of cumulative incidence data, the arthritis-only category was assigned a phenocopy probability of .02—that is, a .02 probability of having arthritis while not carrying the disease allele.

All the individuals with skin rash were biopsied, and the granulomatous nature of the skin rash was verified. Nonetheless, since the skin rash in the family had to be distinguished from sarcoidosis, and since there was no single definitive criterion to distinguish between granulomatous skin rash in the family and sarcoid/sarcoidlike granulomatous skin rash, the incidence of sarcoidosis was used to establish a phenocopy probability. In the absence of cumulative incidence data, we used the simplification that incidence multiplied by life expectancy was equal to cumulative incidence. Sarcoidosis has an incidence of 6.1–10.2/100,000/year (Henke et al. 1986; Scharkoff 1987), and therefore a phenocopy probability of .00635 was allowed. Because of the location of the inflammation in uveitis, no biopsy confirmation of the nature of the eye inflammation could be obtained. The incidence of uveitis is ~17 cases/100,000/year (Baarsma 1992), and the uveitis-only category was assigned a phenocopy probability of .01275. The combination of skin rash together with uveitis occurs in ~8% of uveitis patients. Consequently, the category of uveitis and skin rash was assigned a phenocopy probability of .00128, which is ~10% of that of uveitis alone. Those individuals with all three symptoms were considered to be definitely affected. The penetrance parameters for all liability classes in the final model are shown in table 2.

Another consideration for the linkage analyses was the allele frequency of the disease and marker alleles. Blau syndrome was first described in 1985 (Blau 1985). Since then, only two families with similar symptoms have been described in the literature (Pastores et al.

**Table 2**

**Penetrance Parameters for Liability Classes of Analysis Model**

| | | | PENETRANCE OF | | |
|---|---|---|---|---|---|
| | | | | Affected Genotype | |
| | | | Unaffected Genotype: b/b | B/b | B/B |
| Unaffected individual, age: | | | | | |
| 0–10 years | | | 1 | .81 | .81 |
| 11–15 years | | | 1 | .47 | .47 |
| 16–20 years | | | 1 | .19 | .19 |
| 21–30 years | | | 1 | .07 | .07 |
| ⩾31 years | | | 1 | .05 | .05 |
| Unrelated, in-law (all ages) | | | 1 | 0 | 0 |
| Affected individual, symptoms: | | | | | |
| Arthritis | Uveitis | Skin Rash | | | |
| + | − | − | .02 | 1 | 1 |
| − | + | − | .01275 | 1 | 1 |
| − | − | + | .00635 | 1 | 1 |
| − | + | + | .00128 | 1 | 1 |
| + | + | + | 0 | 1 | 1 |

1990; de Chadarevian et al. 1993). Even if we allow for the possibily of many undiscovered families, the assumption of a disease-allele frequency of .0002, or ~4 affected individuals in 10,000, is conservative. The marker-allele frequencies were of greater concern. Because the family has origins in the Netherlands, the program ILINK (Lathrop and Lalouel 1984; Lathrop et al. 1984) was used to estimate the allele frequencies at $\theta = .5$ for each locus. For most loci there were ~30–34 alleles from the founders that furnished the estimates. There were insufficient data to treat the allele frequencies as nuisance parameters in the analyses. Comparison with known allele frequencies that were available for some loci revealed overall similarity (data not shown).

The initial search for the Blau disease susceptibility locus included analyses of polymorphic markers in and around a number of potential candidate genes, especially those of the extracellular matrix (not shown). Since HLA loci were reported to be associated with RA (Nepom and Erlich 1991), we also performed linkage analyses with previous HLA typing data (Raphael et al. 1993). In two-point analyses, one HLA locus, DRb3, gave a positive LOD score; however, all others gave a negative LOD score (table 3). All HLA loci gave negative LOD scores in multipoint analysis. After either excluding or finding no evidence for linkage at such candidate loci, we undertook a genomewide search with 240 amplifiable dinucleotide-repeat markers spread at ~10–20-cM intervals among all autosomes. The programs of the SLINK package (Ott 1989; Weeks et al. 1990*b*; also see Ott 1991; Terwilliger and Ott 1994) were used to estimate, by Monte Carlo simulations of the pedigree under the analysis model, the ELOD or expected LOD score as the average LOD score at a given $\theta$. The simulations and analyses were performed under the assumption that there were four alleles of equal frequency that yield a PIC value of .75, similar to that of many VNTR markers. The ELOD at $\theta = .1$ was 1.78 and at $\theta = .05$ was 2.54. Markers representing approximately half of the human genome were tested before a LOD score >1.78 was detected in the pericentromeric region of chromosome 16.

Two-point linkage analyses in which the above, complex model and the estimated allele frequencies of a series of chromosome 16 markers were used generated the results shown in table 3. The highest LOD score was obtained with D16S298 at $\theta = .05$ (with ILINK, the maximum LOD score [$Z_{max}$] is at $\theta = .04$). Multipoint linkage analyses were performed by LINKMAP (Lathrop and Lalouel 1984; Lathrop et al. 1984) and with order and intermarker distances as published elsewhere (Shen et al. 1994). Data from a series of analyses with the disease locus versus three marker loci were plotted (fig. 3). The support interval was calculated by subtracting 1 from the $Z_{max}$. By the $Z_{max} - 1$ criterion there was support for linkage over a region of ~14.3 cM, ~10.5 cM p-terminal and ~3.8 cM q-terminal of the centromere. The region of support on the p arm extends from ~6.2 cM p-terminal to ~4.3 cM centromeric of D16S298, and the region on the q arm extends

## Table 3

**Two-Point LOD Scores for Loci on Chromosome 16 and in HLA Locus on Chromosome 6**

| | LOD Score at θ = | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Locus | 0 | .001 | .01 | .05 | .10 | .20 | .30 | .40 |
| HABP1 | −∞ | −7.839 | −4.493 | −1.879 | −.821 | −.017 | .189 | .147 |
| D16S292 | −∞ | −8.600 | −6.198 | −3.766 | −2.311 | −.754 | −.111 | .051 |
| D16S294 | −2.264 | −2.134 | −1.484 | −.565 | −.173 | .084 | .104 | .053 |
| D16S420 | −∞ | −6.561 | −3.886 | −1.236 | −.082 | .693 | .687 | .299 |
| D16S401 | −3.298 | −3.234 | −2.872 | −2.083 | −1.407 | −.631 | −.235 | −.041 |
| D16S409 | −.506 | −.476 | −.274 | .096 | .260 | .340 | .291 | .173 |
| D16S298 | 3.446 | 3.471 | 3.619 | <u>3.738</u> | 3.556 | 2.863 | 1.938 | .852 |
| SPN | 1.422 | 1.473 | 1.820 | 2.454 | 2.621 | 2.327 | 1.659 | .773 |
| D16S411 | 2.217 | 2.228 | 2.306 | 2.453 | 2.410 | 2.007 | 1.381 | .613 |
| D16S308 | .131 | .191 | .611 | 1.470 | 1.825 | 1.785 | 1.290 | .569 |
| D16S416 | 1.989 | 1.986 | 1.955 | 1.816 | 1.634 | 1.241 | .800 | .313 |
| D16S419 | 2.210 | 2.206 | 2.170 | 2.007 | 1.798 | 1.358 | .887 | .411 |
| D16S408 | −2.771 | −2.672 | −2.038 | −.647 | .121 | .655 | .635 | .321 |
| D16S400 | −∞ | −3.675 | −2.330 | −1.031 | −.459 | −.041 | .032 | −.008 |
| D16S265 | −5.850 | −1.632 | −.264 | 1.116 | 1.630 | 1.725 | 1.309 | .620 |
| D16S301 | −6.691 | −4.059 | −2.861 | −1.533 | −.833 | −.245 | −.054 | −.010 |
| D16S266 | 1.303 | 1.306 | 1.333 | 1.366 | 1.315 | 1.078 | .726 | .291 |
| D16S305 | −∞ | −8.793 | −6.265 | −3.759 | −2.464 | −1.154 | −.486 | −.138 |
| HLA A | −4.719 | −3.440 | −1.990 | −.718 | −.140 | .301 | .346 | .210 |
| HLA B | −10.528 | −5.931 | −4.667 | −2.203 | −1.069 | −.178 | .111 | .142 |
| HLA Bw | −3.447 | −1.305 | −.334 | .245 | .395 | .389 | .251 | .085 |
| HLA C | −11.403 | −6.168 | −3.403 | −1.249 | −.423 | .140 | .236 | .167 |
| HLA DRβ1 | −4.012 | −2.327 | −1.273 | −.431 | −.084 | .192 | .265 | .188 |
| HLA DRβ3 | .893 | .891 | .869 | .774 | .655 | .421 | .211 | .058 |
| HLA DQα1 | −3.936 | −2.243 | −1.189 | −.349 | −.012 | .224 | .268 | .186 |
| HLA DQβ1 | −4.811 | −3.120 | −2.055 | −1.160 | −.750 | −.348 | −.128 | −.024 |
| HLA DPα1 | −2.954 | −1.545 | −.561 | .113 | .349 | .446 | .351 | .175 |
| HLA DPβ1 | −10.528 | −7.715 | −4.993 | −2.191 | −1.051 | −.151 | .137 | .154 |

Note.—The highest LOD score obtained is underlined.

from ~0.9 cM centromeric to ~2.9 cM q-terminal of the marker D16S416. The most likely location of the Blau syndrome locus was close to D16S298.

To verify that the two-point $Z_{max}$ of 3.75 at θ = .04 for D16S298 was not due to either modeling errors or biases in data collection, scrutiny, or maximization over two models, simulations were performed with the complex model shown in table 2 and the ILINK estimates of allele frequencies for D16S298, under the assumption of absence of linkage in one series and presence of linkage in another. In the absence of linkage, analysis of the same 100,000 replicates once under each model yielded 12 LOD scores >3.70. The empirical *P* value for the observed linkage is .00012. Several considerations arose when we interpreted the empirical *P* value. First, with what statistic should it be compared for significance? Although the theory of likelihood-ratio tests predicts that, asymptotically, in the absence of linkage, 2log10 $Z_{max}$ follows a $\chi^2$ distribution with 1 df, the distribution can be quite different, depending on the pedigree structure (Ott 1991). It has been shown that the significance, α, is equal to the probability that $Z_{max}$ is greater or equal to a critical LOD score, $Z_0$, and that the latter probability is $\leq 10^{-z^0}$ (see Ott 1991), which provides a statistic against which to compare our empirical *P* value. Second, >240 markers were tested in the genome screen, and therefore the significance would have to be adjusted for multiple tests. It previously had been shown that the correction for the multiple tests from multiple markers is not equivalent to the Bonferroni correction, since many of the markers are not independent but linked (Lander and Botstein 1989; Lander and Kruglyak 1995). After applying the suggested correction (Lander and Botstein 1989; Lander and Kruglyak 1995) while allowing for a genome length of 33 Morgans in 22 autosomes, we found that a LOD score of ~3.55 is necessary for an overall significance of .03 (or a LOD score of slightly >3.3 for an overall significance of .05). Third, simulation will asymptotically approach the true *P* value for the model, in a large number of replicates. If linkage is not a chance event, then one expects very few LOD scores greater than $Z_{max}$, which should exceed $Z_0$. Since LOD scores greater than $Z_{max}$ are or should be very rare events,

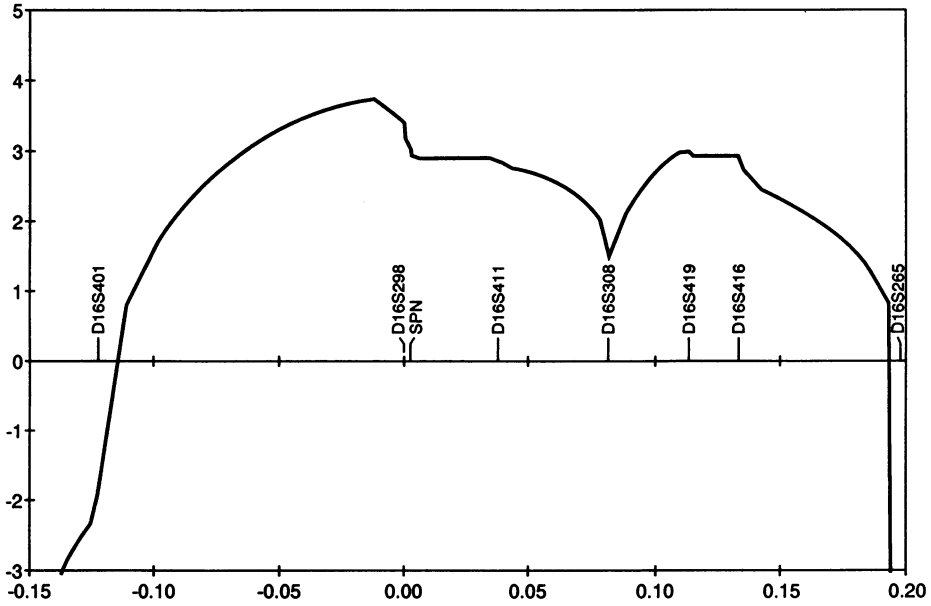


**Figure 3** Results of multipoint analysis of the Blau disease susceptibility gene versus a series of markers on chromosome 16. The graph depicts the location scores (multipoint LOD scores), on the *Y*-axis, versus the map distance (in cM [Kosambi]), on the *X*-axis.

there is considerable error in the estimate of *P;* consequently, it is imperative that not only the *P* value but also the upper bound of a confidence interval for the *P* value be $\leq 10^{-z_0}$.

Taking these considerations into account, we chose $Z_0$ as 3.55, the LOD score required for proof of linkage at an overall significance of .03 (Lander and Kruglyak 1995). A confidence interval with a 97% confidence coefficient was constructed (Ott 1991) for 12 of 100,000 observations ($P = .00012$); and the interval had an upper bound of .0002057. The upper bound does not overlap with the formal $P = .0002987$ ($10^{-3.55}$) that is considered evidence of linkage at an overall significance of .03. Since, under the simulation and analysis using both models, obtaining a LOD score of $\geq 3.70$ was so rare an event that the upper bound of the confidence interval did not overlap with the formal criterion, the linkage observed with D16S298 can be considered significant.

The simulations assuming the presence of linkage were performed such that the simulated marker locus was inherited in the manner of the haplotypes in figure 2. A total of 111 of 2,000 replicates yielded a LOD score >3.70. Analysis of the effect of altering the penetrances and phenocopy probabilities indicated that the number of replicates with a LOD score >3.70 could be increased to 988 by decreasing the age at onset, thereby effectively increasing the informativeness of unaffected individuals, and by increasing the phenocopy probabilities for the arthritis-only and uveitis-only classes (table 2), thereby decreasing the effect of recombinant individuals III-9 and IV-25. Scrutiny of recombinants has an effect similar to reclassification of phenotypes or varying diagnostic schemes, in that it can resolve recombinants. Reclassification of individuals III-5, III-9, and IV-25 (fig. 2) increased the number of replicates with LOD scores >3.70 to 1,482 when the complex model for the analysis was used (without altering age-at-onset penetrances or phenocopy probabilities). Reclassifying only individual IV-29 to affected in the appropriate class yielded 287 replicates with a LOD score of >3.70. These results of reclassification of individuals' phenotypes were expected, since it removes recombinants; and it was shown that varying diagnostic schemes had a more profound effect on results than did varying model parameters (Weeks et al. 1990*a*).

Maximization of the LOD score by either fitting the model parameters to the data or reclassifying individuals or both would have resulted in a much higher $Z_{max}$. The number of replicates with a LOD score greater than the $Z_{max}$ under a maximized model assuming the presence of linkage would become a rare event similar to the rare event of observing such a high LOD score in the absence of linkage. Rarely observing replicates that exceed $Z_{max}$ could be an indication that the analysis is biased. The simulations thus served to verify that the model used was plausible and that the results were not biased by fitting the model to the data, by preferential scrutiny of recombinants, or by maximization over two models.

## Discussion

Parametric linkage analyses are influenced by the parameter estimates used for the analysis models. It has been shown, however, that misspecification of the model will not result in failure to detect linkage, if it exists,

although it will lead to overestimation of the recombination fraction (Clerget-Darpoux et al. 1986; Risch and Giuffra 1990, 1992; also see Ott 1991; Terwilliger and Ott 1994). Great care therefore was taken in establishing the analysis model for Blau syndrome. The model used to analyze linkage in the Blau syndrome pedigree took into account initial considerations regarding phenotypic variability and age-at-onset differences among affected members. The first consideration was addressed by assigning each category of affected individual to a separate liability class with phenocopy probability appropriate to how well the symptoms could be differentiated from similar symptoms in the general population. Therefore, an individual with only arthritis was considered 50 times more likely to be carrying the Blau syndrome allele than to have arthritis due to some other cause (calculated as the ratio of B/b penetrance over b/b penetrance; see table 2). On the other hand, an individual with only skin rash was considered 157 times more likely to be carrying the Blau syndrome allele than not to be carrying it. The second consideration, variable age at onset, was addressed by assigning five age classes, with the youngest unaffecteds being only ~1.25 times as likely not to be carrying the disease allele as to be carrying it. Those >30 years of age were 20 times more likely not to be carrying the disease allele. The family has origins in the Netherlands but with admixture from the North American population. The concern was, therefore, that the allele frequencies would be different both from the available frequencies and from the frequencies determined from a control group, either North American or Netherlands. Therefore, allele-frequency estimates for each locus were derived by the program ILINK.

Our data are consistent with a disease susceptibility locus in the pericentromeric region of chromosome 16, but they cannot exclude the possibility that an additional locus may exist that is permissive or modulatory. In light of the reported association between the HLA-DR4 locus and a number of inflammatory arthridites (Jabs et al. 1985; Gao et al. 1990; Nepom and Erlich 1991; Weyand et al. 1992*b*) and granulomatous arteritis (Weyand et al. 1992*a*), we undertook linkage analysis of the HLA locus in the present family. The data reported in table 3 clearly indicate that no single HLA locus or haplotype is linked to the disease. Multilocus analyses decreased the LOD scores for all HLA loci. It is, nonetheless, possible that one or more HLA haplotypes may be permissive for the development of Blau syndrome (Greenberg 1993). Within the scope of the current study we did not have sufficient independent individuals to test for such a relationship.

Blau syndrome is a subset of a larger family of granulomatoses that demonstrate some of the classical inflammatory features of other autoimmune diseases, such as RA, as well as other nonautoimmune inflammatory diseases, such as sarcoidosis. It is the only human model of inherited, multisystem inflammatory disease; as such, it provides a unique and important opportunity to define a genetic defect and the mechanism that is responsible for tissue-specific, self-directed inflammation. In light of what is currently known about the immunobiology of the disease, the product of a putative disease-susceptibility gene is likely to be a component of the extra- or pericellular matrix, whose alteration may force the breakdown of self-tolerance and, ultimately, may lead to the development of autoimmune inflammation. Potential candidates in this category that map in the Blau interval include sialophorin (also known as "CD43"), the major sialoglycoprotein on the surface of a variety of cell types, including granulocytes (Bazil and Strominger 1993), and the CD11 integrin complex (Corbi et al. 1988), which includes the α-subunit of the lymphocyte function–associated antigen and the leukocyte complement receptor type 3, both of which are involved in cell adhesion functions. Additional candidates are CD19 (Maloney and Lingwood 1994), involved in B-lymphocyte function, and the interleukin-4 receptor (IL4R; Pritchard et al. 1991). The ligand for IL4R is the pleiotropic cytokine IL4, which counts among its biological functions the regulation of isotype switching in immunoglobulin molecules and, therefore, has been implicated in a variety of autoimmune, allergic, and inflammatory responses (Beckmann et al. 1992, and references therein). An examination of positional candidates, a positional cloning effort, or both, to identify the disease-susceptibility gene, will benefit from fine mapping with additional Blau syndrome families.

## Acknowledgments

The authors wish to thank the family members for their cooperation. The authors are also grateful to Drs. D. J. Prockop and J. Uitto, for allowing some of this work to be performed in their laboratories, and to Dr. S. Hsu, for assistance with HLA haplotype and allele frequencies. This work was supported by the Thomas Jefferson University Dean's Fund, the St. Christopher's Hospital Fund for Academic Development, and a generous grant from the Arthritis Foundation.

## References

Aho K, Heliovaara M, Sievers K, Maatela J, Isomäki H (1989) Clinical arthritis associated with positive radiological and serological findings in Finnish adults. Rheumatol Int 9:7–11

Baarsma GS (1992) The epidemiology and genetics of endogenous uveitis: a review. Curr Eye Res Suppl 2:1–9

Bazil V, Strominger JL (1993) CD43, the major sialoglycoprotein of human leukocytes, is proteolytically cleaved from the

surface of stimulated lymphocytes and granulocytes. Proc Natl Acad Sci USA 90:3792–3796

Beckmann MP, Cosman D, Fanslow W, Maliszewski CR, Lyman SD (1992) The interleukin-4 receptor: structure, function, and signal transduction. Chem Immunol 51:107–134

Blau EB (1985) Familial granulomatous arthritis, iritis, and rash. J Pediatr 107:689–693

Chan KW, Felson DT, Yood RA, Walker AM (1993) Incidence of rheumatoid arthritis in central Massachusetts. Arthritis Rheum 36:1691–1696

Clerget-Darpoux F, Bonaiti-Pellie C, Hochez J (1986) Effects of misspecifying genetic parameters in lod score analysis. Biometrics 42:393–399

Corbi AL, Larson RS, Kishimoto TK, Springer TA, Morton CC (1988) Chromosomal location of the genes encoding the leukocyte adhesion receptors LFA-1, Mac-1 and p150.95: identification of a gene cluster involved in cell adhesion. J Exp Med 167:1597–1607

Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53:252–263

de Chadarevian J-P, Raphael SA, Murphy GF (1993) Histologic, ultrastructural, and immunocytochemical features of the granulomas seen in a child with the syndrome of familial granulomatous arthritis, uveitis, and rash. Arch Pathol Lab Med 117:1050–1052

Dwarkadas S, Schaffer AA, Cottingham RW Jr, Cox AL, Keleher P, Zwaenepoel W (1994) Parallelization of general linkage analysis problems. Hum Hered 44:121–127

Felson DT (1993) Epidemiology of the rheumatic diseases, In: McCarthy DJ, Koopman WJ (eds) Arthritis and allied conditions, 12th ed. Lea & Febiger, Philadelphia, pp 17–47

Gao X, Olsen NJ, Pincus T, Stastny P (1990) HLA-DR alleles with naturally occurring amino acid substitutions and risk for development of rheumatoid arthritis. Arthritis Rheum 33:936–946

Greenberg DA (1993) Linkage analysis of "necessary" disease loci versus "susceptibility" loci. Am J Hum Genet 52:135–143

Henke CE, Henke G, Elveback LR, Beard CM, Ballard DJ, Kurland LT (1986) The epidemiology of sarcoidosis in Rochester, Minnesota: a population-based study of incidence and survival. Am J Epidemiol 123:840–845

Jabs DA, Houk LJ, Bias WB, Arnett FC (1985) Familial granulomatous synovitis, uveitis and cranial neuropathies. Am J Med 78:801–804

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

Lathrop GM, Lalouel JM (1984) Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 36:460–465

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lawrence RC, Hochberg MC, Kelsey JL, McDuffie FC, Medsger TA Jr, Felts WR, Shulman LE (1989) Estimates of the prevalence of selected arthritic and musculoskeletal diseases in the United States. J Rheumatol 16:427–441

Laxer RM, Cameron BJ, Chaisson D, Smith LR, Stein LD (1986) The camptodactyly-arthropathy-pericarditis syndrome: case report and literature review. Arthritis Rheum 29:439–444

Linos A, Worthington JW, O'Fallon WM, Kurland LT (1980) The epidemiology of rheumatoid arthritis in Rochester Minnesota: a study of incidence, prevalence and mortality. Am J Epidemiol 111:87–98

Maloney MD, Lingwood CA (1994) CD19 has a potential CD77 (globotriaosyl ceramide)-binding site with a sequence similarity to verotoxin B-subunits: implication of molecular mimicry for B cell adhesion and enterohemorrhagic *E. coli* pathogenesis. J Exp Med 180:191–201

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Nepom GT, Erlich H (1991) MHC class-II molecules and autoimmunity. Annu Rev Immunol 9:493–525

North AF Jr, Fink CW, Gibson WM, Levinson JE, Schuchter SL, Howard WK, Johnson NH, et al (1970) Sarcoid arthritis in children. Am J Med 48:449–455

O'Sullivan JB, Catchcart ES (1972) The prevalence of rheumatoid arthritis: follow-up evaluation of the effect of criteria on rates in Sudbury, Massachusetts. Ann Intern Med 76: 573–577

Ott J (1989) Computer simulation methods in human linkage analysis. Proc Natl Acad Sci USA 86:4175–4178

——— (1991) Analysis of human genetic linkage. Johns Hopkins University Press, Baltimore

Pastores GM, Michels VV, Stickler GB, Su WPD, Nelson AM, Bovenmyer DA (1990) Autosomal dominant granulomatous arthritis, uveitis, skin rash, and synovial cysts. J Pediatr 117: 403–408

Pritchard MA, Baker E, Whitmore SA, Sutherland GR, Idzerda RL, Park LS, Cosman D, et al (1991) The interleukin 4 receptor gene (IL4R) maps to 16p11.2-16p12.1 in human and to the distal region of mouse chromosome 7. Genomics 10:801–806

Raphael SA, Blau EB, Zhang WH, Hsu SH (1993) Analysis of a large kindred with Blau syndrome for HLA, autoimmunity, and sarcoidosis. Am J Dis Child 147:842–848

Risch N, Giuffra L (1990) Multipoint linkage analysis of genetically complex traits. Am J Hum Genet Suppl 47:A197

——— (1992) Model misspecification and multipoint linkage analysis. Hum Hered 42:77–92

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354

Schaffer AA, Gupta SK, Shiriram K, Cottingham RW Jr (1994) Avoiding recomputation in linkage analysis. Hum Hered 44:225–237

Scharkoff T (1987) Apropos of the present level of epidemiologic knowledge on sarcoidosis. Sarcoidosis 4:152–154

Shen Y, Kozman HM, Thompson A, Phillips HA, Holman K, Nancarrow J, Lane S, et al (1994) A PCR-based genetic linkage map of human chromosome 16. Genomics 22:68–78

Terwilliger JD, Ott J (1994) Handbook of genetic linkage. Johns Hopkins University Press, Baltimore

Weber L (1990) Informativeness of human $(dC\text{-}dA)_n/(dG\text{-}dT)_n$ polymorphisms. Genomics 7:524–530

Weber JL, May PE (1991) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Weeks DE, Lehner T, Squires-Wheeler E, Kaufmann C, Ott J (1990*a*) Measuring the inflation of the lod score due to its maximization over model parameter values in human genetic linkage analysis. Genet Epidemiol 7:237–243

Weeks DE, Ott J, Lathrop GM (1990*b*) SLINK, a general simulation program for linkage analysis. Am J Hum Genet Suppl 47:A204

Weyand CM, Hicok KC, Hunder GG, Goronzy JJ (1992*a*) The HLA-DRB1 locus as a genetic component in giant cell arteritis: mapping of a disease-linked sequence motif to the antigen binding site of the HLA-DR molecule. J Clin Invest 90:2355–2361

Weyand CM, Xie C, Goronzy JJ (1992*b*) Homozygosity for the HLA-DRB1 allele selects for extraarticular manifestations in rheumatoid arthritis. J Clin Invest 89:2033–2039